RESISTANCE TRAINING AND MUSCLE-BRAIN CROSSTALK

Implications for Cognitive Decline in Aging and Spinal Cord Injury

W.A.J. VINTS

ISBN: 978-94-6522-058-1

Cover design and lay-out: © evelienjagtman.com
Printing: Ridderprint

Printing of this thesis was kindly supported by Adelante Zorggroep, Livit Ottobock Care, Hanssen Footcare, and Heckert & van Lierop.

RESISTANCE TRAINING AND MUSCLE-BRAIN CROSSTALK

Implications for Cognitive Decline in Aging and Spinal Cord Injury

DISSERTATION

to obtain the degree of Doctor at Maastricht University
on the authority of the Rector Magnificus Prof. Dr. Pamela Habibović
and to obtain the degree of Doctor in Natural Sciences, Biology at the Lithuanian Sports University
on the authority of the Rector Magnificus Prof. Diana Rėklaitienė
in accordance with the decision of the College of Deans,
to be defended in public
on Thursday 22nd of May, 2025 at 4.00 PM in Maastricht, the Netherlands
and on Tuesday 27th of May, 2025 at 12.00 PM in Kaunas, Lithuania

by

Wouter Arthur Johan Vints

2025

The research in this thesis was conducted in 2020-2024 under the Regulations of the Double Doctoral Programme of the Lithuanian Sports University, Department of Health Promotion and Rehabilitation and University of Tartu and Maastricht University, Care and Public Health Research Institute CAPHRI, Department of Rehabilitation Medicine. CAPHRI participates in the Netherlands School of Primary Care Research CaRe.

Supervisors
Prof. N. Masiulis, Lithuanian Sports University (LSU)
Prof. Dr. J.A. Verbunt, Maastricht University (UM)

Co-supervisors
Prof. O. Levin, Lithuanian Sports University (LSU)/KU Leuven
Dr. C.C.M. van Laake-Geelen, Adelante Hoensbroek

Assessment Committee Maastricht University
Prof. Dr. R. Smeets, Maastricht University, Maastricht, the Netherlands, chair
Prof. K. Meijer, Maastricht University, Maastricht, the Netherlands
Prof. A. Ratkevicius, Lithuanian Sports University, Kaunas, Lithuania
Dr. J. Stolwijk, De Hoogstraat Revalidatie, Utrecht, The Netherlands
Prof. C. Voelcker-Rehage, University of Münster, Münster, Germany

Assessment Committee Lithuanian Sports University
Prof. Tomas Venckūnas, Lithuanian Sports University, Natural Sciences, Biology - N 010, chair
Prof. Priit Kaasik, University of Tartu, Natural Sciences, Biology - N 010
Prof. Osvaldas Rukšėnas, Vilnius University, Natural Sciences, Biology - N 004
Prof. Džilda Veličkienė, Lithuanian University of Health Sciences, Medical and Health Sciences, Medicine M 001
Assoc. Prof. Dr. Miglė Bacevičienė, Lithuanian Sports University, Medical and Health Sciences, Public Health - M 004

The work in this thesis was partially supported by grants from the Lithuanian Research Council (Lietuvos Mokslo Taryba), grant numbers: S-SEN-20-5 and S-MIP-21-37.

The dissertation is available at the library of the Lithuanian Sports University and Maastricht University.

Address: Sporto street 6, LT-44221 Kaunas, Lithuania
Address: Grote Looiersstraat 17, 6211 JH Maastricht, the Netherlands

LIETUVOS SPORTO UNIVERSITETAS

Wouter Arthur Johan Vints

JĖGOS TRENIRAVIMAS IR RAUMENŲ-SMEGENŲ SĄVEIKA

SMEGENŲ SILPIMO PASEKMĖS SENĖJIMO IR NUGAROS SMEGENŲ SUŽALOJIMO ATVEJAIS

Daktaro disertacija
Gamtos mokslai, biologija (N 010)

KAUNAS 2025

Disertacija rengta 2020–2024 metais Lietuvos sporto universitete ir Mastrichto universitete (nyderlandų kalba – *Universiteit Maastricht*, UM) pagal Lietuvos sporto universiteto (LSU) ir Tartu universiteto bendros biologijos mokslo krypties doktorantūros reglamentą ir Mastrichto universiteto Visuomenės sveikatos tyrimų dvigubos doktorantūros studijų nuostatus. CAPHRI (angl. *Care and Public Health Research Institute*, liet. *Sveikatos priežiūros ir visuomenės sveikatos tyrimų institutas*), Reabilitacinės medicinos skyrius.

Moksliniai vadovai
prof. N. Masiulis (Lietuvos sporto universitetas, gamtos mokslai, biologija – N 010)
prof. dr. J. A. Verbunt (Mastrichto universitetas, CAPHRI, reabilitacinė medicina)

Moksliniai konsultantai
prof. O. Levin (Lietuvos sporto universitetas, gamtos mokslai, biologija – N 010)
dr. C. C. M. van Laake-Geelen (Adelante Hoensbroek, reabilitacinė medicina)

Disertacijos gynimo taryba UM
Pirmininkas:
prof. Dr. R. Smeets (Mastrichto universitetas, Mastrichtas, Nyderlandai)

Nariai:
prof. K. Meijer (Mastrichto universitetas, Mastrichtas, Nyderlandai)
prof. A. Ratkevicius (Lietuvos sporto universitetas, Kaunas, Lietuva)
dr. J. Stolwijk (De Hoogstraat Revalidatie, Utrechtas, Nyderlandai)
prof. C. Voelcker-Rehage (Miunsterio universitetas, Miunsteris, Vokietija)

Disertacijos gynimo taryba LSU
Pirmininkas:
Prof. dr. Tomas Venckūnas (Lietuvos sporto universitetas, Gamtos mokslai, biologija N010)

Nariai:
Prof. dr. Priit Kaasik (Tartu universitetas, Gamtos mokslai, biologija N010).
Prof. dr. Osvaldas Rukšėnas (Vilniaus universitetas, gamtos mokslai, biologija N010)
Prof. dr. Džilda Veličkienė (Lietuvos sveikatos mokslų universitetas, medicinos ir sveikatos mokslai, medicina M001)
Doc. dr. Miglė Bacevičienė (Lietuvos sporto universitetas, Medicinos ir sveikatos mokslai, visuomenės sveikata M004)

Šio darbo tyrimai buvo iš dalies finansuoti Lietuvos mokslo tarybos (LMT), dotacijų numeriai: S-SEN-20-5 ir S-MIP-21-37.

Su disertacija galima susipažinti Lietuvos sporto universiteto ir Mastrichto universiteto bibliotekose.

Adresas: Sporto g. 6, 44221 Kaunas, Lietuva.
Adresas: Grote Looiersstraat 17, 6211 JH Mastrichtas, Nyderlandai.

TABLE OF CONTENTS

GENERAL INTRODUCTION

1. Muscle-brain crosstalk

The brain is constantly receiving and sending messages from and towards the body. The primary route of communication is through the central and peripheral nervous systems. For example, the brain can order the muscle to contract (e.g., via descending signals from brain areas involved in motor control) and the muscle can send the brain feedback about how much it is contracted and how it is positioned (e.g., via ascending signal from muscle spindles) [1]. Nervous system messages are carried along the length of neurons as electrical waves and from one neuron to the next as a chemical signal to reach their destination in a matter of milliseconds [1][2]. The junctions between two neurons are called synapses and the chemical signals that are being transmitted between synapses are called neurotransmitters [1]. For a chemical response to create a new electrical signal in the next neuron, the release of neurotransmitters needs to be sufficiently large or the neuron receiving this chemical signal needs to be sufficiently responsive. Interestingly, the central nervous system has the ability to adapt and learn by altering its responsiveness to a specific signal (i.e. by long-term synaptic potentiation) and by adding new and removing old neuronal connections (i.e. by synaptogenesis, which is the formation of new synapses on an existing neuron, or by neurogenesis, which is the formation of new neurons). These alterations in neuronal connections and their responsiveness are called neuroplasticity (see Figure 1) [3].

Another route of communication between body and brain is via the blood circulation. Only specific blood factors can enter and/or leave the brain, either because they are sufficiently small to diffuse through the blood-brain barrier or because they make use of transporters or receptor-mediated endocytosis [4]. These blood factors can originate from any bodily organ, including muscle cells, liver tissue, the gastrointestinal tract, fat tissue, bone cells and peripheral nervous tissue [5]. Within the brain, these blood factors can bind to receptors that are expressed by cells in the central nervous system and activate specific signaling pathways, causing brain cells to respond or change their behavior [6]. This way, these blood factors can either promote or hinder an optimal neural environment for neuroplastic processes, thereby enhancing or reducing the brain's adaptability [5].

Of particular interest for this dissertation, is the blood-bound communication from the muscle towards the brain, as this is considered to play a role in the beneficial effect of physical exercise on brain health and cognitive function [7]. The muscle-derived factors, released during and following physical exercise, are called myokines [8][9]. A research article published in 2019 suggested that these myokines can facilitate neuroplasticity within the brain [9]. With this 'myokine hypothesis', a whole new research field emerged with the goal to better understand the relationship between physical exercise and cognitive function. Recently, it was discovered that other bodily systems also release

blood factors in response to physical exercise, which signal other systems, including the brain. As a result, it is now more accurate to refer to this as the 'exerkine hypothesis'. Exerkines are all peptides, metabolites, and nucleic acids released in to the bloodstream during and following physical exercise, including myokines (muscle tissue), hepatokines (liver tissue), adipokines (fat tissue), osteokines (bone tissue) and neurokines (nervous tissue) [5].

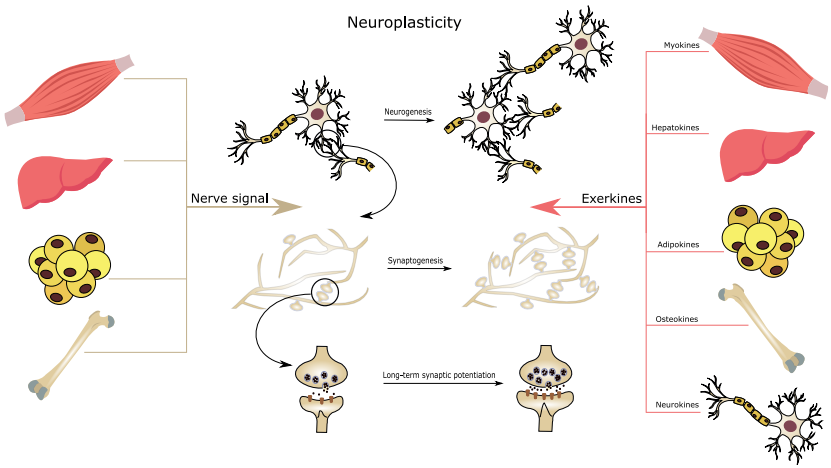


Figure 1. Forms of neuroplasticity at different levels of the nervous system and a depiction of the signals influencing neuroplasticity. Neurogenesis results in the formation of new neurons. Synaptogenesis results the formation of new synapses between two neurons. Long-term synaptic potentiation (LTP) results in an increase of excitatory neurotransmitters and their receptors in a synapse. All of these processes increase the chance that a message will reach its intended destination.

2. Relevance of the topic

The search for a better understanding of the mechanism behind the beneficial effects of physical exercise on cognitive function has gained increased attention recently, driven by the growing number of older adults living with age-related cognitive impairments [10]. This is a consequence of the worldwide increase in life expectancy, which is prognosed to cause a tripling of the population's proportion of people aged above 80 between 2020 and 2050. By 2030, one in six people worldwide will be over 60 years old [11]. While overall life expectancy is increasing, evidence suggests that healthy life expectancy has remained relatively constant. This implies that people live longer with chronic conditions, placing high demands on our health care systems [12]. Hence,

there is an urgent need to shift our focus from extending life to extending healthy life expectancies and towards prevention. A study comparing causes of chronic care-demanding diseases revealed that cognitive deficits and dementias were the fastest increasing cause of disability for older adults between 2000 and 2016, increasing faster than one would expect based only on population aging [10]. This discrepancy is most probably due to the increased amount of people living with risk factors of cognitive decline, partly attributed to unhealthy life choices [13]. For example, sedentary behavior, diabetes mellitus and obesity are well known modifiable risk factors of dementia [14]. Among adults, the global prevalence of sedentary behavior has increased about 5% between 2005 and 2017 [15], type 2 diabetes mellitus increased with 50% between 1990 and 2015 [16] and the worldwide prevalence of obesity has tripled between 1975 and 2014 [17]. Physical activity has the potential to reduce these numbers and prevent or delay cognitive decline [14][18]. Interestingly, lifelong physical activity has also been associated to increased total brain volume [19] and N-acetylaspartate to creatine (NAA/Cr) levels in the frontal cortex [20]. NAA is a neurometabolite suggested to reflect increased neural density [21]. In addition to lifelong physical activity levels, even short-term physical exercise interventions lasting a few months have been shown to decrease or prevent age-related hippocampal volume loss in older adults [22] and preliminary evidence suggest that 12 weeks of endurance [23] exercise training may help preserve healthy neurometabolic levels. Overall, total brain volume, especially regional volumes of the frontal and hippocampal cortex [24], and NAA levels [25][26] decrease with advancing age, and both are suggested to partly explain age-related cognitive decline [25][27]. In addition to intracerebral changes, (cognitive) aging was found to be associated with chronic low-grade inflammatory state, called 'inflammaging' [28], and a decrease in circulating neurotrophic factors [29]. Neurotrophic signaling is critical to neuroplasticity and the rise in inflammatory markers impairs normal neurotrophic signaling [30]. This age-related change in circulating inflammatory and neurotrophic factors was reported to correlate with age-related changes in brain volume, neurometabolites and cognitive function [31][32][33]. Again, physical exercise interventions were shown to reverse this age-related process, causing the release of exerkines with anti-inflammatory [34] and neurotrophic effects [35][36][37]. Using this knowledge, a theoretical framework was developed as depicted in Figure 2. However, the scientific literature lacks a comprehensive understanding of this framework. With this dissertation, the interrelationships within this framework were explored, addressing some of the remaining knowledge gaps concerning the beneficial effect of physical exercise on cognitive function in older adults.

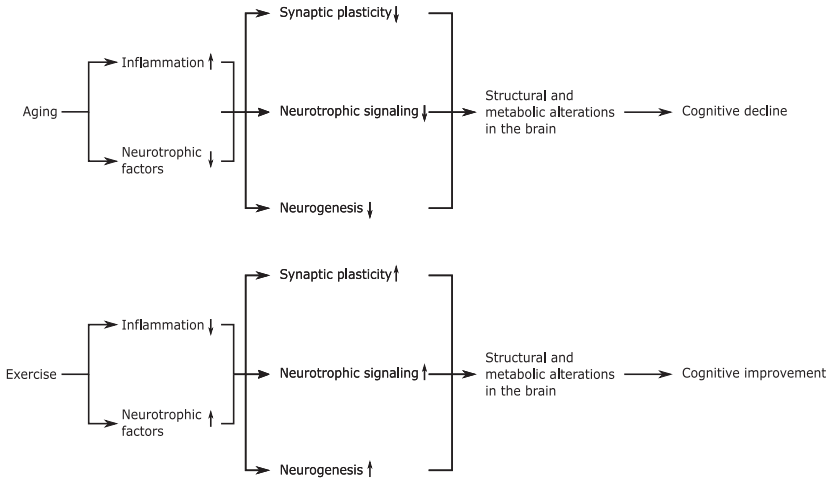


Figure 2. Theoretical frameworks of the aging and exercise-related changes resulting in cognitive decline and cognitive improvement, respectively, illustrating their similarities. Interrelationships within this framework need further investigation by future research.

3. Populations studied

During this dissertation, the effects of physical exercise and the role of exerkines were explored in two populations, older adults and individuals with spinal cord injury (SCI). Researchers have mainly focused on studying individuals who are at risk of cognitive impairment and neurodegenerative diseases, especially adults aged above 60 years old [38][39]. However, many questions remain unanswered. For instance, it is still unclear whether the effect of resistance exercise on cognitive function is influenced by the cognitive status of the older adults. Therefore, the effects of resistance exercise were compared in older adults with different cognitive statuses, categorized as either low or high risk of mild cognitive impairment, based on a general cognitive screening using the Montreal Cognitive Assessment (MoCA). Based on a meta-analysis on cardiovascular exercise interventions, it was hypothesized that larger effect sizes would be found for participants with high risk of mild cognitive impairment [40].

A population recently identified as being at risk for accelerated cognitive aging is individuals with SCI [41]. Literature indicates that individuals with SCI, excluding those with associated traumatic brain injury, exhibit significant impairments across several cognitive domains even at a young age [42] and face an elevated risk of developing Alzheimer's dementia, which is twice that of the general population [43][44]. An important question is whether increasing physical activity earlier in life can reduce the dementia risk in this

population. Research in able-bodied individuals has demonstrated that midlife physical activity can reduce or delay cognitive decline and lower the risk of dementia in later life [45]. However, no previous research has specifically evaluated the potential benefits of physical exercise for individuals with SCI [41]. Research in this population is challenging due to the significant heterogeneity in clinical presentations and the difficulty to recruit large, homogeneous samples.

4. Applied interventions

The intervention studies included in this dissertation have applied acute (i.e. a single bout) or chronic resistance exercise in older adults or acute neuromuscular electrical stimulation (NMES) in individuals with SCI. The acute resistance exercise intervention was of very high intensity (90-100% of 1 repetition maximum, 1RM). This intensity is usually applied to improve muscle strength. The chronic resistance exercise intervention progressed from moderate (70-75% 1RM) to high (80-85% 1RM) intensity over the course of 12 weeks. These intensities are generally used for improving muscle size and strength. Both the acute and chronic exercise interventions included lower limb resistance exercises (including squats, leg press, leg extensions, leg curls and calf raises). Of note, most of the existing evidence about the effect of physical exercise on cognitive function is currently derived from studies with cardiovascular exercise interventions (such as running, cycling or other types of endurance training) [46], while also resistance exercise was found to enhance cognitive and brain outcomes [47][48]. Interestingly, on the one hand, some studies suggest that resistance exercise effects on brain health and cognition may to a larger extent be associated with the release of myokines compared to cardiovascular exercise effects. On the other hand, the effect of cardiovascular exercise on brain health and cognition may to a larger extent be attributed to benefits in cardiovascular health or energy metabolism compared to the effect of resistance exercise [49]. Moreover, two network meta-analyses provided evidence that the effect of resistance exercise on cognitive function is superior to other exercise modalities in older adults and older adults with mild cognitive impairment or dementia [46][50]. Furthermore, it was argued that higher intensity exercise would induce a larger release of myokines [51][52][53][54] and could therefore induce larger effects on brain and cognitive outcomes. For this reason, we designed one study to evaluate the acute effects of high-intensity resistance exercise in order to enhance our mechanistic understanding of responses to this type of training. From a mechanistic perspective, some researchers suggest that the benefits of acute exercise effects may accumulate over time, leading to chronic effects [55]. At present, the literature lacks sufficient evidence from human studies to support this hypothesis. Yet, some evidence to support the link between acute and chronic exercise effects can

be derived from animal research. Animal studies suggest that acute exercise gives rise to (short-lasting) functional changes at the brain mediated by exerkines which signify a temporary enhancement in the responsiveness of neuroplastic processes. In contrast, chronic exposure to physical exercise is suggested to induce structural changes within the central nervous system, which are the result of longer-lasting changes in neuroplastic responsiveness [5][56]. However, even if future evidence in humans supports the idea that acute effects of physical exercise (mediated by exerkines) can lead to longer-lasting changes with repeated exposure, it is important to recognize that other mechanisms have also been proposed to produce chronic effects of physical exercise (for an overview of existing hypothesis, see the discussion section of this dissertation).

For the acute NMES session, a control condition was compared with low-intensity (40mA) or high intensity (100mA) electrical stimulation on the gluteal and hamstring muscles of individuals with SCI. There were multiple reasons for choosing NMES in this population. First, individuals with spinal cord injury have often difficulty to engage sufficiently in physical exercise [57]. This can be due to several barriers such as a lack of access or transportation options to exercise facilities, lack of background knowledge or professional guidance about exercising with a spinal cord injury, physical exercise-induced symptoms or complaints or limited voluntary muscle activity or range of motion [58]. NMES is a valid muscle training method that can be used at home, even on paretic muscles. Second, neuromuscular electrical stimulation was previously shown to induce a large release of myokines [59]. One study found higher levels of brain-derived neurotrophic factor (BDNF) in a NMES group compared to voluntary exercise with the same integrated force of muscle activation in young adults. They argued that the higher levels of lactate in the NMES group contributed to an increased release of BDNF [59], based on findings from previous studies [60]. Notably, the effect of NMES on cognitive function has never been tested [61].

5. Aim of this dissertation

The primary objective of this thesis was to gain a better understanding of the mechanism underlying the beneficial effect of resistance exercise on cognitive function in older adults, by exploring the role of exerkines on (exercise-induced changes in) neuroplasticity.

It was hypothesized that

- Levels of circulating markers of chronic inflammation would be positively associated with metabolic markers of neuroinflammation and neurodegeneration and brain volume loss.

- Obesity and sarcopenia would be associated with elevated markers of (neuro) inflammation and neurodegeneration, a decrease in brain volume, and cognitive impairment.
- Acute and chronic resistance exercise training would increase the level of circulating neurotrophic factors compared to control.
- chronic resistance exercise would decrease the level of circulating inflammatory factors compared to control.
- Acute and chronic exercise would enhance synaptic plasticity compared to control.
- Chronic resistance exercise would delay, prevent or even reverse age-related loss of hippocampus volume compared to control.
- Chronic resistance exercise would delay, prevent or even reverse age-related changes in neurometabolites in the hippocampus compared to control.
- Acute and chronic resistance exercise training would (transiently) enhance cognitive performance compared to control.
- Acute and chronic effects of resistance exercise on brain functional, metabolic or structural changes would be associated with the exercise-induced increase in neurotrophic factors and decrease in chronic inflammation.
- The abovementioned changes induced by chronic resistance exercise would be more pronounced in older adults with high risk of mild cognitive impairment compared to older adults with low risk of mild cognitive impairment.

Secondarily, this work was extended to individuals with spinal cord injury (SCI), who are at increased risk of age-related cognitive decline.

It was hypothesized that

- acute NMES would increase the level of circulating lactate and neurotrophic factors compared to a control condition.
- acute NMES would transiently increase cognitive performance on an information processing speed task compared to a control condition.
- the abovementioned changes induced by acute NMES would be more pronounced after high intensity NMES compared to low intensity NMES.

References

1. Sivadas A, Broadie K. How does my brain communicate with my body? Front young minds [Internet]. NIH Public Access; 2020 [cited 2024 Jan 26];8:540970. Available from: /pmc/articles/PMC7725213/
2. Buhusi C V., Meck WH. What makes us tick? Functional and neural mechanisms of interval timing. Nat Rev Neurosci [Internet]. Nat Rev Neurosci; 2005 [cited 2024 Jan 26];6:755–65. Available from: https://pubmed.ncbi.nlm.nih.gov/16163383/
3. Lisman JE. Three Ca2+ levels affect plasticity differently: The LTP zone, the LTD zone and no man's land. J Physiol [Internet]. Wiley-Blackwell; 2001 [cited 2020 Oct 5];532:285. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2278561/
4. Ballabh P, Braun A, Nedergaard M. The blood–brain barrier: an overview: Structure, regulation, and clinical implications. Neurobiol Dis. Academic Press; 2004;16:1–13.
5. Vints WAJ, Levin O, Fujiyama H, Verbunt J, Masiulis N. Exerkines and long-term synaptic potentiation: Mechanisms of exercise-induced neuroplasticity. Front Neuroendocrinol [Internet]. Academic Press; 2022 [cited 2022 Mar 15];66:100993. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0091302222000164
6. Alberts B, Johnson A, Lewis J, Raff M, Roberts K, Walter P. General Principles of Cell Communication. Mol Biol Cell [Internet]. 4th ed. New York: Garland Science; 2002. Available from: https://www.ncbi.nlm.nih.gov/books/NBK26813/
7. Erickson KI, Voss MW, Prakash RS, Basak C, Szabo A, Chaddock L, et al. Exercise training increases size of hippocampus and improves memory. Proc Natl Acad Sci U S A [Internet]. National Academy of Sciences; 2011 [cited 2019 May 18];108:3017–22. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21282661
8. Pedersen BK, Steensberg A, Fischer C, Keller C, Keller P, Plomgaard P, et al. Searching for the exercise factor: Is IL-6 a candidate? J Muscle Res Cell Motil [Internet]. J Muscle Res Cell Motil; 2003 [cited 2021 Mar 6]. p. 113–9. Available from: https://pubmed.ncbi.nlm.nih.gov/14609022/
9. Pedersen BK. Physical activity and muscle–brain crosstalk [Internet]. Nat. Rev. Endocrinol. Nature Publishing Group; 2019 [cited 2020 Sep 12]. p. 383–92. Available from: https://pubmed.ncbi.nlm.nih.gov/30837717/
10. Mattiuzzi C, Lippi G. Worldwide disease epidemiology in the older persons. Eur Geriatr Med [Internet]. Springer; 2020 [cited 2022 Jan 3];11:147–53. Available from: https://link.springer.com/article/10.1007/s41999-019-00265-2
11. World Health Organization. Ageing and health [Internet]. 2022 [cited 2024 Feb 14]. Available from: https://www.who.int/news-room/fact-sheets/detail/ageing-and-health
12. Dall TM, Gallo PD, Chakrabarti R, West T, Semilla AP, Storm M V. An Aging Population And Growing Disease Burden Will Require A Large And Specialized Health Care Workforce By 2025. Health Aff [Internet]. Health Affairs; 2013 [cited 2019 Jul 23];32:2013–20. Available from: http://www.healthaffairs.org/doi/10.1377/hlthaff.2013.0714
13. Vints WAJ, Kušleikienė S, Sheoran S, Valatkevičienė K, Gleiznienė R, Himmelreich U, et al. Body fat and components of sarcopenia relate to inflammation, brain volume, and neurometabolism in older adults. Neurobiol Aging [Internet]. 2023;127:1–11. Available from: https://doi.org/10.1016/j.neurobiolaging.2023.02.011
14. Livingston G, Huntley J, Sommerlad A, Ames D, Ballard C, Banerjee S, et al. Dementia prevention, intervention, and care: 2020 report of the Lancet Commission. Lancet Comm [Internet]. Lancet Publishing Group; 2020 [cited 2022 Aug 22];396:413–46. Available from: http://www.thelancet.com/article/S0140673620303676/fulltext

15. López-Valenciano A, Mayo X, Liguori G, Copeland RJ, Lamb M, Jimenez A. Changes in sedentary behaviour in European Union adults between 2002 and 2017. BMC Public Health [Internet]. BioMed Central; 2020 [cited 2022 Mar 6];20:1–10. Available from: https://bmcpublichealth.biomedcentral.com/articles/10.1186/s12889-020-09293-1
16. Khan MAB, Hashim MJ, King JK, Govender RD, Mustafa H, Kaabi J Al. Epidemiology of Type 2 Diabetes – Global Burden of Disease and Forecasted Trends. J Epidemiol Glob Health [Internet]. Springer; 2020 [cited 2024 Aug 28];10:107–11. Available from: /pmc/articles/PMC7310804/
17. Blüher M. Obesity: global epidemiology and pathogenesis. Nat Rev Endocrinol 2019 155 [Internet]. Nature Publishing Group; 2019 [cited 2022 Mar 6];15:288–98. Available from: https://www-nature-com.mu.idm.oclc.org/articles/s41574-019-0176-8
18. Erickson KI, Hillman C, Stillman CM, Ballard RM, Bloodgood B, Conroy DE, et al. Physical Activity, Cognition, and Brain Outcomes: A Review of the 2018 Physical Activity Guidelines [Internet]. Med. Sci. Sports Exerc. Lippincott Williams and Wilkins; 2019 [cited 2021 Feb 20]. p. 1242–51. Available from: /pmc/articles/PMC6527141/
19. Tan ZS, Spartano NL, Beiser AS, DeCarli C, Auerbach SH, Vasan RS, et al. Physical Activity, Brain Volume, and Dementia Risk: The Framingham Study. Journals Gerontol Ser A Biol Sci Med Sci [Internet]. Narnia; 2016 [cited 2019 May 18];72:glw130. Available from: https://academic.oup.com/biomedgerontology/article-lookup/doi/10.1093/gerona/glw130
20. Gonzales MM, Tarumi T, Kaur S, Nualnim N, Fallow BA, Pyron M, et al. Aerobic fitness and the brain: Increased N-acetyl-aspartate and choline concentrations in endurance-trained middle-aged adults. Brain Topogr [Internet]. NIH Public Access; 2013 [cited 2020 Nov 28];26:126–34. Available from: /pmc/articles/PMC3537918/?report=abstract
21. Cheng LL, Newell K, Mallory AE, Hyman BT, Gonzalez RG. Quantification of neurons in Alzheimer and control brains with ex vivo high resolution magic angle spinning proton magnetic resonance spectroscopy and stereology. Magn Reson Imaging [Internet]. Magn Reson Imaging; 2002 [cited 2024 Aug 28];20:527–33. Available from: https://pubmed.ncbi.nlm.nih.gov/12413598/
22. Wilckens KA, Stillman CM, Waiwood AM, Kang C, Leckie RL, Peven JC, et al. Exercise interventions preserve hippocampal volume: A meta-analysis. Hippocampus. 2021;31:335–47.
23. Matura S, Fleckenstein J, Deichmann R, Engeroff T, Füzéki E, Hattingen E, et al. Effects of aerobic exercise on brain metabolism and grey matter volume in older adults: results of the randomised controlled SMART trial. Transl Psychiatry [Internet]. Transl Psychiatry; 2017 [cited 2021 Apr 18];7:e1172. Available from: https://pubmed.ncbi.nlm.nih.gov/28934191/
24. Raz N, Lindenberger U, Rodrigue KM, Kennedy KM, Head D, Williamson A, et al. Regional Brain Changes in Aging Healthy Adults: General Trends, Individual Differences and Modifiers. Cereb Cortex [Internet]. Oxford Academic; 2005 [cited 2021 Apr 17];15:1676–89. Available from: http://academic.oup.com/cercor/article/15/11/1676/296890/Regional-Brain-Changes-in-Aging-Healthy-Adults
25. Cleeland C, Pipingas A, Scholey A, White D. Neurochemical changes in the aging brain: A systematic review. Neurosci. Biobehav. Rev. Elsevier Ltd; 2019. p. 306–19.
26. Maghsudi H, Schütze M, Maudsley AA, Dadak M, Lanfermann H, Ding XQ. Age-related Brain Metabolic Changes up to Seventh Decade in Healthy Humans: Whole-brain Magnetic Resonance Spectroscopic Imaging Study. Clin Neuroradiol [Internet]. Springer; 2020 [cited 2020 Oct 24];30:581–9. Available from: https://doi.org/10.1007/s00062-019-00814-z
27. Fjell AM, Walhovd KB. Structural brain changes in aging: Courses, causes and cognitive consequences [Internet]. Rev. Neurosci. Freund Publishing House Ltd; 2010 [cited 2021 Apr 17]. p. 187–221. Available from: https://pubmed.ncbi.nlm.nih.gov/20879692/

28. Franceschi C, Bonafè M, Valensin S, Olivieri F, De Luca M, Ottaviani E, et al. Inflamm-aging. An evolutionary perspective on immunosenescence. Ann N Y Acad Sci [Internet]. New York Academy of Sciences; 2000 [cited 2020 Oct 25];908:244–54. Available from: https://pubmed.ncbi.nlm.nih.gov/10911963/
29. Tapia-Arancibia L, Aliaga E, Silhol M, Arancibia S. New insights into brain BDNF function in normal aging and Alzheimer disease. Brain Res Rev [Internet]. 2008 [cited 2019 Aug 3];59:201–20. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18708092
30. Bourgognon J-M, Cavanagh J. The role of cytokines in modulating learning and memory and brain plasticity. Brain Neurosci Adv [Internet]. SAGE Publications; 2020 [cited 2021 Feb 3];4:239821282097980. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7750764/
31. Erickson KI, Prakash RS, Voss MW, Chaddock L, Heo S, McLaren M, et al. Brain-derived neurotrophic factor is associated with age-related decline in hippocampal volume. J Neurosci [Internet]. Society for Neuroscience; 2010 [cited 2021 Feb 19];30:5368–75. Available from: https://www.jneurosci.org/content/30/15/5368
32. Vints WAJ, Kušleikienė S, Sheoran S, Šarkinaitė M, Valatkevičienė K, Gleiznienė R, et al. Inflammatory blood biomarker kynurenine is linked with elevated neuroinflammation and neurodegeneration in older adults: evidence from two 1H-MRS post-processing analysis methods. Front Psychiatry [Internet]. Frontiers; 2022 [cited 2022 Mar 17];13:859772. Available from: https://www.frontiersin.org/articles/10.3389/fpsyt.2022.859772/abstract
33. Walker KA, Hoogeveen RC, Folsom AR, Ballantyne CM, Knopman DS, Windham BG, et al. Midlife systemic inflammatory markers are associated with late-life brain volume: The ARIC study. Neurology [Internet]. American Academy of Neurology; 2017 [cited 2024 Aug 28];89:2262–70. Available from: /pmc/articles/PMC5705246/
34. Khalafi M, Akbari A, Symonds ME, Pourvaghar MJ, Rosenkranz SK, Tabari E. Influence of different modes of exercise training on inflammatory markers in older adults with and without chronic diseases: A systematic review and meta-analysis. Cytokine [Internet]. Cytokine; 2023 [cited 2024 Aug 12];169:156303. Available from: https://pubmed.ncbi.nlm.nih.gov/37467710/
35. Marinus N, Hansen D, Feys P, Meesen R, Timmermans A, Spildooren J. The Impact of Different Types of Exercise Training on Peripheral Blood Brain-Derived Neurotrophic Factor Concentrations in Older Adults: A Meta-Analysis [Internet]. Sport. Med. Springer International Publishing; 2019 [cited 2021 Jan 24]. p. 1529–46. Available from: https://pubmed.ncbi.nlm.nih.gov/31270754/
36. Dinoff A, Herrmann N, Swardfager W, Liu CS, Sherman C, Chan S, et al. The effect of exercise training on resting concentrations of peripheral brain-derived neurotrophic factor (BDNF): A meta-analysis. PLoS One [Internet]. Public Library of Science; 2016 [cited 2021 Jan 24];11. Available from: /pmc/articles/PMC5033477/?report=abstract
37. Amiri N, Fathei M, Mosaferi Ziaaldini M. Effects of resistance training on muscle strength, insulin-like growth factor-1, and insulin-like growth factor–binding protein-3 in healthy elderly subjects: a systematic review and meta-analysis of randomized controlled trials. Hormones [Internet]. Springer Science and Business Media Deutschland GmbH; 2021 [cited 2021 Jan 25]; Available from: http://link.springer.com/10.1007/s42000-020-00250-6
38. Ludyga S, Gerber M, Pühse U, Looser VN, Kamijo K. Systematic review and meta-analysis investigating moderators of long-term effects of exercise on cognition in healthy individuals. Nat Hum Behav 2020 46 [Internet]. Nature Publishing Group; 2020 [cited 2021 Sep 4];4:603–12. Available from: https://www.nature.com/articles/s41562-020-0851-8
39. Boa Sorte Silva NC, Barha CK, Erickson KI, Kramer AF, Liu-Ambrose T. Physical exercise, cognition, and brain health in aging. Trends Neurosci [Internet]. Elsevier; 2024 [cited 2024 Jun 26];47:402–17. Available from: http://www.cell.com.mu.idm.oclc.org/article/S0166223624000626/fulltext

40. Talar K, Vetrovsky T, van Haren M, Négyesi J, Granacher U, Váczi M, et al. The effects of aerobic exercise and transcranial direct current stimulation on cognitive function in older adults with and without cognitive impairment: A systematic review and meta-analysis. Ageing Res Rev. Elsevier; 2022;81:101738.
41. Vints WAJ, Levin O, Masiulis N, Verbunt J, van Laake-Geelen CCM. Myokines may target accelerated cognitive aging in people with spinal cord injury: A systematic and topical review. Neurosci Biobehav Rev [Internet]. Pergamon; 2023 [cited 2023 Jan 31];146:105065. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0149763423000349
42. Chiaravalloti ND, Weber E, Wylie G, Dyson-Hudson T, Wecht JM. Patterns of cognitive deficits in persons with spinal cord injury as compared with both age-matched and older individuals without spinal cord injury. J Spinal Cord Med [Internet]. Taylor and Francis Ltd.; 2020 [cited 2021 Mar 26];43:88–97. Available from: /pmc/articles/PMC7006756/
43. Huang SW, Wang W Te, Chou LC, Liou TH, Lin HW. Risk of dementia in patients with spinal cord injury: A nationwide population-based cohort study. J Neurotrauma [Internet]. Mary Ann Liebert Inc.; 2017 [cited 2021 Mar 13];34:615–22. Available from: https://pubmed.ncbi.nlm.nih.gov/27539630/
44. Mahmoudi E, Lin P, Peterson MD, Meade MA, Tate DG, Kamdar N. Traumatic Spinal Cord Injury and Risk of Early and Late Onset Alzheimer's Disease and Related Dementia: Large Longitudinal Study. Arch Phys Med Rehabil. W.B. Saunders; 2021;102:1147–54.
45. Chang M, Jonsson P V., Snaedal J, Bjornsson S, Saczynski JS, Aspelund T, et al. The Effect of Midlife Physical Activity on Cognitive Function Among Older Adults: AGES—Reykjavik Study. Journals Gerontol Ser A Biol Sci Med Sci [Internet]. Oxford University Press; 2010 [cited 2024 Jul 9];65A:1369–74. Available from: /pmc/articles/PMC2990266/
46. Gallardo-Gómez D, del Pozo-Cruz J, Noetel M, Álvarez-Barbosa F, Alfonso-Rosa RM, del Pozo Cruz B. Optimal dose and type of exercise to improve cognitive function in older adults: A systematic review and bayesian model-based network meta-analysis of RCTs. Ageing Res Rev. Elsevier; 2022;76:101591.
47. Northey JM, Cherbuin N, Pumpa KL, Smee DJ, Rattray B. Exercise interventions for cognitive function in adults older than 50: A systematic review with meta-Analysis. Br. J. Sports Med. BMJ Publishing Group; 2018. p. 154–60.
48. Turner DT, Hu MX, Generaal E, Bos D, Ikram MK, Heshmatollah A, et al. Physical Exercise Interventions Targeting Cognitive Functioning and the Cognitive Domains in Nondementia Samples: A Systematic Review of Meta-Analyses. J Geriatr Psychiatry Neurol [Internet]. SAGE Publications; 2021 [cited 2024 Jul 9];34:91–101. Available from: /pmc/articles/PMC7859677/
49. Kim S, Choi JY, Moon S, Park DH, Kwak HB, Kang JH. Roles of myokines in exercise-induced improvement of neuropsychiatric function [Internet]. Pflugers Arch. Eur. J. Physiol. Springer Verlag; 2019 [cited 2021 Jan 13]. p. 491–505. Available from: https://link.springer.com/article/10.1007/s00424-019-02253-8
50. Huang X, Zhao X, Li B, Cai Y, Zhang S, Wan Q, et al. Comparative efficacy of various exercise interventions on cognitive function in patients with mild cognitive impairment or dementia: A systematic review and network meta-analysis. J Sport Heal Sci. Elsevier; 2022;11:212–23.
51. Huh JY, Mougios V, Kabasakalis A, Fatouros I, Siopi A, Douroudos II, et al. Exercise-induced irisin secretion is independent of age or fitness level and increased irisin may directly modulate muscle metabolism through AMPK activation. J Clin Endocrinol Metab [Internet]. Endocrine Society; 2014 [cited 2021 Feb 6];99:E2154–61. Available from: https://pubmed.ncbi.nlm.nih.gov/25119310/
52. Müller P, Duderstadt Y, Lessmann V, Müller NG. Lactate and BDNF: Key Mediators of Exercise Induced Neuroplasticity? J Clin Med [Internet]. MDPI AG; 2020 [cited 2021 Jan 9];9:1136. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7230639/

53. Daskalopoulou SS, Cooke AB, Gomez YH, Mutter AF, Filippaios A, Mesfum ET, et al. Plasma irisin levels progressively increase in response to increasing exercise workloads in young, healthy, active subjects. Eur J Endocrinol [Internet]. BioScientifica Ltd.; 2014 [cited 2021 Feb 6];171:343–52. Available from: https://pubmed.ncbi.nlm.nih.gov/24920292/
54. Schwarz AJ, Brasel JA, Hintz RL, Mohan S, Cooper DM. Acute effect of brief low- and high-intensity exercise on circulating insulin-like growth factor (IGF) I, II, and IGF-binding protein-3 and its proteolysis in young healthy men. J Clin Endocrinol Metab [Internet]. The Endocrine Society; 1996 [cited 2021 Jan 6];81:3492–7. Available from: https://academic.oup.com/jcem/article-lookup/doi/10.1210/jcem.81.10.8855791
55. Voss MW, Weng TB, Narayana-Kumanan K, Cole RC, Wharff C, Reist L, et al. Acute exercise effects predict training change in cognition and connectivity. Med Sci Sports Exerc [Internet]. NIH Public Access; 2020 [cited 2023 Sep 1];52:131–40. Available from: /pmc/articles/PMC7753185/
56. El-Sayes J, Harasym D, Turco C V., Locke MB, Nelson AJ. Exercise-Induced Neuroplasticity: A Mechanistic Model and Prospects for Promoting Plasticity. Neuroscientist [Internet]. A.J. Nelson, Department of Kinesiology, McMaster University, Hamilton, ON, Canada; 2019 [cited 2019 Mar 21];25:65–85. Available from: http://journals.sagepub.com/doi/10.1177/1073858418771538
57. Martin Ginis KA, Latimer AE, Arbour-Nicitopoulos KP, Buchholz AC, Bray SR, Craven BC, et al. Leisure Time Physical Activity in a Population-Based Sample of People With Spinal Cord Injury Part I: Demographic and Injury-Related Correlates. Arch Phys Med Rehabil [Internet]. Arch Phys Med Rehabil; 2010 [cited 2021 Mar 14];91:722–8. Available from: https://doi.org/10.1016/j.apmr.2009.12.027
58. Gorgey AS. Exercise awareness and barriers after spinal cord injury. World J Orthop [Internet]. Baishideng Publishing Group Inc; 2014 [cited 2024 Jul 9];5:158–62. Available from: /pmc/articles/PMC4095007/
59. Kimura T, Kaneko F, Iwamoto E, Saitoh S, Yamada T. Neuromuscular electrical stimulation increases serum brain-derived neurotrophic factor in humans. Exp Brain Res [Internet]. Springer Verlag; 2019 [cited 2021 Mar 6];237:47–56. Available from: https://pubmed.ncbi.nlm.nih.gov/30306243/
60. Proia P, di Liegro CM, Schiera G, Fricano A, Di Liegro I. Lactate as a Metabolite and a Regulator in the Central Nervous System. Int J Mol Sci 2016, Vol 17, Page 1450 [Internet]. Multidisciplinary Digital Publishing Institute; 2016 [cited 2023 Nov 1];17:1450. Available from: https://www.mdpi.com/1422-0067/17/9/1450/htm
61. Vints WAJ, Levin O, van Griensven M, Vlaeyen JWS, Masiulis N, Verbunt J, et al. Neuromuscular electrical stimulation to combat cognitive aging in people with spinal cord injury: protocol for a single case experimental design study. BMC Neurol. 2024;24:197.

OUTLINE OF THIS DISSERTATION

OUTLINE OF THIS DISSERTATION

This section describes the different parts included in this dissertation, as well as the aims for each of the individual studies. The study results per chapter are described in the summary section of this dissertation.

Part I of this dissertation discusses in detail the exerkines that are suggested to mediate neuroplastic and cognitive changes of acute and chronic exercise. In this part, the existing literature was compiled with the following aims:

In chapter 1, a narrative review of the existing literature is presented, aiming to describe what is known about the release of exerkines in response to acute or chronic exercise, what is known about their response to cardiovascular or resistance exercise, and what is known about the influence of different exercise protocol characteristics, such as exercise intensity, frequency, duration of a session, and duration of the program, or the influence of study participant characteristics, such as age, gender, medical comorbidities, and participants' fitness level. Furthermore, for each of the exerkines included in this narrative review, the molecular pathways that are known to affect neuroplasticity via the process of long-term synaptic potentiation were provided. This narrative review served as a foundation for this thesis by describing promising blood biomarkers, target populations and exercise protocols for investigating the effect of resistance exercise on cognitive function.

Chapter 2 contains a protocol paper for a comprehensive living systematic review and meta-analysis in which it is planned to investigate which myokines can be considered mediators of the exercise-induced effects on cognitive function in older adults. The main aim of this protocol paper was to inform the research field about ongoing research plans. Within this paper it is described why it is hypothesized that myokines mediate at least part of the effects of exercise on cognitive in older adults. The paper contains a list of 1126 putative myokines, as well as a narrative description of myokines that are commonly considered to impact cognitive function.

Part II of this dissertation describes the relationship between participant characteristics, blood and brain biomarkers and cognitive function in older adults. In this part, findings from cross-sectional analyses were reported with the following aims:

In chapter 3 a cross-sectional study is presented, aimed to investigate if inflammatory blood biomarkers can be used as surrogates for brain inflammation and neurodegeneration by investigating their mutual relationship in a cohort of older adults. Additionally, two post-processing techniques that are commonly used to analyze these brain neurochemical markers were compared in order to evaluate if the choice of post-processing technique influences the conclusions that can be drawn.

Chapter 4 is a cross-sectional study aimed to test our hypotheses that markers of obesity and sarcopenia are associated with increased levels of blood and brain inflammation, lower brain volume and declined cognitive performance. In this study the relationship between participant characteristics, blood and brain biomarker levels and brain volume were evaluated within a cohort of older adults.

Part III of this dissertation reports the effects of resistance exercise training on brain and cognitive outcomes in older adults with or without mild cognitive impairment. In this part, findings from interventional studies were reported with the following aims:

Chapter 5 is a quasi-randomized controlled interventional study aimed to test if a single bout of very high intense resistance exercise could induce transient increases in cognitive performance and postural dual task control in older adults.

Chapter 6 is a randomized controlled trial aimed to test if 12 weeks of resistance exercise increases hippocampus volume and improves neurometabolic status of the hippocampus in older adults compared to a waiting list control group. Additionally, it was explored if these changes were related to changes in blood biomarker levels. Finally, it was examined if the benefit of resistance exercise was larger in older adults with high risk of mild cognitive impairment compared to older adults with a low risk of mild cognitive impairment.

Chapter 7 is a randomized controlled trial aimed to test if 12 weeks of resistance exercise enhances cognitive function in older adults compared to a waiting list control group. Additionally, it was explored if these changes were related to changes in blood biomarker levels. Finally, it was examined if the benefit of resistance exercise was larger in older adults with high risk of mild cognitive impairment compared to older adults with a low risk of mild cognitive impairment.

Chapter 8 is a case series aimed to report pre-to-post COVID-19 neurometabolic and volumetric changes in the hippocampus compared to non-infected controls. Additionally, this it was explored within the group of participants infected with COVID-19 whether neurometabolic and volumetric changes in the hippocampus differed between study participants that were allocated to resistance exercise compared to the waiting list control group.

Part IV of this dissertation concentrates on individuals with spinal cord injury. The following aims were evaluated:

Chapter 9 is an empty systematic and narrative review of the existing literature aimed to prove lack of knowledge on the effect of physical exercise and myokines on cognitive function in individuals with spinal cord injury. Additionally, the review paper aimed to describe the mechanism of accelerated cognitive aging in individuals with spinal cord injury and formulate future directions.

Chapter 10 is a randomized cross-over study aimed to test if a single session of neuromuscular electrical stimulation can induce changes in circulating levels of insulin-like growth factor-1 (IGF-1) and performance on an information processing speed task in individuals with spinal cord injury.

Chapter 11 is a protocol paper aimed to describe the methods of a single-case experimental design study for individuals with spinal cord injury. In this ongoing study it will be tested if 12 weeks of neuromuscular electrical stimulation can induce an elevation in brain-derived neurotrophic factor (BDNF) and boost cognitive performance in individuals with spinal cord injury.

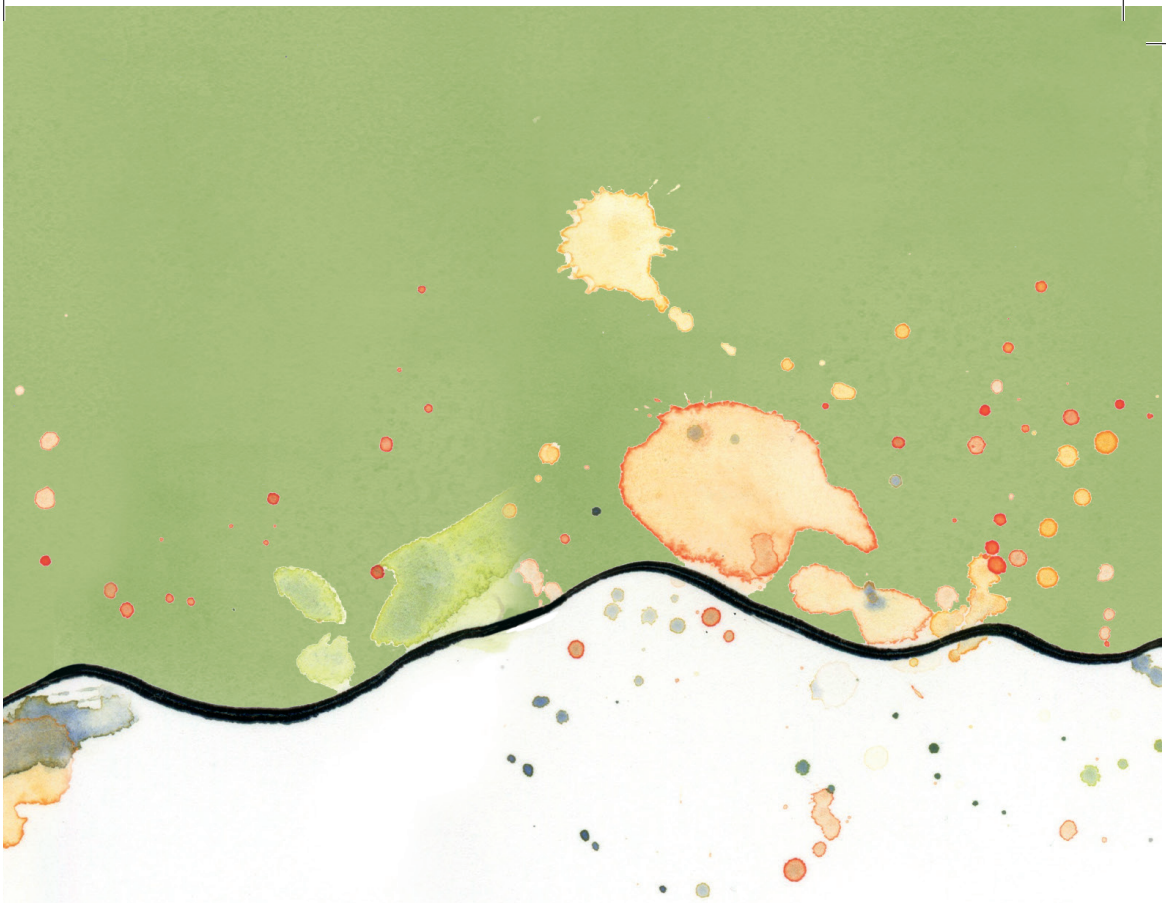
Part V of this dissertation concludes with a general discussion and summary of the new insights that can be derived from this dissertation.

Chapter 12 includes a summary of the dissertation in three languages.

Chapter 13 contains a general discussion on the topic of the dissertation.

Chapter 14 reflects on the impact of the dissertation.

In the appendix, you can find information about the author and a list of publications.



PART I

Mechanisms of exercise-induced neuroplastic and cognitive changes: the role of exerkines in general, and myokines in particular

CHAPTER 1

Exerkines and long-term synaptic potentiation: Mechanisms of exercise-induced neuroplasticity

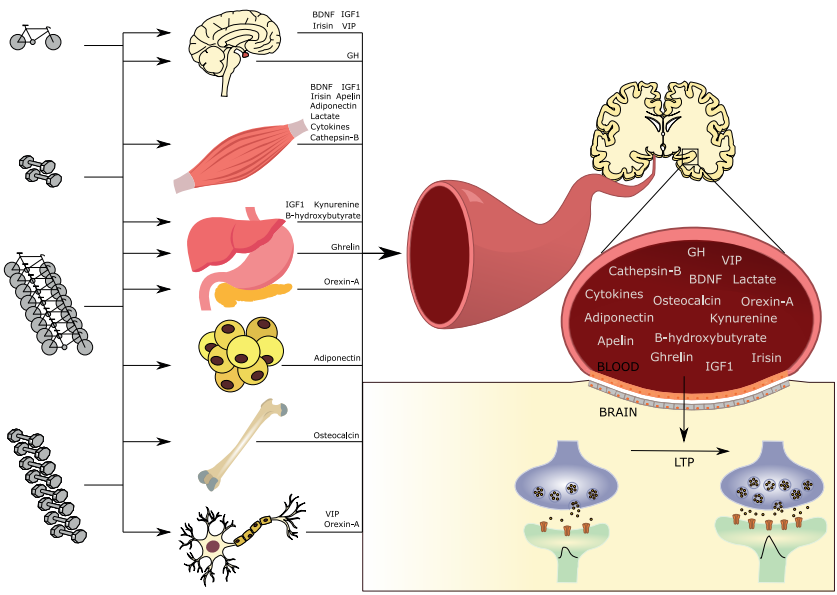
Authors: Wouter A.J. Vints, Oron Levin, Hakuei Fujiyama, Jeanine Verbunt, Nerijus Masiulis

Published in Frontiers in Neuroendocrinology, 2022, Vol. 66, p. 100993
https://doi-org.mu.idm.oclc.org/10.1016/j.yfrne.2022.100993

Abstract

Physical exercise may improve cognitive function by modulating molecular and cellular mechanisms within the brain. We propose that the facilitation of long-term synaptic potentiation (LTP)-related pathways, by products induced by physical exercise (i.e., exerkines), is a crucial aspect of the exercise-effect on the brain. This review summarizes synaptic pathways that are activated by exerkines and may potentiate LTP. For a total of 16 exerkines, we indicated how blood and brain exerkine levels are altered depending on the type of physical exercise (i.e., cardiovascular or resistance exercise) and how they respond to a single bout (i.e., acute exercise) or multiple bouts of physical exercise (i.e., chronic exercise). This information may be used for designing individualized physical exercise programs. Finally, this review may serve to direct future research towards fundamental gaps in our current knowledge regarding the biophysical interactions between muscle activity and the brain at both cellular and system levels.

Graphical abstract



Abbreviations

AEP; auditory evoked potentials; AMPA, amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor; BDNF, brain-derived neurotrophic factor; CamKII, Ca2+-calmodulin-dependent kinase II; cAMP, cyclic adenosine monophosphate; CREB, cAMP-response element binding protein; DAG, diacylglycerol; EEG, electroencephalography; EPSP, excitatory postsynaptic potential; ER, endoplasmic reticulum; ERK, extracellular signal regulated kinase; Erra, estrogen-related receptor-α; FNDC5, fibronectin type III domain-containing protein 5; fMRI, functional magnetic resonance imaging; fNIRS, functional near-infrared spectroscopy; GABA, γ-aminobutyric acid; GH, growth hormone; IGF-1, insulin-like growth factor-1; IPSP, inhibitory postsynaptic potentials; IP3, inositol 1,4,5 triphospate; LTD, long-term synaptic depression; LTP, long-term synaptic potentiation; mRNA, messenger-ribonucleic acid; NMDA, N-methyl-D-aspartate receptor; PGC1α, peroxisome proliferator-activated receptor-γ coactivator 1α; PI3K, phosphoinositide 3-kinase; PKA, protein kinase A; PKC, protein kinase C; PLCγ, phospholipase C-γ; ^{1}H-MRS, proton magnetic resonance spectroscopy; SIRT-1, silent information regulator 1; TMS, transcranial magnetic stimulation; TrkB, tropomyosin-receptor kinase-B receptor; VEP, visually-evoked potentials; VIP, vasoactive intestinal peptide.

1. Introduction

The beneficial effect of physical exercise on cognition first appeared in literature in the 1930s (Burpee and Stroll, 1936; Beise and Peaseley, 1937). A search in PubMed with the terms "exercise" AND "cognition" shows how this topic has exploded in the last decades, reaching over one hundred publications a year in 1998 and over 3000 publications a year in 2020. However, the underlying mechanisms of physical exercise-induced cognitive improvements are still not fully understood, indicating the complexity of the neurophysiological processes that mediate the beneficial effects of physical exercise on the brain (Gomez-Pinilla and Hillman 2013; El-Sayes et al., 2019; Kim et al., 2019). Of critical importance in this respect is neuroplasticity (Martin et al., 2000; Autio et al., 2020). Neuroplasticity refers to the brain's ability to undergo functional and structural changes in response to external or internal stimuli from the environment or organs in the body (Voss et al., 2017). Currently, there is a vast amount of research showing that neuroplasticity could well be induced by acute (i.e., a single bout) or chronic (i.e., a program of multiple bouts) exposure to physical exercise (Knaepen et al., 2010; Svensson et al., 2015; Vilela et al., 2017; Müller et al., 2020).

At the level of the brain, acute exercise studies in humans have discovered transient changes in neurotransmitter levels like glutamate and γ-aminobutyric acid (GABA) immediately following physical exercise, as measured with proton magnetic resonance spectroscopy (^{1}H-MRS) (Maddock et al., 2016). Both glutamate and GABA are important neurotransmitters in the mammalian brain and are known to be primary mediators of long-term synaptic potentiation (LTP) and long-term synaptic depression (LTD) through glutamatergic (see Box 1) and GABAergic pathways. LTP and LTD are neuroplastic processes, which respectively cause strengthening or weakening of excitatory synaptic connections within the brain (Lisman, 2001). Both induce changes in the synapse involving a rapid, short-lasting alteration of the function of existing synaptic proteins by processes such as phosphorylation (i.e., early LTP or LTD) and a slower, longer-lasting change in the availability of synaptic proteins by targeting cell DNA and inducing transcription of new proteins (i.e., late LTP or LTD) (Loprinzi, 2019).

'LTP-like' processes (i.e., the increased efficacy of synaptic neurotransmission through neural networks) are found in many brain regions, playing a critical role in several domains of cognitive function (Martin et al., 2000). For example, disruption of LTP-like processes in the hippocampal, prefrontal, visual, auditory, and motor cortex were respectively suggested to result in an impairment of episodic memory function (Chen et al., 2000; Barnes, 2003), working memory and executive function (Dallérac et al., 2011), visual (Yeap et al., 2008), auditory (O'Donnell et al., 2004), and motor processing (Frantseva et al., 2008). These disruptions can be found with aging (Barnes, 2003), Alzheimer's disease (Chen et al., 2000), major depression (Normann et al., 2007), and other psychiatric (Frantseva et

al., 2008; Yeap et al., 2008) and neurological disorders (Rison and Stanton, 1995; Bliss and Cooke, 2011; Dallérac et al., 2011; Conte et al., 2012). While the direct measurement of LTP requires invasive in vivo or in vitro electrophysiological tests, LTP-like processes can also be assessed with non-invasive techniques. For example, LTP-like processes in the human motor cortex can be assessed with transcranial magnetic stimulation (TMS) (Frantseva et al., 2008). Furthermore, electroencephalography (EEG) (Kirk et al., 2010) can show LTP-like processes in the visual cortex by measuring visually-evoked potentials (VEP) (Yeap et al., 2008), or in the auditory cortex by using auditory evoked potentials (AEP) (O'Donnell et al., 2004).

Physical exercise can induce either short- or long-lasting neuroplastic changes in the brain. Early LTP is considered a candidate mechanism for the brain's short-lasting functional changes that occur during and/or immediately following acute exercise (Crabbe and Dishman, 2004; Yanagisawa et al., 2010; Singh et al., 2014b; van Dongen et al., 2016). These functional brain changes can be detected with TMS (Singh et al., 2014b), EEG (Crabbe and Dishman, 2004), functional near-infrared spectroscopy (fNIRS) (Yanagisawa et al., 2010), or functional magnetic resonance imaging (fMRI) (van Dongen et al., 2016). In addition, late LTP processes are likely activated during and/or shortly after the exposure to acute exercise, but measurable structural changes have only been observed following chronic exercise (Colcombe et al., 2006; Erickson et al., 2011; Gonzales et al., 2013; Haeger et al., 2019; Herold et al., 2019).

Importantly, the pathways activated in the process of late LTP also increase the transcription of growth and survival stimulating factors, such as brain-derived neurotrophic factor (BDNF). The transcription of BDNF was shown both after acute exercise and chronic exercise (Venezia et al., 2017). The resulting increased availability of BDNF may, in turn, upregulate pathways of neurogenesis, increasing the number of neurons in the dentate gyrus of the hippocampus (Cho et al., 2013). These newly formed neurons were described to activate LTP processes more easily (Snyder et al., 2001; Van Praag et al., 2002). Without effortful learning, and thus the activation of LTP, these new neurons do not survive more than three weeks (Shors et al., 2012). This might indicate that newly formed neurons are dependent on the survival-promoting factors which are being released during LTP for further maturation and to be hooked up into functional networks (Shors et al., 2012; Denoth-Lippuner and Jessberger, 2021). A successful process of neurogenesis might explain the biochemical and structural brain changes reported in chronic exercise studies such as increases in N-acetyl aspartate, a neurometabolic marker of neuronal integrity (Gonzales et al., 2013) measured with ^{1}H-MRS, and increases in gray matter volume and white matter microstructural organization (Colcombe et al., 2006) measured with magnetic resonance imaging (MRI). These are interesting findings, as higher levels of N-acetyl aspartate and larger brain volume has been associated with better cognitive functioning in older adults (Fjell and Walhovd, 2010; Cleeland et al., 2019).

In sum, a vast amount of research suggests that both acute and chronic exercise have beneficial effects on the biological mechanisms that mediate neuroplasticity, possibly through a physical exercise-induced enhanced response to LTP induction, which in turn induces functional and structural changes to the brain, improving cognitive function. (Erickson et al., 2011; Broadhouse et al., 2020) Yet, how muscle activity eventually results in the facilitation of LTP is still a topic of debate. An increasingly popular explanation for the mechanism of cognitive enhancement following physical exercise is the exerkine hypothesis. Exerkines are all of the peptides, metabolites, and nucleic acids released into the bloodstream during and following physical exercise. Depending on the organ they are being released from, they are called myokines, adipokines, or hepatokines, respectively referring to physical exercise-induced factors released from muscle, adipose tissue, or the liver (Pedersen, 2019). Some of these exerkines may cross the blood-brain barrier (Kastin and Akerstrom, 1999; Carro et al., 2000; Dogrukol-Ak et al., 2003; Higuchi et al., 2007; Oury et al., 2013; Agudelo et al., 2014; Ribeiro et al., 2014; Takimoto and Hamada, 2014; Yau et al., 2014; Li et al., 2015; Moon et al., 2016; Serra-Millàs, 2016; Wrann, 2016). It is plausible to assume that exerkines which crossed the blood-brain barrier can facilitate signaling pathways that regulate the induction of LTP.

In this narrative review, we elucidate the possible role that the physical exercise-induced enhancement of the LTP process by the release of exerkines may play in improving brain functions, while showing how it fits the currently popular view that exerkines are involved in multiple signaling pathways that mediate neuroplasticity. As a general objective, we aim to generate a framework that structures all relevant information about exerkines that are possibly involved in the regulation of early and late stages of LTP in the human brain. We decided to review existing literature on growth factors, myokines, cytokines, metabolites, hormones, and neuropeptides, including only those that are known to be released or generated during physical exercise and appear to have direct or indirect application for the enhancement of early and/or late LTP (Fig. 1 and 2). We purposefully did not review all exerkines that may cross the blood-brain barrier, as for some of them, empirical evidence suggesting the cellular pathway for the facilitation of LTP is not available, unclear, or conflicting. For every exerkine addressed, we will describe their origin, discuss if the effect is to be expected after acute or chronic exercise and differentiate between cardiovascular or resistance exercise (Table 1). In section two, the process of LTP will be explained in short, focused on the pathways important for discussion in the remainder of the paper. In section three, 16 exerkines of interest will be addressed one by one. We start with growth factors (i.e., brain-derived neurotrophic factor (BDNF), insulin-like growth factor-1 (IGF-1), and growth hormone (GH)), followed by pro- and anti-inflammatory biomarkers (i.e., cytokines and kynurenine), of whom some are myokines. Then, we discuss other myokines (i.e., irisin, cathepsin-B, apelin, and adiponectin) and metabolites (i.e., lactate and β-hydroxybutyrate (BHB)). At last, we describe the remaining

exerkines that could not be placed in any of the other groups (i.e., osteocalcin, orexin-A, ghrelin, and vasoactive intestinal peptide (VIP)). In sections four and five, we summarize the most interesting conclusions to be drawn from this comprehensive review paper and propose how this information can be used for future research.

2. Long-term synaptic potentiation

BOX 1. The long-term synaptic potentiation process (LTP)

LTP is mediated primarily through glutamatergic pathways. In glutamatergic synapses, a signal in the form of an action potential is transmitted from one neuron to the next by the release of glutamate from the presynaptic neuron and the subsequent activation of glutamatergic amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors in the postsynaptic neuron. Postsynaptic AMPA receptor activation causes influx of Na^+, depolarizing the postsynaptic membrane, which is measured as an excitatory postsynaptic potential (EPSP) with patch clamp techniques. If many EPSPs from multiple excitatory synapses accumulate, depolarization reaches the threshold for the generation of a new action potential and the neural signal is transmitted. LTP is an activity-dependent process that makes the synapse become more responsive to subsequent stimuli, increasing the chance an action potential will be generated in the postsynaptic neuron. The activity-dependence lies in the fact that repeated excitatory stimulation causes the synaptic connections to become more and more strengthened (Lisman, 2001; Vaynman et al., 2003; Marsden et al., 2010). LTP is likely dependent on the activation of Ca^{2+}-sensing signaling pathways, which is (at least in part) mediated by activation of pre- and postsynaptic N-methyl-D-aspartate (NMDA) receptors. These Ca^{2+} permeable receptors require membrane depolarization and activation by glutamate to open (Fig. 1). In the *presynaptic neuron*, depolarization of the presynaptic membrane by an action potential first activates voltage-gated Ca^{2+} channels. This facilitates Ca^{2+}-dependent exocytosis of glutamate-containing synaptic vesicles. Consequently, Ca^{2+} influx further increases by the opening of NMDA receptors, which results in the activation of pathways involved in LTP, see section 2. These pathways are mainly thought to involve the increased release of glutamate upon activation by a subsequent action potential. This may result from an increased number of glutamate-containing vesicles available in the reserve pool, an increased number of vesicles being transported from the reserve pool towards the releasable pool, and an increased number of vesicles being released upon Ca^{2+} influx (Loprinzi, 2019). At the *postsynaptic excitatory neuron*, glutamatergic AMPA receptor activation causes influx of Na^+, depolarizing the postsynaptic membrane. Consequently, NMDA receptors become activated, allowing Ca^{2+} to flow into the cell. Postsynaptic Ca^{2+}-sensing pathways result in the activation of kinases, which through phosphorylation induce an increased activity and number of glutamatergic AMPA receptors. This way, the EPSP level in response to a subsequent release of presynaptic glutamate will be enhanced (Lisman, 2001).

BOX 1. Continued

Of note, another form of neural plasticity that is mediated through glutamatergic pathways is long-term synaptic depression (LTD). Similar to LTP, LTD is also activated by Ca^{2+} influx inside the cell. In contrast to LTP, phosphatases and not kinases have the overhand during LTD processes at the excitatory glutamatergic synapse. This results in a weakening of glutamatergic synaptic connections, by for example, internalization of AMPA receptors and a decrease in the number of glutamates containing vesicles (Collingridge et al., 2010). LTD has a low intracellular Ca^{2+} threshold, and is typically induced by a prolonged modest increase in Ca^{2+}. In contrast, the induction of LTP requires a brief, but higher amplitude of intracellular Ca^{2+} increase (Yang et al., 1999). An in between zone is also expected to exist, where the amplitude and/or duration of the Ca^{2+} influx is not sufficient for the induction of neither LTP nor LTD (Lisman, 2001). Several pathways may cause that LTD and not LTP would be induced by a Ca^{2+} increase, as is described in more detail by Collingridge et al. (2010). One influential pathway on LTP/LTD we would like to mention is through inhibitory inputs from GABAergic synapses. GABA receptors are Cl^- channels, which hyperpolarize the postsynaptic membrane upon opening, called inhibitory postsynaptic potentials (IPSP). This may cause membrane depolarization by Na^+ not to reach the threshold for opening of NMDA receptors at the glutamatergic synapse upon AMPA activation and Ca^{2+} levels to remain low (Marsden et al., 2010; Mele et al., 2016). Studies have shown that high GABAergic input causes LTD to be induced more readily. This way, a certain concentration of intracellular Ca^{2+} may induce LTD, when in the absence of GABAergic input it would induce LTP or neither LTP nor LTP (Steele and Mauk, 1999). A detailed discussion on the LTD process and the interplay between LTP and LTD is outside the scope of this review paper, but is described in more detail by others, e.g. (Steele and Mauk, 1999; Yang et al., 1999; Lisman, 2001; Collingridge et al., 2010; Marsden et al., 2010; Mele et al., 2016).

For the sake of clarity, we briefly report the most important intracellular pre- and postsynaptic pathways involved in LTP (Fig. 1). At the *presynaptic* neuron, activation of N-methyl-D-aspartate (NMDA)-type ionotropic glutamate receptors induces Ca^{2+}-triggered autophosphorylation of_Ca^{2+}-calmodulin-dependent kinase II (CamKII). In turn, CamKII activates synapsin I by phosphorylation and mediates the transcription of synapsin I via phosphorylation of the transcription factor cyclic adenosine monophosphate (cAMP)-response element binding protein (CREB) (Vaynman et al., 2003; Murray and Holmes, 2011). Synapsin I controls the fraction of synaptic vesicles available for release. After activation of synapsin I, synaptic vesicles from the reserve pool are transferred to the releasable pool. Moreover, elevated synapsin I levels at the presynaptic terminal are thought to increase the rate of synaptic vesicle recycling and formation. This is important to prevent synaptic fatigue due to vesicular rundown on subsequent stimulation (Vaynman et al., 2003; Gerth et al., 2017). In addition, calcium-sensitive adenylyl cyclases activate cAMP-dependent protein kinase A (PKA). PKA is also capable of phosphorylating synapsin I (Chenouard et al., 2020). For a more elaborate overview of presynaptic mechanisms, we refer to the review of Yang and Calakos (2013).

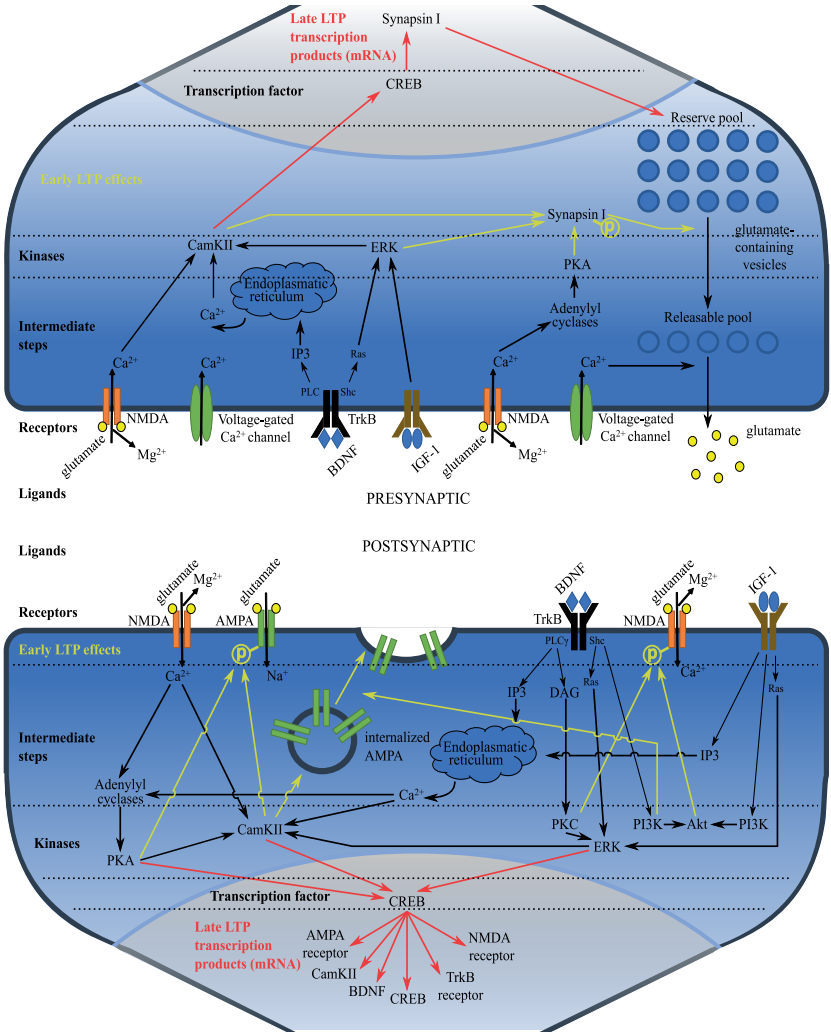


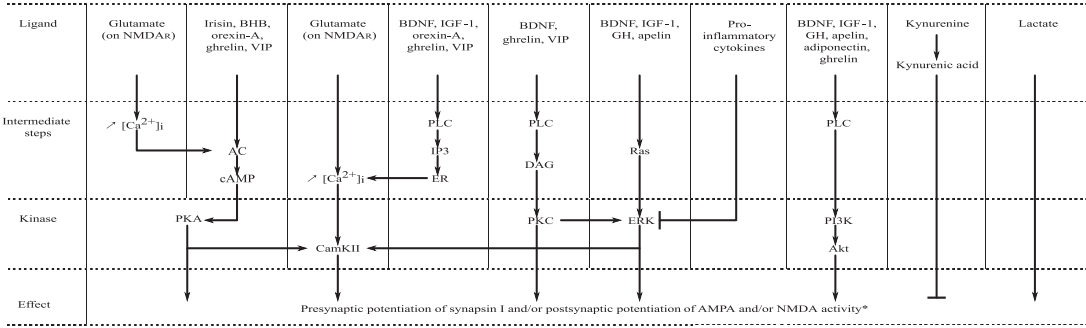
Figure 1. Visual representation of the early and late LTP pathways in the pre- and post-synaptic excitatory neuron

Abbreviations: Ca2+, calcium; AMPA, amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor; BDNF, brain-derived neurotrophic factor; CamKII, Ca2+-calmodulin-dependent kinase II; CREB, cAMP-response element binding protein; DAG, diacylglycerol; ERK, extracellular signal regulated kinase; IGF-1, insulin-like growth factor-1; IP3, inositol 1,4,5 triphospate; LTP, long-term synaptic potentiation; mRNA, messenger-ribonucleic acid; NMDA, N-methyl-D-aspartate; PI3K, phosphoinositide 3-kinase; PKA, protein kinase A; PKC, protein kinase C; PLC, phospholipase C; TrkB, tropomyosin-receptor kinase-B receptor.

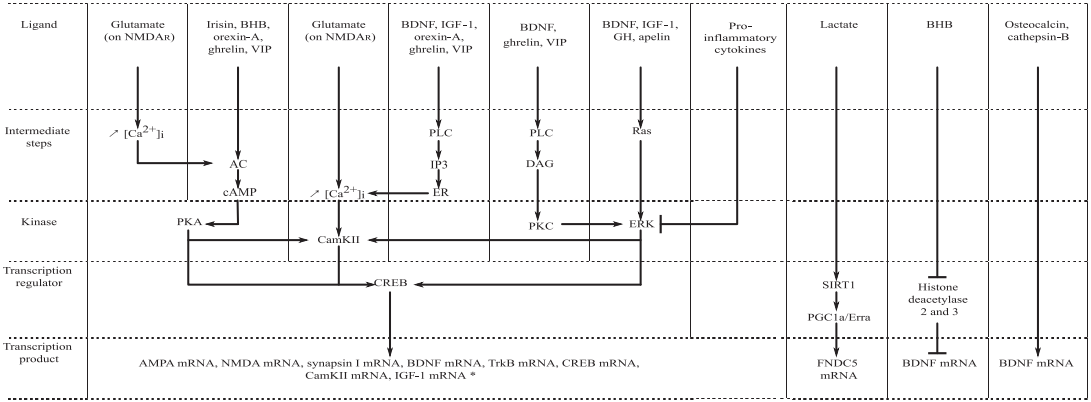
At the *postsynaptic* neuron, NMDA-dependent Ca^{2+} influx also activates CamKII. Here, CamKII phosphorylates amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) receptors, making them more easily activated. Also, CamKII enhances surface AMPA receptor levels by inducing exocytosis of internalized receptors in the membrane surface (Lu et al., 2001) and de novo synthesis of AMPA receptors via activation of the transcription factor CREB (Middei et al., 2013). Next, PKA stimulates CamKII activity by inhibiting protein phosphatase-1, it potentiates AMPA receptors by phosphorylation (Roche et al., 1996), and also activates CREB (Winder and Sweatt, 2001).

In addition to NMDA-receptors, several exerkines, such as BDNF and IGF-1, have been found to activate pre- and postsynaptic intracellular pathways with similar effects (Fig. 1). These exerkine-induced pathways can transiently potentiate the response of presynaptic synapsin I or postsynaptic AMPA receptor activity during the activation of a subsequent LTP process by phosphorylation (i.e., early LTP) or by inducing transcription of synapsin I and AMPA receptors by activating the transcription factor CREB (i.e., late LTP) (Vaynman et al., 2003; Prescott et al., 2006; Kim et al., 2010; Murray and Holmes, 2011; Molina et al., 2012; Ribeiro et al., 2014; Wang et al., 2019). Furthermore, some exerkines were reported to indirectly increase the effect of the following LTP induction. For example, by potentiating NMDA receptors, or by inducing transcription or enhancing activity of proteins critical for LTP, like NMDA receptors, downstream products like CamKII and CREB, or BDNF, IGF-1 and their receptors (Fig. 2) (Carro et al., 2001; Vaynman et al., 2003; Ding et al., 2006; Yang et al., 2009; Kim et al., 2010; Molina et al., 2012; Yang et al., 2014; Wang et al., 2019). Other exerkines may play a regulatory role by modulating synaptic transmission (e.g., kynurenine) (Rózsa et al., 2008; Potter et al., 2010; Demeter et al., 2013; Vécsei et al., 2013) or transcription of other exerkines (e.g., lactate and osteocalcin) (Wrann et al., 2013; Khrimian et al., 2017; Nicolini et al., 2020). These findings underscore how physical exercise may facilitate pathways involved in the LTP process by increasing the circulating levels of these exerkines. We will discuss these exerkine-induced pathways in more detail below.

Early LTP



Late LTP



◂ **Figure 2.** Schematic overview of the exerkine-mediated pathways associated with early and late LTP in the pre- and postsynaptic excitatory neuron.
* Pre- and postsynaptic pathways are not considered separately. In addition, for some of the exerkines, the intermediate steps are currently not completely understood. We refer to the accompanying text for more details.
Abbreviations: [Ca2+]i, intracellular calcium concentration; AC, adenylyl cyclase; AMPA, amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor; BDNF, brain-derived neurotrophic factor; BHB, β-hydroxybutyrate; CamKII, Ca2+-calmodulin-dependent kinase II; cAMP, cyclic adenosine monophosphate; CREB, cAMP-response element binding protein; DAG, diacylglycerol; ER, endoplasmic reticulum; ERK, extracellular signal regulated kinase; Erra, oestrogen-related receptor-α; FNDC5, fibronectin type III domain-containing protein 5; GH, growth hormone; IGF-1, insulin-like growth factor-1; IP3, inositol 1,4,5 triphospate; LTP, long-term synaptic potentiation; mRNA, messenger-ribonucleic acid; NMDAR, N-methyl-D-aspartate receptor; PGC1α, peroxisome proliferator-activated receptor-γ coactivator 1α; PI3K, phosphoinositide 3-kinase; PKA, protein kinase A; PKC, protein kinase C; PLC, phospholipase C; SIRT-1, silent information regulator 1; TrkB, tropomyosin-receptor kinase-B receptor; VIP, vasoactive intestinal peptide.

3. Exerkines with the potential to alter LTP-related pathways

In this section, we will describe the role of 16 different exerkines on LTP. These include growth factors, myokines, cytokines, metabolites, hormones, and neuropeptides that are released during physical exercise and have the potential to alter LTP-related pathways. When evidence on the exerkine-mediated facilitation or impairment of LTP-related pathways was not available, unclear or conflicting, the exerkine was not included in this paper, even if the concerned exerkine was known to cross the blood-brain barrier. We will report how the included exerkines are upregulated by physical exercise and the organ of their origin. Furthermore, we will highlight the possible synaptic pathways that they may activate (Fig. 2). Finally, we will discuss if the effect is to be expected after acute or chronic exercise and differentiate between cardiovascular or resistance exercise (Table 1). Findings from both animal and human studies are included. A detailed description of study characteristics including mean age of the subjects, gender of the subjects, healthy/unhealthy study population, fitness level of the subjects, physical exercise duration, physical exercise intensity, and the direction of the (subgroup specific) significant/insignificant changes of the levels of a certain exerkine following acute/chronic cardiovascular/resistance exercise of all physical exercise studies described in this section is provided in Supplementary Table S1 (for blood exerkine levels) and S2 (for brain exerkine levels). Table 1 can be considered a short summary of Supplementary Table S1 and S2.

Table 1. Overview of exerkines that influence LTP

Peripheral versus central measurements:		BLOOD				BRAIN			
Acute versus chronic exercise:		Acute		Chronic		Acute		Chronic	
Cardiovascular (C) versus resistance (R) exercise:		C	R	C	R	C	R	C	R
Growth factors	BDNF	↗[d,f,g]	↗[d,f,g]	↗	↗[a]	↗	NA	↗	↗
	IGF-1	↔	↗	↔[a]	↔[a,g]	↗*	X	↗	↗
	GH	↗[a]	↗[a]	↗	X	NA	NA	↗	NA
Pro-inflammatory markers	IL-1β, TNFα, IL-6	↗	↗	↔[o]	↘	↗	NA	↔[o]	↘
	Kynurenine	↗[a]	↗	X	↘[c]	NA	NA	NA	NA
Anti-inflammatory markers	IL-4, IL-10	↗	↗	↗	↗	↗	NA	↗	↗
Myokines[±]	Irisin	↗[e]	↗	↔*[a]	↔[a,d,i,w]	NA	NA	↗	NA
	Cathepsin-B	X*	X*	↗*	↗	NA	NA	↗*	X*
	Apelin	↗	↗	↔[a,c,w]	↔[c,w]	NA	NA	NA	NA
	Adiponectin	↔	X	↗[w]	↗[i,w]	NA	NA	NA	NA
Metabolites	Lactate	↗[i]	↗[i]	NA	NA	↗[i]	NA	↗*	NA
	β-hydroxybutyrate	↗	↗*	↗	↗*[w]	↗[h]	NA	↗	NA
Other exerkines[±]	Osteocalcin	↗[a,g]	X	↗	↗	NA	NA	NA	NA
	Orexin-A	↗	NA	↗*	NA	↗	NA	NA	NA
	Ghrelin	↔[i]	↘	↗[w]	↗	NA	NA	NA	NA
	VIP	↗	NA	↗	NA	NA	NA	NA	NA

The direction of significant changes in exerkine levels were reported as follows: '↗' at least one study found a significant increase and no studies were found that reported significant decreases; '↘' at least one study found a significant decrease and no studies were found that reported significant increases; '↔' inconsistent, with studies indicating both significant increases and decreases. 'X', no significant effect reported by any of the studies found; 'NA', no studies available. '*', was used to mark studies were the arrow's direction was only based on data from 1 study.

It is indicated whether studies have measured these alterations in biomarker levels in peripheral circulation (BLOOD) or behind the blood-brain barrier (BRAIN), after Acute or Chronic exercise, and cardiovascular (C) or resistance (R) exercise. ± All of these factors are considered exerkines. We categorized the exerkines based on how they are most commonly referred to. It should be noted that BDNF, IGF-1, IL-6, etc. can also be considered myokines, as they are to some extent also released from muscle tissue.

Caution is needed when interpreting this table, as findings may be population- or exercise-specific. We refer to the accompanying text and Supplementary table 1 and 2 for more details. In addition, letter codes were used to highlight the most important particularities: Significant different results have been reported with 'a', older **a**ge (↗ BDNF, IGF-1, kynurenine, irisin, apelin; ↘ GH; X total osteocalcin) or younger age (↘ IGF-1); 'c', **c**onditions such as cancer (↘ kynurenine) or insulin resistance (↘ apelin); 'd', longer exercise **d**uration in minutes (↗ BDNF) or weeks (<12 weeks ↗ irisin, > 16 weeks ↘ irisin); 'f', higher cardiorespiratory **f**itness level (↗ BDNF); 'g', female **g**ender (↗ IGF-1) male gender (↗ BDNF, total osteocalcin); 'h', exercise-induced **h**ypoglycemia (↗ β-hydroxybutyrate); 'i', higher exercise **i**ntensity (↗ irisin, adiponectin, lactate) or lower intensity (↘ irisin, ↗ ghrelin); 'o', excessive chronic exercise or **o**vertraining (↗ pro-inflammatory factors); 'w', exercise-associated **w**eight loss (↗ irisin, ghrelin, adiponectin; ↘ apelin).

Abbreviations: BDNF, brain-derived neurotrophic factor; IGF-1, insulin-like growth factor-1; IL, interleukin; GH, growth hormone; TNFα, tumor necrosis factor alpha; VIP, vasoactive intestinal peptide.

3.1 Growth factors

3.1.1 Brain-derived neurotrophic factor (BDNF)

BDNF is recognized as a growth factor with a wide repertoire of neurotrophic and neuroprotective properties in the CNS and the periphery (Knaepen et al., 2010). It can be produced by neurons (Lessmann et al., 2003), astrocytes (Numakawa et al., 2010), and endothelial cells (Wang et al., 2006) within the brain. During physical exercise, an increase in circulating BDNF levels may result from the release of BDNF from skeletal muscle cells or platelets (Antony and Li, 2020; Le Blanc et al., 2020; Farmer et al., 2021). Skeletal muscle cells may synthesize (Matthews et al., 2009) and release BDNF into the circulation (Máderová et al., 2019) in response to physical exercise. Platelets were found to contain 99% of circulating BDNF (Radka et al., 1996). The number of circulating BDNF-containing platelets is increased during physical exercise when the activation of the sympathetic system causes splenic constriction (Ahmadizad and El-Sayed, 2003; Stewart et al., 2003). At last, increased shear stress due to acceleration of blood flow during physical exercise causes the release of platelet-derived BDNF (Fujimura et al., 2002). The increased shear stress was also reported in the cerebral vasculature during physical exercise (Jorgensen et al., 1992). However, Pardridge et al. (1994) found that peripherally administered BDNF was rapidly cleared from the systemic circulation and was not able to be delivered to the brain. As was reviewed by Serra-Millàs (2016), the relationship between peripheral and central levels of BDNF remains a topic of discussion. While some studies suggest peripherally administered BDNF may cross the blood-brain barrier or have beneficial effects on central nervous system functioning, several other studies reported blood-brain barrier permeability for BDNF to be poor or absent (Serra-Millàs, 2016). It is possible that the central increases in BDNF availability following physical exercise, as were found in animal studies (see below), merely result from the stimulation of central BDNF synthesis. Other exerkines may be important mediators of this effect. This will be substantiated by pathways of the other exerkines discussed in sections 3.1.2 till 3.5.4, some of whom were found to induce the hippocampal release of BDNF during physical exercise.

<u>Pathway</u>

Evidence from studies including animal models suggests that the physical exercise-induced increase of BDNF levels in the brain enhances the response to LTP induction by electrophysiological stimulation (Novkovic et al., 2015; Miao et al., 2021). For example, in wild type and heterozygote $BDNF^{+/-}$ mice, 5 weeks of voluntary physical exercise was found to elevate BDNF levels compared with sedentary controls and enhance LTP activity, measured with in vitro electrophysiological recordings. In the $BDNF^{+/-}$ mice, LTP was initially impaired, but physical exercise restored it to the level of sedentary wild type mice (Novkovic et al., 2015). Another study reported a dose-dependent enhancement of synaptic responses to electrophysiological stimuli after BDNF administration on

slices of the anterior cingulate cortex of male mice in vitro (Miao et al., 2021). BDNF acts via tropomyosin-receptor kinase-B (TrkB) receptors in the postsynaptic density of the excitatory synapse (Fig. 1, 2). TrkB receptors mediate many signaling cascades involved both in early and late LTP (Müller et al., 2020). Specifically, BDNF binding to the TrkB receptor causes dimerization and autophosphorylation of the receptor. Consequently, docking sites for Src homology 2 domain-containing adapter protein (Shc) and phospholipase C-γ (PLCγ) emerge. Shc is coupled with Ras and phosphoinositide 3-kinase (PI3K) signaling cascades. *Ras* activates extracellular signal regulated kinase (ERK), a member of the mitogen-activated protein kinases (MAPK) that may, in turn, activate several other pathways by phosphorylating its target (Murray and Holmes, 2011). For example, on rat hippocampal slices, inhibition of ERK was found to prevent the phosphorylation of CamKII, which is crucial in the LTP process (Giovannini et al., 2001). ERK also activates CREB (Finkbeiner et al., 1997) and following a physical exercise-induced rise in BDNF, CREB was seen to induce its own gene expression, thereby increasing the number of CREB molecules (Vaynman et al., 2003). Moreover, CREB was found to induce the upregulation of BDNF in response to Ca^{2+} influx in the postsynaptic cell (Shieh and Ghosh, 1999), CamKII during the process of LTP (Giovannini et al., 2001), NMDA receptor transcription following administration of BDNF (Caldeira et al., 2007) and TrkB receptors following physical exercise in response to increased BDNF levels (Vaynman et al., 2003). Ras also promotes the activation of PI3K. In turn, *PI3K* activates Akt, which counteracts pro-apoptotic proteins, stimulating survival (Murray and Holmes, 2011). Both Ras and PI3K signaling cascades were found to lead to the phosphorylation of NMDA receptors following administration of BDNF on cultured mouse hippocampi, increasing NMDA receptor open probability (Xu et al., 2006). Furthermore, PI3K was involved in increasing surface AMPA receptor expression during LTP in cultured hippocampal neurons (Man et al., 2003). Results from Vaynman et al. (2003) suggested that the interplay between the TrkB and NMDA receptor signaling cascades is crucial for the CREB-mediated transcription of BDNF, TrkB, CREB and synapsin I mRNA, as blocking of any of these two receptors fully abrogated the physical exercise-induced increases in these transcripts (Vaynman et al., 2003). Next to the Shc induced pathways, *PLCγ* will promote another pathway starting with the catalyzation of lipids to inositol 1,4,5 triphospate (IP3). IP3 binds to receptors on the endoplasmic reticulum, triggering calcium release (Yamamoto et al., 2000). This calcium release enhances LTP through activation of the CamKII and PKA mediated pathways similarly as upon activation of NMDA-receptors. Furthermore, IP3 activity is required to keep AMPA receptors clustered at the postsynaptic membrane, as shown on hippocampal slices (Arendt et al., 2010). PLCγ also induces an increase in diacylglycerol (DAG), which regulates protein kinase C (PKC). In turn, PKC might be required for the ERK cascade (Murray and Holmes, 2011) and was found to potentiate AMPA receptors by phosphorylation in cultured neurons during LTP (Roche et al., 1996).

BDNF also binds TrkB receptors at the *presynaptic* neuron of the excitatory synapse (Fig. 1, 2). Here, in vitro examination found that ERK signaling activates synapsin I by phosphorylation, targeting synaptic vesicles from the reserve pool toward the releasable pool (Jovanovic et al., 1996). Moreover, BDNF-mediated activation of the PLC/IP3 pathway will increase presynaptic intracellular Ca^{2+}-levels. This increases CamKII signaling and results in CREB-mediated transcription of synapsin I. Synapsin I levels were found to increase following cardiovascular exercise, which was abrogated after blocking CamKII (Vaynman et al., 2003; Murray and Holmes, 2011).

Acute exercise effect

Both acute cardiovascular and resistance exercise were found to transiently increase circulating BDNF in a meta-analysis that included 47 studies on cardiovascular exercise and eight studies on resistance exercise (Dinoff et al., 2017). Dinoff et al. (2017) reported that physical exercise with a duration of more than 30 minutes induced higher elevations of circulating BDNF than shorter physical exercise bouts. They also found that plasma BDNF measurements increased more in response to physical exercise compared to serum measurements and that studies including more males had greater effect sizes than those where the majority of participants were females. With approximately three-quarters of all participants in the included acute exercise studies being males, subgroup analysis revealed that only in males significant increases in circulating BDNF were found (Dinoff et al., 2017). This might be due to that women already have higher basal serum BDNF levels than men (Glud et al., 2019). It was reported that estrogen levels influence circulating BDNF levels and BDNF signaling pathways (Harte-Hargrove et al., 2013; Dong et al., 2017). Furthermore, Dinoff et al. (2017) found no significant difference in effect sizes associated with age, with most acute exercise studies including young adults. At last, higher cardiorespiratory fitness was associated with greater increases in circulating BDNF (Dinoff et al., 2017).

In the brain, studies in male rats showed that acute voluntary wheel running induced elevated levels of hippocampal BDNF (Oliff et al., 1998; Takimoto and Hamada, 2014).

Chronic exercise effect

While Knaepen et al. (2010) concluded in their review that chronic exercise is rather unlikely to elevate basal BDNF concentration in healthy adults, more recent meta-analyses did find small effects in favor of a peripheral BDNF rise of baseline levels in response to regular cardiovascular exercise (Szuhany et al., 2015; Dinoff et al., 2016). Moreover, in a systematic review including older adults with cognitive decline, serum levels of BDNF significantly rose after chronic cardiovascular training (de Assis and de Almondes, 2017). However, this was not confirmed in a more recent meta-analysis including older adults with or without cognitive decline (Marinus et al., 2019). The latter meta-analysis, including eight resistance training and four combined cardiovascular and resistance training studies, stated that in order to increase

baseline BDNF levels, resistance training is an essential component of the physical exercise program in older adults (Marinus et al., 2019). In contrast, the meta-analysis of Dinoff et al. (2016), including healthy adults of all ages, did not find an effect of chronic resistance training on resting circulating BDNF levels. Therefore, this effect is probably age-specific (Table 1), although future studies are needed to confirm the inference we make here. Moreover, Dinoff et al. (2016) did not report effect differences dependent on physical exercise intervention characteristics such as duration, frequency, or intensity. In addition, there was no difference between BDNF rises measured in serum or plasma. Finally, age, gender, and body mass index were not related to the effect found after chronic exercise (Dinoff et al., 2016).

Brain levels of BDNF, TrkB and CREB in male rat hippocampus did also increase after chronic cardiovascular (Vaynman et al., 2003; Berchtold et al., 2005; Cassilhas et al., 2012) and resistance training (Tang et al., 2017; Vilela et al., 2017). It was shown that 3 weeks of running resulted in elevated hippocampal BDNF levels until 2 weeks after cessation of physical exercise (Berchtold et al., 2010). In the study of Tang et al. (2017), the resistance trained male diabetic rats showed a higher upregulation of TrkB and CREB genes than cardiovascular trained diabetic rats.

3.1.2 Insulin-like growth factor-1 (IGF-1)

IGF-1 plays a role in enhancing insulin action (Moses et al., 1996), and decreased levels of IGF-1 are associated with age-related sarcopenia (Mak and Rotwein, 2006; Bian et al., 2020). It is secreted both centrally and peripherally and may cross the blood-brain barrier (Carro et al., 2000). The central release has been shown in regions of the brain involved in postnatal neurogenesis, e.g. hippocampus, cerebellum, and olfactory bulb (Wrigley et al., 2017). IGF-1 release in the brain was inconsistently indicated as being regulated by growth hormone (GH) (Furigo et al., 2018) or being GH-independent (Lupu et al., 2001). Peripherally, GH is considered to mediate the main release of IGF1- from the liver (Schwander et al., 1983). During physical exercise, circulating IGF-1 levels were found to increase rapidly in some studies, which indicates it is most likely released from IGF-1 stores and not mediated by GH-induced transcription (Berg and Bang, 2004). It was suggested that muscle cells contain such IGF-1 stores that are released upon muscle contraction (Pedersen, 2019). Furthermore, IGF-1 mRNA expression was found to be upregulated in contracting muscles independently of GH (Berg and Bang, 2004). However, the increase in circulating IGF-1 levels is only inconsistently reported. A possible explanation was given by Carro et al. (2000) who indicated that after acute cardiovascular exercise brain IGF-1 levels increased, while circulating levels did not. They suggested that physical exercise might increase the uptake of IGF-1 in the brain (and other target organs) in association with its release from muscle and liver into the blood stream, keeping circulating IGF-1 levels relatively stable. Depending on the strength of this increased uptake, researchers might find increased, unchanged, or decreased circulating IGF-1 levels after physical exercise (Carro et al., 2000).

Pathway

In vitro examinations by Zheng and Quirion (2004) and Ding et al. (2006) using hippocampal cultured neurons showed that the IGF-1 receptor shares downstream signaling cascades with the TrkB receptor in pre- and postsynaptic excitatory neurons (Fig. 1, 2). Hence, similarly to BDNF, IGF-1 is thought to activate PI3K/Akt, IP3/CamKII, and Ras/ERK pathways. Zheng and Quirion (2004) indicated IGF-1 causes rapid and sustained activation of Akt signaling, while it mediated only transient ERK signaling. The inverse was observed for BDNF, i.e. a transient activation of Akt signaling and a sustained activation of ERK signaling. Furthermore, systemic injection of IGF-1 or physical exercise-induced elevations of IGF-1 was found to increase transcription of hippocampal BDNF (Carro et al., 2001; Ding et al., 2006) and IGF-1 (Ding et al., 2006) and intracerebroventricular administration of IGF-1 reversed the age-related decline in the number of NMDA receptors (Sonntag et al., 2000). Blocking the IGF-1 receptor partly disrupted the physical exercise-induced increase of hippocampal BDNF levels and decreased memory recall performance (Ding et al., 2006). To our knowledge, no studies have directly assessed the effect of physical exercise-induced IGF-1 on the increased response to induction of LTP.

Acute exercise effect

Several reviews and meta-analyses confirmed that acute resistance exercise may increase circulating IGF-1 (Berg and Bang, 2004; de Alcantara Borba et al., 2020; Gulick et al., 2020), while the findings concerning acute cardiovascular exercise are equivocal (de Alcantara Borba et al., 2020; Gulick et al., 2020).

In the brain, there was evidence to suggest that acute cardiovascular exercise increases IGF-1 levels (Carro et al., 2000). This was not confirmed in a more recent study (Takimoto and Hamada, 2014). To the best of our knowledge, there are only two studies that examined the effect of acute resistance exercise on brain IGF-1 levels and signaling. One studied male rats (Fernandes et al., 2016) while the other used female rats (Kelty et al., 2019). Both failed to find an effect. Fernandes et al. (2016) indicated that this may have been caused by the time of sample collection, which was 24 hours after exercise, while circulating IGF-1 levels after acute exercise typically return back to baseline after 15 to 30 minutes post-exercise (Rubin et al., 2005; West et al., 2009; Rojas Vega et al., 2010; Tsai et al., 2015). Also in the study of Kelty et al. (2019), rats were killed and brain tissue was collected only 24 hours after the resistance exercise session.

Chronic exercise effect

In a systematic review by Stein and colleagues (2018), circulating levels of IGF-1 were not found to be elevated after chronic cardiovascular exercise in older adults. Only one out of five studies found increased IGF-1 levels after cardiovascular exercise, while one even reported a decrease. This review included only two resistance training studies (Stein et al., 2018). Both of

them showed increased IGF-1 levels (Cassilhas et al., 2007; Tsai et al., 2015; Stein et al., 2018). More recent meta-analyses confirmed that IGF-1 may increase following resistance training, but only in women more than 40 years old, while at younger age IGF-1 levels might even decrease following resistance training (Jiang et al., 2020; Ye et al., 2020; Amiri et al., 2021).

Finally, evidence from animal models suggests that physical exercise training had no differential effect on the levels of IGF-1 in the brain as a function of gender. Specifically, it was found that hippocampal IGF-1 levels increased both in male and female rats following chronic cardiovascular training (Ding et al., 2006; Gomes et al., 2009; Wong-Goodrich et al., 2010; Cassilhas et al., 2012) and resistance (Cassilhas et al., 2012; Kelty et al., 2019) exercise.

3.1.3 Growth hormone (GH)

GH is produced by the pituitary gland in response to GH-releasing hormone or somatostatin release from the hypothalamus. Most of the GH release occurs during sleep (Sonntag et al., 2005), but it is also released from the pituitary gland during physical exercise (Galbo, 1993). GH is thought to play a role in the post-exercise repair of tissues and synthesis of new tissues by stimulating protein anabolism. Furthermore, it helps to prepare the individual for future physical exercise bouts by enhancing the synthesis of gluconeogenic and lipolytic enzymes (Galbo, 1993). Decreased GH levels are related to age-related sarcopenia in human subjects (Bian et al., 2020). While studies before the year 2000 hypothesized that all of the cognitive effects of GH were mediated through its induction of IGF-1-synthesis, since then, some studies have indicated direct effects of GH on cognition (Sonntag et al., 2005).

Pathway

In the hippocampus, GH was found to induce dimerization of its receptor upon binding, which in turn activates Janus kinase 2 (JAK2). Activated JAK2 induces signaling cascades including PI3K/Akt and Ras/ERK. Via ERK, GH can also induce CREB activation. These pathways are very similar to the IGF-1 pathways and are shared with some of the BDNF pathways. GH receptor activates these pathways via JAK-mediated phosphorylation of non-receptor tyrosine kinases. In contrast, IGF-1 and BDNF receptors are receptor tyrosine kinases that can phosphorylate signaling molecules by themselves (Lobie et al., 2000). It was shown that a period of daily GH injections facilitated LTP, restored age-related and sleep deprivation-induced alterations in NMDA receptor-dependent synaptic transmission, and enhanced AMPA receptor activity, as shown on CA1 hippocampal slices (Kim et al., 2010; Molina et al., 2012). At last, GH can stimulate neural IGF-1 signaling by inducing its transcription (Furigo et al., 2018). We found no studies that examined the direct link between physical exercise-related increases in GH and the physical exercise-induced facilitation of LTP. Hence, this relationship can only be inferred from models that investigated the effect of GH administration (Kim et al., 2010; Molina et al., 2012).

Acute exercise effect

Both acute cardiovascular and resistance exercise were found to induce increases in GH levels in a systematic review (Wideman et al., 2002). It was indicated that this effect occurred both in men and women with similar levels being attained during physical exercise, but the increase from baseline was higher in men, with women having higher baseline GH levels. Older adults of both sexes showed an attenuated GH response to acute exercise. Furthermore, higher intensity of cardiovascular or higher volume of resistance exercise was suggested to induce larger GH increases (Wideman et al., 2002). Furthermore, resistance exercise induced higher acute responses than cardiovascular exercise (Consitt et al., 2007).

Chronic exercise effect

A systematic review reported that some studies found increased baseline GH levels after chronic cardiovascular exercise, but not following chronic resistance training (Wideman et al., 2002). The authors indicated that resistance exercise only induces an acute elevation of GH. However, resistance training studies that examine baseline 24h GH measurements (as is advised) remain scarce (Wideman et al., 2002). In the brain, Blackmore and colleagues provided suggestive findings for the induction of GH signaling and activation of neural precursor cells in the subventricular zone after chronic cardiovascular exercise. They showed that in the absence of GH signaling, by administration of GH antagonist or in GH receptor null female mice, cardiovascular exercise training no longer resulted in the activation of neural precursor cells (Blackmore et al., 2009, 2012).

3.2 Pro- and anti-inflammatory markers

3.2.1 Cytokines

Cytokines play a key role in immune responses. Cytokines such as interleukin-1β (IL-1β), IL-2, IL-8, IL-12, IL-15, IL-18, tumor necrosis factor-α (TNF-α), and interferon-γ (IFN-γ) are considered to be markers of pro-inflammatory action. On the contrary, the cytokines IL-4 and IL-10 have anti-inflammatory effects (Dai et al., 2013; Svensson et al., 2015; Agudelo et al., 2018). IL-6 activates both pro-inflammatory and anti-inflammatory processes. It is suggested to have a controlling function in inflammation (Smith and Miles, 2000). Some of these cytokines (e.g. IL-1β, IL-6, and TNF-α) are known to be released by muscle fibers into the bloodstream and, as such, are expected to play a role in the regulation of physical exercise-induced pro-and anti-inflammatory processes. Peripheral and central inflammatory levels are linked via blood-borne and neural routes of communication. As a result, peripheral inflammation may activate microglial cells in the brain. These innate immune cells react to inflammatory signals by de novo synthesis of inflammatory cytokines, further increasing inflammation within the brain (Barrientos et al., 2015).

Chronic elevation of (neuro-)inflammatory markers has previously been linked to obesity, metabolic syndrome, aging, cognitive decline, and many neurodegenerative disorders like Alzheimer's dementia (Kruse et al., 1993; Cotman et al., 2007; Yudkin, 2007; Sartori et al., 2012; Woods et al., 2012; Su et al., 2019).

Adipose tissue, especially visceral fat, is considered one of the largest contributors to systemic inflammation (Yudkin, 2007; Woods et al., 2012). Furthermore, inflammatory markers are secreted by senescent cells. These are old, damaged cells that, as a protective mechanism, have become locked into cell-cycle arrest to prevent the spread of damage and potential malignant transformation. In association, they exhibit altered secretory activity (Coppé et al., 2010; Hernandez-Segura et al., 2017). The number of these cells gradually increases as we get older (Dimri et al., 1995). The age-related development of a chronic inflammatory status is also found in the brain (Sartori et al., 2012). Indeed, magnetic resonance spectroscopy studies have shown age-related increases in neuro-inflammatory markers in the brain (i.e., myoinositol and choline) (Glanville et al., 1989; Urenjak et al., 1993). Furthermore, stereological findings have indicated increased numbers of glial cells in the frontal and temporal cortex with age (Terry et al., 1987). These glial cells change into their pro-inflammatory phenotype in older adults (Perry et al., 2007; Cohen and Torres, 2019). It was suggested that these changes underlie, at least in part, the process of age-related cognitive decline (Bourgognon and Cavanagh, 2020).

Pathway

The effect of IL-1β, IL-6 and TNF-α, which are the most studied cytokines, on LTP and learning were recently reviewed by Bourgognon and Cavanagh (2020). They describe that the effect is dependent of the intensity and duration of the inflammatory activity. Low brain cytokine levels may exert beneficial effects, while high or long-lasting elevations are detrimental to the LTP process. The latter is typically reported in older adults and neurodegenerative diseases (Bourgognon and Cavanagh, 2020; Ross et al., 2003). At the cellular level, a non-exhaustive summation of the pathways these cytokines interfere in (probably both in a beneficial or detrimental way depending on their concentration) are: the BDNF and IGF-1 signaling pathways, MAPK pathways both involved in synaptic plasticities such as the postsynaptic ERK pathway and those involved in cell damage or cell death such as c-jun N-terminal kinase (JNK) and p38 pathway, and the presynaptic ERK-mediated phosphorylation of synapsin I that induces glutamate release (Bourgognon and Cavanagh, 2020). As an example, excessively high-intense chronic cardiovascular exercise was found to suppress LTP during in vivo recordings in the hippocampal CA1 area in rats, in association with the increased expression of inflammatory factors IL-1β and TNF-α and induced activation of microglial cells. In addition, the physical exercise paradigm increased levels of phosphorylated JNK, ERK and p38 (Sun et al., 2017). Other studies on mice reported that the detrimental effect of elevated IL-1β on LTP could be

abrogated by the administration of the anti-inflammatory cytokine IL-10 (Lynch et al., 2004; Lenz et al., 2020). While this study did not examine the effect of physical exercise, other studies have reported the circulating level of IL-10 to increase following cardiovascular exercise, e.g., Gomes da Silva et al. (2013).

Acute exercise effect

Acute bouts of cardiovascular and resistance exercise were associated with increased circulating levels of both pro- and anti-inflammatory cytokines (Flynn et al., 2007; Koch, 2010; Johnson et al., 2020). The balance between the pro- and anti-inflammatory response to physical exercise is dependent on several factors, including the individual's health status, intensity or duration of physical exercise, and glucose availability (Flynn et al., 2007). In addition, pro-inflammatory cytokines may increase less following acute exercise in physical exercise-trained individuals, as was reported after six weeks of cardiovascular training (Fonseca et al., 2021). Overall, the regulation of peripheral inflammation by physical exercise is a complex process and will not be addressed in detail in this review paper. For further reading, we refer to other review articles (Cotman et al., 2007; Woods et al., 2012; Su et al., 2019; Scheffer and Latini, 2020).

At brain levels, both pro- and anti-inflammatory cytokines also increased in response to acute cardiovascular exercise (Packer et al., 2010; Lovatel et al., 2013; Packer and Hoffman-Goetz, 2015; Nogueira et al., 2020). However, the link between physical exercise-induced peripheral and central inflammation is not clear. For example, in a study where IL-6 levels in human plasma and cerebrospinal fluid were measured after acute cardiovascular exercise at 60% of VO2 max, there was an increase in plasma IL-6 without accompanying cerebrospinal fluid IL-6 increase (Steensberg et al., 2006). A more recent study, using a panel with 92 cytokines and chemokines to measure inflammatory markers in cerebrospinal fluid and plasma reported a modest increase in inflammatory markers in cerebrospinal fluid after acute vigorous intensity exercise (Isung et al., 2021). However, after correction for multiple comparisons, only three cerebrospinal fluid and 12 plasma proteins were significantly changed. In line with Steensberg et al. (2006), changes in cerebrospinal fluid IL-6 levels were nonsignificant (Isung et al., 2021). Steensberg et al. (2006) suggested that IL-6 may not reach the brain via the cerebrospinal fluid, but through alternative routes such as via the hypothalamus, which does not have a blood-brain barrier, via afferent nerves, or from local release by endothelial cells or the pituitary gland (Steensberg et al., 2006).

Chronic exercise effect

Chronic cardiovascular and resistance exercises were found to reduce blood and brain pro-inflammatory cytokines and elicit anti-inflammatory effects in an impressive array of human and animal research (Flynn et al., 2007; Gibbons et al., 2014; Kim, 2014; Chupel

et al., 2017; Liu et al., 2020; Roh et al., 2020). In humans, findings from resistance exercise studies suggested that lower levels of pre-exercise circulating pro-inflammatory factors were associated with better gains in muscle strength (Forti et al., 2014; Hangelbroek et al., 2018; Grosicki et al., 2020). In contrast to the anti-inflammatory effect of chronic exercise, the cytokine hypothesis of overtraining by Smith states that an inadequate recovery between physical exercise bouts would lead to chronic inflammation, associated with fatigue and depression indicative of overtraining (Smith, 2000). Only a limited amount of human studies investigated the effect of overtraining on pro-inflammatory markers, probably due to ethical considerations (Izquierdo et al., 2009; Main et al., 2009; Main et al., 2010; Halson et al., 2003). Most studies measured inflammatory markers immediately after cardiovascular exercise, which needs to be considered an acute exercise effect in excessively trained human subjects. These studies report increased elevations of pro-inflammatory cytokines following a bout of resistance or cardiovascular exercise in overtrained persons (Izquierdo et al., 2009; Main et al., 2009; Main et al., 2010). We also found one study that reported increased morning pro-inflammatory markers before physical exercise in male cyclists during an intense training program (Halson et al., 2003).

On the brain level, animal studies found that chronic exercise decreased microglial activation in the hypothalamus of obese mice (Barrientos et al., 2011; Yi et al., 2012) and in the hippocampus of aged mice (Kohman et al., 2013). Chronic cardiovascular (Liu et al., 2013; Bobinski et al., 2015) and resistance (Liu et al., 2020) exercise decreased central pro-inflammatory cytokines in male rats, and chronic cardiovascular (Gomes da Silva et al., 2013) and resistance exercise (Liu et al., 2020) increased the level of the anti-inflammatory cytokine IL-10 in the hippocampus of healthy old male rats and frontal cortex of male Alzheimer dementia mice, respectively. In contrast, maximal intensity cardiovascular exercise on seven consecutive days increased the expression of inflammatory factors IL-1β and TNF-α in the hippocampus of rats and induced the activation of microglial cells (Sun et al., 2017). In healthy human subjects, observations from a recent study by Isung et al. (2021) showed that chronic exercise has only a small effect on inflammation-related protein levels in the cerebrospinal fluid.

3.2.2 Kynurenine

Kynurenine is converted from tryptophan by the enzyme indoleamine 2,3 dioxygenase in the liver (Capuron et al., 2011). Pro-inflammatory cytokines, like IL-1β, TNF-α and IFN-γ have been shown to upregulate indoleamine 2,3 dioxygenase (Allison et al., 2017). In correspondence with high systemic inflammatory cytokine levels, high circulating kynurenine levels were found to be associated with reduced memory performance (Solvang et al., 2019). During physical exercise, the activity of kynurenine aminotransferase is enhanced. This enzyme converts kynurenine into kynurenic acid, which is unable to cross the blood-brain barrier (Agudelo et al., 2014).

Pathway

Within the brain, kynurenine can be metabolized into quinolinic acid by macrophages and microglia, or into kynurenic acid by astrocytes. Quinolinic acid leads to overactivation of NMDA receptors, which contributes to excitotoxic neural damage (Vécsei et al., 2013). Furthermore, it was found to have neuroinflammatory action (Stone and Darlington, 2013). In contrast, kynurenic acid was found to be an antagonist of NMDA and α7 nicotinic acetylcholine receptors (Potter et al., 2010). The latter receptors exist on presynaptic glutamatergic synapses and increase glutamate release from presynaptic neurons upon activation (Vécsei et al., 2013). Similar to inflammatory cytokines, electrophysiological recordings on rat hippocampal slices in the CA1 region showed that perfusion of low concentrations of kynurenic acid was beneficial, while high concentrations were detrimental for LTP (Rózsa et al., 2008). Only perfusion of low concentrations was found to increase AMPA receptor activity (Prescott et al., 2006). In addition, low concentrations of kynurenic acid preferentially antagonized extrasynaptic NMDA receptors, sparing synaptic NMDA and AMPA receptors, while high concentrations completely antagonized both extrasynaptic and synaptic glutamatergic receptors (Rózsa et al., 2008; Demeter et al., 2013; Vécsei et al., 2013). Kynurenic acid was not found to influence the number of NMDA or AMPA receptors (Potter et al., 2010). Of note, none of these studies examined if physical exercise-induced elevations or reductions of kynurenine, quinolinic acid, or kynurenic acid have an influence on LTP.

Acute exercise effect

A recent review paper from Joisten and colleagues (2020) that includes their own work reported the effect of acute and chronic exercise on kynurenine. After acute cardiovascular and resistance exercise, circulating kynurenine levels increased, but this elevation was associated with increased kynurenine aminotransferase and kynurenic acid levels (Joisten et al., 2020). In their own work, Joisten et al. (2020) discovered that the kynurenine aminotransferase pathway was elevated to a higher extent following cardiovascular exercise compared with resistance exercise. From the literature review was derived that kynurenic acid/kynurenine ratios increased immediately and 60 minutes after cardiovascular and immediately after resistance exercise, and kynurenine levels decreased compared with pre-exercise 60 minutes after resistance or cardiovascular exercise (Joisten et al., 2020). Another study recently reported that acute sprint interval exercise resulted in increased levels of kynurenine 60 min after physical exercise in old but not in young healthy human subjects. The elevation of kynurenine in older adults was followed by increased levels of kynurenic acid 24h later (Trepci et al., 2020).

Chronic exercise effect

Chronic cardiovascular exercise was also found to upregulate muscle kynurenine aminotransferase activity in mice (Agudelo et al., 2014; Ieraci et al., 2020) and humans (Agudelo et al., 2014; Allison et al., 2019). Agudelo et al. (2014) showed this resulted in increased conversion of kynurenine into kynurenic acid. One other study confirmed decreased kynurenine levels following cardiovascular exercise in mice that received kynurenine injections (Su et al., 2020). In contrast, most studies in humans have only reported trends of decreased circulating kynurenine levels or no effect after chronic cardiovascular or resistance exercise, as recently reviewed by Joisten et al. (2020) and confirmed in more recent studies (e.g., Isung et al. 2021). However, we found two studies that reported a decrease in circulating kynurenine levels after chronic resistance exercise in breast or pancreatic cancer patients, who had elevated baseline levels compared to healthy subjects (Zimmer et al., 2019; Pal et al., 2021). These results are suggestive to assume kynurenine levels only decrease following chronic exercise in conditions where they were elevated at baseline. In subjects with normal baseline levels, the upregulation of kynurenine aminotransferase activity seems only to keep levels in balance when physical exercise bouts tend to increase kynurenine levels.

3.3 Myokines

3.3.1 Irisin

Irisin was initially best known for turning white adipose tissue into brown adipose tissue (Boström et al., 2012). Furthermore, it was suggested to be a marker for muscle mass (Ruan et al., 2020). Irisin is cleaved from the membrane protein FNDC5. This membrane protein is upregulated after activation of the transcriptional regulators: peroxisome proliferator-activated receptor-γ coactivator 1α (PGC1α) and estrogen-related receptor-α (ERRα) (Olesen et al., 2010; Wrann et al., 2013). Physical exercise enhances the PGC1α/ERRα-induced expression of FNDC5 not only in muscle, but also in the hippocampus (Wrann et al., 2013; Wrann, 2016). It is suggested that peripheral, physical exercise-induced irisin can pass through the blood-brain barrier (Wrann, 2016; Lourenco et al., 2019). Finally, observations from an animal model showed that administration of irisin in the hippocampus increased the response to LTP induction by electrophysiological stimuli (Mohammadi et al., 2019).

Pathway

Physical exercise was found to upregulate FNDC5/irisin expression and improve LTP in a mouse Alzheimer's dementia model. Downregulating FNDC5/irisin with lentivirus-mediated short hairpin RNA knockdown centrally or with anti-FNDC5 antibodies peripherally caused LTP not to improve following chronic exercise (Lourenco et al., 2019). Physical exercise-induced irisin was found to increase BDNF levels and is thought to affect

neurotransmission and/or regulation of LTP in the brain by stimulating the cAMP/PKA/CREB pathway (Wrann et al., 2013; Lourenco et al., 2019). Greater physical exercise-induced increases of irisin were correlated with higher physical exercise-induced BDNF levels (Nicolini et al., 2020). However, it remains unknown which neuronal receptor induces this pathway after being activated by irisin (Chen and Gan, 2019).

Acute exercise effect

A systematic review reported that both acute cardiovascular and acute resistance exercise may induce a transient increase in irisin levels, as the authors found in six out of eight included studies (Rodrigues et al., 2016). The two studies that did not find a significant effect used cardiovascular exercise (Pekkala et al., 2013)(Aydin et al., 2013). Tsuchiya et al. (2015) indicated that resistance exercise induces a larger irisin response than cardiovascular exercise alone or resistance and cardiovascular exercise combined. Kraemer et al. (2014) compared young men with women during the early follicular phase and mid-luteal phase of the menstrual cycle, but did not find any differences. Higher intensity cardiovascular exercise was associated with higher levels of irisin (Daskalopoulou et al., 2014; Huh et al., 2014), but there was no significant difference for age or fitness level (Huh et al., 2014).

Chronic exercise effect

Wrann et al. (2013) showed that irisin levels could be increased by chronic cardiovascular exercise both in blood and brain. Multiple animal studies using cardiovascular training confirmed their finding (Wrann, 2016; Uysal et al., 2018; Lourenco et al., 2019; Gruhn et al., 2021). In human studies, we found only two studies that showed increases in irisin levels in men following cardiovascular exercise (Boström et al., 2012; Miyamoto-Mikami et al., 2015) while others indicated no significant effect (Pekkala et al., 2013; Norheim et al., 2014; Kim et al., 2016) and one showed decreases following sprint training in young physically active men (Tsuchiya et al., 2016). Miyamoto-Mikami et al. (2015) found only significant increases in irisin levels in middle-aged/older men and not in the young subgroup. Despite that Wrann (2016) stated that resistance training would probably not induce FNDC5 expression, since resistance exercise was found to activate a different isoform of PCC-1α than cardiovascular exercise (PCC-1α4 instead of PCC-1α1), a recent meta-analysis of randomized controlled trials included three resistance training studies which showed significant irisin level increases (Cosio et al., 2021). Furthermore, there were two resistance training studies reporting significant decreases and two reporting nonsignificant effects. Overall, the meta-analysis concluded that the effect of chronic resistance exercise on circulating irisin was a nonsignificant positive trend. However, subgroup analysis showed significant increases for older adults when % body fat decreased during the intervention period and when the intervention was less or equal to 12 weeks. There was a significant decrease when resistance training lasted longer than 4 months or when less than 80%

of the sessions were supervised by a professional (Cosio et al., 2021). Both studies with significant decreases had a duration of approximately 6 months, with low intense physical exercise sessions and without progression in intensity (Hecksteden et al., 2013; Scharhag-Rosenberger et al., 2014). Subgroup analysis showed no differences for gender (Cosio et al., 2021). One pilot study reported an increase in circulating irisin in their resistance training group compared to their cardiovascular training group and control group following 8 weeks in obese subjects (Kim et al., 2016).

3.3.2 Cathepsin-B

Cathepsin-B is a lysosomal cysteine protease. During physical exercise, it is released from skeletal muscle cells. Cathepsin-B was found to pass through the blood-brain barrier and induce an increase in brain levels of BDNF. This was associated with improved memory function (Moon et al., 2016). However, cathepsin-B is also suggested to be a major driver for inflammatory brain diseases, neurodegenerative disorders, and brain aging associated with cognitive decline, as reviewed by Hook et al. (2020). Other authors have even advised to search for specific inhibitors of cathepsin-B as a therapeutic approach against neurodegeneration (Nakanishi, 2020).

Pathway

Cathepsin-B administration was reported to induce an increase in BDNF mRNA and protein levels on hippocampal progenitor cells in culture (Moon et al., 2016). The downstream signaling cascades that caused transcription of BDNF are currently unknown. A direct effect of physical exercise-induced cathepsin-B on LTP has not yet been investigated.

Acute exercise effect

A single bout of high-intensity interval exercise (Nicolini et al., 2020) or resistance exercise (Johnson et al., 2020) did not alter cathepsin-B levels in healthy young male adults.

Chronic exercise effect

Evidence for chronic exercise-induced changes in cathepsin-B levels is inconsistent. Moon et al. (2016) showed increased levels after cardiovascular training both peripherally and in the brain, while this was not confirmed by other authors (Gourgouvelis et al., 2018; Mees et al., 2019; Nicolini et al., 2019; Pena et al., 2020). Chronic resistance exercise was found to elevate cathepsin-B mRNA levels in muscle tissue (Norheim et al., 2011) and increase circulating levels in obese females (Kim and Kang, 2020). Again, other studies only found non-significant trends or no effect in female mice or humans (Pena et al., 2020; Micielska et al., 2021).

3.3.3 Apelin

Apelin is synthesized in many tissues, such as muscle, adipose tissue, and the brain (Masoumi et al., 2018; Wysocka et al., 2018; Halon-Golabek et al., 2019). It was reported to improve glucose homeostasis. Apelin levels were found to be increased in obesity and diabetes mellitus. This is suggested to be a compensatory mechanism to decrease insulin resistance (Boucher et al., 2005; Bertrand et al., 2015). Furthermore, apelin was suggested to be a biomarker for the diagnosis of aging-associated sarcopenia (Vinel et al., 2018). Pro-apelin is cleaved into apelin-36, and then further processed into shorter isoforms. Apelin-13 may represent the adipose tissue-derived isoform. Apelin-13 synthesis was found to be upregulated in adipose tissue of male obese mice (Shin et al., 2013). It is not clear which is the most expressed isoform in muscle tissue, but most researchers use non-specific measurements of apelin (Bae et al., 2019). Muscle-derived apelin might also be able to cross the blood-brain barrier, as intraperitoneal injections have been shown to increase apelin concentrations in the hypothalamus (Higuchi et al., 2007). However, none of the physical exercise studies we found searched for central apelin levels.

Pathway

Apelin administration to brain-derived glial cells increased BDNF levels in vitro. Inhibition of the apelin receptor downregulated BDNF mRNA expression, indicating apelin might promote BDNF-mediated LTP facilitation (Kwak et al., 2019). Another study also found that one week of daily intracerebroventricular injection of apelin increased hippocampal BDNF levels, as measured in vitro 24 hours after the last injection on hippocampal slices. In addition, this study also discovered that an antagonist of the TrkB-receptor blocked the ameliorative effect of apelin on memory performance in rats (Shen et al., 2019). Furthermore, apelin has been shown to act via the PI3K and ERK signaling pathways in the hippocampus. The beneficial effect of intracerebroventricular apelin administration on depression and memory of stressed rats was blocked by pretreatment with PI3K or ERK1/2 inhibitors (Li et al., 2016). At last, apelin is considered an anti-inflammatory agent counteracting the elevation of neuroinflammatory markers such as IL-1β and TNF-α, as occurring following brain injury (Masoumi et al., 2018). No studies searched for a causal link between physical exercise-induced elevations of apelin and the facilitation of LTP.

Acute exercise effect

Some studies reported that an acute bout of endurance (Bilski et al., 2016; Son et al., 2019; Dundar et al., 2019a; Kon et al., 2020), sprint interval (Kon et al., 2019), or resistance exercise (Kechyn et al., 2015; Fortunato et al., 2018) significantly increased apelin plasma levels. But levels did not significantly change in other studies (Waller et al., 2019).

Chronic exercise effect

A recent meta-analysis from Bae and colleagues (2019), including nine studies, showed that circulating apelin levels increased following physical exercise. They reported that all four studies including participants with a mean age between 50-60 years old showed significant increases. In contrast, only one of the five studies including younger adults could replicate these results. Only two studies included resistance exercise, with one reporting non-significant changes and the other reporting a decrease in apelin levels (Bae et al., 2019). In rat studies, apelin levels increased following chronic cardiovascular and resistance exercise (Zhang et al., 2006; Ji et al., 2016; Son et al., 2017; Vinel et al., 2018; Kwak et al., 2019; Sabouri et al., 2020). However, decreases following chronic cardiovascular or resistance exercise were also reported. Some studies in obese women reported declines of (non-isoform specific) apelin levels linked to physical exercise-induced weight loss (Sheibani et al., 2012; Jang et al., 2019), but physical exercise-induced decreases in apelin levels were more consistently linked to physical exercise-associated improvements of insulin resistance (Krist et al., 2013; Bertrand et al., 2015; Delavar and Heidarianpour, 2016; Otero-Díaz et al., 2018; Kolahdouzi et al., 2019; Nam et al., 2020). Moreover, it was shown that insulin directly drives the upregulation of adipocyte-derived apelin in a state of hyperinsulinemia (Boucher et al., 2005; Yang et al., 2015), while muscle tissue expresses apelin only following physical exercise (Yang et al., 2015). As apelin has a beneficial effect on glucose homeostasis, adipocyte-derived apelin might have a role in limiting insulin resistance when it is already present, while muscle-derived apelin has the potential to prevent it.

3.3.4 Adiponectin

Adiponectin is mainly released from adipose tissue. However, during physical exercise, it is also expressed and released from skeletal muscle (Dai et al., 2013). Circulating adiponectin levels were lower in obese adults (Yang et al., 2002). Adiponectin was found to be able to cross the blood-brain barrier and mediate hippocampal neurogenesis (Yau et al., 2014; Li et al., 2015). Moreover, it is considered an anti-inflammatory marker with beneficial effects on cardiovascular and metabolic disorders (Ouchi and Walsh, 2007).

Pathway

Intracerebroventricular injection adiponectin was found to facilitate LTP in anesthetized rats (Pousti et al., 2018). Wang et al. (2019) showed that administration of adiponectin increased AMPA and NMDA surface expression on hippocampal slices. However, the intracellular signaling pathway activated by adiponectin remains unclear. It was suggested that adiponectin might enhance NMDA-receptor function via the PI3K/Akt pathway in the hippocampus (Pousti et al., 2018), as this pathway was activated following intracerebroventricular adiponectin injection in an Alzheimer's rat model (Xu et al., 2018). Furthermore, multiple studies have

shown that adiponectin has an anti-inflammatory effect on the brain (Forny-Germano et al., 2019). Although these studies may suggest that adiponectin can mediate the exercise-cognition effect, no studies have currently provided direct evidence that changes in circulating adiponectin levels following physical exercise facilitate LTP.

Acute exercise effect

In systematic reviews, acute cardiovascular exercise was found to increase adiponectin levels (Simpson and Singh, 2008; Bouassida et al., 2010). Simpson and Singh (2008) suggested that high-intensity exercise is required for the modulation of adiponectin levels. Bouassida et al. (2010) indicated that increases are only found following physical exercise bouts of less than 60 min. We found only two studies that examined the effect of acute resistance exercise. Both did not show significant changes in adiponectin levels (Mansouri et al., 2011; Ihalainen et al., 2017).

Chronic exercise effect

A meta-analysis showed that adiponectin expression increased following chronic cardiovascular exercise, but not following resistance exercise in prediabetic and diabetic adults (Becic et al., 2018). A more recent meta-analysis also reported an overall increase of adiponectin levels following chronic exercise, but only included two studies with resistance exercise. In one of the two studies, resistance exercise induced significant increases in adiponectin levels (Rahimi et al., 2021). However, quite some other studies not included in these meta-analyses did report significant increases in adiponectin levels after chronic resistance exercise (Fatouros et al., 2005; Ihalainen et al., 2017; Galbreath et al., 2018; Montrezol et al., 2019; Park et al., 2019) or combined cardiovascular and resistance exercise protocols (Markofski et al., 2014; Dieli-Conwright et al., 2018a; Dieli-Conwright et al., 2018b; Ghayomzadeh et al., 2020). Of interest, Davis et al. (2015) reported that the combination of cardiovascular and resistance exercise was better at increasing adiponectin levels than resistance exercise alone. This might be explained by the finding from others that adiponectin increase was linked with fat loss (Bouassida et al., 2010; Christiansen et al., 2010; Kelly et al., 2014).

3.4 Metabolites

3.4.1 Lactate

Acute high-intensity exercise increases muscle-derived lactate levels (Saucedo Marquez et al., 2015; Albesa-Albiol et al., 2019). Next, lactate may cross the blood-brain barrier via monocarboxylate transporters. Interestingly, these transporters were found to be rapidly upregulated during an acute bout of physical exercise (Takimoto and Hamada, 2014). Brain lactate levels were found to remain elevated more than 40min after vigorously intense physical exercise, while blood lactate levels had already dropped back to baseline

(Maddock et al., 2011). Brain lactate can also arise from astrocyte metabolism (Müller et al., 2020). Lactate is transferred via monocarboxylate transporters from astrocytes towards neurons when energy demand is high, such as during memory formation. Pharmacological inhibition of monocarboxylate transporter 2, the transporter that is found on neurons to admit lactate, impairs long-term memory formation (Newman et al., 2011).

Pathway

Increased blood lactate levels were found to correlate with circulating BDNF, IGF-1, GH, and VEGF (Schiffer et al., 2011; Salgueiro et al., 2014; Kujach et al., 2020). Furthermore, lactate was found to increase the hippocampal levels of transcriptional coactivator PGC1α and its transcriptional product, FNDC5/irisin. As described in section 3.3.1, FNDC5/irisin is known to induce BDNF expression (Wrann et al., 2013). Lactate acts by activating silent information regulator 1 (SIRT-1), a class III histone deacetylase (El Hayek et al., 2019). El Hayek et al. (2019) discovered in male mice that SIRT-1 is activated by the NADH molecules that originate from the conversion of lactate back to pyruvate. Moreover, both protein and mRNA levels of SIRT-1 were increased following physical exercise and lactate infusion. The same effect was found after intraperitoneal injections of lactate at concentrations that induced increases in hippocampal lactate levels of the same level as found after physical exercise (El Hayek et al., 2019).

In addition, lactate was found to potentiate active NMDA receptors in cultured cortical neurons, thereby increasing the response of downstream signaling pathways, which are involved in the LTP process (Yang et al., 2014). Lactate can also be used in the tricarboxycyclic acid cycle to produce intermediates that can be used for de novo synthesis of amino acid neurotransmitters such as glutamate and GABA (Kleppner and Tobin, 2002).

Finally, lactate may also reduce neuroinflammation by changing microglia toward their anti-inflammatory phenotype, see section 3.2.1 (Errea et al., 2016). Lactate causes the addition of a lactyl group to the lysine amino-acid residues in the tails of histone proteins (i.e., histone lactylation) which stimulates genes of the anti-inflammatory phenotype in microglia (Zhang et al., 2019).

Lactate seems to activate several pathways associated with LTP. It can be used as a precursor to the excitatory neurotransmitter glutamate and the inhibitory neurotransmitter GABA (Kleppner and Tobin, 2002), and physical exercise-induced elevations of lactate were reported to cause SIRT-1 activation. The physical exercise-induced activation of PGC1α and, in turn, FNDC5/irisin, which induces BDNF synthesis, were found to be dependent on SIRT-1 activation (El Hayek et al., 2019). As increased levels of BDNF are linked with the facilitation of LTP, the effect of lactate can be inferred. However, none of these studies examined the direct link between the physical exercise-induced facilitation of LTP and lactate.

Acute exercise effect

Both acute high-intensity cardiovascular exercise and resistance exercise are capable of increasing blood lactate levels at intensities above the anaerobic threshold (Saucedo Marquez et al., 2015; Albesa-Albiol et al., 2019). Animal studies confirmed acute cardiovascular exercise-induced increases in brain lactate levels in the cortex, hippocampus and hypothalamus (Takimoto and Hamada, 2014). In humans, a difference between the carotid artery and jugular vein lactate levels indicated lactate is used within the brain during acute cardiovascular exercise (Ide et al., 1999; 2000). Moreover, magnetic resonance studies showed increased brain lactate levels after high-intensity cardiovascular exercise in the visual cortex (Maddock et al., 2011).

Chronic exercise effect

No effect on baseline levels of lactate after chronic exercise is to be expected. However, one study showed that mice with free access to a running wheel for 30 days had higher in vitro hippocampal lactate concentrations than sedentary controls. Therefore, they suggested that lactate accumulates following chronic cardiovascular exercise in the hippocampus of male mice (El Hayek et al., 2019).

3.4.2 β-hydroxybutyrate (BHB)

Ketone bodies, like BHB, are increased in the circulation and brain after fasting, dieting, and prolonged physical exercise (Mitchell et al., 1995). Ketone bodies are produced in the liver. They are used as an energy source under conditions of reduced glucose levels (Mitchell et al., 1995). Similar to lactate, also BHB penetrates the blood-brain barrier through the monocarboxylate transporter (Takimoto and Hamada, 2014). As described in section 3.4.1, the activation of these transporters was found to increase following acute exercise (Takimoto and Hamada, 2014). Of interest, BHB administration was found to improve cognitive function in rats (Murray et al., 2016; Hernandez et al., 2018).

Pathway

BHB acts as a direct class I histone deacetylase inhibitor. It prevents the recruitment of histone deacetylase 2 and 3 to the BDNF promoter I. This way, BDNF gene transcription was increased following BHB administration or the elevation of circulating BHB levels after chronic exercise interventions, as was shown on mice and rat hippocampal cultures (Sleiman et al., 2016; Hu et al., 2018; Lan et al., 2018). Moreover, it acts via the cAMP/PKA pathway to activate CREB (Hu et al., 2018). Incubation of hippocampal slices with BHB increased excitatory synaptic transmission, which is related to LTP facilitation (Sleiman et al., 2016). While Sleiman et al. (2016) both reported increased hippocampal BHB levels following physical exercise and facilitation of LTP following administration of BHB on hippocampal slices, they did not directly measure LTP activity following physical exercise.

Acute exercise effect

An acute bout of prolonged cardiovascular exercise was found to increase BHB blood (Mitchell et al., 1995; Nybo et al., 2003; Kim et al., 2013) and brain (Nybo et al., 2003; Takimoto and Hamada, 2014) levels in animal and humans (arterial and jugular venous blood level differences). All of these studies used male animals or human subjects. Nybo et al. (2003) reported that the effect was absent when exercising subjects received carbohydrate supplementation. One study showed that acute resistance exercise also increased BHB levels in men (Tsekouras et al., 2009).

Chronic exercise effect

In old male mice, BHB serum levels increased following a 4-week physical exercise program in endurance trained, but not in resistance trained animals (Kwak et al., 2021). However, in combination with a low calorie diet, both cardiovascular and resistance training were found to increase BHB levels (Jo et al., 2019; Vieira et al., 2021). Furthermore, elastic band exercise, which was considered a hybrid form of physical exercise between cardiovascular and resistance training, increased BHB serum levels in women with low pre-exercise BHB concentrations (Kwak et al., 2021). Finally, BHB was found to accumulate in the hippocampus of male mice after a chronic cardiovascular exercise program (Sleiman et al., 2016; Lan et al., 2018). Non-significant trends of increased blood and brain BHB levels after chronic cardiovascular exercise are also reported (Béland-Millar et al., 2020).

3.5 Other exerkines

3.5.1 Osteocalcin

Osteocalcin is a bone-derived hormone. It is secreted by osteoblasts during bone resorption. It can be found in blood in active (uncarboxylated) and decarboxylated forms (Khrimian et al., 2017). The uncarboxylated form of osteocalcin was found to cross the blood-brain barrier. There, it enhances the synthesis of monoamine neurotransmitters (serotonin and catecholamines), but inhibits the synthesis of GABA. This was found to favor learning in adult mice (Oury et al., 2013). In mice, baseline osteocalcin serum levels were found to decrease with age (Mera et al., 2016). Recent studies in humans also discovered an association between lower levels of osteocalcin and brain atrophy and cognitive performance decline (Puig et al., 2016; Fang et al., 2018). Bradburn and colleagues (2016) reported that lower levels of plasma osteocalcin were only associated with cognitive decline in older women, but not in older men or young adults (Bradburn et al., 2016). However, another study including mainly older women (85% female, 15% male) with cognitive decline did not find an association between cognitive function and total or uncarboxylated osteocalcin (Ross et al., 2018). For more information on the osteocalcin-cognition link, see review papers by Shan et al. (2019) and Nakamura et al. (2020).

Pathway

Osteocalcin was shown to bind to the G-protein-coupled receptor (Gpcr158) on cultured hippocampal neurons (Khrimian et al., 2017). Osteocalcin treatment on these cultured hippocampal neurons resulted in an enhancement of hippocampal BDNF and BDNF mRNA expression and fastened trafficking of BDNF-containing vesicles to synapses. The peripheral injection of osteocalcin also improved memory function in old mice (Khrimian et al., 2017). Moreover, greater physical exercise-induced increases in the active, uncarboxylated form of osteocalcin were found to be associated with higher serum BDNF levels in young healthy men (Nicolini et al., 2020). However, no studies examined the direct effect of physical exercise-induced elevations in circulating osteocalcin on LTP activity.

Acute exercise effect

The circulating levels of uncarboxylated osteocalcin were found to double during acute cardiovascular exercise in young mice, whereas in older mice, the response was much lower (Mera et al., 2016). In humans, a bout of moderate to high-intensity cardiovascular exercise was found to increase uncarboxylated osteocalcin in males (Levinger et al., 2014a; Nicolini et al., 2020b; Smith et al., 2021) and females (Jürimäe et al., 2016; Smith et al., 2021). Smith et al. (2021) reviewed studies with middle-aged and older adults reporting total, uncarboxylated, and carboxylated osteocalcin levels following acute exercise. They described that total osteocalcin levels increased more in middle-aged than in older adults and more in men than in women (Smith et al., 2021). Osteocalcin levels did not change following acute resistance exercise (Levinger et al., 2011; Rogers et al., 2011). The recent systematic review of Smith et al. (2021) only included one study on acute resistance exercise. Hence, more studies are needed to confirm that acute resistance exercise is incapable of increasing circulating osteocalcin.

Chronic exercise effect

A meta-analytic study showed that both chronic cardiovascular and resistance exercise were found to increase basal levels of circulating uncarboxylated osteocalcin (Rahimi et al., 2021). In the study of Lester et al. (2009), only resistance exercise or resistance exercise combined with cardiovascular exercise, but not cardiovascular exercise alone, resulted in increased levels of osteocalcin. Furthermore, baseline uncarboxylated osteocalcin levels were positively associated with muscle strength, which might indicate that chronic resistance exercise is the better approach to induce uncarboxylated osteocalcin (Karlsson et al., 1995; Levinger et al., 2014b).

3.5.2 Orexin-A/Hypocretin-1

Orexin-A is synthesized by neurons in the hypothalamus (Chieffi et al., 2017) or gastrointestinal tract (Nakabayashi et al., 2003), and by the pancreatic islets (Dall'Aglio et al., 2010). Orexin-A levels are decreased in obese and sedentary humans, whereas high levels are associated with improved cognitive performance (Polito et al., 2020). The origin of the peripheral rise in orexin A levels induced by physical exercise is not well known. However, it has been suggested to be induced by sympathetic nervous system activation (Messina et al., 2016). It may be released in the bloodstream from the pituitary (Tsunematsu and Yamanaka, 2012), leak from cerebrospinal fluid (Chieffi et al., 2017), or diffuse through the blood-brain barrier (Kastin and Akerstrom, 1999).

Pathway

Hippocampal orexin-A infusion was reported to enhance the response to LTP induction by electrophysiological stimuli in vivo in anesthetized rats, which was blocked by a specific orexin-A receptor-1 antagonist (Wayner et al., 2004). The same antagonist also decreased LTP in freely moving rats, as measured with two electrodes over the perforant pathway (i.e., the connectional route from the entorhinal cortex to the hippocampal formation) (Akbari et al., 2011). In a mouse model in which orexin-producing neurons degenerate by three months of age, in vitro hippocampal LTP magnitude and the level of phosphorylated CREB were decreased. This suggests a role of orexin-A in CREB-mediated transcription (Yang et al., 2013). In vitro electrophysiological recordings with and without administration of orexin receptor 1+ and 2, and PLC and PKA antagonists suggested that orexin-A mediates this effect on LTP by the PLC-pathway via orexin receptor-1 and cAMP/PKA-pathway via orexin receptor-2 (Lu et al., 2016). Currently, a possible link between the physical exercise-induced increase of orexin-A and the facilitation of LTP-related pathways can only be inferred from these models, as no studies exist that examined the direct link between those two.

Acute exercise effect

An acute bout of cardiovascular exercise was found to increase circulating orexin-A levels in young sedentary men (Messina et al., 2016) and cerebrospinal fluid levels in animals (Wu et al., 2002; Martins et al., 2004).

Chronic exercise effect

We found only one study that showed that chronic cardiovascular exercise increases circulating Orexin-A levels in healthy middle-aged men and men with metabolic syndrome (Monda et al., 2020).

3.5.3 Ghrelin

Ghrelin is mainly produced in the stomach before meals and released into circulation (Cummings et al., 2001). It stimulates appetite and enhances the secretion of GH from the pituitary gland (Kojima et al., 1999). Peripheral ghrelin may cross the blood-brain barrier, but it may also be synthesized in the brain itself (Ribeiro et al., 2014). It has been shown to have neuroprotective properties (Santos et al., 2017) and enhance the response to LTP induction by electrophysiological stimuli in the hippocampus (Diano et al., 2006; Chen et al., 2011).

Pathway

Ghrelin binds to the growth hormone secretagogue type 1a receptor in the pituitary (Kojima et al., 1999), where it induces the release of GH (see section 3.1.3) and in the hippocampus (Guan et al., 1997), where it increases memory retention (Diano et al., 2006; Chen et al., 2011). Intraperitoneal injection of ghrelin resulted in hippocampal elevations of IGF-1 and IGF-1 mRNA levels (see section 3.1.2). In cultured rat hippocampal neurons, Ribeiro et al. (2014) showed that GHS-1a receptors are found on the excitatory synapse. GHS-1a receptor activation by ghrelin administration resulted in the increase and phosphorylation of AMPA receptors in the postsynaptic density, enhancing excitatory synaptic transmission. This effect was mediated by the PLC/IP3, PLC/PKC, PLC/PI3K, and cAMP/PKA-pathways (Ribeiro et al., 2014). No studies were found that assessed the influence of physical exercise-induced ghrelin on LTP-related pathways.

Acute exercise effect

Autio et al. (2020) recently reviewed the effect of physical exercise on ghrelin levels. They indicated that acute cardiovascular and resistance exercise lowered circulating ghrelin levels in some studies. In contrast, Erdmann et al. (2007) suggested a role of physical exercise intensity, showing increased ghrelin levels after low-intensity cardiovascular exercise below the aerobic threshold. Also Toshinai et al. (2007) showed intensity dependent effects on ghrelin levels in healthy men, with higher intensity physical exercise inducing a greater suppression of ghrelin levels, associated with higher adrenalin and noradrenalin levels.

Chronic exercise effect

Chronic cardiovascular and resistance exercise were found to increase baseline plasma ghrelin levels (Ravussin et al., 2001; Martins et al., 2010; Kim et al., 2014; Moraes et al., 2015; Dundar et al., 2019b; Tremblay et al., 2019) and cardiovascular exercise increased 24h measurements of ghrelin (i.e., the sum of all serum ghrelin levels measured in blood obtained every 20 minutes for a duration of 24 hours) (Leidy et al., 2007). Some authors reported that only those with significant weight loss had increased ghrelin levels after chronic exercise (Leidy et al., 2004; Foster-Schubert et al., 2005; Scheid et al., 2011).

3.5.4 Vasoactive intestinal peptide (VIP)

VIP is a peptide with vasodilatory function, which is secreted by nerve endings in the gastrointestinal tract, heart, lungs, thyroid, urinary bladder, kidney, genital organs, and brain (Said and Mutt, 1970; Henning and Sawmiller, 2001). VIP was found to cross the blood-brain barrier only unidirectionally from blood towards the brain (Dogrukol-Ak et al., 2003). Within the brain, it may potentiate LTP-related pathways (Cunha-Reis and Caulino-Rocha, 2020).

Pathway

In the hippocampus, VIP is known to activate the VIP receptor 1, VAPC1, and VIP receptor 2, VAPC2. VAPC1 activated PLC/IP3 and PLC/PKC-signaling, while VAPC2 induced the cAMP/PKA-pathway in hippocampal CA1 pyramidal cells (Cunha-Reis et al., 2005). In vitro administration of VIP on CA1 cells activated these receptors and resulted in increased synaptic transmission by enhancing NMDA currents (Yang et al., 2009). Inhibition of PKC or PKA attenuated the VIP-mediated enhancement of synaptic transmission (Cunha-Reis et al., 2005). For a review concerning the facilitating action of VIP on LTP and LTP-related pathways, we refer to Cunha-Reis and Caulino-Rocha (2020). However, we found no studies that assessed the direct effect of physical exercise-induced VIP elevations on LTP-related pathways.

Acute exercise effect

A bout of cardiovascular exercise until exhaustion, submaximal muscular exercise, and an acute bout of low-intensity cardiovascular exercise of long duration were found to increase circulating VIP levels in men (Galbo et al., 1979; Woie et al., 1986; Rolandi et al., 1988; MacLaren et al., 1995). The acute exercise-associated rise in circulating VIP was suggested to result from the overflow of the peptide at skeletal muscle blood vessels, where it acts as a potent vasodilator (Woie et al., 1986).

Chronic exercise effect

A five-day period of physical exercise with calorie deficiency and sleep deprivation induced increases in VIP levels in male military cadets (Øktedalen et al., 1983a, 1983b). However, calorie compensation lowered the VIP increase (Øktedalen et al., 1983a). An eight-week program of low-intensity cardiovascular exercise did not induce increases in VIP serum levels (Amirazodi et al., 2019).

4. Concluding remarks

This review describes current evidence for the role of exerkines in mediating the neurophysiological processes leading to LTP that occur in the brain following physical exercise. It is important to note that we only reported a small fraction of all the processes that exerkines may induce in the brain. Furthermore, we discussed only LTP processes at the glutamatergic excitatory synapse and did not refer in our review to mechanisms and pathways of neurogenesis, LTD-related processes, or processes at the GABAergic inhibitory synapse. Yet, LTP and neurogenesis are somewhat related, as neurogenesis may be boosted by the growth factors that are synthesized in neurons during LTP (Cho et al., 2013), and newly formed neurons appear to depend on LTP for their survival and maturation (Shors et al., 2012; Denoth-Lippuner and Jessberger, 2021). In comparison with LTP and the modulatory effects of exerkines on pathways at the glutamatergic synapse, evidence for exerkine effects on LTD or changes in the GABAergic synapse is limited. However, some processes and pathways that were described in this review may also be implicated in up- or downregulation of GABAergic transmission, e.g., studies have reported an effect of lactate on GABA levels (Maddock et al., 2016; Coxon et al., 2018), and of BDNF on GABAergic modulation (Vaz et al., 2011). Furthermore, evidence is still lacking regarding the effect that physical exercise-induced elevations of GABA concentrations in cortical neurons, measured with ^{1}H-MRS, may have on GABAergic neurotransmission (Maddock et al., 2016). On the one hand, lactate may be converted to GABA, which is expected to increase its availability in presynaptic terminals and strengthen GABA-mediated inhibitory control (Kleppner and Tobin, 2002; Maddock et al., 2016). On the other hand, findings generated from studies involving non-invasive brain stimulation methods such as TMS demonstrated an overall downregulation of GABAergic activity following an acute bout of cardiovascular exercise (e.g., Singh et al., 2014a; Mooney et al., 2016; Stavrinos and Coxon, 2017; O'Leary et al., 2018; for a review see Levin et al. 2021).

Although the LTP process has extensively been studied, for example, in relation to neuroplasticity, its relationship with exerkines needs further exploration. Most studies involve animal models and have investigated the effect of administration of a specific exerkine on the alteration of LTP-related pathways, but do not offer direct evidence that the physical exercise-induced increase of this exerkine may also alter LTP-related pathways. More specifically, only for three of the 16 exerkines presented in this review (BDNF, irisin, and pro-inflammatory cytokines), we found evidence suggesting that the physical exercise-related change in circulating exerkine levels was associated with the facilitation or impairment of LTP activity. In mice, elevated levels of BDNF (Novkovic et al., 2015) and irisin (Lourenco et al., 2019) following chronic exercise facilitated LTP activity and elevated levels of the pro-inflammatory cytokines TNF-α and IL-1β following seven days of daily maximal cardiovascular exercise were reported to have detrimental effects

on LTP activity (Sun et al., 2017). In other studies, the exerkine-effect on LTP activity was only reported following in vitro administration of the exerkine. However, the physical exercise-induced elevation of only four of the 16 exerkines included in this review (IGF-1, BHB, lactate, and irisin) was found to activate the transcription of one of the exerkines with known physical exercise-induced facilitatory effect on LTP (i.e., BDNF or irisin). For example, BDNF transcription in rodent brain was associated with physical exercise-induced elevations of IGF-1 (Ding et al., 2006), irisin (Wrann et al., 2013), and BHB (Sleiman et al., 2016) following chronic exercise and with the physical exercise-induced elevation of IGF-1 (Carro et al., 2001) following acute exercise. Furthermore, neural synthesis of irisin was suggested to be an effect of physical exercise-induced elevations in lactate, measured after 30 days of voluntary physical exercise in mice(El Hayek et al., 2019). Of note, physical exercise-induced elevations in irisin were both found to enhance the response to electrophysiological stimulation of LTP (Lourenco et al., 2019) and to mediate hippocampal BDNF transcription (Wrann et al., 2013). Thus, the faciliatory effect of irisin on LTP activity may be indirect by the induction of a rise in BDNF levels. The circulating levels of the ten remaining exerkines were found to be altered by physical exercise, but at present, none of the studies measured their role in the physical exercise-induced facilitation of LTP activity. Current evidence about the role of these exerkines on LTP is mainly derived from studies, in which these exerkines were administered in vitro and subsequent changes in LTP activity (in case of GH, kynurenine, adiponectin, orexin-A, ghrelin, and VIP) or BDNF levels (in case of cathepsin-B, apelin, and osteocalcin) were found (Wayner et al., 2004; Diano et al., 2006; Rózsa et al., 2008; Kim et al., 2010; Chen et al., 2011; Molina et al., 2012; Moon et al., 2016; Khrimian et al., 2017; Pousti et al., 2018; Kwak et al., 2019; Cunha-Reis and Caulino-Rocha, 2020; Nicolini et al., 2020).

Of note, there were some inconsistencies in the reported effect of physical exercise on exerkine levels. Some differences might be due to the discrepancy in the quantification of biomarkers, timing of sample collection, pre-analytic sample processing, the analytical method, and calculation of other factors (Son et al., 2018). As an example, studies have described how BDNF levels may differ between measurements due to circadian variability, the time between blood collection and centrifugation, or whether BDNF was measured in serum or in plasma (Cain et al., 2017; Gejl et al., 2019). Of importance, the time between the last physical exercise bout and sample collection is often not clearly denoted in chronic exercise studies. However, this is critical to differentiate between changes in exerkine levels that represent acute exercise effects in trained individuals and changes in baseline exerkine levels as a function of chronic exercise. Acute exercise may transiently change exerkine levels lasting for minutes up to more than 24 hours after physical exercise (Garneau et al., 2020). To accurately measure longer-lasting changes in baseline exerkine levels induced by chronic exercise, we would advise having at least one, but preferably two or more full rest days between blood sample collection and the last physical exercise

bout. Chronic intervention studies should also consider adding follow-up measurements several months after the end of the intervention in order to examine whether exerkine levels return to their pre-intervention levels or remain elevated.

In addition to the differences in blood sampling methods, there is a large heterogeneity in the physical exercise protocols and study subjects' characteristics, which vary according to the study's objectives. It is interesting to learn which type of physical exercise works best to change a certain exerkine in a certain population as this may lead to the design of individualized physical exercise protocols. The ultimate goal for individualized physical exercise training is to find a physical exercise protocol that works best to improve performance or prevent a specific type of cognitive or motor deficit in a specific population. In young and healthy older adults, the primary aim may be the improvement or acquisition of certain skills that has been shown to be associated with LTP induction during the memory consolidation phase (e.g., Statton et al., 2015; for a review, see Wanner et al., 2020). For older adults with neurodegenerative disorders or abnormal cognitive decline, physical exercise may have more specific functional/therapeutic goals (e.g., inhibit inflammation or improve cardiovascular function). In the following paragraphs, we will discuss what we can learn from the literature that was summarized in this review paper. As we did not review the link between certain domains of cognitive function and specific exerkines, our discussion will be limited to the effect of physical exercise and subject characteristics on the release of these exerkines into circulation. Where possible, the association between these physical exercises and subject characteristics and LTP will be highlighted.

First, different modes of physical exercise (i.e., cardiovascular versus resistance exercise) are expected to activate different regulatory pathways. Kim and colleagues (2019) argued that resistance exercise may be better in promoting the release of myokines, while cardiovascular exercise may have a greater influence on other exerkines such as adiponectin (Davis et al., 2015; Kim et al., 2019). Compared with resistance exercise, the beneficial effect of cardiovascular exercise on the brain may, to a greater extent, be attributed to improvements in cardiovascular function or changes in energy metabolism, such as increased delivery of nutrients and oxygen (Kim et al., 2019). From our review, it becomes clear that the evidence on exerkine release during and following acute and chronic resistance exercise is limited compared to cardiovascular exercise. Therefore, it is not possible to draw final conclusions. However, it was argued that resistance training is an essential component of the physical exercise program to boost BDNF levels in older (Marinus et al., 2019) and osteocalcin levels in young adults (Lester et al., 2009). Acute or chronic resistance exercise was preferred to boost IGF-1 (de Alcantara Borba et al., 2020; Gulick et al., 2020; Jiang et al., 2020) and acute resistance exercise resulted in larger irisin level increases than cardiovascular exercise (Tsuchiya et al., 2015). In contrast, adiponectin levels were found to increase to a greater extent if physical exercise

contained a component of cardiovascular exercise, compared with resistance exercise alone (Davis et al., 2015).

Second, physical exercise intensity was reported to influence the release of exerkines. Acute exercise of higher intensity was associated with higher circulating levels of lactate (Saucedo Marquez et al., 2015; Albesa-Albiol et al., 2019), irisin (Daskalopoulou et al., 2014; Huh et al., 2014) and adiponectin (Simpson and Singh, 2008). While some studies reported that BDNF levels were higher following acute high-intense versus low-intense physical exercise, e.g., Schmolesky et al. (2013), the meta-analysis of Dinoff et al. (2017) only found a nonsignificant positive trend ($p = 0.085$) between BDNF levels and higher physical exercise intensity. In general, the release of exerkines is expected to require a certain physical exercise intensity before protein synthesis is activated. However, higher intensity is not always better. For example, ghrelin levels increased more following low-intense acute exercise compared with high-intense physical exercise in the study of Toshinai et al. (2007). Furthermore, chronic high-intense physical exercise without the necessary recovery periods (i.e., overtraining) was associated with increased levels of pro-inflammatory cytokines, with detrimental effects on LTP (Sun et al., 2017).

Third, longer physical exercise duration was associated with higher levels of BDNF following acute exercise intervention. Also in chronic exercise interventions, the length of the intervention may influence the change in baseline exerkine levels. For example, a meta-analysis reporting the effect of resistance exercise on irisin levels described that irisin levels significantly increased in interventions lasting less than 12 weeks and decreased in physical exercise interventions lasting longer than 16 weeks (Cosio et al., 2021). Two studies had reported decreased irisin levels. Both were not only of long duration (6 months or more), but also used low-intense physical exercise sessions without progression in intensity (Hecksteden et al., 2013; Scharhag-Rosenberger et al., 2014). Hence, it is possible that the physical exercise intensity level, known to affect irisin response (Daskalopoulou et al., 2014; Huh et al., 2014), may have had a higher impact on the irisin levels than the physical exercise duration.

Fourth, age is considered to play an important role in how our body responds to physical exercise. Furthermore, the effect of age is widely studied with respect to LTP. For example, in old compared with young rodents, in vitro radioligand binding studies have shown a significant age-related loss of postsynaptic glutamatergic receptors, especially of the NMDA subtype, which is critical for the LTP process (Kito et al., 1990; Cohen and Müller, 1992). In addition, in vitro electrophysiological studies found LTP induction deficits in hippocampal slices of old rats compared with their younger counterparts (Deupree et al., 1993; Moore et al., 1993). Increasing age is also linked with a decrease in the baseline levels of myokines and growth factors, with BDNF as the cornerstone (Tapia-Arancibia et al., 2008; Erickson et al., 2010; El-Sayes et al., 2019). However, higher physical exercise-induced elevations were found in older adults for IGF-1 following chronic resistance exercise (Jiang

et al., 2020; Ye et al., 2020; Amiri et al., 2021), for irisin following chronic cardiovascular (Miyamoto-Mikami et al., 2015) and resistance exercise (Cosio et al., 2021), and for apelin following chronic cardiovascular exercise (Bae et al., 2019). In contrast, GH (Wideman et al., 2002) and total osteocalcin (Smith et al., 2021) increase following acute exercise were lower in older adults compared with young or middle-aged adults. Furthermore, in the process of aging, persons gradually progress into a more pro-inflammatory state. For example, the pro-inflammatory cytokine IL-1β was found to be increased in old rats, and the concentration of dentate gyrus IL-1β was inversely related to the level of hippocampal LTP measured in vivo (Murray and Lynch, 1998). From a mechanistic perspective, chronic inflammation was found to damage neurons and impair neurotrophic factor signaling (Cotman et al., 2007; Bourgognon and Cavanagh, 2020; Scheiblich et al., 2020). Of note, older adults may also be more vulnerable to the pro-inflammatory effects of acute high-intense physical exercise. For example, Trepci et al. (2020) found increased levels of the inflammatory marker kynurenine 60 min after acute sprint interval exercise in old but not in young healthy human subjects (Trepci et al., 2020).

Fifth, gender differences may influence the effect of physical exercise. As with aging, also gender may influence the baseline levels of certain exerkines. As a result, significant pre-to-post physical exercise changes are more easily found in the gender with the lowest baseline levels (Glud et al., 2019). This was reported for BDNF (Dinoff et al., 2017); baseline BDNF levels were found to be influenced by estrogen levels, with women having higher basal serum BDNF levels than men (Harte-Hargrove et al., 2013; Dong et al., 2017; Glud et al., 2019). In addition, we found studies reporting higher acute exercise-induced increases of BDNF (Dinoff et al., 2017) and total osteocalcin (Smith et al., 2021) in men and higher chronic resistance exercise-induced increases of IGF-1 in women (Jiang et al., 2020; Ye et al., 2020; Amiri et al., 2021). It is remarkable to note that the majority of studies were conducted using male human subjects or animals. Therefore, while some studies reported gender-related differences in exerkine responses following physical exercise, gender-related differences are unknown for most of them.

Sixth, it was reported that if the physical exercise intervention induced weight loss, the circulating levels of irisin (Cosio et al., 2021), adiponectin (Bouassida et al., 2010; Christiansen et al., 2010; Kelly et al., 2014), and ghrelin (Leidy et al., 2004; Foster-Schubert et al., 2005; Scheid et al., 2011) increased, while apelin levels of obese women decreased in association with significant weight loss (Sheibani et al., 2012; Jang et al., 2019). However, decreases in apelin levels were more consistently associated with the improvements in insulin sensitivity caused by physical exercise (Krist et al., 2013; Bertrand et al., 2015; Delavar and Heidarianpour, 2016; Otero-Díaz et al., 2018; Kolahdouzi et al., 2019; Nam et al., 2020). More specifically, it is thought that adipocyte-derived apelin is positively associated with insulin resistance (Boucher et al., 2005; Yang et al., 2015). It remains unclear if the muscle-derived isoform of apelin would also be responsive to changes in insulin sensitivity. Glucose metabolism

also plays a role in the release of BHB, with higher levels of BHB found following acute exercise sessions that cause hypoglycemia and long physical exercise sessions without carbohydrate supplementation (Nybo et al., 2003), or in chronic exercise in association with low calorie diet (Jo et al., 2019; Vieira et al., 2021). Importantly, diabetes mellitus and obesity are both also associated with a pro-inflammatory state (Yudkin, 2007; Woods et al., 2012; Pedersen, 2017) which may affect LTP (Murray and Lynch, 1998; Sun et al., 2017; Bourgognon and Cavanagh, 2020). Furthermore, these cardiovascular risk profiles were linked with cognitive decline (Jefferson et al., 2015; Viticchi et al., 2015; Chatterjee et al., 2016) and with structural brain alterations (Cox et al., 2019). Future studies should address whether the cognitive decline in persons with obesity, diabetes mellitus or other cardiovascular risk factors is related to impairments in LTP and the extent by which LTP is compromised by elevated levels of pro-inflammatory cytokines.

5. Future directions

We reviewed a total of 16 different exerkines that were linked to the LTP process. However, the number of myokines currently discovered alone exceeds 600 (Görgens et al., 2015). Researchers should keep exploring the specific bioactivity of exerkines on body systems. Especially, their effect on the central nervous system remains largely undescribed. Unfortunately, technical issues limit the investigation of exerkine effects on the human brain. Only some exerkines can be measured in humans with noninvasive techniques such as ^{1}H-MRS, e.g., lactate and BHB (Dacko and Lange, 2019). Invasive alternatives that may be used in patient groups, but are not commonly used in research, are cerebrospinal fluid measurements (e.g., Steensberg et al., 2006; Isung et al., 2021) and carotid artery versus jugular vein differences (e.g., Ide et al., 1999; 2000). Hence, most evidence for exerkine changes in the brain arises from animal studies. However, most studies included in this review, which examined the effect of exerkines on LTP, did not measure the physical exercise-induced elevation, but administered the exerkine in vitro on brain slices or by the use of intravenous injections. More clinical and preclinical (physical exercise) research is needed to increase understanding of the effects that exerkines have on the brain and LTP activity. In addition to the exerkines presented in this review, other review papers have presented exerkines that are worth further investigation, as their effect on the LTP process is currently unclear (Woodbury and Ikezu, 2014; Morland et al., 2017; Pedersen, 2019; Autio et al., 2020; Kwon et al., 2020; Scheffer and Latini, 2020).

There is a relatively lower amount of studies examining resistance exercise effects compared with cardiovascular exercise effects. For example, in rodent studies, which are crucial to increase insight into the neurophysiological pathways that are modulated by physical exercise or exerkines, the resistance exercise protocol (most often weighted ladder

climbing) is much less used than the cardiovascular exercise protocol (i.e., treadmill running). More specifically, we found only two studies that examined brain exerkine levels after acute resistance exercise (Fernandes et al., 2016; Kelty et al., 2019). In addition, studies comparing resistance and cardiovascular protocols are needed to make final decisions on differences between cardiovascular and resistance exercise effects. These studies comparing both protocols are scarce, e.g., Tang et al. (2017), Joisten et al. (2020), Tsuchiya et al. (2015), Davis et al. (2015), Lester et al. (2009). Also, only for some exerkines there are sufficient high-quality studies to make valuable comparisons in meta-analytic studies, like for BDNF (Dinoff et al., 2016, 2017), IGF-1 (de Alcantara Borba et al., 2020; Gulick et al., 2020; Jiang et al., 2020; Ye et al., 2020; Amiri et al., 2021), and osteocalcin (Rahimi et al., 2021). But again, the reason for this is the limited number of studies using resistance exercise protocols. In sum, we need more clinical and preclinical research to focus on the effect of exerkines following resistance exercise on cognitive improvements and changes in LTP activity. Especially as more and more evidence suggests that resistance exercise might be the preferred physical exercise mode to boost certain of the exerkines (Tsuchiya et al., 2015; Kim et al., 2019; Marinus et al., 2019; de Alcantara Borba et al., 2020; Gulick et al., 2020; Jiang et al., 2020).

This review did not focus on specific disorders that may cause acute or progressive deficits in cognitive function, such as neurodegenerative disorders, stroke, or traumatic brain injury. Yet, more insight into the effect of physical exercise in these specific cases would be of value for rehabilitation practitioners. In addition, certain exerkines may benefit specific domains of cognitive function. This was not discussed in this review, as we focused specifically on the alteration of LTP-related pathways. The combined evaluation of which physical exercise protocol would be most optimal to target specific exerkines and the evaluation of which exerkine could benefit a specific cognitive function may direct researchers towards the design of individualized physical exercise programs that can be implemented as a treatment strategy. Due to the large heterogeneity in possible physical exercise protocols, many more studies will be needed before such physical exercise treatment can be designed. For example, physical exercise characteristics that should be considered are intensity, duration, frequency, and the amount or size of the muscles used. Also physical exercise type can be further divided into specific sports. While running might be only slightly different from cycling, bigger differences can be expected when using hybrid forms of physical exercise between cardiovascular and resistance training, like elastic band exercises (Kwak et al., 2021), or types of physical exercise that require memorizing movement patterns like dancing (Kimura and Hozumi, 2012) or Tai Chi (Wayne et al., 2014), or a combination of physical and cognitive training (Netz, 2019). At last, increasing insight on the effects of exerkines on our body may lead to the design of pharmacological pills containing exerkines to mimic the effects of physical exercise. This may be especially useful in those unable to perform physical exercise at a sufficient duration and intensity, as recently reviewed by Gubert and Hannan (2021).

6. Summary

We reviewed physical exercise-induced circulating factors (i.e., exerkines) and their effect on LTP-related pathways (Fig. 2). For each of these exerkines we assessed the physical exercise and subject characteristics that influence the alterations of exerkine levels in the circulation and the brain following acute or chronic, cardiovascular or resistance exercise (Table 1). By combining and structuring evidence from a large and rapidly increasing amount of research, this review summarizes what is already sufficiently known and where research is limited. This knowledge may serve to guide researchers towards designing an individualized physical exercise treatment to improve cognitive health. The beneficial effect of physical exercise on cognitive function is only one of the many reasons to promote physical activity for people of all ages, especially in adults with cognitive decline. This is very important in a society that faces the prospect of an aging population.

Acknowledgements

We thank Dr. Aivaras Ratkevicius for proof reading the article.

Declarations of interest

This work was supported by the Research Council of Lithuania (grant number S-SEN-20-5). The sponsor was not involved in study design; collection, analysis and interpretation of the data; in writing of the report; nor in the decision to submit the article for publication.

Reference list

Agudelo, L.Z., Femenía, T., Orhan, F., Porsmyr-Palmertz, M., Goiny, M., Martinez-Redondo, V., Correia, J.C., Izadi, M., Bhat, M., Schuppe-Koistinen, I., Pettersson, A.T., Ferreira, D.M.S., Krook, A., Barres, R., Zierath, J.R., Erhardt, S., Lindskog, M., Ruas, J.L., 2014. Skeletal muscle PGC-1α1 modulates kynurenine metabolism and mediates resilience to stress-induced depression. Cell 159, 33–45. https://doi.org/10.1016/j.cell.2014.07.051

Agudelo, L.Z., Ferreira, D.M.S., Cervenka, I., Bryzgalova, G., Dadvar, S., Jannig, P.R., Pettersson-Klein, A.T., Lakshmikanth, T., Sustarsic, E.G., Porsmyr-Palmertz, M., Correia, J.C., Izadi, M., Martínez-Redondo, V., Ueland, P.M., Midttun, Ø., Gerhart-Hines, Z., Brodin, P., Pereira, T., Berggren, P.O., Ruas, J.L., 2018. Kynurenic Acid and Gpr35 Regulate Adipose Tissue Energy Homeostasis and Inflammation. Cell Metab. 27, 378-392.e5. https://doi.org/10.1016/j.cmet.2018.01.004

Ahmadizad, S., El-Sayed, M.S., 2003. The effects of graded resistance exercise on platelet aggregation and activation. Med. Sci. Sports Exerc. 35, 1026–1032. https://doi.org/10.1249/01.MSS.0000069406.54766.C6

Akbari, E., Motamedi, F., Davoodi, F.G., Noorbakhshnia, M., Ghanbarian, E., 2011. Orexin-1 receptor mediates long-term potentiation in the dentate gyrus area of freely moving rats. Behav. Brain Res. 216, 375–380. https://doi.org/10.1016/j.bbr.2010.08.017

Albesa-Albiol, L., Serra-Payá, N., Garnacho-Castaño, M.A., Guirao Cano, L., Pleguezuelos Cobo, E., Maté-Muñoz, J.L., Garnacho-Castaño, M. V., 2019. Ventilatory efficiency during constant-load test at lactate threshold intensity: Endurance versus resistance exercises. PLoS One 14, e0216824. https://doi.org/10.1371/journal.pone.0216824

Allison, D.J., Josse, A.R., Gabriel, D.A., Klentrou, P., Ditor, D.S., 2017. Targeting inflammation to influence cognitive function following spinal cord injury: A randomized clinical trial. Spinal Cord 55, 26–32. https://doi.org/10.1038/sc.2016.96

Allison, D.J., Nederveen, J.P., Snijders, T., Bell, K.E., Kumbhare, D., Phillips, S.M., Parise, G., Heisz, J.J., 2019. Exercise training impacts skeletal muscle gene expression related to the kynurenine pathway. Am. J. Physiol. - Cell Physiol. 316, C444–C448. https://doi.org/10.1152/ajpcell.00448.2018

Amirazodi, M., Mehrabi, A., Kordestani, Z., Habibi, H., Divsalar, K., 2019. The Effect of Eight Weeks of Low Intensity Aerobic Exercise on Serum Levels of VIP and ET-1 Hormones in the Non-Athlete Healthy Elderly Women and Patients with Coronary Artery Disease. Rep. Heal. Care 5, 24–32.

Amiri, N., Fathei, M., Mosaferi Ziaaldini, M., 2021. Effects of resistance training on muscle strength, insulin-like growth factor-1, and insulin-like growth factor–binding protein-3 in healthy elderly subjects: a systematic review and meta-analysis of randomized controlled trials. Hormones. https://doi.org/10.1007/s42000-020-00250-6

Antony, R., Li, Y., 2020. BDNF secretion from C2C12 cells is enhanced by methionine restriction. Biochem. Biophys. Res. Commun. 533, 1347–1351. https://doi.org/10.1016/J.BBRC.2020.10.017

Arendt, K.L., Royo, M., Fernández-Monreal, M., Knafo, S., Petrok, C.N., Martens, J.R., Esteban, J.A., 2010. PIP 3 controls synaptic function by maintaining AMPA receptor clustering at the postsynaptic membrane. Nat. Neurosci. 13, 36–44. https://doi.org/10.1038/nn.2462

Autio, J., Stenbäck, V., Gagnon, D.D., Leppäluoto, J., Herzig, K.-H., 2020. (Neuro) Peptides, Physical Activity, and Cognition. J. Clin. Med. 9, 2592. https://doi.org/10.3390/jcm9082592

Aydin, Suleyman, Aydin, Suna, Kuloglu, T., Yilmaz, M., Kalayci, M., Sahin, I., Cicek, D., 2013. Alterations of irisin concentrations in saliva and serum of obese and normal-weight subjects, before and after 45 min of a Turkish bath or running. Peptides 50, 13–18. https://doi.org/10.1016/j.peptides.2013.09.011

Bae, J.H., Kwak, S.E., Lee, J.H., Yangjie, Z., Song, W., 2019. Does exercise-induced apelin affect sarcopenia? A systematic review and meta-analysis. Hormones 18, 383–393. https://doi.org/10.1007/s42000-019-00157-x

Barnes, C.A., 2003. Long-term potentiation and the ageing brain. Philos. Trans. R. Soc. B Biol. Sci. https://doi.org/10.1098/rstb.2002.1244

Barrientos, R.M., Frank, M.G., Crysdale, N.Y., Chapman, T.R., Ahrendsen, J.T., Day, H.E.W., Campeau, S., Watkins, L.R., Patterson, S.L., Maier, S.F., 2011. Little exercise, big effects: Reversing aging and infection-induced memory deficits, and underlying processes. J. Neurosci. 31, 11578–11586. https://doi.org/10.1523/jneurosci.2266-11.2011

Barrientos, R.M., Kitt, M.M., Watkins, L.R., Maier, S.F., 2015. Neuroinflammation in the normal aging hippocampus. Neuroscience. https://doi.org/10.1016/j.neuroscience.2015.03.007

Becic, T., Studenik, C., Hoffmann, G., 2018. Exercise Increases Adiponectin and Reduces Leptin Levels in Prediabetic and Diabetic Individuals: Systematic Review and Meta-Analysis of Randomized Controlled Trials. Med. Sci. 6, 97. https://doi.org/10.3390/medsci6040097

Beise, D., Peaseley, V., 1937. The relation of reaction time, speed, and agility of big muscle groups to certain sport skills. Res. Q. Am. Phys. Educ. Assoc. 8, 133–142. https://doi.org/10.1080/23267402.1937.10761808

Béland-Millar, A., Takimoto, M., Hamada, T., Messier, C., 2020. Brain and muscle adaptation to high-fat diets and exercise: Metabolic transporters, enzymes and substrates in the rat cortex and muscle. Brain Res. 1749, 147126. https://doi.org/10.1016/j.brainres.2020.147126

Berchtold, N.C., Castello, N., Cotman, C.W., 2010. Exercise and time-dependent benefits to learning and memory. Neuroscience 167, 588–597. https://doi.org/10.1016/j.neuroscience.2010.02.050

Berchtold, N.C., Chinn, G., Chou, M., Kesslak, J.P., Cotman, C.W., 2005. Exercise primes a molecular memory for brain-derived neurotrophic factor protein induction in the rat hippocampus. Neuroscience 133, 853–861. https://doi.org/10.1016/j.neuroscience.2005.03.026

Berg, U., Bang, P., 2004. Exercise and Circulating Insulin-Like Growth Factor I. Horm. Res. Paediatr. 62, 50–58. https://doi.org/10.1159/000080759

Bertrand, C., Valet, P., Castan-Laurell, I., 2015. Apelin and energy metabolism. Front. Physiol. 6, 115. https://doi.org/10.3389/fphys.2015.00115

Bian, A., Ma, Y., Zhou, X., Guo, Y., Wang, W., Zhang, Y., Wang, X., 2020. Association between sarcopenia and levels of growth hormone and insulin-like growth factor-1 in the elderly. BMC Musculoskelet. Disord. 21, 214. https://doi.org/10.1186/s12891-020-03236-y

Bilski, J., Jaworek, J., Pokorski, J., Nitecki, J., Nitecka, E., Pokorska, J., Mazur-Baily, A., Szklarczyk, J., 2016. Effects of time of day and the wingate test on appetite perceptions, food intake and plasma levels of adipokines. J Physiol Pharmacol 67, 667–676.

Blackmore, D.G., Golmohammadi, M.G., Large, B., Waters, M.J., Rietze, R.L., 2009. Exercise Increases Neural Stem Cell Number in a Growth Hormone-Dependent Manner, Augmenting the Regenerative Response in Aged Mice. Stem Cells 27, 2044–2052. https://doi.org/10.1002/stem.120

Blackmore, D.G., Vukovic, J., Waters, M.J., Bartlett, P.F., 2012. GH Mediates Exercise-Dependent Activation of SVZ Neural Precursor Cells in Aged Mice. PLoS One 7, 49912. https://doi.org/10.1371/journal.pone.0049912

Bliss, T.V.P., Cooke, S.F., 2011. Long-term potentiation and long-term depression: A clinical perspective. Clinics 66, 3–17. https://doi.org/10.1590/S1807-59322011001300002

Bobinski, F., Ferreira, T.A.A., Córdova, M.M., Dombrowski, P.A., Cunha, C. da, Santo, C.C. do E., Poli, A., Pires, R.G.W., Martins-Silva, C., Sluka, K.A., Santos, A.R.S., 2015. Role of brainstem serotonin in analgesia produced by low-intensity exercise on neuropathic pain following sciatic nerve injury in mice. Pain 156, 2595. https://doi.org/10.1097/J.PAIN.0000000000000372

Boström, P., Wu, J., Jedrychowski, M.P., Korde, A., Ye, L., Lo, J.C., Rasbach, K.A., Boström, E.A., Choi, J.H., Long, J.Z., Kajimura, S., Zingaretti, M.C., Vind, B.F., Tu, H., Cinti, S., Højlund, K., Gygi, S.P., Spiegelman, B.M., 2012. A PGC1-α-dependent myokine that drives brown-fat-like development of white fat and thermogenesis. Nature 481, 463–468. https://doi.org/10.1038/nature10777

Bouassida, A., Chamari, K., Zaouali, M., Feki, Y., Zbidi, A., Tabka, Z., 2010. Review on leptin and adiponectin responses and adaptations to acute and chronic exercise. Br. J. Sports Med. https://doi.org/10.1136/bjsm.2008.046151

Boucher, J., Masri, B., Daviaud, D., Gesta, S., Guigné, C., Mazzucotelli, A., Castan-Laurell, I., Tack, I., Knibiehler, B., Carpéné, C., Audigier, Y., Saulnier-Blache, J.-S., Valet, P., 2005. Apelin, a Newly Identified Adipokine Up-Regulated by Insulin and Obesity. Endocrinology 146, 1764–1771. https://doi.org/10.1210/en.2004-1427

Bourgognon, J.-M., Cavanagh, J., 2020. The role of cytokines in modulating learning and memory and brain plasticity. Brain Neurosci. Adv. 4, 239821282097980. https://doi.org/10.1177/2398212820979802

Bradburn, S., McPhee, J., Bagley, L., Sipila, S., Stenroth, L., Narici, M., Pääsuke, M., Gapeyeva, H., Osborne, G., Sassano, L., Meskers, C., Maier, A., Hogrel, J., Barnouin, Y., Butler-Browne, G., Murgatroyd, C., 2016. Association between osteocalcin and cognitive performance in healthy older adults. Age Ageing 45, 844–849. https://doi.org/10.1093/ageing/AFW137

Broadhouse, K.M., Singh, M.F., Suo, C., Gates, N., Wen, W., Brodaty, H., Jain, N., Wilson, G.C., Meiklejohn, J., Singh, N., Baune, B.T., Baker, M., Foroughi, N., Wang, Y., Kochan, N., Ashton, K., Brown, M., Li, Z., Mavros, Y., Sachdev, P.S., Valenzuela, M.J., 2020. Hippocampal plasticity underpins long-term cognitive gains from resistance exercise in MCI. NeuroImage Clin. 25, 102182. https://doi.org/10.1016/j.nicl.2020.102182

Burpee, R.H., Stroll, W., 1936. Measuring Reaction Time of Athletes. Res. Q. Exerc. Sport 7, 110–118. https://doi.org/10.1080/23267402.1936.10761762

Cain, S.W., Chang, A.M., Vlasac, I., Tare, A., Anderson, C., Czeisler, C.A., Saxena, R., 2017. Circadian Rhythms in Plasma Brain-derived Neurotrophic Factor Differ in Men and Women. J. Biol. Rhythms 32, 75–82. https://doi.org/10.1177/0748730417693124

Caldeira, M. V., Melo, C. V., Pereira, D.B., Carvalho, R.F., Carvalho, A.L., Duarte, C.B., 2007. BDNF regulates the expression and traffic of NMDA receptors in cultured hippocampal neurons. Mol. Cell. Neurosci. 35, 208–219. https://doi.org/10.1016/j.mcn.2007.02.019

Capuron, L., Schroecksnadel, S., Féart, C., Aubert, A., Higueret, D., Barberger-Gateau, P., Layé, S., Fuchs, D., 2011. Chronic low-grade inflammation in elderly persons is associated with altered tryptophan and tyrosine metabolism: Role in neuropsychiatric symptoms. Biol. Psychiatry 70, 175–182. https://doi.org/10.1016/j.biopsych.2010.12.006

Carro, E., Nuñez, A., Busiguina, S., Torres-Aleman, I., 2000. Circulating insulin-like growth factor I mediates effects of exercise on the brain. J. Neurosci. 20, 2926–2933. https://doi.org/10.1523/jneurosci.20-08-02926.2000

Carro, E., Trejo, J.L., Busiguina, S., Torres-Aleman, I., 2001. Circulating insulin-like growth factor I mediates the protective effects of physical exercise against brain insults of different etiology and anatomy. J. Neurosci. 21, 5678–5684.

Cassilhas, R.C., Lee, K.S., Fernandes, J., Oliveira, M.G.M., Tufik, S., Meeusen, R., De Mello, M.T., 2012. Spatial memory is improved by aerobic and resistance exercise through divergent molecular mechanisms. Neuroscience 202, 309–317. https://doi.org/10.1016/j.neuroscience.2011.11.029

Cassilhas, R.C., Viana, V.A.R., Grassmann, V., Santos, R.T., Santos, R.F., Tufik, S., Mello, M.T., 2007. The Impact of Resistance Exercise on the Cognitive Function of the Elderly. Med. Sci. Sport. Exerc. 39, 1401–1407. https://doi.org/10.1249/mss.0b013e318060111f

Chatterjee, S., Peters, S.A.E., Woodward, M., Arango, S.M., Batty, G.D., Beckett, N., Beiser, A., Borenstein, A.R., Crane, P.K., Haan, M., Hassing, L.B., Hayden, K.M., Kiyohara, Y., Larson, E.B., Li, C.Y., Ninomiya, T., Ohara, T., Peters, R., Russ, T.C., Seshadri, S., Strand, B.H., Walker, R., Xu, W., Huxley, R.R., 2016. Type 2diabetes as a risk factor for dementia in women compared with men: A pooled analysis of 2.3 million people comprising more than 100,000 cases of dementia. Diabetes Care 39, 300–307. https://doi.org/10.2337/dc15-1588

Chen, L., Xing, T., Wang, M., Miao, Y., Tang, M., Chen, J., Li, G., Ruan, D.Y., 2011. Local infusion of ghrelin enhanced hippocampal synaptic plasticity and spatial memory through activation of phosphoinositide 3-kinase in the dentate gyrus of adult rats. Eur. J. Neurosci. 33, 266–275. https://doi.org/10.1111/j.1460-9568.2010.07491.x

Chen, Q.-S., Kagan, B.L., Hirakura, Y., Xie, C.-W., 2000. Impairment of hippocampal long-term potentiation by Alzheimer amyloid β-peptides. J. Neurosci. Res. 60, 65–72. https://doi.org/10.1002/(SICI)1097-4547(20000401)60:1<65::AID-JNR7>3.0.CO;2-Q

Chen, X., Gan, L., 2019. An exercise-induced messenger boosts memory in Alzheimer's disease. Nat. Med. 25, 20–21. https://doi.org/10.1038/s41591-018-0311-4

Chenouard, N., Xuan, F., Tsien, R.W., 2020. Synaptic vesicle traffic is supported by transient actin filaments and regulated by PKA and NO. Nat. Commun. 11, 1–16. https://doi.org/10.1038/s41467-020-19120-1

Chieffi, S., Messina, G., Villano, I., Messina, A., Esposito, M., Monda, V., Valenzano, A., Moscatelli, F., Esposito, T., Carotenuto, M., Viggiano, A., Cibelli, G., Monda, M., 2017. Exercise influence on hippocampal function: Possible involvement of orexin-a. Front. Physiol. 8, 85. https://doi.org/10.3389/fphys.2017.00085

Cho, T., Ryu, J.K., Taghibiglou, C., Ge, Y., Chan, A.W., Liu, L., Lu, J., McLarnon, J.G., Wang, Y.T., 2013. Long-Term Potentiation Promotes Proliferation/Survival and Neuronal Differentiation of Neural Stem/Progenitor Cells. PLoS One 8. https://doi.org/10.1371/journal.pone.0076860

Christiansen, T., Paulsen, S.K., Bruun, J.M., Ploug, T., Pedersen, S.B., Richelsen, B., 2010. Diet-induced weight loss and exercise alone and in combination enhance the expression of adiponectin receptors in adipose tissue and skeletal muscle, but only diet-induced weight loss enhanced circulating adiponectin. J. Clin. Endocrinol. Metab. 95, 911–919. https://doi.org/10.1210/jc.2008-2505

Chupel, M.U., Direito, F., Furtado, G.E., Minuzzi, L.G., Pedrosa, F.M., Colado, J.C., Ferreira, J.P., Filaire, E., Teixeira, A.M., 2017. Strength Training Decreases Inflammation and Increases Cognition and Physical Fitness in Older Women with Cognitive Impairment. Front. Physiol. 8, 377. https://doi.org/10.3389/fphys.2017.00377

Cleeland, C., Pipingas, A., Scholey, A., White, D., 2019. Neurochemical changes in the aging brain: A systematic review. Neurosci. Biobehav. Rev. https://doi.org/10.1016/j.neubiorev.2019.01.003

Cohen, J., Torres, C., 2019. Astrocyte senescence: Evidence and significance. Aging Cell. https://doi.org/10.1111/acel.12937

Cohen, S.A., Müller, W.E., 1992. Age-related alterations of NMDA-receptor properties in the mouse forebrain: partial restoration by chronic phosphatidylserune treatment. Brain Res. 584, 174–180. https://doi.org/10.1016/0006-8993(92)90892-D

Colcombe, S.J., Erickson, K.I., Scalf, P.E., Kim, J.S., Prakash, R., McAuley, E., Elavsky, S., Marquez, D.X., Hu, L., Kramer, A.F., 2006. Aerobic Exercise Training Increases Brain Volume in Aging Humans. Journals Gerontol. Ser. A Biol. Sci. Med. Sci. 61, 1166–1170. https://doi.org/10.1093/gerona/61.11.1166

Collingridge, G.L., Peineau, S., Howland, J.G., Wang, Y.T., 2010. Long-term depression in the CNS. Nat. Rev. Neurosci. 11, 459–473. https://doi.org/10.1038/nrn2867

Consitt, L.A., Bloomer, R.J., Wideman, L., 2007. The effect of exercise type on immunofunctional and traditional growth hormone. Eur. J. Appl. Physiol. 100, 321–330. https://doi.org/10.1007/s00421-007-0431-x

Conte, A., Belvisi, D., Bologna, M., Ottaviani, D., Fabbrini, G., Colosimo, C., Williams, D.R., Berardelli, A., 2012. Abnormal Cortical Synaptic Plasticity in Primary Motor Area in Progressive Supranuclear Palsy. Cereb. Cortex 22, 693–700. https://doi.org/10.1093/cercor/bhr149

Coppé, J.P., Desprez, P.Y., Krtolica, A., Campisi, J., 2010. The senescence-associated secretory phenotype: The dark side of tumor suppression. Annu. Rev. Pathol. Mech. Dis. https://doi.org/10.1146/annurev-pathol-121808-102144

Cosio, P.L., Crespo-Posadas, M., Velarde-Sotres, Á., Pelaez, M., 2021. Effect of Chronic Resistance Training on Circulating Irisin: Systematic Review and Meta-Analysis of Randomized Controlled Trials. Int. J. Environ. Res. Public Health 18, 1–16. https://doi.org/10.3390/IJERPH18052476

Cotman, C.W., Berchtold, N.C., Christie, L.-A., 2007. Exercise builds brain health: key roles of growth factor cascades and inflammation. TRENDS Neurosci. 30, 464–472. https://doi.org/10.1016/j.tins.2007.06.011

Cox, S.R., Lyall, D.M., Ritchie, S.J., Bastin, M.E., Harris, M.A., Buchanan, C.R., Fawns-Ritchie, C., Barbu, M.C., de Nooij, L., Reus, L.M., Alloza, C., Shen, X., Neilson, E., Alderson, H.L., Hunter, S., Liewald, D.C., Whalley, H.C., McIntosh, A.M., Lawrie, S.M., Pell, J.P., Tucker-Drob, E.M., Wardlaw, J.M., Gale, C.R., Deary, I.J., 2019. Associations between vascular risk factors and brain MRI indices in UK Biobank. Eur. Heart J. 40, 2290–2300. https://doi.org/10.1093/eurheartj/ehz100

Coxon, J.P., Cash, R.F.H.H., Hendrikse, J.J., Rogasch, N.C., Stavrinos, E., Suo, C., Yücel, M., 2018. GABA concentration in sensorimotor cortex following high-intensity exercise and relationship to lactate levels. J. Physiol. 596, 691–702. https://doi.org/10.1113/JP274660

Crabbe, J.B., Dishman, R.K., 2004. Brain electrocortical activity during and after exercise: A quantitative synthesis. Psychophysiology 41, 563–574. https://doi.org/10.1111/J.1469-8986.2004.00176.X

Cummings, D.E., Purnell, J.Q., Frayo, R.S., Schmidova, K., Wisse, B.E., Weigle, D.S., 2001. A Preprandial Rise in Plasma Ghrelin Levels Suggests a Role in Meal Initiation in Humans. Diabetes 50, 1714–1719. https://doi.org/10.2337/diabetes.50.8.1714

Cunha-Reis, D., Caulino-Rocha, A., 2020. VIP Modulation of Hippocampal Synaptic Plasticity: A Role for VIP Receptors as Therapeutic Targets in Cognitive Decline and Mesial Temporal Lobe Epilepsy. Front. Cell. Neurosci. https://doi.org/10.3389/fncel.2020.00153

Cunha-Reis, D., Ribeiro, J.A., Sebastião, A.M., 2005. VIP enhances synaptic transmission to hippocampal CA1 pyramidal cells through activation of both VPAC1 and VPAC2 receptors. Brain Res. 1049, 52–60. https://doi.org/10.1016/j.brainres.2005.04.077

Dacko, M., Lange, T., 2019. Improved detection of lactate and β-hydroxybutyrate using MEGA-sLASER at 3 T. NMR Biomed. 32. https://doi.org/10.1002/nbm.4100

Dai, Y., Pang, J., Gong, H., Fan, W., Zhang, T.M., 2013. Roles and tissue source of adiponectin involved in lifestyle modifications. Journals Gerontol. - Ser. A Biol. Sci. Med. Sci. 68, 117–128. https://doi.org/10.1093/gerona/gls131

Dall'Aglio, C., Pedini, V., Scocco, P., Boiti, C., Ceccarelli, P., 2010. Immunohistochemical evidence of Orexin-A in the pancreatic beta cells of domestic animals. Res. Vet. Sci. 89, 147–149. https://doi.org/10.1016/j.rvsc.2010.03.003

Dallérac, G.M., Vatsavayai, S.C., Cummings, D.M., Milnerwood, A.J., Peddie, C.J., Evans, K.A., Walters, S.W., Rezaie, P., Hirst, M.C., Murphy, K.P.S.J., 2011. Impaired long-term potentiation in the prefrontal cortex of Huntington's disease mouse models: Rescue by D1 dopamine receptor activation. Neurodegener. Dis. 8, 230–239. https://doi.org/10.1159/000322540

Daskalopoulou, S.S., Cooke, A.B., Gomez, Y.H., Mutter, A.F., Filippaios, A., Mesfum, E.T., Mantzoros, C.S., 2014. Plasma irisin levels progressively increase in response to increasing exercise workloads in young, healthy, active subjects. Eur. J. Endocrinol. 171, 343–352. https://doi.org/10.1530/EJE-14-0204

Davis, G.R., Stephens, J.M., Nelson, A.G., 2015. Effect of 12 weeks of periodized resistance training upon total plasma adiponectin concentration in healthy young men. J. Strength Cond. Res. 29, 3097–3104. https://doi.org/10.1519/JSC.0000000000000894

de Alcantara Borba, D., da Silva Alves, E., Rosa, J.P.P., Facundo, L.A., Costa, C.M.A., Silva, A.C., Narciso, F.V., Silva, A., de Mello, M.T., 2020. Can IGF-1 serum levels really be changed by acute physical exercise? A systematic review and meta-analysis. J. Phys. Act. Heal. 17, 575–584. https://doi.org/10.1123/jpah.2019-0453

de Assis, G.G., de Almondes, K.M., 2017. Exercise-dependent BDNF as a modulatory factor for the executive processing of individuals in course of cognitive decline. A systematic review. Front. Psychol. https://doi.org/10.3389/fpsyg.2017.00584

Delavar, R., Heidarianpour, A., 2016. The effect of aerobic exercise training on plasma apelin levels and pain threshold in T1DM rats. Iran. Red Crescent Med. J. 18. https://doi.org/10.5812/ircmj.31737

Demeter, I., Nagy, K., Farkas, T., Kis, Z., Kocsis, K., Knapp, L., Gellert, L., Fülöp, F., Vecsei, L., Toldi, J., 2013. Paradox effects of kynurenines on LTP induction in the Wistar rat. An in vivo study. Neurosci. Lett. 553, 138–141. https://doi.org/10.1016/j.neulet.2013.08.028

Denoth-Lippuner, A., Jessberger, S., 2021. Formation and integration of new neurons in the adult hippocampus. Nat. Rev. Neurosci. 1–14. https://doi.org/10.1038/s41583-021-00433-z

Deupree, D., Bradley, J., Turner, D., 1993. Age-related alterations in potentiation in the CA1 region in F344 rats. Neurobiol. Aging 14, 249–258. https://doi.org/10.1016/0197-4580(93)90009-Z

Diano, S., Farr, S.A., Benoit, S.C., McNay, E.C., Da Silva, I., Horvath, B., Gaskin, F.S., Nonaka, N., Jaeger, L.B., Banks, W.A., Morley, J.E., Pinto, S., Sherwin, R.S., Xu, L., Yamada, K.A., Sleeman, M.W., Tschöp, M.H., Horvath, T.L., 2006. Ghrelin controls hippocampal spine synapse density and memory performance. Nat. Neurosci. 9, 381–388. https://doi.org/10.1038/nn1656

Dieli-Conwright, C.M., Courneya, K.S., Demark-Wahnefried, W., Sami, N., Lee, K., Buchanan, T.A., Spicer, D. V., Tripathy, D., Bernstein, L., Mortimer, J.E., 2018a. Effects of aerobic and resistance exercise on metabolic syndrome, sarcopenic obesity, and circulating biomarkers in overweight or obese survivors of breast cancer: A randomized controlled trial. J. Clin. Oncol. 36, 875–883. https://doi.org/10.1200/JCO.2017.75.7526

Dieli-Conwright, C.M., Parmentier, J.H., Sami, N., Lee, K., Spicer, D., Mack, W.J., Sattler, F., Mittelman, S.D., 2018b. Adipose tissue inflammation in breast cancer survivors: effects of a 16-week combined aerobic and resistance exercise training intervention. Breast Cancer Res. Treat. 168, 147–157. https://doi.org/10.1007/s10549-017-4576-y

Dimri, G.P., Lee, X., Basile, G., Acosta, M., Scott, G., Roskelley, C., Medrano, E.E., Linskens, M., Rubelj, I., Pereira-Smith, O., Peacocke, M., Campisi, J., 1995. A biomarker that identifies senescent human cells in culture and in aging skin in vivo. Proc. Natl. Acad. Sci. U. S. A. 92, 9363–9367. https://doi.org/10.1073/pnas.92.20.9363

Ding, Q., Vaynman, S., Akhavan, M., Ying, Z., Gomez-Pinilla, F., 2006. Insulin-like growth factor I interfaces with brain-derived neurotrophic factor-mediated synaptic plasticity to modulate aspects of exercise-induced cognitive function. Neuroscience 140, 823–833. https://doi.org/10.1016/j.neuroscience.2006.02.084

Dinoff, A., Herrmann, N., Swardfager, W., Lanctôt, K.L., 2017. The effect of acute exercise on blood concentrations of brain-derived neurotrophic factor in healthy adults: a meta-analysis. Eur. J. Neurosci. https://doi.org/10.1111/ejn.13603

Dinoff, A., Herrmann, N., Swardfager, W., Liu, C.S., Sherman, C., Chan, S., Lanctôt, K.L., 2016. The effect of exercise training on resting concentrations of peripheral brain-derived neurotrophic factor (BDNF): A meta-analysis. PLoS One 11. https://doi.org/10.1371/journal.pone.0163037

Dogrukol-Ak, D., Banks, W.A., Tuncel, N., Tuncel, M., 2003. Passage of vasoactive intestinal peptide across the blood-brain barrier. Peptides 24, 437–444. https://doi.org/10.1016/S0196-9781(03)00059-7

Dong, F., Zhang, Q., Kong, W., Chen, J., Ma, J., Wang, L., Wang, Y., Liu, Y., Li, Y., Wen, J., 2017. Regulation of endometrial cell proliferation by estrogen-induced BDNF signaling pathway. Gynecol. Endocrinol. 33, 485–489. https://doi.org/10.1080/09513590.2017.1295439

Dundar, A., Kocahan, S., Arslan, C., 2019a. Effects of different loading exercises on apelin levels and physical and hematologic parameters of swimmers. Horm. Mol. Biol. Clin. Investig. 38. https://doi.org/10.1515/hmbci-2018-0070

Dundar, A., Kocahan, S., Sahin, L., 2019b. Associations of apelin, leptin, irisin, ghrelin, insulin, glucose levels, and lipid parameters with physical activity during eight weeks of regular exercise training. Arch. Physiol. Biochem. https://doi.org/10.1080/13813455.2019.1635622

El-Sayes, J., Harasym, D., Turco, C. V., Locke, M.B., Nelson, A.J., 2019. Exercise-Induced Neuroplasticity: A Mechanistic Model and Prospects for Promoting Plasticity. Neuroscientist 25, 65–85. https://doi.org/10.1177/1073858418771538 LK - http://limo.libis.be/resolver?&sid=EMBASE&issn=10894098&id=doi:10.1177%2F1073858418771538&atitle=Exercise-Induced+Neuroplasticity%3A+A+Mechanistic+Model+and+Prospects+for+Promoting+Plasticity&stitle=Neuroscientist&title=Neuroscientist&volume=25&issue=1&spage=65&epage=85&aulast=El-Sayes&aufirst=Jenin&auinit=J.&aufull=El-Sayes+J.&coden=NROSF&isbn=&pages=65-85&date=2019&auinit1=J&auinitm=

El Hayek, L., Khalifeh, M., Zibara, V., Abi Assaad, R., Emmanuel, N., Karnib, N., El-Ghandour, R., Nasrallah, P., Bilen, M., Ibrahim, P., Younes, J., Abou Haidar, E., Barmo, N., Jabre, V., Stephan, J.S., Sleiman, S.F., 2019. Lactate mediates the effects of exercise on learning and memory through sirt1-dependent activation of hippocampal brain-derived neurotrophic factor (BDNF). J. Neurosci. 39, 2369–2382. https://doi.org/10.1523/jneurosci.1661-18.2019

Erdmann, J., Tahbaz, R., Lippl, F., Wagenpfeil, S., Schusdziarra, V., 2007. Plasma ghrelin levels during exercise - Effects of intensity and duration. Regul. Pept. 143, 127–135. https://doi.org/10.1016/j.regpep.2007.05.002

Erickson, K.I., Prakash, R.S., Voss, M.W., Chaddock, L., Heo, S., McLaren, M., Pence, B.D., Martin, S.A., Vieira, V.J., Woods, J.A., McAuley, E., Kramer, A.F., 2010. Brain-derived neurotrophic factor is associated with age-related decline in hippocampal volume. J. Neurosci. 30, 5368–5375. https://doi.org/10.1523/jneurosci.6251-09.2010

Erickson, K.I., Voss, M.W., Prakash, R.S., Basak, C., Szabo, A., Chaddock, L., Kim, J.S., Heo, S., Alves, H., White, S.M., Wojcicki, T.R., Mailey, E., Vieira, V.J., Martin, S.A., Pence, B.D., Woods, J.A., McAuley, E., Kramer, A.F., 2011. Exercise training increases size of hippocampus and improves memory. Proc. Natl. Acad. Sci. U. S. A. 108, 3017–22. https://doi.org/10.1073/pnas.1015950108

Errea, A., Cayet, D., Marchetti, P., Tang, C., Kluza, J., Offermanns, S., Sirard, J.-C., Rumbo, M., 2016. Lactate Inhibits the Pro-Inflammatory Response and Metabolic Reprogramming in Murine Macrophages in a GPR81-Independent Manner. PLoS One 11, e0163694. https://doi.org/10.1371/journal.pone.0163694

Fang, H., Xu, X., Xu, R., Zhen, Y., Xu, G., Li, Y., 2018. Decreased serum undercarboxylated osteocalcin is associated with cognitive impairment in male patients with type 2 diabetes. J. Diabetes Complications 32, 56–60. https://doi.org/10.1016/j.jdiacomp.2017.09.004

Farmer, C.A., Thurm, A.E., Honnekeri, B., Kim, P., Swedo, S.E., Han, J.C., 2021. The contribution of platelets to peripheral BDNF elevation in children with autism spectrum disorder. Sci. Reports 2021 111 11, 1–6. https://doi.org/10.1038/s41598-021-97367-4

Fatouros, I.G., Tournis, S., Leontsini, D., Jamurtas, A.Z., Sxina, M., Thomakos, P., Manousaki, M., Douroudos, I., Taxildaris, K., Mitrakou, A., 2005. Leptin and adiponectin responses in overweight inactive elderly following resistance training and detraining are intensity related. J. Clin. Endocrinol. Metab. 90, 5970–5977. https://doi.org/10.1210/jc.2005-0261

Fernandes, J., Soares, J.C.K., do Amaral Baliego, L.G.Z., Arida, R.M., 2016. A single bout of resistance exercise improves memory consolidation and increases the expression of synaptic proteins in the hippocampus. Hippocampus 26, 1096–1103. https://doi.org/10.1002/hipo.22590

Finkbeiner, S., Tavazoie, S.F., Maloratsky, A., Jacobs, K.M., Harris, K.M., Greenberg, M.E., 1997. CREB: A major mediator of neuronal neurotrophin responses. Neuron 19, 1031–1047. https://doi.org/10.1016/S0896-6273(00)80395-5

Fjell, A.M., Walhovd, K.B., 2010. Structural brain changes in aging: Courses, causes and cognitive consequences. Rev. Neurosci. https://doi.org/10.1515/revneuro.2010.21.3.187

Flynn, M.G., McFarlin, B.K., Markofski, M.M., 2007. State of the Art Reviews: The Anti-Inflammatory Actions of Exercise Training. Am. J. Lifestyle Med. 1, 220–235. https://doi.org/10.1177/1559827607300283

Fonseca, T.R., Mendes, T.T., Ramos, G.P., Cabido, C.E.T., Morandi, R.F., Ferraz, F.O., Miranda, A.S., Mendonça, V.A., Teixeira, A.L., Silami-Garcia, E., Nunes-Silva, A., Teixeira, M.M., 2021. Aerobic Training Modulates the Increase in Plasma Concentrations of Cytokines in response to a Session of Exercise. J. Environ. Public Health 2021, 1–13. https://doi.org/10.1155/2021/1304139

Forny-Germano, L., De Felice, F.G., Do Nascimento Vieira, M.N., 2019. The role of leptin and adiponectin in obesity-associated cognitive decline and Alzheimer's disease. Front. Neurosci. 13, 1027. https://doi.org/10.3389/fnins.2018.01027

Forti, L., Njemini, R., Beyer, I., Eelbode, E., Meeusen, R., Mets, T., Bautmans, I., 2014. Strength training reduces circulating interleukin-6 but not brain-derived neurotrophic factor in community-dwelling elderly individuals. Age (Dordr). 36, 9704. https://doi.org/10.1007/S11357-014-9704-6

Fortunato, A.K., Pontes, W.M., De Souza, D.M.S., Prazeres, J.S.F., Marcucci-Barbosa, L.S., Santos, J.M.M., Veira, É.L.M., Bearzoti, E., De Castro Pinto, K.M., Talvani, A., Da Silva, A.N., 2018. Strength training session induces important changes on physiological, immunological, and inflammatory biomarkers. J. Immunol. Res. 2018. https://doi.org/10.1155/2018/9675216

Foster-Schubert, K.E., McTiernan, A., Frayo, R.S., Schwartz, R.S., Rajan, K.B., Yasui, Y., Tworoger, S.S., Cummings, D.E., 2005. Human Plasma Ghrelin Levels Increase during a One-Year Exercise Program. J. Clin. Endocrinol. Metab. 90, 820–825. https://doi.org/10.1210/jc.2004-2081

Frantseva, M. V., Fitzgerald, P.B., Chen, R., Moller, B., Daigle, M., Daskalakis, Z.J., 2008. Evidence for Impaired Long-Term Potentiation in Schizophrenia and Its Relationship to Motor Skill Leaning. Cereb. Cortex 18, 990–996. https://doi.org/10.1093/cercor/bhm151

Fujimura, H., Altar, C.A., Chen, R., Nakamura, T., Nakahashi, T., Kambayashi, J.I., Sun, B., Tandon, N.N., 2002. Brain-derived neurotrophic factor is stored in human platelets and released by agonist stimulation. Thromb. Haemost. 87, 728–734. https://doi.org/10.1055/s-0037-1613072

Furigo, I.C., Melo, H.M., Lyra e Silva, N.M., Ramos-Lobo, A.M., Teixeira, P.D.S., Buonfiglio, D.C., Wasinski, F., Lima, E.R., Higuti, E., Peroni, C.N., Bartolini, P., Soares, C.R.J., Metzger, M., de Felice, F.G., Donato, J., 2018. Brain STAT5 signaling modulates learning and memory formation. Brain Struct. Funct. 223, 2229–2241. https://doi.org/10.1007/s00429-018-1627-z

Galbo, H., 1993. Hormonal and metabolic adaptation to exercise. Thieme Medical Publishers, New York.

Galbo, H., Hilsted, J., Fahrenkrug, J., Schaffalitzky De Muckadell, O.B., 1979. Fasting and prolonged exercise increase vasoactive intestinal polypeptide (VIP) in plasma. Acta Physiol. Scand. 105, 374–377. https://doi.org/10.1111/j.1748-1716.1979.tb06353.x

Galbreath, M., Campbell, B., Labounty, P., Bunn, J., Dove, J., Harvey, T., Hudson, G., Gutierrez, J.L., Levers, K., Galvan, E., Jagim, A., Greenwood, L., Cooke, M.B., Greenwood, M., Rasmussen, C., Kreider, R.B., 2018. Effects of adherence to a higher protein diet on weight loss, markers of health, and functional capacity in older women participating in a resistance-based exercise program. Nutrients 10. https://doi.org/10.3390/nu10081070

Garneau, L., Parsons, S.A., Smith, S.R., Mulvihill, E.E., Sparks, L.M., Aguer, C., 2020. Plasma Myokine Concentrations After Acute Exercise in Non-obese and Obese Sedentary Women. Front. Physiol. 11. https://doi.org/10.3389/fphys.2020.00018

Gejl, A.K., Enevold, C., Bugge, A., Andersen, M.S., Nielsen, C.H., Andersen, L.B., 2019. Associations between serum and plasma brain-derived neurotrophic factor and influence of storage time and centrifugation strategy. Sci. Reports 2019 91 9, 1–9. https://doi.org/10.1038/s41598-019-45976-5

Gerth, F., Jäpel, M., Pechstein, A., Kochlamazashvili, G., Lehmann, M., Puchkov, D., Onofri, F., Benfenati, F., Nikonenko, A.G., Fredrich, K., Shupliakov, O., Maritzen, T., Freund, C., Haucke, V., 2017. Intersectin associates with synapsin and regulates its nanoscale localization and function. Proc. Natl. Acad. Sci. U. S. A. 114, 12057–12062. https://doi.org/10.1073/pnas.1715341114

Ghayomzadeh, M., Earnest, C.P., Hackett, D., SeyedAlinaghi, S.A., Navalta, J.W., Gholami, M., Hosseini Rouzbahani, N., Mohraz, M., Voltarelli, F.A., 2020. Combination of resistance and aerobic exercise for six months improves bone mass and physical function in HIV infected individuals: A randomized controlled trial. Scand. J. Med. Sci. Sport. 31. https://doi.org/10.1111/sms.13871

Gibbons, T.E., Pence, B.D., Petr, G., Ossyra, J.M., Mach, H.C., Bhattacharya, T.K., Perez, S., Martin, S.A., McCusker, R.H., Kelley, K.W., Rhodes, J.S., Johnson, R.W., Woods, J.A., 2014. Voluntary wheel running, but not a diet containing (-)-epigallocatechin-3-gallate and β-alanine, improves learning, memory and hippocampal neurogenesis in aged mice. Behav. Brain Res. 272, 131–140. https://doi.org/10.1016/j.bbr.2014.05.049

Giovannini, M.G., Blitzer, R.D., Wong, T., Asoma, K., Tsokas, P., Morrison, J.H., Iyengar, R., Landau, E.M., 2001. Mitogen-activated protein kinase regulates early phosphorylation and delayed expression of Ca2+/calmodulin-dependent protein kinase II in long-term potentiation. J. Neurosci. 21, 7053–7062. https://doi.org/10.1523/jneurosci.21-18-07053.2001

Glanville, N.T., Byers, D.M., Cook, H.W., Spence, M.W., Palmer, F.B.S.C., 1989. Differences in the metabolism of inositol and phosphoinositides by cultured cells of neuronal and glial origin. Biochim. Biophys. Acta (BBA)/Lipids Lipid Metab. 1004, 169–179. https://doi.org/10.1016/0005-2760(89)90265-8

Glud, M., Christiansen, T., Larsen, L.H., Richelsen, B., Bruun, J.M., 2019. Changes in Circulating BDNF in relation to Sex, Diet, and Exercise: A 12-Week Randomized Controlled Study in Overweight and Obese Participants. J. Obes. 2019. https://doi.org/10.1155/2019/4537274

Gomes da Silva, S., Simões, P.S.R., Mortara, R.A., Scorza, F.A., Cavalheiro, E.A., da Graça Naffah-Mazzacoratti, M., Arida, R.M., 2013. Exercise-induced hippocampal anti-inflammatory response in aged rats. J. Neuroinflammation 10, 827. https://doi.org/10.1186/1742-2094-10-61

Gomes, R.J., De Oliveira, C.A.M., Ribeiro, C., Mota, C.S.D.A., Moura, L.P., Tognoli, L.M.M.C., Leme, J.A.C.D.A., Luciano, E., De Mello, M.A.R., 2009. Effects of exercise training on hippocampus concentrations of insulin and IGF-1 in diabetic rats. Hippocampus 19, 981–987. https://doi.org/10.1002/hipo.20636

Gomez-Pinilla, F., Hillman, C., 2013. The influence of exercise on cognitive abilities. Compr. Physiol. 3, 403–428. https://doi.org/10.1002/cphy.c110063

Gonzales, M.M., Tarumi, T., Kaur, S., Nualnim, N., Fallow, B.A., Pyron, M., Tanaka, H., Haley, A.P., 2013. Aerobic fitness and the brain: Increased N-acetyl-aspartate and choline concentrations in endurance-trained middle-aged adults. Brain Topogr. 26, 126–134. https://doi.org/10.1007/s10548-012-0248-8

Görgens, S.W., Eckardt, K., Jensen, J., Drevon, C.A., Eckel, J., 2015. Exercise and Regulation of Adipokine and Myokine Production, in: Progress in Molecular Biology and Translational Science. Elsevier B.V., pp. 313–336. https://doi.org/10.1016/bs.pmbts.2015.07.002

Gourgouvelis, J., Yielder, P., Clarke, S.T., Behbahani, H., Murphy, B., 2018. You can't fix what isn't broken: Eight weeks of exercise do not substantially change cognitive function and biochemical markers in young and healthy adults. PeerJ 2018. https://doi.org/10.7717/peerj.4675

Grosicki, G., Barrett, B., Englund, D., Liu, C., Travison, T., Cederholm, T., Koochek, A., von Berens, A., Gustafsson, T., Benard, T., Reid, K., Fielding, R., 2020. Circulating Interleukin-6 Is Associated with Skeletal Muscle Strength, Quality, and Functional Adaptation with Exercise Training in Mobility-Limited Older Adults. J. frailty aging 9, 57–63. https://doi.org/10.14283/JFA.2019.30

Gruhn, K., Siteneski, A., Camargo, A., Freitas, A.E., Olescowicz, G., Brocardo, P.S., Rodrigues, A.L.S., 2021. Physical exercise stimulates hippocampal mTORC1 and FNDC5/irisin signaling pathway in mice: Possible implication for its antidepressant effect. Behav. Brain Res. 400. https://doi.org/10.1016/j.bbr.2020.113040

Guan, X.M., Yu, H., Palyha, O.C., McKee, K.K., Feighner, S.D., Sirinathsinghji, D.J.S., Smith, R.G., Van Der Ploeg, L.H.T., Howard, A.D., 1997. Distribution of mRNA encoding the growth hormone secretagogue receptor in brain and peripheral tissues. Mol. Brain Res. 48, 23–29. https://doi.org/10.1016/S0169-328X(97)00071-5

Gubert, C., Hannan, A.J., 2021. Exercise mimetics: harnessing the therapeutic effects of physical activity. Nat. Rev. Drug Discov. 2021 1–18. https://doi.org/10.1038/S41573-021-00217-1

Gulick, C.N., Peddie, M., Rehrer, N.J., 2020. Does Exercise Impact Insulin-like Growth Factor 1? : Systematic Review & Meta-Analysis. Open J. Heal. Sci. Med. 1, 104. https://doi.org/10.0000/OJHSM.1000104

Haeger, A., Costa, A.S., Schulz, J.B., Reetz, K., 2019. Cerebral changes improved by physical activity during cognitive decline: A systematic review on MRI studies. NeuroImage Clin. https://doi.org/10.1016/j.nicl.2019.101933

Halon-Golabek, M., Borkowska, A., Herman-Antosiewicz, A., Antosiewicz, J., 2019. Iron Metabolism of the Skeletal Muscle and Neurodegeneration. Front. Neurosci. https://doi.org/10.3389/fnins.2019.00165

Halson, S.L., Lancaster, G.I., Jeukendrup, A.E., Gleeson, M., 2003. Immunological Responses to Overreaching in Cyclists. Med. Sci. Sport. Exerc. 35, 854–861.

Hangelbroek, R., Knuiman, P., Tieland, M., de Groot, L., 2018. Attenuated strength gains during prolonged resistance exercise training in older adults with high inflammatory status. Exp. Gerontol. 106, 154–158. https://doi.org/10.1016/J.EXGER.2018.02.008

Harte-Hargrove, L., MacLusky, N.J., Scharfman, H.E., 2013. BDNF-estrogen interactions in hippocampal mossy fiber pathway: implications for normal brain function and disease. Neuroscience 239, 46–66. https://doi.org/10.1016/j.neuroscience.2012.12.029

Hecksteden, A., Wegmann, M., Steffen, A., Kraushaar, J., Morsch, A., Ruppenthal, S., Kaestner, L., Meyer, T., 2013. Irisin and exercise training in humans – Results from a randomized controlled training trial. BMC Med. 11, 1–8. https://doi.org/10.1186/1741-7015-11-235

Henning, R.J., Sawmiller, D.R., 2001. Vasoactive intestinal peptide: Cardiovascular effects. Cardiovasc. Res. https://doi.org/10.1016/S0008-6363(00)00229-7

Hernandez-Segura, A., de Jong, T. V., Melov, S., Guryev, V., Campisi, J., Demaria, M., 2017. Unmasking Transcriptional Heterogeneity in Senescent Cells. Curr. Biol. 27, 2652-2660.e4. https://doi.org/10.1016/j.cub.2017.07.033

Hernandez, A.R., Hernandez, C.M., Campos, K., Truckenbrod, L., Federico, Q., Moon, B., McQuail, J.A., Maurer, A.P., Bizon, J.L., Burke, S.N., 2018. A Ketogenic Diet Improves Cognition and Has Biochemical Effects in Prefrontal Cortex That Are Dissociable From Hippocampus. Front. Aging Neurosci. 10, 391. https://doi.org/10.3389/fnagi.2018.00391

Herold, F., Törpel, A., Schega, L., Müller, N.G., 2019. Functional and/or structural brain changes in response to resistance exercises and resistance training lead to cognitive improvements - A systematic review. Eur. Rev. Aging Phys. Act. https://doi.org/10.1186/s11556-019-0217-2

Higuchi, K., Masaki, T., Gotoh, K., Chiba, S., Katsuragi, I., Tanaka, K., Kakuma, T., Yoshimatsu, H., 2007. Apelin, an APJ Receptor Ligand, Regulates Body Adiposity and Favors the Messenger Ribonucleic Acid Expression of Uncoupling Proteins in Mice. Endocrinology 148, 2690–2697. https://doi.org/10.1210/en.2006-1270

Hook, V., Yoon, M., Mosier, C., Ito, G., Podvin, S., Head, B.P., Rissman, R., O'Donoghue, A.J., Hook, G., 2020. Cathepsin B in neurodegeneration of Alzheimer's disease, traumatic brain injury, and related brain disorders. Biochim. Biophys. acta. Proteins proteomics. https://doi.org/10.1016/j.bbapap.2020.140428

Hu, E., Du, H., Zhu, X., Wang, L., Shang, S., Wu, X., Lu, H., Lu, X., 2018. Beta-hydroxybutyrate Promotes the Expression of BDNF in Hippocampal Neurons under Adequate Glucose Supply. Neuroscience 386, 315–325. https://doi.org/10.1016/j.neuroscience.2018.06.036

Huh, J.Y., Mougios, V., Kabasakalis, A., Fatouros, I., Siopi, A., Douroudos, I.I., Filippaios, A., Panagiotou, G., Park, K.H., Mantzoros, C.S., 2014. Exercise-induced irisin secretion is independent of age or fitness level and increased irisin may directly modulate muscle metabolism through AMPK activation. J. Clin. Endocrinol. Metab. 99, E2154–E2161. https://doi.org/10.1210/jc.2014-1437

Ide, K., Horn, A., Secher, N.H., 1999. Cerebral metabolic response to submaximal exercise. J. Appl. Physiol. 87, 1604–1608. https://doi.org/10.1152/jappl.1999.87.5.1604

Ide, K., Schmalbruch, I.K., Quistorff, B., Horn, A., Secher, N.H., 2000. Lactate, glucose and O2 uptake in human brain during recovery from maximal exercise. J. Physiol. 522 Pt 1, 159–64. https://doi.org/10.1111/j.1469-7793.2000.t01-2-00159.xm

Ieraci, A., Beggiato, S., Ferraro, L., Barbieri, S.S., Popoli, M., 2020. Kynurenine pathway is altered in BDNF Val66Met knock-in mice: Effect of physical exercise. Brain. Behav. Immun. 89, 440–450. https://doi.org/10.1016/j.bbi.2020.07.031

Ihalainen, J.K., Ahtiainen, J.P., Walker, S., Paulsen, G., Selänne, H., Hämäläinen, M., Moilanen, E., Peltonen, H., Mero, A.A., 2017. Resistance training status modifies inflammatory response to explosive and hypertrophic resistance exercise bouts. J. Physiol. Biochem. 73, 595–604. https://doi.org/10.1007/s13105-017-0590-0

Isung, J., Granqvist, M., Trepci, A., Huang, J., Schwieler, L., Kierkegaard, M., Erhardt, S., Jokinen, J., Piehl, F., 2021. Differential effects on blood and cerebrospinal fluid immune protein markers and kynurenine pathway metabolites from aerobic physical exercise in healthy subjects. Sci. Rep. 11, 1–13. https://doi.org/10.1038/s41598-021-81306-4

Izquierdo, M., Ibañez, J., Calbet, J.A.L., Navarro-Amezqueta, I., González-Izal, M., Idoate, F., Häkkinen, K., Kraemer, W.J., Palacios-Sarrasqueta, M., Almar, M., Gorostiaga, E.M., 2009. Cytokine and hormone responses to resistance training. Eur. J. Appl. Physiol. 107, 397–409. https://doi.org/10.1007/S00421-009-1139-X

Jang, S.H., Paik, I.Y., Ryu, J.H., Lee, T.H., Kim, D.E., 2019. Effects of aerobic and resistance exercises on circulating apelin-12 and apelin-36 concentrations in obese middle-aged women: A randomized controlled trial. BMC Womens. Health 19, 23. https://doi.org/10.1186/s12905-019-0722-5

Jefferson, A.L., Hohman, T.J., Liu, D., Haj-Hassan, S., Gifford, K.A., Benson, E.M., Skinner, J.S., Lu, Z., Sparling, J., Sumner, E.C., Bell, S., Ruberg, F.L., 2015. Adverse vascular risk is related to cognitive decline in older adults. J. Alzheimer's Dis. 44, 1361–1373. https://doi.org/10.3233/JAD-141812

Ji, W., Gong, L., Wang, J., He, H., Zhang, Y., 2016. Hypoxic Exercise Training Promotes apelin/APJ Expression in Skeletal Muscles of High Fat Diet-Induced Obese Mice. Protein Pept. Lett. 24, 64–70. https://doi.org/10.2174/0929866524666161111111726

Jiang, Q., Lou, K., Hou, L., Lu, Y., Sun, L., Tan, S.C., Low, T.Y., Kord-Varkaneh, H., Pang, S., 2020. The effect of resistance training on serum insulin-like growth factor 1(IGF-1): A systematic review and meta-analysis. Complement. Ther. Med. https://doi.org/10.1016/j.ctim.2020.102360

Jo, E., Worts, P.R., Elam, M.L., Brown, A.F., Khamoui, A. V., Kim, D.H., Yeh, M.C., Ormsbee, M.J., Prado, C.M., Cain, A., Snyder, K., Kim, J.S., 2019. Resistance training during a 12-week protein supplemented VLCD treatment enhances weight-loss outcomes in obese patients. Clin. Nutr. 38, 372–382. https://doi.org/10.1016/j.clnu.2017.12.015

Johnson, T.K., Belcher, D.J., Sousa, C.A., Carzoli, J.P., Visavadiya, N.P., Khamoui, A. V., Whitehurst, M., Zourdos, M.C., 2020. Low-volume acute multi-joint resistance exercise elicits a circulating brain-derived neurotrophic factor response but not a cathepsin b response in well-trained men. Appl. Physiol. Nutr. Metab. 45, 1332–1338. https://doi.org/10.1139/apnm-2019-0854

Joisten, N., Kummerhoff, F., Koliamitra, C., Schenk, A., Walzik, D., Hardt, L., Knoop, A., Thevis, M., Kiesl, D., Metcalfe, A.J., Bloch, W., Zimmer, P., 2020. Exercise and the Kynurenine pathway: Current state of knowledge and results from a randomized cross-over study comparing acute effects of endurance and resistance training. Exerc. Immunol. Rev. 26, 24–42.

Jorgensen, L.G., Perko, G., Secher, N.H., 1992. Regional cerebral artery mean flow velocity and blood flow during dynamic exercise in humans. J. Appl. Physiol. 73, 1825–1830. https://doi.org/10.1152/jappl.1992.73.5.1825

Jovanovic, J.N., Benfenati, F., Siow, Y.L., Sihra, T.S., Sanghera, J.S., Pelech, S.L., Greengard, P., Czernik, A.J., 1996. Neurotrophins stimulate phosphorylation of synapsin I by MAP kinase and regulate synapsin I-actin interactions. Proc. Natl. Acad. Sci. U. S. A. 93, 3679–3683. https://doi.org/10.1073/pnas.93.8.3679

Jürimäe, J., Vaiksaar, S., Purge, P., Jürimäe, T., 2016. Adiponectin and osteocalcin responses to rowing exercise, and the relationship to substrate oxidation in female rowers. Acta Physiol. Hung. 103, 220–230. https://doi.org/10.1556/036.103.2016.2.9

Karlsson, M.K., Vergnaud, P., Delmas, P.D., Obrant, K.J., 1995. Indicators of bone formation in weight lifters. Calcif. Tissue Int. 56, 177–180. https://doi.org/10.1007/BF00298605

Kastin, A.J., Akerstrom, V., 1999. Orexin A but Not Orexin B Rapidly Enters Brain from Blood by Simple Diffusion. J. Pharmacol. Exp. Ther. 289.

Kechyn, S., Barnes, G., Howard, L., 2015. Assessing dynamic changes in plasma apelin concentration in response to maximal exercise in man, in: European Respiratory Journal. European Respiratory Society (ERS), p. PA2316. https://doi.org/10.1183/13993003.congress-2015.pa2316

Kelly, K.R., Navaneethan, S.D., Solomon, T.P.J., Haus, J.M., Cook, M., Barkoukis, H., Kirwan, J.P., 2014. Lifestyle-induced decrease in fat mass improves adiponectin Secretion in Obese Adults. Med. Sci. Sports Exerc. 46, 920–926. https://doi.org/10.1249/MSS.0000000000000200

Kelty, T.J., Schachtman, T.R., Mao, X., Grigsby, K.B., Childs, T.E., Olver, T.D., Michener, P.N., Richardson, R.A., Roberts, C.K., Booth, F.W., 2019. Resistance-exercise training ameliorates LPS-induced cognitive impairment concurrent with molecular signaling changes in the rat dentate gyrus. J. Appl. Physiol. 127, 254–263. https://doi.org/10.1152/japplphysiol.00249.2019

Khrimian, L., Obri, A., Ramos-Brossier, M., Rousseaud, A., Moriceau, S., Nicot, A.S., Mera, P., Kosmidis, S., Karnavas, T., Saudou, F., Gao, X.B., Oury, F., Kandel, E., Karsenty, G., 2017. Gpr158 mediates osteocalcin's regulation of cognition. J. Exp. Med. 214, 2859–2873. https://doi.org/10.1084/jem.20171320

Kim, B., Kang, S., 2020. Regular leisure-time physical activity is effective in boosting neurotrophic factors and alleviating menopause symptoms. Int. J. Environ. Res. Public Health 17, 1–10. https://doi.org/10.3390/ijerph17228624

Kim, E., Grover, L.M., Bertolotti, D., Green, T.L., 2010. Growth hormone rescues hippocampal synaptic function after sleep deprivation. Am. J. Physiol. - Regul. Integr. Comp. Physiol. 298, R1588. https://doi.org/10.1152/ajpregu.00580.2009

Kim, H.-J., Lee, H.-J., So, B., Son, J.S., Yoon, D., Song, Wook, Song, W, 2016. Effect of Aerobic Training and Resistance Training on Circulating Irisin Level and Their Association With Change of Body Composition in Overweight/Obese Adults: a Pilot Study. Physiol. Res 65, 271–279. https://doi.org/10.33549/physiolres.932997

Kim, H.H., Kim, Y.J., Lee, S.Y., Jeong, D.W., Lee, J.G., Yi, Y.H., Cho, Y.H., Choi, E.J., Kim, H.J., 2014. Interactive effects of an isocaloric high-protein diet and resistance exercise on body composition, ghrelin, and metabolic and hormonal parameters in untrained young men: A randomized clinical trial. J. Diabetes Investig. 5, 242–247. https://doi.org/10.1111/jdi.12148

Kim, K.B., 2014. Effect of different training mode on Interleukin-6 (IL-6) and C-reactive protein (CRP) in type 2 diabetes mellitus (T2DM) patients. J. Exerc. Nutr. Biochem. 18, 371–378. https://doi.org/10.5717/jenb.2014.18.4.371

Kim, K.H., Kim, S.H., Min, Y.K., Yang, H.M., Lee, J.B., Lee, M.S., 2013. Acute Exercise Induces FGF21 Expression in Mice and in Healthy Humans. PLoS One 8, 63517. https://doi.org/10.1371/journal.pone.0063517

Kim, S., Choi, J.Y., Moon, S., Park, D.H., Kwak, H.B., Kang, J.H., 2019. Roles of myokines in exercise-induced improvement of neuropsychiatric function. Pflugers Arch. Eur. J. Physiol. https://doi.org/10.1007/s00424-019-02253-8

Kimura, K., Hozumi, N., 2012. Investigating the acute effect of an aerobic dance exercise program on neuro-cognitive function in the elderly. Psychol. Sport Exerc. 13, 623–629. https://doi.org/10.1016/j.psychsport.2012.04.001

Kirk, I.J., McNair, N.A., Hamm, J.P., Clapp, W.C., Mathalon, D.H., Cavus, I., Teyler, T.J., 2010. Long-term potentiation (LTP) of human sensory-evoked potentials. Wiley Interdiscip. Rev. Cogn. Sci. 1, 766–773. https://doi.org/10.1002/WCS.62

Kito, S., Miyoshi, R., Nomoto, T., 1990. Influence of age on NMDA receptor complex in rat brain studied by in vitro autoradiography. J. Histochem. Cytochem. 38, 1725–1731. https://doi.org/10.1177/38.12.2147708

Kleppner, S.R., Tobin, A.J., 2002. GABA, in: Ramachadran, V.S. (Ed.), Encyclopedia of the Human Brain. Academic Press, California, pp. 353–367.

Knaepen, K., Goekint, M., Heyman, E.M., Meeusen, R., 2010. Neuroplasticity - Exercise-Induced Response of Peripheral Brain-Derived Neurotrophic Factor: A Systematic Review of Experimental Studies in Human Subjects. Sport. Med. 40, 765–801. https://doi.org/10.2165/11534530-000000000-00000

Koch, A.J., 2010. Immune Response to Resistance Exercise. Am. J. Lifestyle Med. 4, 244–252. https://doi.org/10.1177/1559827609360190

Kohman, R.A., Bhattacharya, T.K., Wojcik, E., Rhodes, J.S., 2013. Exercise reduces activation of microglia isolated from hippocampus and brain of aged mice. J. Neuroinflammation 10, 114. https://doi.org/10.1186/1742-2094-10-114

Kojima, M., Hosoda, H., Date, Y., Nakazato, M., Matsuo, H., Kangawa, K., 1999. Ghrelin is a growth-hormone-releasing acylated peptide from stomach. Nature 402, 656–660. https://doi.org/10.1038/45230

Kolahdouzi, S., Baghadam, M., Kani-Golzar, F.A., Saeidi, A., Jabbour, G., Ayadi, A., De Sousa, M., Zouita, A., Abderrahmane, A. Ben, Zouhal, H., 2019. Progressive circuit resistance training improves inflammatory biomarkers and insulin resistance in obese men. Physiol. Behav. 205, 15–21. https://doi.org/10.1016/j.physbeh.2018.11.033

Kon, M., Ebi, Y., Nakagaki, K., 2019. Effects of acute sprint interval exercise on follistatin-like 1 and apelin secretions. Arch. Physiol. Biochem. https://doi.org/10.1080/13813455.2019.1628067

Kon, M., Tanimura, Y., Yoshizato, H., 2020. Effects of acute endurance exercise on follistatin-like 1 and apelin in the circulation and metabolic organs in rats. Arch. Physiol. Biochem. https://doi.org/10.1080/13813455.2020.1764050

Kraemer, R.R., Shockett, P., Webb, N.D., Shah, U., Castracane, V.D., 2014. A transient elevated irisin blood concentration in response to prolonged, moderate aerobic exercise in young men and women. Horm. Metab. Res. 46, 150–154. https://doi.org/10.1055/s-0033-1355381

Krist, J., Wieder, K., Klöting, N., Oberbach, A., Kralisch, S., Wiesner, T., Schön, M.R., Gärtner, D., Dietrich, A., Shang, E., Lohmann, T., Dreßler, M., Fasshauer, M., Stumvoll, M., Blüher, M., 2013. Effects of weight loss and exercise on apelin serum concentrations and adipose tissue expression in human obesity. Obes. Facts 6, 57–69. https://doi.org/10.1159/000348667

Kruse, B., Hanefeld, F., Christen, H.J., Bruhn, H., Michaelis, T., Hänicke, W., Frahm, J., 1993. Alterations of brain metabolites in metachromatic leukodystrophy as detected by localized proton magnetic resonance spectroscopy in vivo. J. Neurol. 241, 68–74. https://doi.org/10.1007/BF00869766

Kujach, S., Olek, R.A., Byun, K., Suwabe, K., Sitek, E.J., Ziemann, E., Laskowski, R., Soya, H., 2020. Acute Sprint Interval Exercise Increases Both Cognitive Functions and Peripheral Neurotrophic Factors in Humans: The Possible Involvement of Lactate. Front. Neurosci. 13. https://doi.org/10.3389/fnins.2019.01455

Kwak, S.E., Bae, J.H., Lee, J.H., Shin, H.E., Zhang, D., Cho, S.C., Song, W., 2021. Effects of exercise-induced beta-hydroxybutyrate on muscle function and cognitive function. Physiol. Rep. 9, e14497. https://doi.org/10.14814/phy2.14497

Kwak, S.E., Cho, S.C., Bae, J.H., Lee, J., Shin, H.E., Di Zhang, D., Lee, Y. Il, Song, W., 2019. Effects of exercise-induced apelin on muscle function and cognitive function in aged mice. Exp. Gerontol. 127, 110710. https://doi.org/10.1016/j.exger.2019.110710

Kwon, J.H., Moon, K.M., Min, K.-W., 2020. Exercise-Induced Myokines can Explain the Importance of Physical Activity in the Elderly: An Overview. Healthcare 8, 378. https://doi.org/10.3390/healthcare8040378

Lan, Y., Huang, Z., Jiang, Y., Zhou, X., Zhang, J., Zhang, D., Wang, B., Hou, G., 2018. Strength exercise weakens aerobic exerciseinduced cognitive improvements in rats. PLoS One 13. https://doi.org/10.1371/journal.pone.0205562

Le Blanc, J., Fleury, S., Boukhatem, I., Bélanger, J.-C., Welman, M., Lordkipanidzé, M., 2020. Platelets Selectively Regulate the Release of BDNF, But Not That of Its Precursor Protein, proBDNF. Front. Immunol. 0, 2994. https://doi.org/10.3389/FIMMU.2020.575607

Leidy, H.J., Dougherty, K.A., Frye, B.R., Duke, K.M., Williams, N.I., 2007. Twenty-four-hour ghrelin is elevated after calorie restriction and exercise training in non-obese women. Obesity 15, 446–455. https://doi.org/10.1038/oby.2007.542

Leidy, H.J., Gardner, J.K., Frye, B.R., Snook, M.L., Schuchert, M.K., Richard, E.L., Williams, N.I., 2004. Circulating ghrelin is sensitive to changes in body weight during a diet and exercise program in normal-weight young women, in: Journal of Clinical Endocrinology and Metabolism. J Clin Endocrinol Metab, pp. 2659–2664. https://doi.org/10.1210/jc.2003-031471

Lenz, M., Eichler, A., Kruse, P., Strehl, A., Rodriguez-Rozada, S., Goren, I., Yogev, N., Frank, S., Waisman, A., Deller, T., Jung, S., Maggio, N., Vlachos, A., 2020. Interleukin 10 Restores Lipopolysaccharide-Induced Alterations in Synaptic Plasticity Probed by Repetitive Magnetic Stimulation. Front. Immunol. 11, 3291. https://doi.org/10.3389/FIMMU.2020.614509

Lessmann, V., Gottmann, K., Malcangio, M., 2003. Neurotrophin secretion: current facts and future prospects. Prog. Neurobiol. 69, 341–374. https://doi.org/10.1016/S0301-0082(03)00019-4

Lester, M.E., Urso, M.L., Evans, R.K., Pierce, J.R., Spiering, B.A., Maresh, C.M., Hatfield, D.L., Kraemer, W.J., Nindl, B.C., 2009. Influence of exercise mode and osteogenic index on bone biomarker responses during short-term physical training. Bone 45, 768–776. https://doi.org/10.1016/j.bone.2009.06.001

Levin, O., Netz, Y., Ziv, G., 2021. Behavioral and Neurophysiological Aspects of Inhibition—The Effects of Acute Cardiovascular Exercise. J. Clin. Med. 10, 282. https://doi.org/10.3390/JCM10020282

Levinger, I., Jerums, G., Stepto, N.K., Parker, L., Serpiello, F.R., McConell, G.K., Anderson, M., Hare, D.L., Byrnes, E., Ebeling, P.R., Seeman, E., 2014a. The effect of acute exercise on undercarboxylated osteocalcin and insulin sensitivity in obese men. J. Bone Miner. Res. 29, 2571–2576. https://doi.org/10.1002/jbmr.2285

Levinger, I., Scott, D., Nicholson, G.C., Stuart, A.L., Duque, G., McCorquodale, T., Herrmann, M., Ebeling, P.R., Sanders, K.M., 2014b. Undercarboxylated osteocalcin, muscle strength and indices of bone health in older women. Bone 64, 8–12. https://doi.org/10.1016/j.bone.2014.03.008

Levinger, I., Zebaze, R., Jerums, G., Hare, D.L., Selig, S., Seeman, E., 2011. The effect of acute exercise on undercarboxylated osteocalcin in obese men. Osteoporos. Int. 22, 1621–1626. https://doi.org/10.1007/s00198-010-1370-7

Li, A., Yau, S., Machado, S., Yuan, T.-F., So, K.-F., 2015. Adult neurogenic and antidepressant effects of adiponectin: a potential replacement for exercise? CNS Neurol. Disord. - Drug Targets 14, 1129–1144. https://doi.org/10.2174/1871527315666151111125533

Li, E., Deng, H., Wang, B., Fu, W., You, Y., Tian, S., 2016. Apelin-13 exerts antidepressant-like and recognition memory improving activities in stressed rats. Eur. Neuropsychopharmacol. 26, 420–430. https://doi.org/10.1016/j.euroneuro.2016.01.007

Lisman, J.E., 2001. Three Ca2+ levels affect plasticity differently: The LTP zone, the LTD zone and no man's land. J. Physiol. 532, 285. https://doi.org/10.1111/j.1469-7793.2001.0285f.x

Liu, W., Sheng, H., Xu, Y., Liu, Y., Lu, J., Ni, X., 2013. Swimming exercise ameliorates depression-like behavior in chronically stressed rats: relevant to proinflammatory cytokines and IDO activation. Behav. Brain Res. 242, 110–116. https://doi.org/10.1016/J.BBR.2012.12.041

Liu, Y., Chu, J.M.T., Yan, T., Zhang, Y., Chen, Y., Chang, R.C.C., Wong, G.T.C., 2020. Short-term resistance exercise inhibits neuroinflammation and attenuates neuropathological changes in 3xTg Alzheimer's disease mice. J. Neuroinflammation 17. https://doi.org/10.1186/s12974-019-1653-7

Lobie, P.E., Zhu, T., Graichen, R., Goh, E.L.K., 2000. Growth hormone, insulin-like growth factor I and the CNS: Localization, function and mechanism of action. Growth Horm. IGF Res. https://doi.org/10.1016/S1096-6374(00)80010-6

Loprinzi, P.D., 2019. The Effects of Exercise on Long-Term Potentiation: A Candidate Mechanism of the Exercise-Memory Relationship. OBM Neurobiol. 3, 1–1. https://doi.org/10.21926/obm.neurobiol.1902026

Lourenco, M. V., Frozza, R.L., de Freitas, G.B., Zhang, H., Kincheski, G.C., Ribeiro, F.C., Gonçalves, R.A., Clarke, J.R., Beckman, D., Staniszewski, A., Berman, H., Guerra, L.A., Forny-Germano, L., Meier, S., Wilcock, D.M., de Souza, J.M., Alves-Leon, S., Prado, V.F., Prado, M.A.M., Abisambra, J.F., Tovar-Moll, F., Mattos, P., Arancio, O., Ferreira, S.T., De Felice, F.G., 2019. Exercise-linked FNDC5/irisin rescues synaptic plasticity and memory defects in Alzheimer's models. Nat. Med. 25, 165–175. https://doi.org/10.1038/s41591-018-0275-4

Lovatel, G., Elsner, V., Bertoldi, K., Vanzella, C., Moysés Fdos, S., Vizuete, A., Spindler, C., Cechinel, L., Netto, C., Muotri, A., Siqueira, I., 2013. Treadmill exercise induces age-related changes in aversive memory, neuroinflammatory and epigenetic processes in the rat hippocampus. Neurobiol. Learn. Mem. 101, 94–102. https://doi.org/10.1016/j.nlm.2013.01.007

Lu, G.L., Lee, C.H., Chiou, L.C., 2016. Orexin A induces bidirectional modulation of synaptic plasticity: Inhibiting long-term potentiation and preventing depotentiation. Neuropharmacology 107, 168–180. https://doi.org/10.1016/j.neuropharm.2016.03.005

Lu, W.Y., Man, H.Y., Ju, W., Trimble, W.S., MacDonald, J.F., Wang, Y.T., 2001. Activation of synaptic NMDA receptors induces membrane insertion of new AMPA receptors and LTP in cultured hippocampal neurons. Neuron 29, 243–254. https://doi.org/10.1016/S0896-6273(01)00194-5

Lupu, F., Terwilliger, J.D., Lee, K., Segre, G. V., Efstratiadis, A., 2001. Roles of growth hormone and insulin-like growth factor 1 in mouse postnatal growth. Dev. Biol. 229, 141–162. https://doi.org/10.1006/dbio.2000.9975

Lynch, A.M., Walsh, C., Delaney, A., Nolan, Y., Campbell, V.A., Lynch, M.A., 2004. Lipopolysaccharide-induced increase in signalling in hippocampus is abrogated by IL-10 - A role for IL-1β? J. Neurochem. 88, 635–646. https://doi.org/10.1046/J.1471-4159.2003.02157.X

MacLaren, D.P., Raine, N.M., O'Connor, A.M., Buchanan, K.D., 1995. Human gastrin and vasoactive intestinal polypeptide responses to endurance running in relation to training status and fluid ingested. Clin. Sci. 89, 137–143. https://doi.org/10.1042/cs0890137

Maddock, R.J., Casazza, G.A., Buonocore, M.H., Tanase, C., 2011. Vigorous exercise increases brain lactate and Glx (glutamate+glutamine): A dynamic 1H-MRS study. Neuroimage 57, 1324–1330. https://doi.org/10.1016/j.neuroimage.2011.05.048

Maddock, R.J., Casazza, G.A., Fernandez, D.H., Maddock, M.I., 2016. Acute Modulation of Cortical Glutamate and GABA Content by Physical Activity. J. Neurosci. 36, 2449–2457. https://doi.org/10.1523/JNEUROSCI.3455-15.2016

Máderová, D., Krumpolec, P., Slobodová, L., Schön, M., Tirpáková, V., Kovaničová, Z., Klepochová, R., Vajda, M., Šutovský, S., Cvečka, J., Valkovič, L., Turčáni, P., Kršňák, M., Sedliak, M., Tsai, C.L., Ukropcová, B., Ukropec, J., 2019. Acute and regular exercise distinctly modulate serum, plasma and skeletal muscle BDNF in the elderly. Neuropeptides 78. https://doi.org/10.1016/j.npep.2019.101961

Main, L.C., Dawson, B., Grove, J.R., Landers, G.J., Goodman, C., 2009. Impact of Training on Changes in Perceived Stress and Cytokine Production. Res. Sport. Med. 17, 112–123. https://doi.org/10.1080/15438620802689757

Main, L.C., Dawson, B., Heel, K., Grove, J.R., Landers, G.J., Goodman, C., 2010. Relationship Between Inflammatory Cytokines and Self-Report Measures of Training Overload. Res. Sport. Med. 18, 127–139. https://doi.org/10.1080/15438621003627133

Mak, R.H., Rotwein, P., 2006. Myostatin and insulin-like growth factors in uremic sarcopenia: The yin and yang in muscle mass regulation. Kidney Int. https://doi.org/10.1038/sj.ki.5001622

Man, H.Y., Wang, Q., Lu, W.Y., Ju, W., Ahmadian, G., Liu, L., D'Souza, S., Wong, T.P., Taghibiglou, C., Lu, J., Becker, L.E., Pei, L., Liu, F., Wymann, M.P., MacDonald, J.F., Wang, Y.T., 2003. Activation of PI3-kinase is required for AMPA receptor insertion during LTP of mEPSCs in cultured hippocampal neurons. Neuron 38, 611–624. https://doi.org/10.1016/S0896-6273(03)00228-9

Mansouri, M., Keshtkar, A., Hasani-Ranjbar, S., Soleymani, E.F., Tabatabaei-Malazy, O., Omidfar, K., Larijani, B., 2011. The impact of one session resistance exercise on plasma adiponectin and RBP4 concentration in trained and untrained healthy young men. Endocr. J. 58, 861–868. https://doi.org/10.1507/endocrj.EJ11-0046

Marinus, N., Hansen, D., Feys, P., Meesen, R., Timmermans, A., Spildooren, J., 2019. The Impact of Different Types of Exercise Training on Peripheral Blood Brain-Derived Neurotrophic Factor Concentrations in Older Adults: A Meta-Analysis. Sport. Med. https://doi.org/10.1007/s40279-019-01148-z

Markofski, M.M., Carrillo, A.E., Timmerman, K.L., Jennings, K., Coen, P.M., Pence, B.D., Flynn, M.G., 2014. Exercise Training Modifies Ghrelin and Adiponectin Concentrations and Is Related to Inflammation in Older Adults. Journals Gerontol. Ser. A Biol. Sci. Med. Sci. 69, 675–681. https://doi.org/10.1093/gerona/glt132

Marsden, K.C., Shemesh, A., Bayer, K.U., Carroll, R.C., 2010. Selective translocation of Ca2+/calmodulin protein kinase IIα (CaMKIIα) to inhibitory synapses. Proc. Natl. Acad. Sci. U. S. A. 107, 20559–20564. https://doi.org/10.1073/pnas.1010346107

Martin, S.J., Grimwood, P.D., Morris, R.G.M., 2000. Synaptic Plasticity and Memory: An Evaluation of the Hypothesis. Annu. Rev. Neurosci. 23, 649–711.

Martins, C., Kulseng, B., King, N.A., Holst, J.J., Blundell, J.E., 2010. The effects of exercise-induced weight loss on appetite-related peptides and motivation to eat. J. Clin. Endocrinol. Metab. 95, 1609–1616. https://doi.org/10.1210/jc.2009-2082

Martins, P.J.F., D'Almeida, V., Pedrazzoli, M., Lin, L., Mignot, E., Tufik, S., 2004. Increased hypocretin-1 (orexin-a) levels in cerebrospinal fluid of rats after short-term forced activity. Regul. Pept. 117, 155–158. https://doi.org/10.1016/j.regpep.2003.10.003

Masoumi, J., Abbasloui, M., Parvan, R., Mohammadnejad, D., Pavon-Djavid, G., Barzegari, A., Abdolalizadeh, J., 2018. Apelin, a promising target for Alzheimer disease prevention and treatment. Neuropeptides. https://doi.org/10.1016/j.npep.2018.05.008

Matthews, V.B., Åström, M.B., Chan, M.H.S., Bruce, C.R., Krabbe, K.S., Prelovsek, O., Åkerström, T., Yfanti, C., Broholm, C., Mortensen, O.H., Penkowa, M., Hojman, P., Zankari, A., Watt, M.J., Bruunsgaard, H., Pedersen, B.K., Febbraio, M.A., 2009. Brain-derived neurotrophic factor is produced by skeletal muscle cells in response to contraction and enhances fat oxidation via activation of AMP-activated protein kinase. Diabetologia 52, 1409–1418. https://doi.org/10.1007/s00125-009-1364-1

Mees, L.M., Coulter, M.M., Chrenek, M.A., Motz, C.T., Landis, E.G., Boatright, J.H., Pardue, M.T., 2019. Low-intensity exercise in mice is sufficient to protect retinal function during light-induced retinal degeneration. Investig. Ophthalmol. Vis. Sci. 60, 1328–1335. https://doi.org/10.1167/iovs.18-25883

Mele, M., Leal, G., Duarte, C.B., 2016. Role of GABA-A-R trafficking in the plasticity of inhibitory synapses. J. Neurochem. 139, 997–1018. https://doi.org/10.1111/jnc.13742

Mera, P., Laue, K., Ferron, M., Confavreux, C., Wei, J., Galán-Díez, M., Lacampagne, A., Mitchell, S.J., Mattison, J.A., Chen, Y., Bacchetta, J., Szulc, P., Kitsis, R.N., De Cabo, R., Friedman, R.A., Torsitano, C., McGraw, T.E., Puchowicz, M., Kurland, I., Karsenty, G., 2016. Osteocalcin Signaling in Myofibers Is Necessary and Sufficient for Optimum Adaptation to Exercise. Cell Metab. 23, 1078–1092. https://doi.org/10.1016/j.cmet.2016.05.004

Messina, G., Di Bernardo, G., Monda, M., Monda, V., Messina, A., Chieffi, S., Galderisi, U., Viggiano, A., De Luca, V., 2016. Exercise increases the level of plasma orexin A in humans. J Basic Clin Physiol Pharmacol 27, 611. https://doi.org/10.1515/jbcpp-2015-0133

Miao, H.-H., Miao, Z., Pan, J.-G., Li, X.-H., Zhuo, M., 2021. Brain-derived neurotrophic factor produced long-term synaptic enhancement in the anterior cingulate cortex of adult mice. Mol. Brain 14, 140. https://doi.org/10.1186/S13041-021-00853-Z

Micielska, K., Kortas, J.A., Gmiat, A., Jaworska, J., Kozlowska, M., Lysak-Radomska, A., Rodziewicz-Flis, E., Zychowska, M., Ziemann, E., 2021. Habitually inactive physically – a proposed procedure of counteracting cognitive decline in women with diminished insulin sensitivity through a high-intensity circuit training program. Physiol. Behav. 229, 113235. https://doi.org/10.1016/j.physbeh.2020.113235

Middei, S., Houeland, G., Cavallucci, V., Ammassari-Teule, M., D'Amelio, M., Marie, H., 2013. CREB is necessary for synaptic maintenance and learning-induced changes of the ampa receptor GluA1 subunit. Hippocampus 23, 488–499. https://doi.org/10.1002/hipo.22108

Mitchell, G.A., Kassovska-Bratinova, S., Boukaftane, Y., Robert, M.F., Wang, S.P., Ashmarina, L., Lambert, M., Lapierre, P., Potier, E., 1995. Medical aspects of ketone body metabolism. Clin. Investig. Med.

Miyamoto-Mikami, E., Sato, K., Kurihara, T., Hasegawa, N., Fujie, S., Fujita, S., Sanada, K., Hamaoka, T., Tabata, I., Iemitsu, M., 2015. Endurance Training-Induced Increase in Circulating Irisin Levels Is Associated with Reduction of Abdominal Visceral Fat in Middle-Aged and Older Adults. PLoS One 10, e0120354. https://doi.org/10.1371/JOURNAL.PONE.0120354

Mohammadi, S., Oryan, S., Komaki, A., Eidi, A., Zarei, M., 2019. Effects of intra-dentate gyrus microinjection of myokine irisin on long-term potentiation in male rats. Arq. Neuropsiquiatr. 77, 881–887. https://doi.org/10.1590/0004-282X20190184

Molina, D.P., Ariwodola, O.J., Linville, C., Sonntag, W.E., Weiner, J.L., Brunso-Bechtold, J.K., Adams, M.M., 2012. Growth hormone modulates hippocampal excitatory synaptic transmission and plasticity in old rats. Neurobiol. Aging 33, 1938–1949. https://doi.org/10.1016/j.neurobiolaging.2011.09.014

Monda, V., Sessa, F., Ruberto, M., Carotenuto, M., Marsala, G., Monda, M., Cambria, M.T., Astuto, M., Distefano, A., Messina, G., 2020. Aerobic exercise and metabolic syndrome: The role of sympathetic activity and the redox system. Diabetes, Metab. Syndr. Obes. Targets Ther. 13, 2433–2442. https://doi.org/10.2147/DMSO.S257687

Montrezol, F.T., Marinho, R., Mota, G.D.F.A.D., D'Almeida, V., De Oliveira, E.M., Gomes, R.J., Medeiros, A., 2019. ACE Gene Plays a Key Role in Reducing Blood Pressure in the Hyperintensive Elderly after Resistance Training. J. Strength Cond. Res. 33, 1119–1129. https://doi.org/10.1519/JSC.0000000000002355

Moon, H.Y., Becke, A., Berron, D., Becker, B., Sah, N., Benoni, G., Janke, E., Lubejko, S.T., Greig, N.H., Mattison, J.A., Duzel, E., van Praag, H., 2016. Running-Induced Systemic Cathepsin B Secretion Is Associated with Memory Function. Cell Metab. 24, 332–340. https://doi.org/10.1016/j.cmet.2016.05.025

Mooney, R.A., Coxon, J.P., Cirillo, · John, Glenny, H., Gant, N., Byblow, W.D., Cirillo, J., Glenny, H., Gant, N., Byblow, W.D., R.A., M., J.P., C., J., C., H., G., N., G., W.D., B., Mooney, R.A., Coxon, J.P., Cirillo, J., Glenny, H., Gant, N., Byblow, W.D., 2016. Acute aerobic exercise modulates primary motor cortex inhibition. Exp. Brain Res. 234, 3669–3676. https://doi.org/10.1007/s00221-016-4767-5

Moore, C., Browning, M., Rose, G., 1993. Hippocampal plasticity induced by primed burst, but not long-term potentiation, stimulation is impaired in area CA1 of aged Fischer 344 rats. Hippocampus 3, 57–66. https://doi.org/10.1002/HIPO.450030106

Moraes, C., Marinho, S., Lobo, J.C., Stockler-Pinto, M.B., Barros, A.F., Jacobson, L.V., Da Nobrega, A.C.L., Rosa, M.L.G., Denise, M., 2015. Effects of resistance exercise training on acyl-ghrelin and obestatin levels in hemodialysis patients. Ren. Fail. 37, 851–857. https://doi.org/10.3109/0886022X.2015.1033634

Morland, C., Andersson, K.A., Haugen, Ø.P., Hadzic, A., Kleppa, L., Gille, A., Rinholm, J.E., Palibrk, V., Diget, E.H., Kennedy, L.H., Stølen, T., Hennestad, E., Moldestad, O., Cai, Y., Puchades, M., Offermanns, S., Vervaeke, K., Bjørås, M., Wisløff, U., Storm-Mathisen, J., Bergersen, L.H., 2017. Exercise induces cerebral VEGF and angiogenesis via the lactate receptor HCAR1. Nat. Commun. 8, 1–9. https://doi.org/10.1038/ncomms15557

Moses, A.C., Young, S.C.J., Morrow, L.A., O'Brien, M., Clemmons, D.R., 1996. Recombinant human insulin-like growth factor I increases insulin sensitivity and improves glycemic control in type II diabetes. Diabetes 45, 91–100. https://doi.org/10.2337/diab.45.1.91

Müller, P., Duderstadt, Y., Lessmann, V., Müller, N.G., 2020. Lactate and BDNF: Key Mediators of Exercise Induced Neuroplasticity? J. Clin. Med. 9, 1136. https://doi.org/10.3390/jcm9041136

Murray, A.J., Knight, N.S., Cole, M.A., Cochlin, L.E., Carter, E., Tchabanenko, K., Pichulik, T., Gulston, M.K., Atherton, H.J., Schroeder, M.A., Deacon, R.M.J., Kashiwaya, Y., King, M.T., Pawlosky, R., Rawlins, J.N.P., Tyler, D.J., Griffin, J.L., Robertson, J., Veech, R.L., Clarke, K., 2016. Novel ketone diet enhances physical and cognitive performance. FASEB J. 30, 4021–4032. https://doi.org/10.1096/fj.201600773R

Murray, C.A., Lynch, M.A., 1998. Evidence That Increased Hippocampal Expression of the Cytokine Interleukin-1 Is a Common Trigger for Age-and Stress-Induced Impairments in Long-Term Potentiation. J. Neurosci. 18, 2974–2981.

Murray, P.S., Holmes, P. V., 2011. An overview of brain-derived neurotrophic factor and implications for excitotoxic vulnerability in the hippocampus. Int. J. Pept. https://doi.org/10.1155/2011/654085

Nakabayashi, M., Suzuki, T., Takahashi, K., Totsune, K., Muramatsu, Y., Kaneko, C., Date, F., Takeyama, J., Darnel, A.D., Moriya, T., Sasano, H., 2003. Orexin-A expression in human peripheral tissues. Mol. Cell. Endocrinol. 205, 43–50. https://doi.org/10.1016/S0303-7207(03)00206-5

Nakamura, M., Imaoka, M., Takeda, M., 2020. Interaction of bone and brain: osteocalcin and cognition. Int. J. Neurosci. 1–9. https://doi.org/10.1080/00207454.2020.1770247

Nakanishi, H., 2020. Microglial cathepsin B as a key driver of inflammatory brain diseases and brain aging. Neural Regen. Res. 15, 25. https://doi.org/10.4103/1673-5374.264444

Nam, J.S., Ahn, C.W., Park, H.J., Kim, Y.S., 2020. Semaphorin 3 C is a Novel Adipokine Representing Exercise-Induced Improvements of Metabolism in Metabolically Healthy Obese Young Males. Sci. Rep. 10. https://doi.org/10.1038/s41598-020-67004-7

Netz, Y., 2019. Is There a Preferred Mode of Exercise for Cognition Enhancement in Older Age?-A Narrative Review. Front. Med. 6, 57. https://doi.org/10.3389/fmed.2019.00057

Newman, L.A., Korol, D.L., Gold, P.E., 2011. Lactate produced by glycogenolysis in astrocytes regulates memory processing. PLoS One 6, e28427. https://doi.org/10.1371/journal.pone.0028427

Nicolini, C., Michalski, B., Toepp, S.L., Turco, C. V., D'Hoine, T., Harasym, D., Gibala, M.J., Fahnestock, M., Nelson, A.J., 2020. A Single Bout of High-intensity Interval Exercise Increases Corticospinal Excitability, Brain-derived Neurotrophic Factor, and Uncarboxylated Osteolcalcin in Sedentary, Healthy Males. Neuroscience 437, 242–255. https://doi.org/10.1016/j.neuroscience.2020.03.042

Nicolini, C., Toepp, S., Harasym, D., Michalski, B., Fahnestock, M., Gibala, M.J., Nelson, A.J., 2019. No changes in corticospinal excitability, biochemical markers, and working memory after six weeks of high-intensity interval training in sedentary males. Physiol. Rep. 7. https://doi.org/10.14814/phy2.14140

Nogueira, J.E., de Deus, J.L., Amorim, M.R., Batalhão, M.E., Leão, R.M., Carnio, E.C., Branco, L.G.S., 2020. Inhaled molecular hydrogen attenuates intense acute exercise-induced hippocampal inflammation in sedentary rats. Neurosci. Lett. 715. https://doi.org/10.1016/j.neulet.2019.134577

Norheim, F., Langleite, T.M., Hjorth, M., Holen, T., Kielland, A., Stadheim, H.K., Gulseth, H.L., Birkeland, K.I., Jensen, J., Drevon, C.A., 2014. The effects of acute and chronic exercise on PGC-1α, irisin and browning of subcutaneous adipose tissue in humans. FEBS J. 281, 739–749. https://doi.org/10.1111/febs.12619

Norheim, F., Raastad, T., Thiede, B., Rustan, A.C., Drevon, C.A., Haugen, F., 2011. Proteomic identification of secreted proteins from human skeletal muscle cells and expression in response to strength training. Am. J. Physiol. - Endocrinol. Metab. 301, E1013–E1021. https://doi.org/10.1152/ajpendo.00326.2011

Normann, C., Schmitz, D., Fürmaier, A., Döing, C., Bach, M., 2007. Long-Term Plasticity of Visually Evoked Potentials in Humans is Altered in Major Depression. Biol. Psychiatry 62, 373–380. https://doi.org/10.1016/j.biopsych.2006.10.006

Novkovic, T., Mittmann, T., Manahan-Vaughan, D., 2015. BDNF contributes to the facilitation of hippocampal synaptic plasticity and learning enabled by environmental enrichment. Hippocampus 25, 1–15. https://doi.org/10.1002/HIPO.22342

Numakawa, T., Suzuki, S., Kumamaru, E., Adachi, N., Richards, M., Kunugi, H., 2010. BDNF function and intracellular signaling in neurons. Histol. Histopathol. 25, 237–258. https://doi.org/10.14670/HH-25.237

Nybo, L., Møller, K., Pedersen, B.K., Nielsen, B., Secher, N.H., 2003. Association between fatigue and failure to preserve cerebral energy turnover during prolonged exercise. Acta Physiol. Scand. 179, 67–74. https://doi.org/10.1046/j.1365-201X.2003.01175.x

O'Donnell, B.F., Vohs, J.L., Hetrick, W.P., Carroll, C.A., Shekhar, A., 2004. Auditory event-related potential abnormalities in bipolar disorder and schizophrenia. Int. J. Psychophysiol. 53, 45–55. https://doi.org/10.1016/j.ijpsycho.2004.02.001

O'Leary, T., Collett, J., Morris, M., 2018. High-intensity exhaustive exercise reduces long-interval intracortical inhibition. Exp. brain Res. 236, 3149–3158. https://doi.org/10.1007/S00221-018-5364-6

Øktedalen, O., Opstad, P.K., Fahrenkrug, J., Fonnum, F., 1983a. Plasma concentration of vasoactive intestinal polypeptide during prolonged physical exercise, calorie supply deficiency, and sleep deprivation. Scand. J. Gastroenterol. 18, 1057–1062. https://doi.org/10.3109/00365528309181840

Øktedalen, O., Opstad, P.K., Schaffalitzky de Muckadell, O.B., Fausa, O., Flaten, O., 1983b. Basal hyperchlorhydria and its relation to the plasma concentrations of secretin, vasoactive intestinal polypeptide (VIP) and gastrin during prolonged strain. Regul. Pept. 5, 235–244. https://doi.org/10.1016/0167-0115(83)90254-9

Olesen, J., Kiilerich, K., Pilegaard, H., 2010. PGC-1α-mediated adaptations in skeletal muscle. Pflugers Arch. Eur. J. Physiol. https://doi.org/10.1007/s00424-010-0834-0

Oliff, H.S., Berchtold, N.C., Isackson, P., Cotman, C.W., 1998. Exercise-induced regulation of brain-derived neurotrophic factor (BDNF) transcripts in the rat hippocampus. Mol. Brain Res. 61, 147–153. https://doi.org/10.1016/S0169-328X(98)00222-8

Otero-Díaz, B., Rodríguez-Flores, M., Sánchez-Muñoz, V., Monraz-Preciado, F., Ordoñez-Ortega, S., Becerril-Elias, V., Baay-Guzmán, G., Obando-Monge, R., García-García, E., Palacios-González, B., Villarreal-Molina, M.T., Sierra-Salazar, M., Antuna-Puente, B., 2018. Exercise Induces White Adipose Tissue Browning Across the Weight Spectrum in Humans. Front. Physiol. 9. https://doi.org/10.3389/fphys.2018.01781

Ouchi, N., Walsh, K., 2007. Adiponectin as an anti-inflammatory factor. Clin. Chim. Acta. https://doi.org/10.1016/j.cca.2007.01.026

Oury, F., Khrimian, L., Denny, C.A., Gardin, A., Chamouni, A., Goeden, N., Huang, Y.Y., Lee, H., Srinivas, P., Gao, X.B., Suyama, S., Langer, T., Mann, J.J., Horvath, T.L., Bonnin, A., Karsenty, G., 2013. Maternal and offspring pools of osteocalcin influence brain development and functions. Cell 155, 228–241. https://doi.org/10.1016/j.cell.2013.08.042

Packer, N., Hoffman-Goetz, L., 2015. Acute exercise increases hippocampal TNF-α, Caspase-3 and Caspase-7 expression in healthy young and older mice. J. Sports Med. Phys. Fitness 55, 368–376.

Packer, N., Pervaiz, N., Hoffman-Goetz, L., 2010. Does exercise protect from cognitive decline by altering brain cytokine and apoptotic protein levels? A systematic review of the literature. Exerc Immunol Rev 16, 138–162.

Pal, A., Zimmer, P., Clauss, D., Schmidt, M.E., Ulrich, C.M., Wiskemann, J., Steindorf, K., 2021. Resistance Exercise Modulates Kynurenine Pathway in Pancreatic Cancer Patients. Int. J. Sports Med. 42, 33–42. https://doi.org/10.1055/a-1186-1009

Pardridge, W.M., Kang, Y.-S., Buciak, J.L., 1994. Transport of Human Recombinant Brain-Derived Neurotrophic Factor (BDNF) Through the Rat Blood-Brain Barrier in Vivo Using Vector-Mediated Peptide Drug Delivery. Pharm. Res. 1994 115 11, 738–746. https://doi.org/10.1023/A:1018940732550

Park, K.M., Park, S.C., Kang, S., 2019. Effects of resistance exercise on adipokine factors and body composition in pre-and postmenopausal women. J. Exerc. Rehabil. 15, 676–682. https://doi.org/10.12965/jer.1938368.184

Pedersen, B.K., 2019. Physical activity and muscle–brain crosstalk. Nat. Rev. Endocrinol. https://doi.org/10.1038/s41574-019-0174-x

Pedersen, B.K., 2017. Anti-inflammatory effects of exercise: role in diabetes and cardiovascular disease. Eur. J. Clin. Invest. https://doi.org/10.1111/eci.12781

Pekkala, S., Wiklund, P.K., Hulmi, J.J., Ahtiainen, J.P., Horttanainen, M., Pöllänen, E., Mäkelä, K.A., Kainulainen, H., Häkkinen, K., Nyman, K., Alén, M., Herzig, K.-H., Cheng, S., 2013. Are skeletal muscle FNDC5 gene expression and irisin release regulated by exercise and related to health? J. Physiol. 591, 5393–5400. https://doi.org/10.1113/JPHYSIOL.2013.263707

Pena, G.S., Paez, H.G., Johnson, T.K., Halle, J.L., Carzoli, J.P., Visavadiya, N.P., Zourdos, M.C., Whitehurst, M.A., Khamoui, A. V., 2020. Hippocampal Growth Factor and Myokine Cathepsin B Expression following Aerobic and Resistance Training in 3xTg-AD Mice. Int. J. Chronic Dis. 2020, 1–11. https://doi.org/10.1155/2020/5919501

Perry, V.H., Cunningham, C., Holmes, C., 2007. Systemic infections and inflammation affect chronic neurodegeneration. Nat. Rev. Immunol. https://doi.org/10.1038/nri2015

Polito, R., Monda, V., Nigro, E., Messina, A., Di Maio, G., Giuliano, M.T., Orrù, S., Imperlini, E., Calcagno, G., Mosca, L., Mollica, M.P., Trinchese, G., Scarinci, A., Sessa, F., Salerno, M., Marsala, G., Buono, P., Mancini, A., Monda, M., Daniele, A., Messina, G., 2020. The Important Role of Adiponectin and Orexin-A, Two Key Proteins Improving Healthy Status: Focus on Physical Activity. Front. Physiol. https://doi.org/10.3389/fphys.2020.00356

Potter, M.C., Elmer, G.I., Bergeron, R., Albuquerque, E.X., Guidetti, P., Wu, H.Q., Schwarcz, R., 2010. Reduction of endogenous kynurenic acid formation enhances extracellular glutamate, hippocampal plasticity, and cognitive behavior. Neuropsychopharmacology 35, 1734–1742. https://doi.org/10.1038/npp.2010.39

Pousti, F., Ahmadi, R., Mirahmadi, F., Hosseinmardi, N., Rohampour, K., 2018. Adiponectin modulates synaptic plasticity in hippocampal dentate gyrus. Neurosci. Lett. 662, 227–232. https://doi.org/10.1016/j.neulet.2017.10.042

Prescott, C., Weeks, A.M., Staley, K.J., Partin, K.M., 2006. Kynurenic acid has a dual action on AMPA receptor responses. Neurosci. Lett. 402, 108–112. https://doi.org/10.1016/j.neulet.2006.03.051

Puig, J., Blasco, G., Daunis-i-Estadella, J., Moreno, M., Molina, X., Alberich-Bayarri, A., Xifra, G., Pedraza, S., Ricart, W., Fernández-Aranda, F., Fernández-Real, J.M., 2016. Lower serum osteocalcin concentrations are associated with brain microstructural changes and worse cognitive performance. Clin. Endocrinol. (Oxf). 84, 756–763. https://doi.org/10.1111/CEN.12954

Radka, S.F., Holst, P.A., Fritsche, M., Altar, C.A., 1996. Presence of brain-derived neurotrophic factor in brain and human and rat but not mouse serum detected by a sensitive and specific immunoassay. Brain Res. 709, 122–130. https://doi.org/10.1016/0006-8993(95)01321-0

Rahimi, G.R.M., Niyazi, A., Alaee, S., 2021. The effect of exercise training on osteocalcin, adipocytokines, and insulin resistance: a systematic review and meta-analysis of randomized controlled trials. Osteoporos. Int. https://doi.org/10.1007/s00198-020-05592-w

Ravussin, E., Tschöp, M., Morales, S., Bouchard, C., Heiman, M.L., 2001. Plasma Ghrelin Concentration and Energy Balance: Overfeeding and Negative Energy Balance Studies in Twins. J. Clin. Endocrinol. Metab. 86, 4547–4547. https://doi.org/10.1210/jcem.86.9.8003

Ribeiro, L.F., Catarino, T., Santos, S.D., Benoist, M., Van Leeuwen, J.F., Esteban, J.A., Carvalho, A.L., 2014. Ghrelin triggers the synaptic incorporation of AMPA receptors in the hippocampus. Proc. Natl. Acad. Sci. U. S. A. 111, E149–E158. https://doi.org/10.1073/pnas.1313798111

Rison, R.A., Stanton, P.K., 1995. Long-term potentiation and N-methyl-d-aspartate receptors: Foundations of memory and neurologic disease? Neurosci. Biobehav. Rev. 19, 533–552. https://doi.org/10.1016/0149-7634(95)00017-8

Roche, K.W., O'Brien, R.J., Mammen, A.L., Bernhardt, J., Huganir, R.L., 1996. Characterization of multiple phosphorylation sites on the AMPA receptor GluR1 subunit. Neuron 16, 1179–1188. https://doi.org/10.1016/S0896-6273(00)80144-0

Rodrigues, A.C., Ferreira, E.F., Carneiro-Júnior, M.A., Natali, A.J., Bressan, J., 2016. Effects of exercise on the circulating concentrations of irisin in healthy adult individuals: A review. Sci. Sports 31, 251–260. https://doi.org/10.1016/J.SCISPO.2016.07.005

Rogers, R.S., Dawson, A.W., Wang, Z., Thyfault, J.P., Hinton, P.S., 2011. Acute response of plasma markers of bone turnover to a single bout of resistance training or plyometrics. J. Appl. Physiol. 111, 1353–1360. https://doi.org/10.1152/japplphysiol.00333.2011

Roh, H.-T., Cho, S.-Y., So, W.-Y., 2020. A Cross-Sectional Study Evaluating the Effects of Resistance Exercise on Inflammation and Neurotrophic Factors in Elderly Women with Obesity. J. Clin. Med. 9, 842. https://doi.org/10.3390/JCM9030842

Rojas Vega, S., Knicker, A., Hollmann, W., Bloch, W., Strüder, H.K., 2010. Effect of resistance exercise on serum levels of growth factors in humans. Horm. Metab. Res. 42, 982–986. https://doi.org/10.1055/S-0030-1267950

Rolandi, E., Reggiani, E., Franceschini, R., Arras, G.B., Cataldi, A., De Lucia, F., Barreca, T., 1988. Prolactin release induced by physical exercise is independent from peripheral vasoactive intestinal polypeptide secretion. Ann. Clin. Res. 20, 428–430.

Ross, F.M., Allan, S.M., Rothwell, N.J., Verkhratsky, A., 2003. A dual role for interleukin-1 in LTP in mouse hippocampal slices. J. Neuroimmunol. 144, 61–67. https://doi.org/10.1016/J.JNEUROIM.2003.08.030

Ross, R.D., Shah, R.C., Leurgans, S., Bottiglieri, T., Wilson, R.S., Sumner, D.R., 2018. Circulating Dkk1 and TRAIL Are Associated With Cognitive Decline in Community-Dwelling, Older Adults With Cognitive Concerns. J. Gerontol. A. Biol. Sci. Med. Sci. 73, 1694. https://doi.org/10.1093/gerona/glx252

Rózsa, É., Robotka, H., Vécsei, L., Toldi, J., 2008. The Janus-face kynurenic acid. J. Neural Transm. 115, 1087–1091. https://doi.org/10.1007/s00702-008-0052-5

Ruan, Q., Huang, Y., Yang, L., Li, J., Gu, W., Bao, Z., Zhang, X., Yu, Z., 2020. Associations of Preoperative Irisin Levels of Paired Cerebrospinal Fluid and Plasma with Physical Dysfunction and Muscle Wasting Severity in Residents of Surgery Wards. J. Nutr. Heal. Aging 24, 412–422. https://doi.org/10.1007/s12603-020-1343-2

Rubin, M.R., Kraemer, W.J., Maresh, C.M., Volek, J.S., Ratamess, N.A., Vanheest, J.L., Silvestre, R., French, D.N., Sharman, M.J., Judelson, D.A., Gómez, A.L., Vescovi, J.D., Hymer, W.C., 2005. High-affinity growth hormone binding protein and acute heavy resistance exercise. Med. Sci. Sports Exerc. 37, 395–403. https://doi.org/10.1249/01.MSS.0000155402.93987.C0

Sabouri, M., Norouzi, J., Zarei, Y., Sangani, M.H., Hooshmand Moghadam, B., 2020. Comparing High-Intensity Interval Training (HIIT) and Continuous Training on Apelin, APJ, NO, and Cardiotrophin-1 in Cardiac Tissue of Diabetic Rats. J. Diabetes Res. 2020. https://doi.org/10.1155/2020/1472514

Said, S.I., Mutt, V., 1970. Polypeptide with broad biological activity: Isolation from small intestine. Science (80-.). 169, 1217–1218. https://doi.org/10.1126/science.169.3951.1217

Salgueiro, R.B., Peliciari-Garcia, R.A., do Carmo Buonfiglio, D., Peroni, C.N., Nunes, M.T., 2014. Lactate activates the somatotropic axis in rats. Growth Horm. IGF Res. 24, 268–270. https://doi.org/10.1016/j.ghir.2014.09.003

Santos, V. V., Stark, R., Rial, D., Silva, H.B., Bayliss, J.A., Lemus, M.B., Davies, J.S., Cunha, R.A., Prediger, R.D., Andrews, Z.B., 2017. Acyl ghrelin improves cognition, synaptic plasticity deficits and neuroinflammation following amyloid β (Aβ1-40) administration in mice. J. Neuroendocrinol. 29. https://doi.org/10.1111/jne.12476

Sartori, A.C., Vance, D.E., Slater, L.Z., Crowe, M., 2012. The impact of inflammation on cognitive function in older adults: Implications for healthcare practice and research. J. Neurosci. Nurs. https://doi.org/10.1097/JNN.0b013e3182527690

Saucedo Marquez, C.M., Vanaudenaerde, B., Troosters, T., Wenderoth, N., 2015. High-intensity interval training evokes larger serum BDNF levels compared with intense continuous exercise. J. Appl. Physiol. 119, 1363–1373. https://doi.org/10.1152/japplphysiol.00126.2015

Scharhag-Rosenberger, F., Meyer, T., Wegmann, M., Ruppenthal, S., Kaestner, L., Morsch, A., Hecksteden, A., 2014. Irisin does not mediate resistance training-induced alterations in resting metabolic rate. Med. Sci. Sports Exerc. 46, 1736–1743. https://doi.org/10.1249/MSS.0000000000000286

Scheffer, D. da L., Latini, A., 2020. Exercise-induced immune system response: Anti-inflammatory status on peripheral and central organs. Biochim. Biophys. Acta - Mol. Basis Dis. https://doi.org/10.1016/j.bbadis.2020.165823

Scheiblich, H., Trombly, M., Ramirez, A., Heneka, M.T., 2020. Neuroimmune Connections in Aging and Neurodegenerative Diseases. Trends Immunol. https://doi.org/10.1016/j.it.2020.02.002

Scheid, J.L., De Souza, M.J., Leidy, H.J., Williams, N.I., 2011. Ghrelin but Not Peptide YY Is Related to Change in Body Weight and Energy Availability. Med. Sci. Sport. Exerc. 43, 2063–2071. https://doi.org/10.1249/MSS.0b013e31821e52ab

Schiffer, T., Schulte, S., Sperlich, B., Achtzehn, S., Fricke, H., Strüder, H.K., 2011. Lactate infusion at rest increases BDNF blood concentration in humans. Neurosci. Lett. 488, 234–237. https://doi.org/10.1016/J.NEULET.2010.11.035

Schmolesky, M.T., Webb, D.L., Hansen, R.A., 2013. The effects of aerobic exercise intensity and duration on levels of brain- derived neurotrophic factor in healthy men. J. Sport. Sci. Med. 12, 502–511.

Schwander, J.C., Hauri, C., Zapf, J., Froesch, E.R., 1983. Synthesis and secretion of insulin-like growth factor and its binding protein by the perfused rat liver: Dependence on growth hormone status. Endocrinology 113, 297–305. https://doi.org/10.1210/endo-113-1-297

Serra-Millàs, M., 2016. Are the changes in the peripheral brain-derived neurotrophic factor levels due to platelet activation? World J. Psychiatry 6, 84. https://doi.org/10.5498/WJP.V6.I1.84

Shan, C., Ghosh, A., Guo, X.Z., Wang, S.M., Hou, Y.F., Li, S.T., Liu, J.M., 2019. Roles for osteocalcin in brain signalling: Implications in cognition- and motor-related disorders. Mol. Brain. https://doi.org/10.1186/s13041-019-0444-5

Sheibani, S., Hanachi, P., Refahiat, M.A., 2012. Effect of Aerobic Exercise on Serum Concentration of Apelin, TNFα and Insulin in Obese Women Serum Concentration of Apelin in Obese Women, Original Article Iranian Journal of Basic Medical Sciences www.mums.ac.ir. Mashhad University of Medical Sciences.

Shen, P., Yue, Q., Fu, W., Tian, S.W., You, Y., 2019. Apelin-13 ameliorates chronic water-immersion restraint stress-induced memory performance deficit through upregulation of BDNF in rats. Neurosci. Lett. 696, 151–155. https://doi.org/10.1016/j.neulet.2018.11.051

Shieh, P.B., Ghosh, A., 1999. Molecular Mechanisms Underlying Activity-Dependent Regulation of BDNF Expression. J Neurobiol 41, 127–134.

Shin, K., Pandey, A., Liu, X.Q., Anini, Y., Rainey, J.K., 2013. Preferential apelin-13 production by the proprotein convertase PCSK3 is implicated in obesity. FEBS Open Bio 3, 328–333. https://doi.org/10.1016/j.fob.2013.08.001

Shors, T.J., Anderson, M.L., Curlik, D.M., Nokia, M.S., 2012. Use it or lose it: How neurogenesis keeps the brain fit for learning. Behav. Brain Res. https://doi.org/10.1016/j.bbr.2011.04.023

Simpson, K.A., Singh, M.A.F., 2008. Effects of Exercise on Adiponectin: A Systematic Review. Obesity 16, 241–256. https://doi.org/10.1038/oby.2007.53

Singh, A.M., Duncan, R.E., Neva, J.L., Staines, W.R., 2014a. Aerobic exercise modulates intracortical inhibition and facilitation in a nonexercised upper limb muscle. BMC Sports Sci. Med. Rehabil. 6, 23. https://doi.org/10.1186/2052-1847-6-23

Singh, A.M., Neva, J.L., Staines, W.R., 2014b. Acute exercise enhances the response to paired associative stimulation-induced plasticity in the primary motor cortex. Exp. Brain Res. 232, 3675–3685. https://doi.org/10.1007/s00221-014-4049-z

Sleiman, S.F., Henry, J., Al-Haddad, R., El Hayek, L., Haidar, E.A., Stringer, T., Ulja, D., Karuppagounder, S.S., Holson, E.B., Ratan, R.R., Ninan, I., Chao, M. V., 2016. Exercise promotes the expression of brain derived neurotrophic factor (BDNF) through the action of the ketone body β- hydroxybutyrate. Elife 5. https://doi.org/10.7554/eLife.15092

Smith, C., Tacey, A., Mesinovic, J., Scott, D., Lin, X., Brennan-Speranza, T.C., Lewis, J.R., Duque, G., Levinger, I., 2021. The effects of acute exercise on bone turnover markers in middle-aged and older adults: A systematic review. Bone. https://doi.org/10.1016/j.bone.2020.115766

Smith, L.L., 2000. Cytokine hypothesis of overtraining: a physiological adaptation to excessive stress? Med. Sci. Sport. Exerc. 32, 317–331.

Smith, L.L., Miles, M.P., 2000. Exercise-Induced Muscle Injury and Inflammation, in: Garret, W.E., Kirkendall, D.T. (Eds.), Exercise and Sport Science. Lippincott Williams & Wilkins, Philadelphia, pp. 401–412.

Snyder, J.S., Kee, N., Wojtowicz, J.M., 2001. Effects of adult neurogenesis on synaptic plasticity in the rat dentate gyrus. J. Neurophysiol. 85, 2423–2431. https://doi.org/10.1152/JN.2001.85.6.2423/ASSET/IMAGES/LARGE/9K0611692006.JPEG

Solvang, S.E.H., Nordrehaug, J.E., Tell, G.S., Nygård, O., McCann, A., Ueland, P.M., Midttun, Ø., Meyer, K., Vedeler, C.A., Aarsland, D., Refsum, H., Smith, A.D., Giil, L.M., 2019. The kynurenine pathway and cognitive performance in community-dwelling older adults. The Hordaland Health Study. Brain. Behav. Immun. 75, 155–162. https://doi.org/10.1016/j.bbi.2018.10.003

Son, J.S., Chae, S.A., Park, B.I., Du, M., Song, W., 2019. Plasma apelin levels in overweight/obese adults following a single bout of exhaustive exercise: A preliminary cross-sectional study. Endocrinol. Diabetes y Nutr. 66, 278–290. https://doi.org/10.1016/j.endinu.2018.12.005

Son, J.S., Chae, S.A., Testroet, E.D., Du, M., Jun, H. pil, 2018. Exercise-induced myokines: a brief review of controversial issues of this decade. Expert Rev. Endocrinol. Metab. https://doi.org/10.1080/17446651.2018.1416290

Son, J.S., Kim, H.-J., Son, Y., Lee, H., Chae, S.A., Seong, J.K., Song, W., 2017. Effects of exercise-induced apelin levels on skeletal muscle and their capillarization in type 2 diabetic rats. Muscle Nerve 56, 1155–1163. https://doi.org/10.1002/mus.25596

Sonntag, W.E., Bennett, S.A., Khan, A.S., Thornton, P.L., Xu, X., Ingram, R.L., Brunso-Bechtold, J.K., 2000. Age and insulin-like growth factor-1 modulate N-methyl-D-aspartate receptor subtype expression in rats. Brain Res. Bull. 51, 331–338. https://doi.org/10.1016/S0361-9230(99)00259-2

Sonntag, W.E., Ramsey, M., Carter, C.S., 2005. Growth hormone and insulin-like growth factor-1 (IGF-1) and their influence on cognitive aging. Ageing Res. Rev. https://doi.org/10.1016/j.arr.2005.02.001

Statton, M.A., Encarnacion, M., Celnik, P., Bastian, A.J., 2015. A single bout of moderate aerobic exercise improves motor skill acquisition. PLoS One 10, e0141393. https://doi.org/10.1371/journal.pone.0141393

Stavrinos, E.L., Coxon, J.P., 2017. High-intensity interval exercise promotes motor cortex disinhibition and early motor skill consolidation. J. Cogn. Neurosci. 29, 593–604. https://doi.org/10.1162/jocn_a_01078

Steele, P.M., Mauk, M.D., 1999. Inhibitory control of LTP and LTD: Stability of synapse strength. J. Neurophysiol. 81, 1559–1566. https://doi.org/10.1152/JN.1999.81.4.1559/ASSET/IMAGES/LARGE/9K0290034006.JPEG

Steensberg, A., Dalsgaard, M.K., Secher, N.H., Pedersen, B.K., 2006. Cerebrospinal fluid IL-6, HSP72, and TNF-α in exercising humans. Brain. Behav. Immun. 20, 585–589. https://doi.org/10.1016/j.bbi.2006.03.002

Stein, A.M., Silva, T.M.V., Coelho, F.G. de M., Arantes, F.J., Costa, J.L.R., Teodoro, E., Santos-Galduróz, R.F., 2018. Physical exercise, IGF-1 and cognition: A systematic review of experimental studies in the elderly. Dement. e Neuropsychol. 12, 114–122. https://doi.org/10.1590/1980-57642018dn12-020003

Stewart, I.B., Warburton, D.E.R., Hodges, A.N.H., Lyster, D.M., McKenzie, D.C., 2003. Cardiovascular and splenic responses to exercise in humans. J. Appl. Physiol. 94, 1619–1626. https://doi.org/10.1152/japplphysiol.00040.2002

Stone, T.W., Darlington, L.G., 2013. The kynurenine pathway as a therapeutic target in cognitive and neurodegenerative disorders. Br. J. Pharmacol. https://doi.org/10.1111/bph.12230

Su, C., Zhao, K., Xia, H., Xu, Y., 2019. Peripheral inflammatory biomarkers in Alzheimer's disease and mild cognitive impairment: a systematic review and meta-analysis. Psychogeriatrics 19, 300–309. https://doi.org/10.1111/psyg.12403

Su, C.H., Chuang, H.C., Hong, C.J., 2020. Physical exercise prevents mice from L-Kynurenine-induced depression-like behavior. Asian J. Psychiatr. 48. https://doi.org/10.1016/j.ajp.2019.101894

Sun, L., Li, X., Wang, F., Zhang, J., Wang, D., Yuan, L., Wu, M., Wang, Z., Qi, J., 2017. High-intensity treadmill running impairs cognitive behavior and hippocampal synaptic plasticity of rats via activation of inflammatory response. J. Neurosci. Res. 95, 1611–1620. https://doi.org/10.1002/JNR.23996

Svensson, M., Lexell, J., Deierborg, T., 2015. Effects of Physical Exercise on Neuroinflammation, Neuroplasticity, Neurodegeneration, and Behavior. Neurorehabil. Neural Repair 29, 577–589. https://doi.org/10.1177/1545968314562108

Szuhany, K.L., Bugatti, M., Otto, M.W., 2015. A meta-analytic review of the effects of exercise on brain-derived neurotrophic factor. J. Psychiatr. Res. 60, 56. https://doi.org/10.1016/J.JPSYCHIRES.2014.10.003

Takimoto, M., Hamada, T., 2014. Acute exercise increases brain region-specific expression of MCT1, MCT2, MCT4, GLUT1, and COX IV proteins. J. Appl. Physiol. 116, 1238–1250. https://doi.org/10.1152/japplphysiol.01288.2013

Tang, L., Kang, Y.T., Yin, B., Sun, L.J., Fan, X.S., 2017. Effects of weight-bearing ladder and aerobic treadmill exercise on learning and memory ability of diabetic rats and its mechanism. Zhongguo Ying Yong Sheng Li Xue Za Zhi 33, 436–440. https://doi.org/10.12047/j.cjap.5570.2017.105

Tapia-Arancibia, L., Aliaga, E., Silhol, M., Arancibia, S., 2008. New insights into brain BDNF function in normal aging and Alzheimer disease. Brain Res. Rev. 59, 201–220. https://doi.org/10.1016/j.brainresrev.2008.07.007

Terry, R.D., DeTeresa, R., Hansen, L.A., 1987. Neocortical cell counts in normal human adult aging. Ann. Neurol. 21, 530–539. https://doi.org/10.1002/ana.410210603

Toshinai, K., Kawagoe, T., Shimbara, T., Tobina, T., Nishida, Y., Mondal, M.S., Yamaguchi, H., Date, Y., Tanaka, H., Nakazato, M., 2007. Acute incremental exercise decreases plasma ghrelin level in healthy men. Horm. Metab. Res. 39, 849–851. https://doi.org/10.1055/s-2007-991177

Tremblay, A., Dutheil, F., Drapeau, V., Metz, L., Lesour, B., Chapier, R., Pereira, B., Verney, J., Baker, J.S., Vinet, A., Walther, G., Obert, P., Courteix, D., Thivel, D., 2019. Long-term effects of high-intensity resistance and endurance exercise on plasma leptin and ghrelin in overweight individuals: the RESOLVE Study. Appl. Physiol. Nutr. Metab. 44, 1172–1179. https://doi.org/10.1139/apnm-2019-0019

Trepci, A., Imbeault, S., Wyckelsma, V.L., Westerblad, H., Hermansson, S., Andersson, D.C., Piehl, F., Venckunas, T., Brazaitis, M., Kamandulis, S., Brundin, L., Erhardt, S., Schwieler, L., 2020. Quantification of Plasma Kynurenine Metabolites Following One Bout of Sprint Interval Exercise: Int. J. Tryptophan Res. 13, 1–12. https://doi.org/10.1177/1178646920978241

Tsai, C.L., Wang, C.H., Pan, C.Y., Chen, F.C., 2015. The effects of long-term resistance exercise on the relationship between neurocognitive performance and GH, IGF-1, and homocysteine levels in the elderly. Front. Behav. Neurosci. 9. https://doi.org/10.3389/fnbeh.2015.00023

Tsekouras, Y.E., Magkos, F., Prentzas, K.I., Basioukas, K.N., Matsama, S.G., Yanni, A.E., Kavouras, S.A., Sidossis, L.S., 2009. A single bout of whole-body resistance exercise augments basal VLDL-triacylglycerol removal from plasma in healthy untrained men. Clin. Sci. 116, 147–156. https://doi.org/10.1042/CS20080078

Tsuchiya, Y., Ando, D., Takamatsu, K., Goto, K., 2015. Resistance exercise induces a greater irisin response than endurance exercise. Metabolism. 64, 1042–1050. https://doi.org/10.1016/j.metabol.2015.05.010

Tsuchiya, Y., Ijichi, T., Goto, K., 2016. Effect of sprint training on resting serum irisin concentration — Sprint training once daily vs. twice every other day. Metabolism 65, 492–495. https://doi.org/10.1016/J.METABOL.2015.12.006

Tsunematsu, T., Yamanaka, A., 2012. The Role of Orexin/Hypocretin in the Central Nervous System and Peripheral Tissues, in: Vitamins and Hormones. Academic Press Inc., pp. 19–33. https://doi.org/10.1016/B978-0-12-394623-2.00002-0

Urenjak, J., Williams, S.R., Gadian, D.G., Noble, M., 1993. Proton nuclear magnetic resonance spectroscopy unambiguously identifies different neural cell types. J. Neurosci. 13, 981–989. https://doi.org/10.1523/jneurosci.13-03-00981.1993

Uysal, N., Yuksel, O., Kizildag, S., Yuce, Z., Gumus, H., Karakilic, A., Guvendi, G., Koc, B., Kandis, S., Ates, M., 2018. Regular aerobic exercise correlates with reduced anxiety and increased levels of irisin in brain and white adipose tissue. Neurosci. Lett. 676, 92–97. https://doi.org/10.1016/j.neulet.2018.04.023

van Dongen, E. V., Kersten, I.H.P., Wagner, I.C., Morris, R.G.M., Fernández, G., 2016. Physical Exercise Performed Four Hours after Learning Improves Memory Retention and Increases Hippocampal Pattern Similarity during Retrieval. Curr. Biol. 26, 1722–1727. https://doi.org/10.1016/J.CUB.2016.04.071

Van Praag, H., Schinder, A.F., Christle, B.R., Toni, N., Palmer, T.D., Gage, F.H., 2002. Functional neurogenesis in the adult hippocampus. Nat. 2002 4156875 415, 1030–1034. https://doi.org/10.1038/4151030a

Vaynman, S., Ying, Z., Gomez-Pinilla, F., 2003. Interplay between brain-derived neurotrophic factor and signal transduction modulators in the regulation of the effects of exercise on synaptic-plasticity. Neuroscience 122, 647–657. https://doi.org/10.1016/j.neuroscience.2003.08.001

Vaz, S.H., Jørgensen, T.N., Cristóvão-Ferreira, S., Duflot, S., Ribeiro, J.A., Gether, U., Sebastião, A.M., 2011. Brain-derived neurotrophic factor (BDNF) enhances GABA transport by modulating the trafficking of GABA transporter-1 (GAT-1) from the plasma membrane of rat cortical astrocytes. J. Biol. Chem. 286, 40464–76. https://doi.org/10.1074/jbc.M111.232009

Vécsei, L., Szalárdy, L., Fülöp, F., Toldi, J., 2013. Kynurenines in the CNS: Recent advances and new questions. Nat. Rev. Drug Discov. https://doi.org/10.1038/nrd3793

Venezia, A.C., Quinlan, E., Roth, S.M., 2017. A single bout of exercise increases hippocampal Bdnf: Influence of chronic exercise and noradrenaline. Genes. Brain. Behav. 16, 811. https://doi.org/10.1111/GBB.12394

Vieira, R.F.L., Muñoz, V.R., Junqueira, R.L., Oliveira, F., Gaspar, R.C., Nakandakari, S.C.B.R., Costa, S. de O., Torsoni, M.A., da Silva, A.S.R., Cintra, D.E., Moura, L.P., Ropelle, E.R., Zaghloul, I., Mekary, R.A., Pauli, J.R., 2021. Time-restricted feeding combined with aerobic exercise training can prevent weight gain and improve metabolic disorders in mice fed a high-fat diet. J. Physiol. JP280820. https://doi.org/10.1113/JP280820

Vilela, T.C., Muller, A.P., Damiani, A.P., Macan, T.P., da Silva, S., Canteiro, P.B., de Sena Casagrande, A., Pedroso, G. dos S., Nesi, R.T., de Andrade, V.M., de Pinho, R.A., 2017. Strength and Aerobic Exercises Improve Spatial Memory in Aging Rats Through Stimulating Distinct Neuroplasticity Mechanisms. Mol. Neurobiol. 54, 7928–7937. https://doi.org/10.1007/s12035-016-0272-x

Vinel, C., Lukjanenko, L., Batut, A., Deleruyelle, S., Pradère, J.P., Le Gonidec, S., Dortignac, A., Geoffre, N., Pereira, O., Karaz, S., Lee, U., Camus, M., Chaoui, K., Mouisel, E., Bigot, A., Mouly, V., Vigneau, M., Pagano, A.F., Chopard, A., Pillard, F., Guyonnet, S., Cesari, M., Burlet-Schiltz, O., Pahor, M., Feige, J.N., Vellas, B., Valet, P., Dray, C., 2018. The exerkine apelin reverses age-associated sarcopenia. Nat. Med. 24, 1360–1371. https://doi.org/10.1038/s41591-018-0131-6

Viticchi, G., Falsetti, L., Buratti, L., Boria, C., Luzzi, S., Bartolini, M., Provinciali, L., Silvestrini, M., 2015. Framingham risk score can predict cognitive decline progression in Alzheimer's disease. Neurobiol. Aging 36, 2940–2945. https://doi.org/10.1016/j.neurobiolaging.2015.07.023

Voss, P., Thomas, M.E., Cisneros-Franco, J.M., de Villers-Sidani, É., 2017. Dynamic Brains and the Changing Rules of Neuroplasticity: Implications for Learning and Recovery. Front. Psychol. 8, 1657. https://doi.org/10.3389/fpsyg.2017.01657

Waller, J.D., McNeill, E.H., Zhong, F., Vervaecke, L.S., Goldfarb, A.H., 2019. Plasma apelin unchanged with acute exercise insulin sensitization. J. Sport. Sci. Med. 18, 537–543.

Wang, H., Ward, N., Boswell, M., Katz, D.M., 2006. Secretion of brain-derived neurotrophic factor from brain microvascular endothelial cells. Eur. J. Neurosci. 23, 1665–1670. https://doi.org/10.1111/J.1460-9568.2006.04682.X

Wang, M., Jo, J., Song, J., 2019. Adiponectin improves long-term potentiation in the 5XFAD mouse brain. Sci. Rep. 9. https://doi.org/10.1038/s41598-019-45509-0

Wanner, P., Cheng, F.H., Steib, S., 2020. Effects of acute cardiovascular exercise on motor memory encoding and consolidation: A systematic review with meta-analysis. Neurosci. Biobehav. Rev. 116, 365–381. https://doi.org/10.1016/j.neubiorev.2020.06.018

Wayne, P.M., Walsh, J.N., Taylor-Piliae, R.E., Wells, R.E., Papp, K. V, Donovan, N.J., Yeh, G.Y., 2014. Effect of tai chi on cognitive performance in older adults: systematic review and meta-analysis. J. Am. Geriatr. Soc. 62, 25–39. https://doi.org/10.1111/jgs.12611

Wayner, M.J., Armstrong, D.L., Phelix, C.F., Oomura, Y., 2004. Orexin-A (Hypocretin-1) and leptin enhance LTP in the dentate gyrus of rats in vivo. Peptides 25, 991–996. https://doi.org/10.1016/j.peptides.2004.03.018

West, D.W.D., Kujbida, G.W., Moore, D.R., Atherton, P., Burd, N.A., Padzik, J.P., De Lisio, M., Tang, J.E., Parise, G., Rennie, M.J., Baker, S.K., Phillips, S.M., 2009. Resistance exercise-induced increases in putative anabolic hormones do not enhance muscle protein synthesis or intracellular signalling in young men. J. Physiol. 587, 5239–5247. https://doi.org/10.1113/JPHYSIOL.2009.177220

Wideman, L., Weltman, J.Y., Hartman, M.L., Veldhuis, J.D., Weltman, A., 2002. Growth hormone release during acute and chronic aerobic and resistance exercise: Recent findings. Sport. Med. https://doi.org/10.2165/00007256-200232150-00003

Winder, D.G., Sweatt, J.D., 2001. Roles of serine/threonine phosphatases in hippocampel synaptic plasticity. Nat. Rev. Neurosci. 2, 461–474. https://doi.org/10.1038/35081514

Woie, L., Kaada, B., Opstad, P.K., 1986. Increase in plasma vasoactive intestinal polypeptide (VIP) in muscular exercise in humans. Gen. Pharmacol. 17, 321–326. https://doi.org/10.1016/0306-3623(86)90047-9

Wong-Goodrich, S.J.E., Pfau, M.L., Flores, C.T., Fraser, J.A., Williams, C.L., Jones, L.W., 2010. Voluntary running prevents progressive memory decline and increases adult hippocampal neurogenesis and growth factor expression after whole-brain irradiation. Cancer Res. 70, 9329–9338. https://doi.org/10.1158/0008-5472.CAN-10-1854

Woodbury, M.E., Ikezu, T., 2014. Fibroblast Growth Factor-2 Signaling in Neurogenesis and Neurodegeneration. J. Neuroimmune Pharmacol. 9, 92–101. https://doi.org/10.1007/s11481-013-9501-5

Woods, J.A., Wilund, K.R., Martin, S.A., Kistler, B.M., 2012. Exercise, inflammation and aging. Aging Dis.

Wrann, C.D., 2016. FNDC5/Irisin – Their Role in the Nervous System and as a Mediator for Beneficial Effects of Exercise on the Brain. Brain Plast. 1, 55–61. https://doi.org/10.3233/bpl-150019

Wrann, C.D., White, J.P., Salogiannnis, J., Laznik-Bogoslavski, D., Wu, J., Ma, D., Lin, J.D., Greenberg, M.E., Spiegelman, B.M., 2013. Exercise induces hippocampal BDNF through a PGC-1α/FNDC5 pathway. Cell Metab. 18, 649–659. https://doi.org/10.1016/j.cmet.2013.09.008

Wrigley, S., Arafa, D., Tropea, D., 2017. Insulin-like growth factor 1: At the crossroads of brain development and aging. Front. Cell. Neurosci. 11, 14. https://doi.org/10.3389/fncel.2017.00014

Wu, M.F., John, J., Maidment, N., Lam, H.A., Siegel, J.M., 2002. Hypocretin release in normal and narcoleptic dogs after food and sleep deprivation, eating, and movement. Am. J. Physiol. - Regul. Integr. Comp. Physiol. 283. https://doi.org/10.1152/ajpregu.00207.2002

Wysocka, M.B., Pietraszek-Gremplewicz, K., Nowak, D., 2018. The role of apelin in cardiovascular diseases, obesity and cancer. Front. Physiol. https://doi.org/10.3389/fphys.2018.00557

Xu, F., Plummer, M.R., Len, G.W., Nakazawa, T., Yamamoto, T., Black, I.B., Wu, K., 2006. Brain-derived neurotrophic factor rapidly increases NMDA receptor channel activity through Fyn-mediated phosphorylation. Brain Res. 1121, 22–34. https://doi.org/10.1016/j.brainres.2006.08.129

Xu, Z.P., Gan, G.S., Liu, Y.M., Xiao, J.S., Liu, H.X., Mei, B., Zhang, J.J., 2018. Adiponectin Attenuates Streptozotocin-Induced Tau Hyperphosphorylation and Cognitive Deficits by Rescuing PI3K/Akt/GSK-3β Pathway. Neurochem. Res. 43, 316–323. https://doi.org/10.1007/s11064-017-2426-2

Yamamoto, K., Hashimoto, K., Isomura, Y., Shimohama, S., Kato, N., 2000. An IP3-assisted form of Ca2+-induced Ca2+ release in neocortical neurons. Neuroreport 11, 535–539. https://doi.org/10.1097/00001756-200002280-00022

Yanagisawa, H., Dan, I., Tsuzuki, D., Kato, M., Okamoto, M., Kyutoku, Y., Soya, H., 2010. Acute moderate exercise elicits increased dorsolateral prefrontal activation and improves cognitive performance with Stroop test. Neuroimage 50, 1702–1710. https://doi.org/10.1016/J.NEUROIMAGE.2009.12.023

Yang, H., Zhao, L., Zhang, J., Tang, C.S., Qi, Y.F., Zhang, J., 2015. Effect of treadmill running on apelin and APJ expression in adipose tissue and skeletal muscle in rats fed a high-fat diet. Int. J. Sports Med. 36, 535–541. https://doi.org/10.1055/s-0034-1398653

Yang, J., Ruchti, E., Petit, J.M., Jourdain, P., Grenningloh, G., Allaman, I., Magistretti, P.J., 2014. Lactate promotes plasticity gene expression by potentiating NMDA signaling in neurons. Proc. Natl. Acad. Sci. U. S. A. 111, 12228–12233. https://doi.org/10.1073/pnas.1322912111

Yang, K., Trepanier, C.H., Li, H., Beazely, M.A., Lerner, E.A., Jackson, M.F., MacDonald, J.F., 2009. Vasoactive intestinal peptide acts via multiple signal pathways to regulate hippocampal NMDA receptors and synaptic transmission. Hippocampus 19, 779–789. https://doi.org/10.1002/hipo.20559

Yang, L., Zou, B., Xiong, X., Pascual, C., Xie, James, Malik, A., Xie, Julian, Sakurai, T., Xie, X.S., 2013. Hypocretin/orexin neurons contribute to hippocampus-dependent social memory and synaptic plasticity in mice. Ann. Intern. Med. 158, 5275–5284. https://doi.org/10.1523/JNEUROSCI.3200-12.2013

Yang, S.N., Tang, Y.G., Zucker, R.S., 1999. Selective induction of LTP and LTD by postsynaptic [Ca2+] i elevation. J. Neurophysiol. 81, 781–787. https://doi.org/10.1152/JN.1999.81.2.781/ASSET/IMAGES/LARGE/9K0290032004.JPEG

Yang, W.-S., Funahashi, T., Matsuzawa, Y., Lee, W.-J., Tanaka, S., Chao, C.-L., Chen, C.-L., Tai, T.-Y., Chuang, M., 2002. Plasma Adiponectin Levels in Overweight and Obese Asians. Obes. Res. 10, 1104–1110. https://doi.org/10.1038/oby.2002.150

Yang, Y., Calakos, N., 2013. Presynaptic long-term plasticity. Front. Synaptic Neurosci. https://doi.org/10.3389/fnsyn.2013.00008

Yau, S.Y., Lia, A., Hooc, R.L.C., Chingb, Y.P., Christied, B.R., Leea, T.M.C., Xuc, A., Soa, K.F., 2014. Physical exercise-induced hippocampal neurogenesis and antidepressant effects are mediated by the adipocyte hormone adiponectin. Proc. Natl. Acad. Sci. U. S. A. 111, 15810–15815. https://doi.org/10.1073/pnas.1415219111

Ye, G., Xiao, Z., Luo, Z., Huang, X., Abdelrahim, M.E.A., Huang, W., 2020. Resistance training effect on serum insulin-like growth factor 1 in the serum: a meta-analysis. Aging Male. https://doi.org/10.1080/13685538.2020.1801622

Yeap, S., Kelly, S.P., Sehatpour, P., Magno, E., Garavan, H., Thakore, J.H., Foxe, J.J., 2008. Visual sensory processing deficits in Schizophrenia and their relationship to disease state. Eur. Arch. Psychiatry Clin. Neurosci. 258, 305–316. https://doi.org/10.1007/s00406-008-0802-2

Yi, C.X., Al-Massadi, O., Donelan, E., Lehti, M., Weber, J., Ress, C., Trivedi, C., Müller, T.D., Woods, S.C., Hofmann, S.M., 2012. Exercise protects against high-fat diet-induced hypothalamic inflammation. Physiol. Behav. 106, 485–490. https://doi.org/10.1016/j.physbeh.2012.03.021

Yudkin, J.S., 2007. Inflammation, obesity, and the metabolic syndrome, in: Hormone and Metabolic Research. © Georg Thieme Verlag KG Stuttgart · New York, pp. 707–709. https://doi.org/10.1055/s-2007-985898

Zhang, D., Tang, Z., Huang, H., Zhou, G., Cui, C., Weng, Y., Liu, W., Kim, S., Lee, S., Perez-Neut, M., Ding, J., Czyz, D., Hu, R., Ye, Z., He, M., Zheng, Y.G., Shuman, H.A., Dai, L., Ren, B., Roeder, R.G., Becker, L., Zhao, Y., 2019. Metabolic regulation of gene expression by histone lactylation. Nature 574, 575–580. https://doi.org/10.1038/s41586-019-1678-1

Zhang, J., Ren, C.X., Qi, Y.F., Lou, L.X., Chen, L., Zhang, L.K., Wang, X., Tang, C., 2006. Exercise training promotes expression of apelin and APJ of cardiovascular tissues in spontaneously hypertensive rats. Life Sci. 79, 1153–1159. https://doi.org/10.1016/j.lfs.2006.03.040

Zheng, W.H., Quirion, R., 2004. Comparative signaling pathways of insulin-like growth factor-1 and brain-derived neurotrophic factor in hippocampal neurons and the role of the PI3 kinase pathway in cell survival. J. Neurochem. 89, 844–852. https://doi.org/10.1111/j.1471-4159.2004.02350.x

Zimmer, P., Schmidt, M.E., Prentzell, M.T., Berdel, B., Wiskemann, J., Kellner, K.H., Debus, J., Ulrich, C., Opitz, C.A., Steindorf, K., 2019. Resistance exercise reduces kynurenine pathway metabolites in breast cancer patients undergoing radiotherapy. Front. Oncol. 9, 962. https://doi.org/10.3389/fonc.2019.00962

CHAPTER 2

Myokines as mediators of exercise-induced cognitive changes in older adults: a protocol for a comprehensive living systematic review and meta-analysis

Authors: Wouter A.J. Vints, Evrim Gökçe, Antoine Langeard, Iuliia Pavlova, Özge Selin Çevik, Mohammad Mosaferi Ziaaldini, Jasemin Todri, Orges Lena, Giorgos K. Sakkas, Suzanne Jak, Ioanna Zorba (Zormpa), Christina Karatzaferi, Oron Levin, Nerijus Masiulis, Yael Netz

Published in Frontiers in Aging Neuroscience, 2023, Vol. 15, p. 1213057
https://doi.org/10.3389/fnagi.2023.1213057

Abstract

Background: The world's population is aging, but life expectancy has risen more than healthy life expectancy (HALE). With respect to brain and cognition, the prevalence of neurodegenerative disorders increases with age, affecting health and quality of life, and imposing significant healthcare costs. Although the effects of physical exercise on cognition in advanced age have been widely explored, in-depth fundamental knowledge of the underlying mechanisms of the exercise-induced cognitive improvements is lacking. Recent research suggests that myokines, factors released into the blood circulation by contracting skeletal muscle, may play a role in mediating the beneficial effect of exercise on cognition. Our goal in this ongoing (living) review is to continuously map the rapidly accumulating knowledge on pathways between acute or chronic exercise-induced myokines and cognitive domains enhanced by exercise.

Method: Randomized controlled studies will be systematically collected at baseline and every six months for at least five years. Literature search will be performed online in Pubmed, EMBASE, PsycINFO, WebOfScience, SportDiscus, LILACS, IBECS, CINAHL, SCOPUS, ICTRP and ClinicalTrials.gov. Risk of bias will be assessed using the Revised Cochrane Risk of Bias Tool (ROB2). A random effects meta-analysis with mediation analysis using Meta-Analytic Structural Equation Modeling (MASEM) will be performed. The primary research question is to what extent exercise-induced myokines serve as mediators of cognitive function. Secondarily, the pooled effect size of specific exercise characteristics (e.g. mode of exercise) or specific older adults' populations (e.g. cognitively impaired) on the relationship between exercise, myokines and cognition will be assessed. The review protocol was registered in PROSPERO (CRD42023416996).

Discussion: Understanding the triad relationship between exercise, myokines and cognition will expand the knowledge on multiple integrated network systems communicating between skeletal muscles and other organs such as the brain, thus mediating the beneficial effects of exercise on health and performance. It may also have practical implications, e.g. if a certain myokine is found to be a mediator between exercise and cognition, the optimal exercise characteristics for inducing this myokine can be prescribed. The living review is expected to improve our state of knowledge and refine exercise regimes for enhancing cognitive functioning in diverse older adults' populations.

Abbreviations

ANGPTL4, angiopoietin-like 4; BAIBA, β-Aminoisobutyric acid; BDNF, brain-derived neurotrophic factor; BMP, bone morphogenetic factor; CFQ, Cognitive failures questionnaire; CTSB, Cathepsin-B; eCOST, European Cooperation in Science and Technology; FGF, Fibroblast growth factor; FKN, fractalkine; FNDC5, Fibronectin type III domain-containing protein 5; GDF-15, growth differentiation factor 15; GH, growth hormone; GRADE, Grading of Recommendations Assessment, Development and Evaluation; HALE, healthy life expectancy; IGF-1, insulin-like growth factor-1; IL-6, interleukin-6; LIF, Leukemia Inhibitory Factor; MASEM, meta-analytic structural equation modeling; MCP1, Monocyte chemoattractant protein 1; MET, metabolic equivalents; Metrnl, meteorin-like protein; MMSE, Mini-Mental State Examination; MoCA, Montreal Cognitive Assessment; PRISMA-P, Preferred Reporting Items for Systematic Review and Meta-analysis Protocols; SDF1, stromal-derived factor 1; SDMs, standardized mean differences; VEGF, vascular endothelial growth factor.

1. Background

1.1 Rationale

Since 1800, life expectancy has increased threefold, rising from 28.5 years to 73.3 years in 2019, with a further increase of 6.6 years in the last 20 years alone, rising from 66.8 years in 2000 to 73.4 years in 2019. However, healthy life expectancy (HALE), which measures the average number of years a person can expect to live in good health, has only increased by 5.4 years in the last two decades, from 58.3 years in 2000 to 63.7 years in 2019 (World Health Organization (WHO), accessed April 7, 2023). With respect to the brain and cognition, advancing age is the main risk factor for neurodegeneration and cognitive decline (Hou et al., 2019). However, in the last 20 years, dementia prevalence has increased faster than one could explain from the increased proportion of older adults in our society alone (Mattiuzzi and Lippi, 2020; Davis et al., 2022), and is prospected almost to triple from 2019 to 2050 (Nichols et al., 2022). This highlights that the issue of a rising number of older adults with cognitive impairment extends beyond the problem of an aging society and suggests an increasing role of other risk factors related to dementia. For example, the prevalence of sedentary behavior, a known risk factor of cognitive decline and dementia has also increased in the last 20 years (López-Valenciano et al., 2020; Livingston et al., 2020). Sedentary behavior is characterized by an energy expenditure ≤1.5 metabolic equivalents (METs), while in a sitting, reclining or lying posture, and it is distinctly different from physical inactivity defined as insufficient physical activity level to meet physical activity recommendations (Tremblay et al., 2017). It has been shown that older adults spend on

average 80% of their time in a seated posture and with 67% being sedentary for more than 8.5 hours per day (Chastin et al., 2021). The repercussions of age-related cognitive decline are far-reaching, affecting not only the individual, but also their family and society as a whole. Cognitive decline has a substantial impact on an individual's quality of life (Bárrios et al., 2013). Moreover, caring for cognitively impaired individuals can be particularly stressful for family caregivers and health care professionals (Kasper, 1990). Furthermore, cognitive decline is the largest contributor of societal, health care system and personal costs related to limitations in functional independence in community dwelling older adults (Falck et al., 2022).

The effects of physical activity on cognition and brain health among the rapidly growing older population have been widely researched and the benefits are well-documented (e.g. Zhao et al., 2022). The literature usually distinguishes between physical activity and exercise, whereby physical activity refers to any bodily movement that is produced by the skeletal muscles and that increases energy expenditure compared to resting. The term exercise, a subcategory of physical activity, refers to planned, structured, and repetitive physical activity, and is more specifically designed to improve certain fitness components, such as cardiorespiratory fitness, flexibility, balance, coordination, strength, and/or power (Bangsbo et al., 2019). Importantly, the effect of exercise on cognition has been reported in healthy older adults (e.g. see review Falck et al., 2019), older adults with mild cognitive impairment (Tam et al., 2022), and older adults with dementia (Zhang et al., 2022). Specifically, physical activity has been shown to have positive effects on executive functions (Chen et al., 2020), information processing speed (Lin et al., 2021), cognitive inhibition (Boucard et al., 2012), cognitive flexibility (Lerche et al., 2018), memory (Wu et al., 2021) and visuospatial ability (Nemoto et al., 2020). Even a single session of exercise (i.e., acute exercise) can transiently improve performance in various cognitive domains (Griebler et al., 2022; Levin et al., 2021). Studies investigating different modes (aerobic, resistance, balance, exergames, coordination, etc.) or doses of exercise (intensities, durations or number of sessions per week) generally conclude that all are effective (e.g. Borde et al., 2015; Levin et al., 2017; Netz, 2019; Gallardo-Gómez et al., 2022).

Neuroimaging techniques developed along the years employed for examining the relationship between brain health, cognition and exercise have significantly expanded the understanding of the effect of chronic exercise (e.g. MRI - Erickson et al., 2011; fMRI - Chen et al., 2019; fNIRS – Eggenberger et al., 2016; EEG - Schättin et al., 2016; PET – Jonasson et al., 2019; 1H-MRS - Sheoran et al., 2023), as well as acute exercise on brain health and cognition (e.g. Hsieh et al., 2018; Callow et al., 2021).

However, in-depth knowledge of the underlying mechanisms of the exercise-induced cognitive improvements is still incomplete (Pedersen, 2019; Liang et al., 2022; Vints et al., 2022b). Furthermore, the knowledge on the effect of physical activity on multiple organ systems is limited (Hawley et al., 2014; Sanford et al., 2020). The increased

metabolic activity produced by contracting skeletal muscles elicits a challenge to the whole-body homeostasis, generating a distress in numerous cells, tissues and organs. To meet this challenge, multiple integrated networks are operated, communicating between the muscles and the other organs, thus mediating the beneficial effects of exercise on health and performance (Hawley et al., 2014). The dynamics of these multiple complex communication pathways is not yet understood. Interestingly, a recently established comprehensive USA National Institute of Health (NIH) project, aiming to map the molecular transducers involved in response to both acute and chronic exercise, has declared that "Exercise provides a robust physiological stimulus that evokes cross-talk among multiple tissues that when repeated regularly improves physiological capacity, benefits numerous organ systems, and decreases the risk for premature mortality" (Sanford et al., 2020).

In the last few years, research on the effect of exercise on cognition is focusing on the cross-talk between bioactive substances released by physical exercise (called "exerkines") and the brain (e.g. Liang et al., 2022; Vints et al., 2022b). Exerkines enable beneficial crosstalk between various systems, organs, and tissues, including regulation of metabolism and inflammatory responses, exertion of protective effects within the central nervous system, and enhancement of cognitive function (Liang et al., 2022).

Skeletal muscle mass comprises one of the most prevalent tissues in the human body, accounting for approximately 40% of the total body weight (Frontera and Ochala, 2015). Besides regulating various homeostatic processes, recent findings indicate that muscle-derived exerkines play an important role in mediating changes in cognitive function in response to physical exercise (Scisciola et al., 2021; Oudbier et al., 2022; Vints et al., 2022b). Muscle-derived signaling molecules can target the central nervous system (Onyango et al., 2021; Pedersen, 2019), eliciting responses from neurons and glial cells. In 2003, the first muscle-derived exerkine, the cytokine interleukin-6 (IL-6), was discovered and named "myokine" (Pedersen et al., 2003). Since then, more muscle-derived proteins were found, identifying skeletal muscle as an endocrine organ, and broadening the term myokine to "all cytokines and other peptides produced, expressed, and released by muscle fibers that exert paracrine, autocrine, or endocrine effects (Pedersen and Febbraio, 2008). Also lactate, who has long be categorized as a myometabolite instead of a myokine, is now referred to as a myokine due to its endocrine effects (Brooks et al., 2023). In addition, the enzyme kynurenine aminotransferase, which is expressed by skeletal muscle, and kynurenine-derived metabolites have been called myokines, although kynurenine itself is produced by the liver (Rai and Demontis, 2022). By now, research has identified over 600 myokines (Görgens et al., 2015). However, their specific bioactivity remains largely undescribed and poorly understood (Lee and Jun, 2019) and most of the myokines likely exert paracrine, not endocrine effects (Weigert et al., 2014). However, some myokines cross the blood-brain barrier and signal directly to brain cells, while others activate specific signaling cascades from outside the brain exerting an indirect effect on the brain. Whether direct or indirect, myokines have been shown to facilitate

the cross-talk between muscle and brain, indicating that they may mediate a muscle-brain endocrine loop (Chen et al., 2021). Hence, many studies have investigated the function of myokines secreted following acute or chronic exercise to better understand how exercise affects cognition. Overall, most work has been done with aerobic type of exercise, while other exercise modes such as resistance exercise, mind-body exercise or multimodal exercise also induce the release of myokines (Solianik et al., 2022; Parada-Sánchez et al., 2022; Vints et al., 2022b). Moreover, clinical evidence indicates that older adults with sarcopenia (a loss of muscle strength and muscle mass and/or physical performance (Cruz-Jentoft et al., 2019)) suffer cognitive impairment (Cipolli et al., 2019; Ramoo et al., 2022) and this is suggested to be linked to myokine levels (Scisciola et al., 2021; Oudbier et al., 2022). Therefore, understanding the association between muscle-derived signaling factors and cognition may be a promising avenue for interventions aimed at promoting healthy aging.

1.2 What myokines are known to impact cognitive function ?

Over 1125 putative myokines have been described in human secretome or transcriptome studies (see section 2.1.5; for a full list, see Appendix C) and more myokines may be discovered in the next years. Several of these myokines have been identified as potential mediators of exercise-induced cognitive function changes. While some of these myokines are commonly examined in human exercise-cognition studies, others are promising candidates that have only been studied in animal or in-vitro studies (see reviews of Piccirillo, 2019; Rai and Demontis, 2022; Vints et al., 2022b).

Here, we introduce some of the myokines that have most frequently been linked to cognition, including: brain-derived neurotrophic factor (BDNF), Fibronectin type III domain-containing protein 5 (FNDC5)/Irisin, Cathepsin B (CTSB), insulin-like growth factor-1 (IGF1), interleukin-6 (IL6), and L-Lactate (Scisciola et al., 2021). For a review paper, which describes the molecular signaling pathways related to neuroplastic processes of these myokines and other exerkines, see Vints et al. (2022b). Within the living meta-analysis, we will assess if there exists evidence for a role of any of the more than 1125 putative myokines in the exercise-cognition relationship.

The neurotrophin known as BDNF is reported to have a predominant role in neuronal growth, repair, and regeneration (Huang and Reichardt, 2001; Hultman et al., 2014; McGregor and English, 2019). There is reliable evidence that both acute and regular exercise significantly affect BDNF levels in diseased and healthy populations (Szuhany et al., 2015). Several studies have reported that a possible mechanism underlying improved cognitive function following exercise may partly be related to muscle-derived BDNF (Huang et al., 2021). Considering that higher BDNF levels positively affect hippocampal functioning and verbal/episodic/spatial memory (Mizuno et al., 2000; Vaynman et al., 2004; Grassi-Oliveira et al., 2008; Canivet et al., 2015), exercise has been seen as a potential candidate for increasing BDNF signaling to improve cognition. Following exercise, BDNF levels have

been shown to increase both in the brain (Seifert et al., 2010), and in muscle (Matthews et al., 2009). Both may lead to increased BDNF levels in the peripheral circulation (Máderová et al., 2019; Huang et al., 2021), as BDNF is reported to cross the blood-brain barrier bi-directionally (Pan et al., 1998). Hence, further research is needed to understand the source of BDNF associated with improved cognition. Indeed, the effect may arise from other myokines that induce brain-derived BDNF following exercise, such as irisin and CTSB.

Irisin is produced from proteolytic cleavage of PGC-1α transmembrane precursor FNDC5 and upregulated following exercise in the skeletal muscle and hippocampal neurons. FNDC5/irisin crosses the blood-brain barrier (Wrann et al., 2013; Islam et al., 2021) and induces the expression of BDNF centrally, thereby participating in improved cognition (Boström et al., 2012). Moreover, irisin deficiency inhibits cognitive performance in exercise and aging (Wrann et al., 2013).

CTSB was demonstrated to increase in the blood circulation after exercise, cross the blood-brain barrier and induce the hippocampal expression of BDNF, accompanied by spatial memory improvement (Moon et al., 2016). Although few studies on the relationship between exercise, CTSB, and cognitive performance have yielded controversial results, increased CTSB and improved cognitive performance have been reported following exercise (Gaitán et al., 2021; Micielska et al., 2021), implicating CTSB as a mediator of the cognitive effects of exercise.

IGF-1, similar to BDNF, is a multifunctional peptide associated with neural development, neurogenesis, synaptogenesis (D'Ercole et al., 2002; Cheng et al., 2003; Guan et al., 2003), and has neuroprotective effects following nerve injury. Carro et al. (2000) demonstrated that IGF-1 crosses the blood-brain barrier, in turn, regulates hippocampal BDNF expression (see also Vints et al. (2022b). In humans, physical activity causes a rapid increase in peripheral IGF-1 levels (Berg and Bang, 2004; Gulick et al., 2020). Additionally, chronic exercise has been associated with increased peripheral IGF-1 levels modulated by age and gender effects (Jiang et al., 2020). Studies focusing on the relationship between exercise, IGF-1 and cognition have demonstrated that exercise improves circulating IGF-1 and cognition, depending on exercise type, duration, and gender (Stein et al., 2018).

IL-6 increases dramatically in response to exercise (Pedersen and Fischer, 2007). In muscle cells, the IL-6 gene remains silent in rest but is activated by muscle contraction (Pedersen et al., 2003). IL-6 can cross the blood-brain barrier (Banks et al., 1994), indicating that muscle-derived IL-6 may affect the brain. IL-6 levels were related to neurometabolic changes reflecting neurodegenerative processes (Vints et al., 2022a). Acute exercise increases peripheral IL-6 (Kuhne et al., 2023), whereas chronic exercise decreases IL-6 levels and improves cognition (Alghadir et al., 2021; Qi et al., 2021). However, due to IL-6 activating pro-inflammatory and anti-inflammatory processes depending on the condition, further research is needed to improve understanding of the exercise-induced neuroinflammation pathway.

L-lactate is the end product of glycolysis, released from many tissues, including skeletal muscle, acting as a signaling molecule in the brain. It plays a role in learning and memory (Suzuki et al., 2011), adult hippocampal neurogenesis (Lev-Vachnish et al., 2019), and modifies neuron excitability (Sada et al., 2015). Following exercise, lactate levels increase in the peripheral circulation and in the hippocampus (Ide et al., 2000). El Hayek et al. (2019) demonstrated that exercise-induced peripheral lactate crosses the blood-brain barrier, and promotes hippocampal BDNF expression, thereby improving learning and memory. Furthermore, lactate can serve as a precursor for glutamate, an excitatory neurotransmitter which is also critical for learning processes (Maddock et al., 2016).

1.3 Objectives

Our goal in this review is to map the possible pathways between exercise-induced myokines and specific cognitive domains enhanced by exercise. By doing so, we aim to contribute to the understanding of the molecular transducers involved in response to both acute and chronic exercise. Specifically, our primary research question is whether exercise-induced myokines serve as mediators of cognitive function in older adults.

Additional sub-questions are:

1. What specific cognitive components are associated with exercise-induced changes in myokine levels?
2. What specific myokines are associated with exercise-induced changes in cognition?
3. To what extent do exercise characteristics (i.e., type of exercise, intensity of exercise intervention duration, frequency of exercise bouts per week, exercise session length) moderate the effect of exercise on myokine levels in exercise-cognition studies?
4. To what extent do participant characteristics (i.e., cognitive health, age, sex, body mass index (BMI), educational level, physical fitness level, and comorbidities) moderate the effect of exercise on myokine levels in exercise-cognition studies?
5. Which of the studied myokines can be considered as mediators of cognition and to what extent do different myokines affect different cognitive components?

Our search in PubMed (without applying filters, see Appendix B) revealed that this topic is relatively new, but rapidly growing. Before 2002, less than 100 articles were published per year on this topic, whereas since 2020, more than 600 articles are being published annually. Considering the rapid pace at which new evidence is being generated, we intend to conduct this review as a living review.

A living review is continually updated in a predetermined period, incorporating relevant new evidence as it becomes available, and is particularly important in fields where research evidence is emerging rapidly, current evidence is uncertain, and new research may change

policy or practice decisions (Elliott et al., 2017). This new evidence synthesis method is being trialed as one of the outcome products of PhysAgeNet, a European Cooperation in Science and Technology (eCOST) network (https://www.cost.eu/actions/CA20104/). PhysAgeNet aims to develop and sustain an international knowledge community about physical exercise at an older age. We plan to update our living systematic search every six months, for a minimal period of five years.

2. Methods and Analysis

We plan to carry out the living systematic review with meta-analysis in the second half of the year 2023. The methods of this systematic review with meta-analysis were registered in the International Prospective Register of Systematic Reviews database (PROSPERO) on the 24th of April, 2023 (registration number CRD42023416996).

Prior to starting this project, we searched PubMed, PROSPERO and Cochrane Central Register of Controlled Trials (CENTRAL) to confirm that no systematic reviews or meta-analyses with the same research questions had recently been published or were currently being conducted. The protocol of this systematic review and meta-analysis is in accordance with the Preferred Reporting Items for Systematic Review and Meta-analysis Protocols (PRISMA-P) guidelines (Shamseer et al., 2015). A PRISMA-P checklist (Moher et al., 2016) is added, see Appendix A.

2.1 Eligibility criteria

Studies will be selected according to the criteria outlined below.

2.1.1 Study designs

To ensure the highest quality of evidence, we will only include randomized controlled trials and exclude non-randomized controlled trials, cross-over studies, or studies that lack pre-to-post exercise comparisons such as case-control studies, or those that do not include a control group, such as longitudinal studies, case reports or case series. Additionally, we will also exclude guidelines and expert opinions.

We will conduct a search for review papers and gray literature, which include article synopses, unpublished studies such as PhD theses or master theses, conference abstracts, and trial registrations. We will evaluate these sources to determine whether they lead to additional randomized controlled trials that meet our inclusion criteria. In case gray literature is retrieved as potentially eligible, we will contact the authors of this literature to request any associated peer-reviewed publications or published data.

2.1.2 Participants

Studies with participants aged 50 years or older with a mean age of 60 years or above will be included, regardless of their health status. Participants may have existing medical conditions or be in good health. Animal studies will be excluded.

2.1.3 Interventions

We will include interventions described as a single bout (acute) or multiple bouts (chronic) of voluntary exercise. We define voluntary exercise as a planned or structured bodily movement done to improve or maintain one or more components of physical fitness (based on the definition of the American College of Sports Medicine, 2001 (Jakicic et al., 2001)). Examples are: cardiorespiratory endurance exercise, high-intensity interval training, resistance exercise, mind-body exercises, balance training, multicomponent exercise, or other specific exercises that do not fit in any of these categories.

We will exclude exercise interventions that do not include a voluntary component (e.g., electrical stimulation, whole-body vibration) or involve solely passive muscle fiber movement (e.g., stretching, manipulations). Additionally, we will exclude exercise interventions that involve a component of cognitive training (e.g. dual task training) or the addition of a nutritional intervention. Finally, we will exclude routine daily activities or occupational tasks that do not meet the definition of exercise based on the American College of Sports Medicine (2001) (Jakicic et al., 2001).

2.1.4 Comparators

Only studies with a control group will be included. The following control groups are eligible for inclusion: passive controls (e.g., waitlist), treatment as usual, active non-exercise controls (stretching, puzzle, computer games not targeting specific cognitive functions, non-exercise recreational activities), non-active non-therapeutic activities (e.g., health education, non-exercise recreational activities). We will exclude studies that solely compare two exercise interventions, including all exercise interventions listed as possibly included interventions in section 2.1.3, without another control group. Studies that use cognitive training or dual tasking interventions as a control condition will also be excluded.

2.1.5 Outcomes

Our main outcome is the mediation effect of exercise-induced myokine level changes on exercise-induced cognitive function changes in older adults. To be eligible for inclusion, studies must report both the myokine and the cognition outcomes.

A total of 1126 secretory proteins, identified as putative myokines, by definition secreted by skeletal muscle and exerting a biological function in a paracrine or endocrine fashion (Whitham and Febbraio, 2016), have been compiled from existing literature. A

comprehensive list, along with corresponding references, can be found in Appendix C. The primary sources for this compilation of myokines include proteomic analysis, secretome analysis and mRNA sequencing studies on human skeletal muscle (Bortoluzzi et al., 2006; Hittel et al., 2009; Norheim et al., 2011; Le Bihan et al., 2012; Raschke et al., 2013; Scheler et al., 2013; Catoire et al., 2014; Hartwig et al., 2014; Pourteymour et al., 2017), some of which specifically studied myokines that are elevated in response to exercise or muscle contraction (Norheim et al., 2011; Raschke et al., 2013; Scheler et al., 2013; Catoire et al., 2014; Pourteymour et al., 2017) and review papers (Engler, 2007; Catoire and Kersten, 2015; Görgens et al., 2015; Kwon et al., 2020; Rai and Demontis, 2022). Notably, lactate and beta-aminoisobutyric acid (BAIBA), categorized most often as myometabolites, but recently also as myokines due to their endocrine effects will also be included (Catoire and Kersten, 2015; Brooks et al., 2023). Additionally, the enzyme kynurenine aminotransferase and kynurenine-derived metabolites have also been referred to as myokines, despite kynurenine itself being produced by the liver, while kynurenine aminotransferase is found on muscle cells (Rai and Demontis, 2022). By employing this inclusive approach, our objective is to generate a comprehensive list of myokines that may potentially impact cognitive function in older adults. However, it is important to acknowledge that for the majority of these putative myokines limited research has been conducted on the exercise-cognition context specifically. Moreover, there exists a lack of comprehensive understanding regarding the biological effects of most discovered putative myokines in general (Lee and Jun, 2019), and most of the myokines likely exert paracrine, not endocrine effects (Weigert et al., 2014). Consequently, we anticipate that our systematic search will likely yield only a concise compilation of myokines relevant to the exercise-cognition field, considering the current state of knowledge. It should be noted that the included putative myokines are allowed to be also released partly from other organs, as long as part of the exercise-induced changes (increase or decrease) in levels in the bloodstream is caused by pathways activated in skeletal muscle tissue. We will exclude myokines that are not released from muscle tissue, even if they have a known effect on the brain, such as growth hormone (GH), Orexin-A, or Ghrelin (see review paper of Vints et al. (2022b) where a schematic overview of exerkines with known effects on neuroplasticity in aerobic and resistance exercise is described). Myokine levels measured in total blood, blood serum, blood plasma or cerebrospinal fluid will be included.

Concerning cognition, we will include intrinsic capacity domains that are particularly vulnerable in older age (fluid cognitive functions, as opposed to crystallized intelligence) based on the Cattell-Horn-Caroll-Miyake taxonomy of cognitive domains (Webb et al., 2018).

The cognitive domains include fluid reasoning (e.g. Raven's progressive matrices), long-term memory (e.g. Rey Auditory Verbal Learning test), short-term memory (e.g. Complex span tasks, Digit span backwards), executive functions (e.g. N-back, Dual-tasking,

Stroop test), processing speed (e.g. Digit symbol substitution task, Choice reaction time), visual processing (e.g. Visual search), global cognitive functioning (e.g. Mini-Mental State Examination (MMSE), Montreal Cognitive Assessment (MoCA), Cognitive failures questionnaire (CFQ)). Cognitive measures will include response time (of all or only the correct answers), throughput (of all or only the correct answers), performance index (100×[accuracy/response time]), accuracy (number or % correct answers), or specific test scores.

2.1.6 Timing of outcome assessments after end of intervention

Concerning the timing of post-exercise assessment, it is critical to distinguish between acute and chronic exercise effects. Any measurement immediately after a single bout of exercise, (irrespective of the time period since the last exercise bout), is generally considered an acute exercise effect. However, in order to measure chronic exercise effects after multiple bouts of exercise, it is advised to measure the outcome more than 24 hours, but preferably more than 48 hours after the last exercise session (Vints et al., 2022b). If outcome assessments were conducted less than or equal to 24 hours after the last exercise bout of an intervention consisting of multiple exercise bouts, we will consider them acute exercise effects in trained individuals. If outcome assessments were conducted more than 24 hours after exercise, we will consider them chronic exercise effects. The timing of the outcome assessment will not be a criterion for inclusion of a study, but the information will be extracted, and the decision whether it is considered acute or chronic effect, will be taken accordingly.

2.1.7 Setting

Included studies will not be restricted to a specific type of setting. Expected settings include community settings (e.g., day care centers, universities, workplace), clinical settings (e.g. hospitals, psychiatric institutions) and home settings (e.g., people's own home, nursing homes, care homes).

2.1.8 Language

We will only include articles written in the English language.

2.2 Information sources

The authors (Wouter Vints, and Ioanna Zorba (Zormpa) - information specialist) will search in PubMed, EMBASE (through Elsevier), PsycInfo (through EBSCO), all databases of Web of Science (excluding MEDLINE), SportDiscus (through EBSCO), LILACS (accessed through Portal Regional da BVS), IBECS (accessed through Portal Regional da BVS), CINAHL (through EBSCO), SCOPUS (Elsevier), International Clinical Trials Registry Platform (ICTRP) accessed through CENTRAL, and ClinicalTrials.gov (CT.gov) accessed through CENTRAL. None of the databases will be restricted by date. The searches will be re-run prior to the final analysis.

Table 1. Details of limitations and filters per database

Database	Limitations/Filters
PubMed	Free terms were searched in the fields of title, abstract and author keywords Limitations/Filters: Randomized Controlled Trial, Humans, English, exclude preprints, Middle Aged OR Aged: 45+ years
Embase	Free terms were searched in the fields of title, abstract and author keywords Limitations/Filters: Randomized Controlled Trial, Human, Aged OR Middle Aged OR Very Elderly
SportDiscus	Free terms were searched in the fields of title, abstract and author keywords Limitations/Filters: English
PsycInfo	Free terms were searched in the fields of title, abstract and author keywords Limitations/Filters: English, Population Group: Human, exclude dissertations, Age Groups: Middle Age (40-64 yrs) OR Aged (65 yrs & older) OR Very Old (85 yrs & older)
LILACS	Free terms were searched in the fields of title, abstract and subject Limitations/Filters: English
IBECS	Free terms were searched in the fields of title, abstract and subject Limitations/Filters: English
Scopus	Free terms were searched in the fields of title, abstract and author keywords Limitations/Filters: Randomized Controlled Trial, English
CINAHL	Free terms were searched in the fields of title, abstract and topic Limitations/Filters: English, Human, exclude MEDLINE records, Middle Aged OR Aged OR Aged 80 and over
Web of Science	Free terms were searched in the fields of title, abstract and indexing Limitations/Filters: English, Article, All databases excluding MEDLINE records
Clinical Trials.gov	Free terms were searched in the fields of title, abstract and author keywords Limitations/Filters: English, Trial
International Clinical Trials Registry Platform	Free terms were searched in the fields of title, abstract and author keywords Limitations/Filters: English, Trial

2.3 Search details

Literature search strategies are developed using free text words and index terms related to: middle or older age AND physical exercise AND cognition AND myokines. Appendix B includes a transcript of the free terms used in the search strategy and the index terms

per database where applicable. The search strategy was translated for each of the used databases in a way that they remained as equal as possible, using the same free text terms, and searching for the most similar index terms. The search strategy was developed by Wouter Vints, who has expertise in the topic of the review with the help of Ioanna Zorba (Zormpa) who works as an information specialist, and was approved by all collaborating authors. Details of the used limitations and filters are provided in table 1.

2.4 Data management (search management and data extraction)

2.4.1 Study selection

Pairs of two authors will be involved in the study selection process. Due to the expected large amount of studies, the workload will be divided between all authors. Two authors will independently, without knowing each other's decisions, screen the eligibility of the studies with a specific publication date, while studies with other publication dates will be screened by different pairs of authors. The first screening step will be based on the title and abstract of the article and in a second step based on the full text article. If the two authors disagree at any step, a third author will evaluate the eligibility criteria and debate among all three authors will be organized to reach an agreement. The software system 'Rayyan' will be used for the study selection process (Ouzzani et al., 2016).

2.4.2 Data extraction

Following the selection process, pairs of two authors will independently extract the following information:

- the name of the first author
- year of publication
- study design
- participant characteristics (number in each group, age [range and mean], sex [%], BMI [range and mean], level of education, physical fitness, main clinical diagnosis and comorbidities)
- exercise characteristics (type of exercise, intensity, duration in weeks or months, frequency, length of one exercise session, follow-up time, home-based vs gym-based, autonomous vs with a trainer [specify the type of trainer e.g. researcher, professional coach])
- control group characteristics (type of control condition)
- cognition (cognitive domain assessed, cognitive test used, how was the outcome measured e.g. as response speed – accuracy – performance index – throughput - specific test score, whether a test was performed by a professional e.g. psychiatrist - nurse - trained personnel - untrained personnel)
- acute effect in trained/untrained individuals, or chronic effect (how long after the last exercise bout was the cognitive outcome measured and the blood collection performed)

- sample size in treatment and control group
- standardized mean differences (SMDs) between control and treatment group on myokines (or the necessary statistics to calculate SMDs)
- SMDs between control and treatment group on cognitive functioning (or the necessary statistics to calculate SMDs)
- bivariate correlations between myokines and cognitive functioning (preferably in the total sample, or else per condition)
- bivariate correlations between different types of myokines (preferably in the total sample, or else per condition)
- bivariate correlations between different types of cognitive functioning (preferably in the total sample, or else per condition).

The exercise characteristics will additionally be used to place the studies in specific categories for subgroup analyses. We will extract information about the type of exercise: cardiorespiratory endurance exercise (such as walking, running, or cycling aimed to improve the aerobic energy systems), high-intensity interval training (including sprint training or other interval-based cardiorespiratory exercise training aimed to improve both aerobic and anaerobic energy systems), resistance exercise (such as weight lifting, strength training, power training, body weighted exercises, elastic bands exercises intended to increase muscular strength/volume/power), mind-body exercises (such as Tai Chi, yoga, motor skill training, or dance aimed to improve mind-body coordination and awareness by a series of controlled movements that focus on the interactions between the brain, body, mind, and behavior), balance training (aimed to improve balance or proprioception), multicomponent exercise (i.e. a combination of at least two of the aforementioned types of exercise), or other specific exercises (e.g. basketball training or competition) that do not fit in any of these categories. We will also extract information about the, intensity of exercise [very light, moderate, vigorous, or near-maximal to maximal intensity based on the American College of Sports Medicine Position Stand, published July 2011 by Garber et al. in Medicine & Science in Sports & Exercise], the intervention duration in weeks (months) [single bout, < 13 weeks (3 months), 13-26 weeks, > 26 weeks (6 months)], the frequency [single bout, < 3 times/week, 3-5 times/week, > 5 times/week], and the exercise session length (< 30 min, 30-60 min, > 60 min)

Participant characteristics will also be extracted to be used in subgroup analysis, including age groups (50-69 years old vs 70 years and over), sex, healthy vs disease, normal cognition *vs.* cognitive impairment at baseline, fitness level at baseline, educational level.

In case of disagreement in the extracted information, a third author will be requested to evaluate the data and debate among all three reviewers will be organized to reach an agreement. In case of missing information, the study investigators will be contacted. When no contact details are available or no reply is received within one month, the missing information will be marked 'not available'.

2.5 Risk of bias assessment

Risk of bias will be assessed using the Cochrane Risk of Bias tool (ROB 2) for randomized controlled trials (Higgins et al., 2011). The possible risk of bias on each of the domains included in these risk of bias tools will be judged as 'high risk', 'low risk' or 'unclear'. The individual results will be compared between two review authors and disagreements will be solved by consulting a third author for arbitration.

2.6 Data synthesis and statistical methods

2.6.1 Meta-analysis with MASEM

If data is appropriate for synthesis, we will conduct a meta-analysis using random effects meta-analyses. We will assess the effect of exercise on each of the myokines and each of the cognitive functioning outcomes that are included in at least three or more included randomized controlled trials.

A path model (see Figure 1) will be evaluated using one-stage meta-analytic structural equation modeling (MASEM). One-stage MASEM is essentially a random-effects multivariate meta-analysis on correlation coefficients, in which the average correlations are restricted to follow the structure of the hypothesized path model. In its simplest form, the random-effects multivariate meta-analytic model decomposes the vector r_k of correlation coefficients for a study *k* in three parts:

$$r_k = \rho_R + u_k + \varepsilon_k, \quad (1)$$

where ρ_R indicates the vector of means of the correlation coefficients over all studies, u_k is a vector of deviations of study k's population correlation coefficients from ρ_R , and ε_k is a vector with the sampling deviations of study k. The covariances of u_k denote the between-studies covariance matrix. The covariances of ε_k represents the within-studies covariance matrix for study k, often denoted V_k , which is estimated for each study, and then treated as known in the final analysis. Estimates of the path coefficients are obtained by restricting ρ_R in Equation 1:

$$\rho_R = \text{vechs}(\mathbf{F}\,(\mathbf{I} - \mathbf{A})^{-1}\,\mathbf{S}\,(\mathbf{I} - \mathbf{A})^{-1T}\,F^T), \quad (2)$$

where using the RAM-formulation (McArdle and McDonald, 1984), **I** is an identity matrix, **F** is a selection matrix with 1's for observed variables and 0's for latent variables, **A** is a square matrix with asymmetric paths such as regression coefficients, **S** is a symmetrical matrix with variances and covariances, and vechs() vectorizes the lower diagonal of its argument. For more explanation and details of this method see Jak and Cheung (2020) and Jak et al., (2021).

This process will be led by co-author Suzanne Jak. The analyses will be conducted using the metaSEM package in R (Cheung, 2015).

Since one-stage MASEM uses correlation coefficients as input, the SMDs on myokines and cognitive functioning will be transformed to point-biserial correlations with a target base-rate of 0.50 (see McGrath and Meyer, 2006). The MASEM will lead to estimates of each of the direct effects in the path model, as well as estimates of the indirect effects of exercise on cognitive functioning through the different myokines. Statistical significance of direct effects will be evaluated using Wald-tests with a significance level of 5%, and statistical significance of mediating (indirect) effects with 95% likelihood-based confidence intervals (Neale and Miller, 1997; Cheung, 2022). A comparison of the strength of effects for different types of myokines and cognitive functions will be performed by constraining the relevant parameters to be equal and evaluating the difference in model's log likelihoods.

Studies on acute exercise effects will be analyzed separately from chronic exercise studies. As a sensitivity analysis, we will also execute random-effects meta-analysis on the SMDs of myokines and cognitive outcomes separately using 'standard' (not SEM) meta-analysis. If there are dependent effect sizes within studies (e.g. multiple operationalization of the same construct) they will be taken into account using robust variance estimation (Hedges et al., 2010).

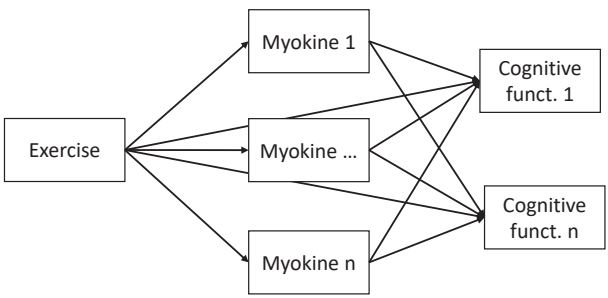


Figure 1. Meta-analytic structural equation modeling (MASEM) model

2.6.2 Heterogeneity

In order to try to explain possible heterogeneity across studies, we will evaluate moderating effects on all path coefficients in the model for all moderators that have enough coded values in studies. The moderating effects will be evaluated separately for each moderator. Moderators under consideration include exercise characteristics such as type of exercise, intensity of exercise, intervention duration in weeks, frequency of exercise sessions per week, exercise session length, participant characteristics including age groups, sex, health status, normal cognition or cognitive impairment at baseline, fitness level at baseline, educational level.

2.6.3 Meta-biases

The potential for reporting bias and publication bias will be explored by visual inspection of funnel plots of the SMDs if ≥10 studies are available. We will also perform Egger's regression tests on the SMD's.

2.6.4 Quality of evidence

The quality of evidence for all outcomes will be judged using the Grading of Recommendations Assessment, Development and Evaluation (GRADE) working group methodology (Akl et al., 2013). The quality of evidence will be assessed across the domains of risk of bias, consistency, directness, precision and publication bias. Additional domains may be considered where appropriate. Quality will be adjudicated as high (further research is very unlikely to change our confidence in the estimate of effect), moderate (further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate), low (further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate), or very low (very uncertain about the estimate of effect). The effect of study quality on the effect sizes will be evaluated using meta-regression.

2.7 Administration, dissemination and updating of the living review

We plan to update the systematic review and meta-analysis for at least 5 years, with the option to extend this period if agreed by the authors. However, we may choose to convert the paper from a living systematic review to a normal systematic review and meta-analysis if the authors collectively determine that the criteria for conducting a living systematic review are no longer applicable. This may occur if there is no longer a significant level of uncertainty in the existing evidence, or if the research field is no longer rapidly evolving with emerging evidence likely to impact the conclusions of the living systematic review.

The project will be managed by one author (Wouter Vints). We plan to rerun the searches every six months and search for new myokines that are not yet included in the review on a yearly basis. The searches will be managed by one author (Wouter Vints) with the support of a librarian (Ioanna Zorba (Zormpa)) upon request. The division of other review tasks will remain as decided for the first review process. Whenever a contributing author wishes to step out of the review update process, he/she will try his/her best to assign a replacement or the replacement will be decided by the other contributing authors.

The decision to update the review paper will be based on a published scheme (Elliott et al., 2017). Updates of the living systematic review and meta-analysis that do not require re-publication will be presented at the website https://www.egrepa.org/. If no new studies are found, only the date of the last search will be updated. If new studies are found, but the new information is likely to have negligible effects on the effect estimates or the certainty

of the evidence, the date of the last search will be updated together with details of the new evidence and a justification for delaying the incorporation of this evidence in the paper. If new studies are found with likely effects on the effect estimates or the certainty of evidence, the date of the last search will be updated, the details of the new evidence will be added and data will be extracted, quality will be assessed and synthesized and the studies will immediately be incorporated in the paper. Once this process is completed, the updated living systematic review and meta-analysis will be submitted for re-publication in an open access international peer-reviewed journal as soon as possible, preferably a journal that has the resources to support a living systematic review. A summary is presented in Table 2.

Table 2 Intended process and publication strategy of the living review

No. of authors maintaining the LSR	15
Search support	Information specialist within the author team (Ioanna Zorba (Zormpa))
Search frequency	Every six months
Communicating review status to reader	On the PhysAgeNet website, the EGREPA website and via an article amendment if the latter is allowed by the journal and its editorial. • https://physagenet.eu/ • https://www.egrepa.org/
Process for integration of new evidence	Full re-publication of the review, with new citation and DOI whenever new information is retrieved that will likely affect the effect estimates or the certainty of evidence.
Trigger for integration of new evidence	Every 12-24 months depending on the impact of the new evidence, according to the decision tree presented by Elliot et al., 2017.

3. Discussion

3.1 Perspectives

This proposed systematic review paper with meta-analysis is expected to make a significant contribution to the existing literature by generating new and valuable knowledge.

Firstly, this will be the first meta-analysis to comprehensively investigate the triad relationship between exercise, myokines and cognition in older adults. The analysis will not be restricted to specific types of exercise, health status, sets of myokines or cognitive function domains. We will use advanced statistical methods that have not been applied in this topic before to assess whether specific myokines mediate the relationship between exercise and cognition.

Secondly, the living mode of this review paper allows for continuous updates, to ensure that the information remains up-to-date and relevant. As the research field progresses, the review will include the latest findings and become a valuable resource for researchers, clinicians, and policymakers.

Lastly, this review paper could have extensive practical implications by providing insights into which myokines are critical for maintaining cognitive health in older adults.

As the review progresses, it may provide more specific information on the value of certain myokines for enhancing particular cognitive domains or treating specific diseases. In addition, we will continuously update the review with information on the optimal exercise characteristics for inducing these myokines such as the mode of exercise, intensity, and other factors. Ultimately, this could help us to individualize exercise programs to meet the specific needs of older adults. The insights gained from this review may extend beyond older adults, providing broader benefits to society. In the future, this knowledge may lead to the design of healthy aging interventions, and even lead to the development of myokine-containing pharmacological pills. Such interventions could be used as add-ons or to mimic the effect of exercise for those unable to participate in optimal exercise interventions (see Gubert and Hannan, 2021).

3.2 Strengths and limitations

One important strength of our study design is its living approach, which enables us to keep this review paper up to date over time. Notably, research has shown that 25% of systematic reviews lose their accuracy and utility within two years (Shojania et al., 2007). An important limitation of most systematic reviews, especially in a rapidly growing research field like the one we are studying, is that they often become outdated quickly. In contrast, our review paper targets a wide range of studies, without restricting our scope to a specific set of myokines, including 1126 myokines at start, cognitive outcomes, exercise interventions or population criteria, except for older age. This approach will provide a comprehensive overview of the research field. Additionally, we will use advanced statistical techniques such as MASEM, to examine the mediating effects of myokines on cognitive changes. This information may drastically change the understanding of the role of myokines in cognitive function changes in older adults with varying health status following exercise.

The limitations of our study design include the expected retrieval of a highly heterogeneous list of studies, which is a common issue in meta-analyses conducted in this research field. The reasons behind this variability include differences in participant characteristics, exercise protocols, blood sampling methods, and cognitive tests used (Vints et al., 2022b). Furthermore, we made a conscious decision to impose fewer restrictions on the inclusion of studies in order to provide a comprehensive overview of the field. However, this decision may increase the heterogeneity of the included studies, which is a potential limitation of our design. In addition, it should be noted that some

studies use low-intensity exercise or balancing exercise as a control condition in their research, but we have decided not to include studies with an exercise control group in our analysis. This decision also constitutes a limitation of our study design. Finally, our search will be limited to the English literature, which could be considered a form of bias. However, this is common practice, as only one third of systematic reviews report to conduct searches without language restriction and only 2% eventually include non-English literature (Jackson and Kuriyama, 2019; Pieper and Puljak, 2021).

4. Conclusion

This protocol outlines the methods for a living systematic review with meta-analysis, which aims to investigate the role of more than 1125 putative myokines as potential mediators in the relationship between exercise and cognitive function in older adults. The existence of a direct cross-talk between muscle and brain via myokine signaling has been demonstrated, indicating the potential for muscle-derived signaling factors to be used as a non-pharmacological intervention to maintain cognitive ability at older age. However, the rapid growth of this research field necessitates continued synthesis to identify the most promising targets and exercise protocols. A living systematic review with meta-analysis can facilitate ongoing research into the specific bioactivity of myokines and their association with cognitive function, leading to a better understanding of the relationship between exercise and cognitive health in older adults.

Conflicts of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author contributions

The first draft of this protocol was written by Wouter Vints, Yael Netz, Evrim Gökçe and Antoine Langeard. The search strategies are developed by Wouter Vints and Ioanna Zorba (Zormpa). Suzanne Jak developed and described the statistical methods. The final version was reviewed and approved by all authors.

Funding

A grant was awarded by eCOST (award number CA20104-dc00d18b-2213e134-470c-11ed-8dfc-06f1f03d9269, date of award 14th of November 2022) in the context of the eCOST Action CA20104, Network on evidence-based physical activity in old age (PhysAgeNet) to support young researchers taking a leading role in research projects. The grant was received by Wouter Vints. The funder was not involved in designing the study or writing of the paper, nor in the decision to submit the article for publication.

Acknowledgements

This publication is based upon work from the eCOST Action CA20104 - Network on evidence-based physical activity in old age (PhysAgeNet), supported by COST (European Cooperation in Science and Technology). https://www.cost.eu/actions/CA20104/, https://physagenet.eu/

References

Akl, E., Mustafa, R., and Wiercioch, N. S. W. (2013). *Handbook for grading the quality of evidence and the strength of recommendations using the GRADE approach*. , eds. H. Schünemann, J. Brozek, G. Guyatt, and A. Oxman Available at: https://gdt.gradepro.org/app/handbook/handbook.html [Accessed April 16, 2023].

Alghadir, A. H., Gabr, S. A., Al-Momani, M., and Al-Momani, F. (2021). Moderate aerobic training modulates cytokines and cortisol profiles in older adults with cognitive abilities. *Cytokine* 138, 155373. doi: 10.1016/J.CYTO.2020.155373.

Bangsbo, J., Blackwell, J., Boraxbekk, C. J., Caserotti, P., Dela, F., Evans, A. B., et al. (2019). Copenhagen Consensus statement 2019: physical activity and ageing. *Br. J. Sports Med.* 53, 856–858. doi: 10.1136/BJSPORTS-2018-100451.

Banks, W. A., Kastin, A. J., and Gutierrez, E. G. (1994). Penetration of interleukin-6 across the murine blood-brain barrier. *Neurosci. Lett.* 179, 53–56. doi: 10.1016/0304-3940(94)90933-4.

Bárrios, H., Narciso, S., Guerreiro, M., Maroco, J., Logsdon, R., and De Mendonça, A. (2013). Quality of life in patients with mild cognitive impairment. *Aging Ment. Health* 17, 287–292. doi: 10.1080/13607863.2012.747083.

Berg, U., and Bang, P. (2004). Exercise and Circulating Insulin-Like Growth Factor I. *Horm. Res. Paediatr.* 62, 50–58. doi: 10.1159/000080759.

Borde, R., Hortobágyi, T., and Granacher, U. (2015). Dose-Response Relationships of Resistance Training in Healthy Old Adults: A Systematic Review and Meta-Analysis. *Sports Med.* 45, 1693–1720. doi: 10.1007/S40279-015-0385-9.

Bortoluzzi, S., Scannapieco, P., Cestaro, A., Danieli, G. A., and Schiaffino, S. (2006). Computational reconstruction of the human skeletal muscle secretome. *Proteins Struct. Funct. Bioinforma.* 62, 776–792. doi: 10.1002/PROT.20803.

Boström, P., Wu, J., Jedrychowski, M. P., Korde, A., Ye, L., Lo, J. C., et al. (2012). A PGC1-α-dependent myokine that drives brown-fat-like development of white fat and thermogenesis. *Nature* 481, 463–468. doi: 10.1038/nature10777.

Boucard, G. K., Albinet, C. T., Bouquet, C. A., Clarys, D., Audiffren, M., and Bugaiska, A. (2012). Impact of Physical Activity on Executive Functions in Aging: A Selective Effect on Inhibition Among Old Adults. *J. Sport Exerc. Psychol.* 34, 808–827. Available at: www.JSEP-Journal.com [Accessed January 22, 2023].

Brooks, G. A., Osmond, A. D., Arevalo, J. A., Duong, J. J., Curl, C. C., Moreno-Santillan, D. D., et al. (2023). Lactate as a myokine and exerkine: drivers and signals of physiology and metabolism. *J. Appl. Physiol.* 134, 529–548. doi: 10.1152/JAPPLPHYSIOL.00497.2022/ASSET/IMAGES/LARGE/JAPPLPHYSIOL.00497.2022_F003.JPEG.

Callow, D. D., Won, J., Alfini, A. J., Purcell, J. J., Weiss, L. R., Zhan, W., et al. (2021). Microstructural Plasticity in the Hippocampus of Healthy Older Adults after Acute Exercise. *Med. Sci. Sports Exerc.* 53, 1928–1936. doi: 10.1249/MSS.0000000000002666.

Canivet, A., Albinet, C. T., André, N., Pylouster, J., Rodríguez-Ballesteros, M., Kitzis, A., et al. (2015). Effects of BDNF polymorphism and physical activity on episodic memory in the elderly: a cross sectional study. *Eur. Rev. Aging Phys. Act.* 12, 15. doi: 10.1186/S11556-015-0159-2.

Carro, E., Nuñez, A., Busiguina, S., and Torres-Aleman, I. (2000). Circulating insulin-like growth factor I mediates effects of exercise on the brain. *J. Neurosci.* 20, 2926–2933. doi: 10.1523/jneurosci.20-08-02926.2000.

Catoire, M., and Kersten, S. (2015). The search for exercise factors in humans. *FASEB J.* 29, 1615–1628. doi: 10.1096/FJ.14-263699.

Catoire, M., Mensink, M., Kalkhoven, E., Schrauwen, P., and Kersten, S. (2014). Identification of human exercise-induced myokines using secretome analysis. *Physiol. Genomics* 46, 256–267. doi: 10.1152/physiolgenomics.00174.2013.

Chastin, S., Gardiner, P. A., Harvey, J. A., Leask, C. F., Jerez-Roig, J., Rosenberg, D., et al. (2021). Interventions for reducing sedentary behaviour in community-dwelling older adults. *Cochrane database Syst. Rev.* 6, CD012784. doi: 10.1002/14651858.CD012784.PUB2.

Chen, F. T., Chen, Y. P., Schneider, S., Kao, S. C., Huang, C. M., and Chang, Y. K. (2019). Effects of Exercise Modes on Neural Processing of Working Memory in Late Middle-Aged Adults: An fMRI Study. *Front. Aging Neurosci.* 11, 224. doi: 10.3389/FNAGI.2019.00224.

Chen, F. T., Etnier, J. L., Chan, K. H., Chiu, P. K., Hung, T. M., and Chang, Y. K. (2020). Effects of Exercise Training Interventions on Executive Function in Older Adults: A Systematic Review and Meta-Analysis. *Sport. Med.* 50, 1451–1467. doi: 10.1007/S40279-020-01292-X/FIGURES/3.

Chen, W., Wang, L., You, W., and Shan, T. (2021). Myokines mediate the cross talk between skeletal muscle and other organs. *J. Cell. Physiol.* 236, 2393–2412. doi: 10.1002/JCP.30033.

Cheng, C. M., Mervis, R. F., Niu, S. L., Salem, N., Witters, L. A., Tseng, V., et al. (2003). Insulin-like growth factor 1 is essential for normal dendritic growth. *J. Neurosci. Res.* 73, 1–9. doi: 10.1002/JNR.10634.

Cheung, M. W. L. (2015). metaSEM: An R package for meta-analysis using structural equation modeling. *Front. Psychol.* 5, 1521. doi: 10.3389/FPSYG.2014.01521/ABSTRACT.

Cheung, M. W. L. (2022). Synthesizing Indirect Effects in Mediation Models With Meta-Analytic Methods. *Alcohol Alcohol.* 57, 5–15. doi: 10.1093/ALCALC/AGAB044.

Cipolli, G. C., Sanches, M. Y., and Aprahamian, I. (2019). Sarcopenia Is Associated with Cognitive Impairment in Older Adults: A Systematic Review and Meta-Analysis. *J. Nutr. Health Aging* 23, 525–531. Available at: https://www.proquest.com/docview/2209105519?pq-origsite=primo&accountid=17215 [Accessed November 16, 2021].

Cruz-Jentoft, A. J., Bahat, G., Bauer, J., Boirie, Y., Bruyère, O., Cederholm, T., et al. (2019). Sarcopenia: revised European consensus on definition and diagnosis. *Age Ageing* 48, 16–31. doi: 10.1093/AGEING/AFY169.

D'Ercole, J. A., Ye, P., and O'Kusky, J. R. (2002). Mutant mouse models of insulin-like growth factor actions in the central nervous system. *Neuropeptides* 36, 209–220. doi: 10.1054/NPEP.2002.0893.

Davis, M. A., Chang, C.-H., Simonton, S., and Bynum, J. P. W. (2022). Trends in US Medicare Decedents' Diagnosis of Dementia From 2004 to 2017. *JAMA Heal. Forum* 3, e220346–e220346. doi: 10.1001/JAMAHEALTHFORUM.2022.0346.

Eggenberger, P., Wolf, M., Schumann, M., and de Bruin, E. D. (2016). Exergame and Balance Training Modulate Prefrontal Brain Activity during Walking and Enhance Executive Function in Older Adults. *Front. Aging Neurosci.* 8, 66. doi: 10.3389/fnagi.2016.00066.

El Hayek, L., Khalifeh, M., Zibara, V., Abi Assaad, R., Emmanuel, N., Karnib, N., et al. (2019). Lactate mediates the effects of exercise on learning and memory through sirt1-dependent activation of hippocampal brain-derived neurotrophic factor (BDNF). *J. Neurosci.* 39, 2369–2382. doi: 10.1523/JNEUROSCI.1661-18.2019.

Elliott, J. H., Synnot, A., Turner, T., Simmonds, M., Akl, E. A., McDonald, S., et al. (2017). Living systematic review: 1. Introduction—the why, what, when, and how. *J. Clin. Epidemiol.* 91, 23–30. doi: 10.1016/j.jclinepi.2017.08.010.

Engler, D. (2007). Hypothesis: Musculin is a hormone secreted by skeletal muscle, the body's largest endocrine organ. Evidence for actions on the endocrine pancreas to restrain the beta-cell mass and to inhibit insulin secretion and on the hypothalamus to co-ordinate the neuroendocrine and appetite responses to exercise. *Acta Biomed.* 78, 156–206. Available at: https://pubmed.ncbi.nlm.nih.gov/17465332/ [Accessed June 6, 2023].

Erickson, K. I., Voss, M. W., Prakash, R. S., Basak, C., Szabo, A., Chaddock, L., et al. (2011). Exercise training increases size of hippocampus and improves memory. *Proc. Natl. Acad. Sci. U. S. A.* 108, 3017–22. doi: 10.1073/pnas.1015950108.

Falck, R. S., Davis, J. C., Best, J. R., Crockett, R. A., and Liu-Ambrose, T. (2019). Impact of exercise training on physical and cognitive function among older adults: a systematic review and meta-analysis. *Neurobiol. Aging* 79, 119–130. doi: 10.1016/J.NEUROBIOLAGING.2019.03.007.

Falck, R. S., Percival, A. G., Tai, D., and Davis, J. C. (2022). International depiction of the cost of functional independence limitations among older adults living in the community: a systematic review and cost-of-impairment study. *BMC Geriatr.* 22, 815. doi: 10.1186/S12877-022-03466-W.

Frontera, W. R., and Ochala, J. (2015). Skeletal muscle: a brief review of structure and function. *Calcif. Tissue Int.* 96, 183–195. doi: 10.1007/S00223-014-9915-Y.

Gaitán, J. M., Moon, H. Y., Stremlau, M., Dubal, D. B., Cook, D. B., Okonkwo, O. C., et al. (2021). Effects of Aerobic Exercise Training on Systemic Biomarkers and Cognition in Late Middle-Aged Adults at Risk for Alzheimer's Disease. *Front. Endocrinol. (Lausanne).* 12, 660181. doi: 10.3389/FENDO.2021.660181.

Gallardo-Gómez, D., del Pozo-Cruz, J., Noetel, M., Álvarez-Barbosa, F., Alfonso-Rosa, R. M., and del Pozo Cruz, B. (2022). Optimal dose and type of exercise to improve cognitive function in older adults: A systematic review and bayesian model-based network meta-analysis of RCTs. *Ageing Res. Rev.* 76, 101591. doi: 10.1016/J.ARR.2022.101591.

Görgens, S. W., Eckardt, K., Jensen, J., Drevon, C. A., and Eckel, J. (2015). Exercise and Regulation of Adipokine and Myokine Production. in *Progress in Molecular Biology and Translational Science* (Elsevier B.V.), 313–336. doi: 10.1016/bs.pmbts.2015.07.002.

Grassi-Oliveira, R., Stein, L. M., Lopes, R. P., Teixeira, A. L., and Bauer, M. E. (2008). Low plasma brain-derived neurotrophic factor and childhood physical neglect are associated with verbal memory impairment in major depression--a preliminary report. *Biol. Psychiatry* 64, 281–285. doi: 10.1016/J.BIOPSYCH.2008.02.023.

Griebler, N., Schröder, N., Artifon, M., Frigotto, M., and Pietta-Dias, C. (2022). The effects of acute exercise on memory of cognitively healthy seniors: A systematic review. *Arch. Gerontol. Geriatr.* 99, 104583. doi: 10.1016/J.ARCHGER.2021.104583.

Guan, J., Bennet, L., Gluckman, P. D., and Gunn, A. J. (2003). Insulin-like growth factor-1 and post-ischemic brain injury. *Prog. Neurobiol.* 70, 443–462. doi: 10.1016/j.pneurobio.2003.08.002.

Gubert, C., and Hannan, A. J. (2021). Exercise mimetics: harnessing the therapeutic effects of physical activity. *Nat. Rev. Drug Discov. 2021* 20, 862–879. doi: 10.1038/S41573-021-00217-1.

Gulick, C. N., Peddie, M., and Rehrer, N. J. (2020). Does Exercise Impact Insulin-like Growth Factor 1? : Systematic Review & Meta-Analysis. *Open J. Heal. Sci. Med.* 1, 104. doi: 10.0000/OJHSM.1000104.

Hartwig, S., Raschke, S., Knebel, B., Scheler, M., Irmler, M., Passlack, W., et al. (2014). Secretome profiling of primary human skeletal muscle cells. *Biochim. Biophys. Acta - Proteins Proteomics* 1844, 1011–1017. doi: 10.1016/J.BBAPAP.2013.08.004.

Hawley, J. A., Hargreaves, M., Joyner, M. J., and Zierath, J. R. (2014). Integrative biology of exercise. *Cell* 159, 738–749. doi: 10.1016/J.CELL.2014.10.029.

Hedges, L. V., Tipton, E., and Johnson, M. C. (2010). Robust variance estimation in meta-regression with dependent effect size estimates. *Res. Synth. Methods* 1, 39–65. doi: 10.1002/JRSM.5.

Higgins, J. P. T., Altman, D. G., Gøtzsche, P. C., Jüni, P., Moher, D., Oxman, A. D., et al. (2011). The Cochrane Collaboration's tool for assessing risk of bias in randomised trials. *BMJ* 343, d5928. doi: 10.1136/BMJ.D5928.

Hittel, D. S., Berggren, J. R., Shearer, J., Boyle, K., and Houmard, J. A. (2009). Increased Secretion and Expression of Myostatin in Skeletal Muscle From Extremely Obese Women. *Diabetes* 58, 30–38. doi: 10.2337/DB08-0943.

Hou, Y., Dan, X., Babbar, M., Wei, Y., Hasselbalch, S. G., Croteau, D. L., et al. (2019). Ageing as a risk factor for neurodegenerative disease. *Nat. Rev. Neurol.* 15, 565–581. doi: 10.1038/S41582-019-0244-7.

Hsieh, S.-S., Huang, C.-J., Wu, C.-T., Chang, Y.-K., and Hung, T.-M. (2018). Acute Exercise Facilitates the N450 Inhibition Marker and P3 Attention Marker during Stroop Test in Young and Older Adults. *J. Clin. Med.* 7. doi: 10.3390/jcm7110391.

Huang, E. J., and Reichardt, L. F. (2001). Neurotrophins: Roles in Neuronal Development and Function. *Annu. Rev. Neurosci.* 24, 736. doi: 10.1146/ANNUREV.NEURO.24.1.677.

Huang, H., Li, W., Qin, Z., Shen, H., Li, X., and Wang, W. (2021). Physical exercise increases peripheral brain-derived neurotrophic factors in patients with cognitive impairment: A meta-analysis. *Restor. Neurol. Neurosci.* 39, 159–171. doi: 10.3233/RNN-201060.

Hultman, R., Kumari, U., Michel, N., and Casey, P. J. (2014). Gαz regulates BDNF-induction of axon growth in cortical neurons. *Mol. Cell. Neurosci.* 58, 53–61. doi: 10.1016/J.MCN.2013.12.004.

Ide, K., Schmalbruch, I. K., Quistorff, B., Horn, A., and Secher, N. H. (2000). Lactate, glucose and O2 uptake in human brain during recovery from maximal exercise. *J. Physiol.* 522 Pt 1, 159–64. doi: 10.1111/j.1469-7793.2000.t01-2-00159.xm.

Islam, H., Neudorf, H., Mui, A. L., and Little, J. P. (2021). Interpreting "anti-inflammatory" cytokine responses to exercise: Focus on interleukin-10. *J. Physiol.* doi: 10.1113/JP281356.

Jackson, J. L., and Kuriyama, A. (2019). How Often Do Systematic Reviews Exclude Articles Not Published in English? *J. Gen. Intern. Med.* 34, 1389. doi: 10.1007/S11606-019-04976-X.

Jak, S., and Cheung, M. W. L. (2020). Meta-analytic structural equation modeling with moderating effects on SEM parameters. *Psychol. Methods* 25, 430–455. doi: 10.1037/MET0000245.

Jak, S., Li, H., Kolbe, L., de Jonge, H., and Cheung, M. W. L. (2021). Meta-analytic structural equation modeling made easy: A tutorial and web application for one-stage MASEM. *Res. Synth. Methods* 12, 590–606. doi: 10.1002/JRSM.1498.

Jakicic, J. M., Clark, K., Coleman, E., Donnelly, J. E., Foreyt, J., Melanson, E., et al. (2001). American College of Sports Medicine position stand. Appropriate intervention strategies for weight loss and prevention of weight regain for adults. *Med. Sci. Sports Exerc.* 33, 2145–2156. doi: 10.1097/00005768-200112000-00026.

Jiang, Q., Lou, K., Hou, L., Lu, Y., Sun, L., Tan, S. C., et al. (2020). The effect of resistance training on serum insulin-like growth factor 1(IGF-1): A systematic review and meta-analysis. *Complement. Ther. Med.* 50, 102360. doi: 10.1016/j.ctim.2020.102360.

Jonasson, L. S., Nyberg, L., Axelsson, J., Kramer, A. F., Riklund, K., and Boraxbekk, C. J. (2019). Higher striatal D2-receptor availability in aerobically fit older adults but non-selective intervention effects after aerobic versus resistance training. *Neuroimage* 202, 116044. doi: 10.1016/J.NEUROIMAGE.2019.116044.

Kasper, J. D. (1990). Cognitive Impairment among Functionally Limited Elderly People in the Community: Future Considerations for Long-Term Care Policy. *Milbank Q.* 68, 109. doi: 10.2307/3350078.

Kuhne, L. A., Ksiezarczyk, A. M., Braumann, K. M., Reer, R., Jacobs, T., Röder, B., et al. (2023). Cardiovascular exercise, learning, memory, and cytokines: Results of a ten-week randomized controlled training study in young adults. *Biol. Psychol.* 176, 108466. doi: 10.1016/J.BIOPSYCHO.2022.108466.

Kwon, J. H., Moon, K. M., and Min, K.-W. (2020). Exercise-Induced Myokines can Explain the Importance of Physical Activity in the Elderly: An Overview. *Healthcare* 8, 378. doi: 10.3390/healthcare8040378.

Le Bihan, M. C., Bigot, A., Jensen, S. S., Dennis, J. L., Rogowska-Wrzesinska, A., Lainé, J., et al. (2012). In-depth analysis of the secretome identifies three major independent secretory pathways in differentiating human myoblasts. *J. Proteomics* 77, 344–356. doi: 10.1016/J.JPROT.2012.09.008.

Lee, J. H., and Jun, H. S. (2019). Role of Myokines in Regulating Skeletal Muscle Mass and Function. *Front. Physiol.* 10, 42. doi: 10.3389/FPHYS.2019.00042.

Lerche, S., Gutfreund, A., Brockmann, K., Hobert, M. A., Wurster, I., Sünkel, U., et al. (2018). Effect of physical activity on cognitive flexibility, depression and RBD in healthy elderly. *Clin. Neurol. Neurosurg.* 165, 88–93. doi: 10.1016/J.CLINEURO.2018.01.008.

Lev-Vachnish, Y., Cadury, S., Rotter-Maskowitz, A., Feldman, N., Roichman, A., Illouz, T., et al. (2019). L-lactate promotes adult hippocampal neurogenesis. *Front. Neurosci.* 13, 403. doi: 10.3389/FNINS.2019.00403/FULL.

Levin, O., Netz, Y., and Ziv, G. (2017). The beneficial effects of different types of exercise interventions on motor and cognitive functions in older age: a systematic review. *Eur. Rev. aging Phys. Act.* 14, 20. doi: 10.1186/s11556-017-0189-z.

Levin, O., Netz, Y., and Ziv, G. (2021). Behavioral and Neurophysiological Aspects of Inhibition—The Effects of Acute Cardiovascular Exercise. *J. Clin. Med.* 10, 282. doi: 10.3390/JCM10020282.

Liang, Y. Y., Zhang, L. D., Luo, X., Wu, L. L., Chen, Z. W., Wei, G. H., et al. (2022). All roads lead to Rome - a review of the potential mechanisms by which exerkines exhibit neuroprotective effects in Alzheimer's disease. *Neural Regen. Res.* 17, 1210–1227. doi: 10.4103/1673-5374.325012.

Lin, T. Y., Hsieh, S. S., Chueh, T. Y., Huang, C. J., and Hung, T. M. (2021). The effects of barbell resistance exercise on information processing speed and conflict-related ERP in older adults: a crossover randomized controlled trial. *Sci. Reports 2021 111* 11, 1–14. doi: 10.1038/s41598-021-88634-5.

Livingston, G., Huntley, J., Sommerlad, A., Ames, D., Ballard, C., Banerjee, S., et al. (2020). Dementia prevention, intervention, and care: 2020 report of the Lancet Commission. *Lancet Comm.* 396, 413–446. doi: 10.1016/S0140-6736(20)30367-6/ATTACHMENT/CEE43A30-904B-4A45-A4E5-AFE48804398D/MMC1.PDF.

López-Valenciano, A., Mayo, X., Liguori, G., Copeland, R. J., Lamb, M., and Jimenez, A. (2020). Changes in sedentary behaviour in European Union adults between 2002 and 2017. *BMC Public Health* 20, 1–10. doi: 10.1186/S12889-020-09293-1/TABLES/3.

Maddock, R. J., Casazza, G. A., Fernandez, D. H., and Maddock, M. I. (2016). Acute Modulation of Cortical Glutamate and GABA Content by Physical Activity. *J. Neurosci.* 36, 2449–2457. doi: 10.1523/JNEUROSCI.3455-15.2016.

Máderová, D., Krumpolec, P., Slobodová, L., Schön, M., Tirpáková, V., Kovaničová, Z., et al. (2019). Acute and regular exercise distinctly modulate serum, plasma and skeletal muscle BDNF in the elderly. *Neuropeptides* 78. doi: 10.1016/j.npep.2019.101961.

Matthews, V. B., Åström, M. B., Chan, M. H. S., Bruce, C. R., Krabbe, K. S., Prelovsek, O., et al. (2009). Brain-derived neurotrophic factor is produced by skeletal muscle cells in response to contraction and enhances fat oxidation via activation of AMP-activated protein kinase. *Diabetologia* 52, 1409–1418. doi: 10.1007/s00125-009-1364-1.

Mattiuzzi, C., and Lippi, G. (2020). Worldwide disease epidemiology in the older persons. *Eur. Geriatr. Med.* 11, 147–153. doi: 10.1007/S41999-019-00265-2/TABLES/4.

McArdle, J. J., and McDonald, R. P. (1984). Some algebraic properties of the Reticular Action Model for moment structures. *Br. J. Math. Stat. Psychol.* 37, 234–251. doi: 10.1111/J.2044-8317.1984.TB00802.X.

McGrath, R. E., and Meyer, G. J. (2006). When effect sizes disagree: the case of r and d. *Psychol. Methods* 11, 386–401. doi: 10.1037/1082-989X.11.4.386.

McGregor, C. E., and English, A. W. (2019). The Role of BDNF in Peripheral Nerve Regeneration: Activity-Dependent Treatments and Val66Met. *Front. Cell. Neurosci.* 12, 522. doi: 10.3389/FNCEL.2018.00522.

Micielska, K., Kortas, J. A., Gmiat, A., Jaworska, J., Kozlowska, M., Lysak-Radomska, A., et al. (2021). Habitually inactive physically – a proposed procedure of counteracting cognitive decline in women with diminished insulin sensitivity through a high-intensity circuit training program. *Physiol. Behav.* 229, 113235. doi: 10.1016/j.physbeh.2020.113235.

Mizuno, M., Yamada, K., Olariu, A., Nawa, H., and Nabeshima, T. (2000). Involvement of brain-derived neurotrophic factor in spatial memory formation and maintenance in a radial arm maze test in rats. *J. Neurosci.* 20, 7116–7121. doi: 10.1523/JNEUROSCI.20-18-07116.2000.

Moher, D., Shamseer, L., Clarke, M., Ghersi, D., Liberati, A., Petticrew, M., et al. (2016). Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. *Rev. Esp. Nutr. Humana y Diet.* 20, 148–160. doi: 10.1186/2046-4053-4-1/TABLES/4.

Moon, H. Y., Becke, A., Berron, D., Becker, B., Sah, N., Benoni, G., et al. (2016). Running-Induced Systemic Cathepsin B Secretion Is Associated with Memory Function. *Cell Metab.* 24, 332–340. doi: 10.1016/j.cmet.2016.05.025.

Neale, M. C., and Miller, M. B. (1997). The use of likelihood-based confidence intervals in genetic models. *Behav. Genet.* 27, 113–120. doi: 10.1023/A:1025681223921/METRICS.

Nemoto, M., Sasai, H., Yabushita, N., Tsuchiya, K., Hotta, K., Fujita, Y., et al. (2020). A Novel Exercise for Enhancing Visuospatial Ability in Older Adults with Frailty: Development, Feasibility, and Effectiveness. *Geriatr. (Basel, Switzerland)* 5, 29. doi: 10.3390/GERIATRICS5020029.

Netz, Y. (2019). Is There a Preferred Mode of Exercise for Cognition Enhancement in Older Age?-A Narrative Review. *Front. Med.* 6, 57. doi: 10.3389/fmed.2019.00057.

Nichols, E., Steinmetz, J. D., Vollset, S. E., Fukutaki, K., Chalek, J., Abd-Allah, F., et al. (2022). Estimation of the global prevalence of dementia in 2019 and forecasted prevalence in 2050: an analysis for the Global Burden of Disease Study 2019. *Lancet Public Heal.* 7, e105–e125. doi: 10.1016/S2468-2667(21)00249-8.

Norheim, F., Raastad, T., Thiede, B., Rustan, A. C., Drevon, C. A., and Haugen, F. (2011). Proteomic identification of secreted proteins from human skeletal muscle cells and expression in response to strength training. *Am. J. Physiol. - Endocrinol. Metab.* 301, E1013–E1021. doi: 10.1152/ajpendo.00326.2011.

Onyango, I. G., Jauregui, G. V., Čarná, M., Bennett, J. P., and Stokin, G. B. (2021). Neuroinflammation in Alzheimer's Disease. *Biomed. 2021, Vol. 9, Page 524* 9, 524. doi: 10.3390/BIOMEDICINES9050524.

Oudbier, S. J., Goh, J., Looijaard, S. M. L. M., Reijnierse, E. M., Meskers, C. G. M., and Maier, A. B. (2022). Pathophysiological Mechanisms Explaining the Association Between Low Skeletal Muscle Mass and Cognitive Function. *Journals Gerontol. Ser. A* 77, 1959–1968. doi: 10.1093/GERONA/GLAC121.

Ouzzani, M., Hammady, H., Fedorowicz, Z., and Elmagarmid, A. (2016). Rayyan - a web and mobile app for systematic reviews. *Syst. Rev.* 6, 210. doi: 10.1186/s13643-016-0384-4.

Pan, W., Banks, W. A., Fasold, M. B., Bluth, J., and Kastin, A. J. (1998). Transport of brain-derived neurotrophic factor across the blood-brain barrier. *Neuropharmacology* 37, 1553–1561. doi: 10.1016/S0028-3908(98)00141-5.

Parada-Sánchez, S. G., Macias-Cervantes, M. H., Pérezvázquez, V., and Vargas-Ortiz, K. (2022). The Effects of Different Types of Exercise on Circulating Irisin Levels in Healthy Individuals and in People With Overweight, Metabolic Syndrome and Type 2 Diabetes. *Physiol. Res.* 71, 457–475. doi: 10.33549/PHYSIOLRES.934896.

Pedersen, B. K. (2019). Physical activity and muscle–brain crosstalk. *Nat. Rev. Endocrinol.* 15, 383–392. doi: 10.1038/s41574-019-0174-x.

Pedersen, B. K., and Febbraio, M. A. (2008). Muscle as an endocrine organ: Focus on muscle-derived interleukin-6. *Physiol. Rev.* 88, 1379–1406. doi: 10.1152/physrev.90100.2007.

Pedersen, B. K., and Fischer, C. P. (2007). Beneficial health effects of exercise--the role of IL-6 as a myokine. *Trends Pharmacol. Sci.* 28, 152–156. doi: 10.1016/J.TIPS.2007.02.002.

Pedersen, B. K., Steensberg, A., Fischer, C., Keller, C., Keller, P., Plomgaard, P., et al. (2003). Searching for the exercise factor: Is IL-6 a candidate? in *Journal of Muscle Research and Cell Motility* (J Muscle Res Cell Motil), 113–119. doi: 10.1023/A:1026070911202.

Piccirillo, R. (2019). Exercise-induced myokines with therapeutic potential for muscle wasting. *Front. Physiol.* 10, 287. doi: 10.3389/FPHYS.2019.00287/BIBTEX.

Pieper, D., and Puljak, L. (2021). Language restrictions in systematic reviews should not be imposed in the search strategy but in the eligibility criteria if necessary. *J. Clin. Epidemiol.* 132, 146–147. doi: 10.1016/j.jclinepi.2020.12.027.

Pourteymour, S., Eckardt, K., Holen, T., Langleite, T., Lee, S., Jensen, J., et al. (2017). Global mRNA sequencing of human skeletal muscle: Search for novel exercise-regulated myokines. *Mol. Metab.* 6, 352–365. doi: 10.1016/J.MOLMET.2017.01.007.

Qi, D., Wong, N. M. L., Shao, R., Man, I. S. C., Wong, C. H. Y., Yuen, L. P., et al. (2021). Qigong exercise enhances cognitive functions in the elderly via an interleukin-6-hippocampus pathway: A randomized active-controlled trial. *Brain. Behav. Immun.* 95, 381–390. doi: 10.1016/J.BBI.2021.04.011.

Rai, M., and Demontis, F. (2022). Muscle-to-Brain Signaling Via Myokines and Myometabolites. *Brain Plast.* 8, 43–63. doi: 10.3233/BPL-210133.

Ramoo, K., Hairi, N. N., Yahya, A., Choo, W. Y., Hairi, F. M., Peramalah, D., et al. (2022). Longitudinal Association between Sarcopenia and Cognitive Impairment among Older Adults in Rural Malaysia. *Int. J. Environ. Res. Public Health* 19, 4723. doi: 10.3390/IJERPH19084723/S1.

Raschke, S., Eckardt, K., Bjørklund Holven, K., Jensen, J., and Eckel, J. (2013). Identification and Validation of Novel Contraction-Regulated Myokines Released from Primary Human Skeletal Muscle Cells. *PLoS One* 8, e62008. doi: 10.1371/JOURNAL.PONE.0062008.

Sada, N., Lee, S., Katsu, T., Otsuki, T., and Inoue, T. (2015). Epilepsy treatment. Targeting LDH enzymes with a stiripentol analog to treat epilepsy. *Science (80-.).* 347, 1362–1367. doi: 10.1126/SCIENCE.AAA1299.

Sanford, J. A., Nogiec, C. D., Lindholm, M. E., Adkins, J. N., Amar, D., Dasari, S., et al. (2020). Molecular Transducers of Physical Activity Consortium (MoTrPAC): Mapping the Dynamic Responses to Exercise. *Cell* 181, 1464–1474. doi: 10.1016/J.CELL.2020.06.004.

Schättin, A., Arner, R., Gennaro, F., and de Bruin, E. D. (2016). Adaptations of Prefrontal Brain Activity, Executive Functions, and Gait in Healthy Elderly Following Exergame and Balance Training: A Randomized-Controlled Study. *Front. Aging Neurosci.* 8, 278. doi: 10.3389/FNAGI.2016.00278.

Scheler, M., Irmler, M., Lehr, S., Hartwig, S., Staiger, H., Al-Hasani, H., et al. (2013). Cytokine response of primary human myotubes in an in vitro exercise model. *Am. J. Physiol. - Cell Physiol.* 305, 877–886. doi: 10.1152/AJPCELL.00043.2013/ASSET/IMAGES/LARGE/ZH00201373480005.JPEG.

Scisciola, L., Fontanella, R. A., Surina, Cataldo, V., Paolisso, G., and Barbieri, M. (2021). Sarcopenia and cognitive function: Role of myokines in muscle brain cross-talk. *Life* 11, 1–12. doi: 10.3390/life11020173.

Seifert, T., Brassard, P., Wissenberg, M., Rasmussen, P., Nordby, P., Stallknecht, B., et al. (2010). Endurance training enhances BDNF release from the human brain. *Am. J. Physiol. - Regul. Integr. Comp. Physiol.* 298, 372–377. doi: 10.1152/ajpregu.00525.2009.

Shamseer, L., Moher, D., Clarke, M., Ghersi, D., Liberati, A., Petticrew, M., et al. (2015). Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015: elaboration and explanation OPEN ACCESS. *BMJ* 349, g7647. doi: 10.1136/bmj.g7647.

Sheoran, S., Vints, W. A. J., Valatkevičienė, K., Kušleikienė, S., Gleiznienė, R., Česnaitienė, V. J., et al. (2023). Strength gains after 12 weeks of resistance training correlate with neurochemical markers of brain health in older adults: a randomized control 1H-MRS study. *GeroScience*, 1–19. doi: 10.1007/S11357-023-00732-6.

Shojania, K. G., Sampson, M., Ansari, M. T., Ji, J., Doucette, S., and Moher, D. (2007). How quickly do systematic reviews go out of date? A survival analysis. *Ann. Intern. Med.* 147, 224–233. doi: 10.7326/0003-4819-147-4-200708210-00179.

Solianik, R., Brazaitis, M., and Cekanauskaite-Krušnauskien, A. (2022). Tai chi effects on balance in older adults: the role of sustained attention and myokines. *J. Sports Med. Phys. Fitness* 62, 1512–1518. doi: 10.23736/S0022-4707.21.12990-1.

Stein, A. M., Silva, T. M. V., Coelho, F. G. de M., Arantes, F. J., Costa, J. L. R., Teodoro, E., et al. (2018). Physical exercise, IGF-1 and cognition: A systematic review of experimental studies in the elderly. *Dement. e Neuropsychol.* 12, 114–122. doi: 10.1590/1980-57642018dn12-020003.

Suzuki, A., Stern, S. A., Bozdagi, O., Huntley, G. W., Walker, R. H., Magistretti, P. J., et al. (2011). Astrocyte-neuron lactate transport is required for long-term memory formation. *Cell* 144, 810–23. doi: 10.1016/j.cell.2011.02.018.

Szuhany, K. L., Bugatti, M., and Otto, M. W. (2015). A meta-analytic review of the effects of exercise on brain-derived neurotrophic factor. *J. Psychiatr. Res.* 60, 56. doi: 10.1016/J.JPSYCHIRES.2014.10.003.

Tam, A. C. Y., Chan, A. W. Y., Cheung, D. S. K., Ho, L. Y. W., Tang, A. S. K., Christensen, M., et al. (2022). The effects of interventions to enhance cognitive and physical functions in older people with cognitive frailty: a systematic review and meta-analysis. *Eur. Rev. Aging Phys. Act.* 19, 19. doi: 10.1186/S11556-022-00299-9.

Tremblay, M. S., Aubert, S., Barnes, J. D., Saunders, T. J., Carson, V., Latimer-Cheung, A. E., et al. (2017). Sedentary Behavior Research Network (SBRN) - Terminology Consensus Project process and outcome. *Int. J. Behav. Nutr. Phys. Act.* 14, 1–17. doi: 10.1186/S12966-017-0525-8/FIGURES/3.

Vaynman, S., Ying, Z., and Gómez-Pinilla, F. (2004). Exercise Induces BDNF and Synapsin I to Specific Hippocampal Subfields. *J. Neurosci. Res.* 76, 356–362. doi: 10.1002/jnr.20077.

Vints, W. A. J., Kušleikienė, S., Sheoran, S., Šarkinaitė, M., Valatkevičienė, K., Gleiznienė, R., et al. (2022a). Inflammatory blood biomarker kynurenine is linked with elevated neuroinflammation and neurodegeneration in older adults: evidence from two 1H-MRS post-processing analysis methods. *Front. Psychiatry* 13, 859772. doi: 10.3389/FPSYT.2022.859772.

Vints, W. A. J., Levin, O., Fujiyama, H., Verbunt, J., and Masiulis, N. (2022b). Exerkines and long-term synaptic potentiation: Mechanisms of exercise-induced neuroplasticity. *Front. Neuroendocrinol.* 66, 100993. doi: 10.1016/J.YFRNE.2022.100993.

Webb, S. L., Loh, V., Lampit, A., Bateman, J. E., and Birney, D. P. (2018). Meta-Analysis of the Effects of Computerized Cognitive Training on Executive Functions: a Cross-Disciplinary Taxonomy for Classifying Outcome Cognitive Factors. *Neuropsychol. Rev.* 28, 232–250. doi: 10.1007/S11065-018-9374-8.

Weigert, C., Lehmann, R., Hartwig, S., and Lehr, S. (2014). The secretome of the working human skeletal muscle—A promising opportunity to combat the metabolic disaster? *PROTEOMICS – Clin. Appl.* 8, 5–18. doi: 10.1002/PRCA.201300094.

Whitham, M., and Febbraio, M. A. (2016). The ever-expanding myokinome: discovery challenges and therapeutic implications. *Nat. Rev. Drug Discov. 2016 1510* 15, 719–729. doi: 10.1038/nrd.2016.153.

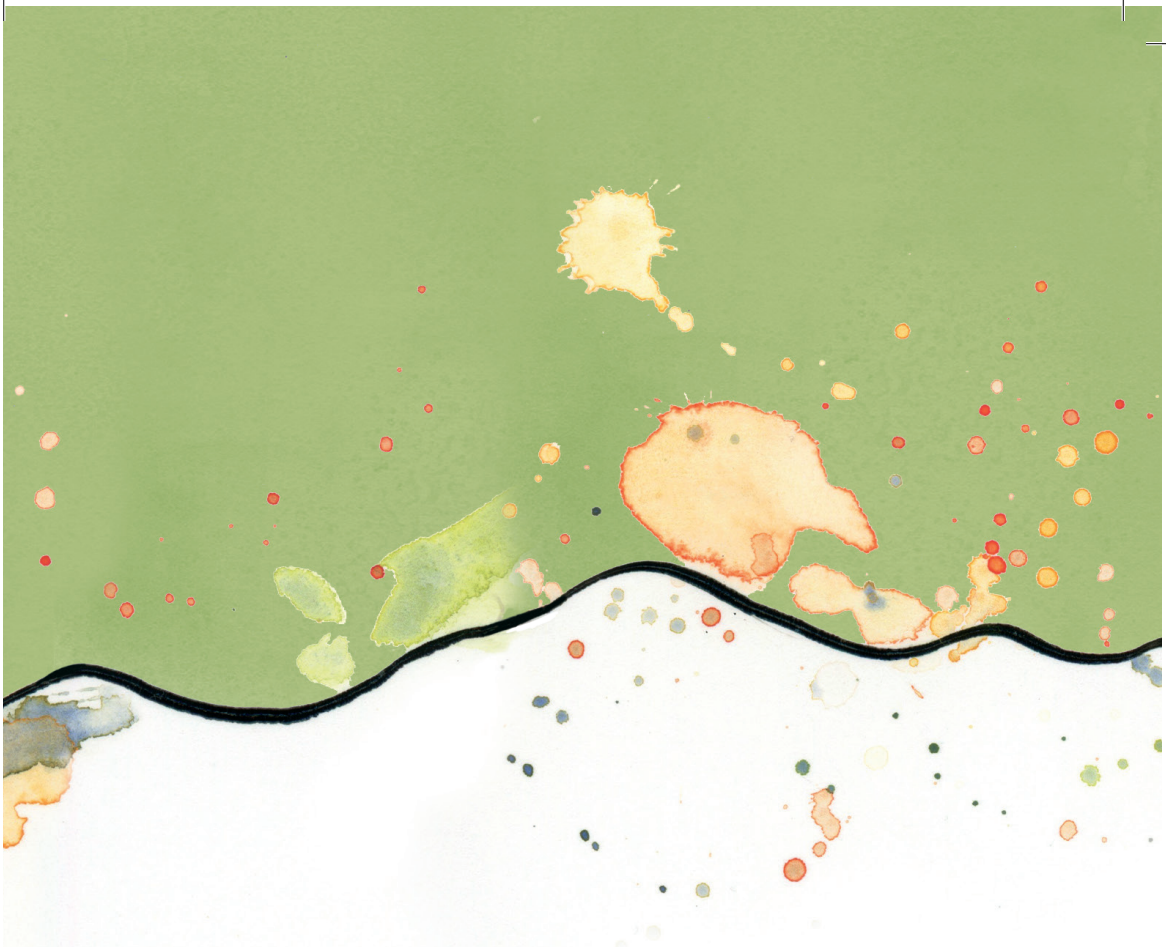
World Health Organization (WHO) (n.d.). Global Health Estimates: Life expectancy and healthy life expectancy. Available at: https://www.who.int/data/gho/data/themes/mortality-and-global-health-estimates/ghe-life-expectancy-and-healthy-life-expectancy [Accessed April 7, 2023].

Wrann, C. D., White, J. P., Salogiannnis, J., Laznik-Bogoslavski, D., Wu, J., Ma, D., et al. (2013). Exercise induces hippocampal BDNF through a PGC-1α/FNDC5 pathway. *Cell Metab.* 18, 649–659. doi: 10.1016/j.cmet.2013.09.008.

Wu, J., Wang, X., Ye, M., Wang, L., and Zheng, G. (2021). Effect of regular resistance training on memory in older adults: A systematic review. *Exp. Gerontol.* 150, 111396. doi: 10.1016/J.EXGER.2021.111396.

Zhang, Y. R., Xu, W., Zhang, W., Wang, H. F., Ou, Y. N., Qu, Y., et al. (2022). Modifiable risk factors for incident dementia and cognitive impairment: An umbrella review of evidence. *J. Affect. Disord.* 314, 160–167. doi: 10.1016/J.JAD.2022.07.008.

Zhao, Y., Li, Y., Wang, L., Song, Z., Di, T., Dong, X., et al. (2022). Physical Activity and Cognition in Sedentary Older Adults: A Systematic Review and Meta-Analysis. *J. Alzheimer's Dis.* 87, 957–968. doi: 10.3233/JAD-220073.



PART II

The relationship between participant characteristics, blood and brain biomarkers and cognitive function in older adults

CHAPTER 3

Inflammatory blood biomarker kynurenine is linked with elevated neuroinflammation and neurodegeneration in older adults: evidence from two 1H-MRS post-processing analysis methods

Authors: Wouter A.J. Vints, Simona Kušleikienė, Samrat Sheoran, Milda Šarkinaitė, Kristina Valatkevičienė, Rymantė Gleiznienė, Mindaugas Kvedaras, Kazimieras Pukėnas, Uwe Himmelreich, Vida J. Česnaitienė, Oron Levin, Jeanine Verbunt, Nerijus Masiulis

Published in Frontiers in Psychiatry, 2022, Vol. 13, p. 859772
https://doi.org/10.3389/fpsyt.2022.859772

Abstract

Rationale and Objectives: Pro-inflammatory processes have been argued to play a role in conditions associated with cognitive decline and neurodegeneration, like aging and obesity. Only a limited amount of studies have tried to measure both peripheral and central biomarkers of inflammation and examined their interrelationship. The primary aim of this study was to examine the hypothesis that chronic peripheral inflammation would be associated with neurometabolic changes that indicate neuroinflammation (the combined elevation of myoinositol and choline), brain gray matter volume decrease and lower cognitive functioning in older adults.

Materials and methods: Seventy-four older adults underwent bio-impedance body composition analysis, cognitive testing with the Montreal Cognitive Assessment (MoCA), blood serum analysis of inflammatory markers interleukin-6 (IL-6) and kynurenine, magnetic resonance imaging (MRI) and proton magnetic resonance spectroscopy (^{1}H-MRS) of the brain. Neurometabolic findings from both Tarquin and LCModel ^{1}H-MRS post-processing software packages were compared. Regions of interest for MRI and ^{1}H-MRS measurements were dorsal posterior cingulate cortex (DPCC), left hippocampal cortex (HPC), left medial temporal cortex (MTC), left primary sensorimotor cortex (SM1) and right dorsolateral prefrontal cortex (DLPFC).

Results: Elevated serum kynurenine levels were associated with signs of neuroinflammation, specifically in the DPCC, left SM1 and right DLPFC, and signs of neurodegeneration, specifically in the left HPC, left MTC and left SM1, after adjusting for age, sex and fat %. Elevated serum IL-6 levels were associated with increased Glx levels in left HPC, left MTC, and right DLPFC, after processing the ^{1}H-MRS data with Tarquin. Overall, the agreement between Tarquin and LCModel results was moderate to strong for tNAA, tCho, mIns and tCr, but weak to very weak for Glx. Peripheral inflammatory markers (IL-6 and kynurenine) were not associated with older age, higher fat %, decreased brain gray matter volume loss or decreased cognitive functioning within a cohort of older adults.

Conclusion: Our results suggest that serum kynurenine may be used as a peripheral inflammatory marker that is associated with neuroinflammation and neurodegeneration, although not linked to cognition. Future studies should consider longitudinal analysis to assess the causal inferences between chronic peripheral and neuroinflammation, brain structural and neurometabolic changes, and cognitive decline in aging.

Abbreviations

^{1}H-MRS, proton magnetic resonance spectroscopy; BMI, body mass index; CRP, C-reactive protein; DLPFC, dorsolateral prefrontal cortex; DPCC, dorsal posterior cingulate cortex; FDR, false discovery rate; Fat %, body fat percentage; Glx, glutamate-glutamine complex; GMV, gray matter volume; HPC, hippocampal cortex; ICC, intraclass correlation coefficient; IL-6, interleukin-6; IL-8, interleukin-8; mIns, myoinositol; MoCA, Montreal Cognitive Assessment; MTC, medial temporal cortex; SM1, primary sensorimotor cortex; tCho, total choline; tCr, total creatine; tNAA, total N-acetyl aspartate; TNF- α, tumor necrosis factor-α.

1. Introduction

Looking back at how the world's population has changed in the last four decades, two major trends have been noticed; 1) the increase in the proportion of older adults and 2) the increase in the prevalence of overweight and obesity. Indeed, first of all, the subpopulation of older adults is rapidly increasing worldwide and is prospected to continue to increase (1). With it, the increasing amount of care-needing elderly places high demands on our health care services (1). At the same time, there is a shift in the prevalence of diseases in the group of older adults showing an increase in chronic care-demanding illnesses. A comparison of diseases in older adults in 2016 showed that dementias displayed the largest increase as a cause of disability and second largest increase as a cause of mortality in the last 16 years (2). Importantly, Mayeux and Stern (2012) hypothesized that the burden of dementia, with Alzheimer's disease as the leading cause of dementia, will double every 20 years until at least 2040 (3). Secondly, a dramatic increase in the prevalence of overweight and obesity has taken place in the last four decades. At this rate, the majority of the world's adult population will be overweight or obese by 2030 (4). Of interest, not only older age, but also obesity has been associated with cognitive decline, as was reported after adjusting for age and educational level (5). Moreover, obese individuals were found to have an increased risk of Alzheimer's disease (6). Furthermore, life-style interventions targeted to reduce obesity, like regular physical exercise and a healthy diet, are also suggested to be promising strategies to prevent cognitive decline and dementia (7).

A common mechanism that has been suggested to be a major player in age- and obesity-related cognitive decline is a chronic state of inflammation (8)(9)(10). Studies have measured increased levels of inflammatory markers and activated immune cells in blood samples and tissue biopsies in old and obese individuals (11)(12). In the context of aging, 'inflammaging' refers to the gradual transition into a chronic pro-inflammatory stage that is reported when people get older (11). It is thought that inflammaging arises at least

partly from the release of inflammatory markers by senescent cells, which accumulate over time (13). The senescence program makes old cells stop dividing to prevent malignant transformation (14), but also alters secretory activity so that they start releasing a range of inflammatory markers and attract and activate immune cells (12). In the context of obesity, chronic inflammation is suggested to arise from adipose cells. Especially from visceral fat, which is considered one of the largest contributors to the release of inflammatory markers in the human body (15)(16).

Of importance, these signs of elevated inflammation can also be found in the brain, both with aging (17) and obesity (18). Within the central nervous system, glial cells, which include microglia and astrocytes, are the main immune cells involved in local neuroinflammation (17). Stereological findings have shown an age-related increase in the number of glial cells in the frontal and temporal cortex (19). Moreover, studies have shown that microglia change to a more proinflammatory phenotype with age (20)(21). In the context of obesity, neuroinflammation in the hypothalamus is consistently reported (9), but also other brain regions are affected, such as the hippocampus, amygdala, cerebral cortex and cerebellum, as has been shown in rodent studies (10)(22)(23)(24). In areas with increased neuroinflammation, activated microglia release inflammatory markers to attract more inflammatory cells and initiate repair mechanisms, further increasing inflammation in the brain (25)(26)(27)(28). This may cause damage to the surrounding neurons and may impair synaptic signaling, which eventually results in cognitive impairment (8)(17)(29).

It is considered that peripheral inflammation and neuroinflammation are closely linked, even though the brain and the rest of the body are separated from each other by the blood-brain barrier. Indeed, peripheral and central inflammatory signals were previously found to interact via blood and neural routes of communication (30)(31)(32)(33)(34)(35)(36)(37). For example, studies have shown that a peripheral inflammatory challenge activated microglial cells and drastically altered neural activity (35)(38). After blocking peripheral cytokine production, neuroinflammation was also altered and this was found to result in cognitive improvements (39)(40). Inversely, traumatic brain injury was reported to induce an inflammatory response in the liver, causing liver damage (41)(42)(43). Recently, the neurobiological link between peripheral inflammation, neuroinflammation and cognitive aging was tested for the first time by Lind et al. (2021). Firstly, in a previous study, they used proton magnetic resonance spectroscopy (^{1}H-MRS) to search for signs of neuroinflammation and discovered a link between cognitive aging and elevated levels of the glia-related neurometabolites total choline (tCho), myoinositol (mIns) and total creatine (tCr) in the anterior cingulate cortex, hippocampus and thalamus (45). Secondly, Lind et al. (2021) correlated these findings to blood levels of the pro-inflammatory markers C-reactive protein (CRP), interleukin (IL) -8 and tumor necrosis factor-α (TNF- α) in young, middle aged and older adults. They reported that circulating levels of CRP and IL-8 were

elevated in older adults. CRP was positively correlated to thalamic mIns levels and IL-8 was positively correlated with tCho in the anterior cingulate cortex as well as with mIns in the hippocampus. Moreover, they observed a negative correlation between CRP levels and visuo-spatial working memory performance (44).

In addition to CRP, IL-8 and TNF-α, other markers of inflammation have previously been linked to aging, obesity and cognitive impairment. For example, IL-6 is extensively studied as a marker of inflammaging and has even been called the "gerontologist's cytokine" (46). IL-6 levels are increased in older and in obese adults (47). A meta-analysis including seven longitudinal studies presented evidence that subjects with high circulating IL-6 levels were 1.42 times more likely to experience global cognitive decline after a 2-7 year follow-up (48). However, IL-6 seems also to be involved in anti-inflammatory actions and is therefore suggested to be an important regulator of the inflammatory response (49). Another very interesting inflammatory marker is kynurenine, as the enzyme indolamine 2,3 dioxygenase, which converts tryptophan into kynurenine in the liver, is upregulated in a state of high inflammation (50). Increased indolamine 2,3 dioxygenase activity and elevated levels of kynurenine were associated with higher levels of CRP, IL-1β, IL-6, IL-8, TNF-α and Interferon-gamma (IFN-γ), while the anti-inflammatory cytokines IL-4 and IL-10 counteracted the increase in kynurenine levels (51)(50)(52)(53)(54). Via this pathway, inflammation results in a reduction in tryptophan, a precursor of serotonin and may cause sickness behavior and depression (55). Furthermore, kynurenine metabolites have been shown to induce dysregulation of glutamatergic pathways, leading to excitotoxic neural damage (56). Higher levels of kynurenine were associated with obesity (57), with cognitive decline in older adults (Solvang et al., 2019) and with striatal and hippocampal volume loss in psychiatric disorders (58). In a Alzheimer mouse model, peripheral inhibition of the kynurenine pathway prevented neurodegeneration and memory deficits (59).

The primary aim of this study was to investigate the association between peripheral inflammatory markers measured from serum samples and signs of neuroinflammation measured with ^{1}H-MRS in a cohort of older adults and secondarily to relate peripheral inflammatory markers to brain gray matter volume (GMV) and cognitive function, as measured with the Montreal Cognitive Assessment (MoCA). The MoCA is considered a sensitive screening tool to evaluate the risk of mild cognitive impairment in the geriatric population (60)(61). We built our analysis starting first with assessing the relationship between the demographic characteristics of the subjects included in our study and cognitive function. Based on existing literature, we also expect to find that older age and higher body fat percentage (fat %) are associated with cognitive deficit (5)(8). Of importance, studies have also shown that cognitive performance depends on the subject's educational level, e.g. (62), and fat % greatly differs between sexes. Also in older adults fat % is generally larger in women than in men (63). Secondly, we will correlate age, fat % and cognition to peripheral inflammatory markers, brain GMV and neurometabolic changes. Here, we hypothesize that older age, higher fat %

and lower cognitive functioning are associated with higher levels of peripheral inflammation, a loss of brain GMV, a decrease in markers of neural integrity and an increase in markers of neuroinflammation. Thirdly, we test the hypothesis that higher levels of peripheral inflammation are associated with brain volume loss, and eventually our study's primary endpoint that peripheral inflammation is associated with neurometabolic changes indicating brain inflammation. This investigation will elaborate on the findings of Lind et al. (2021), since we used different peripheral serum biomarkers, added brain GMV measurements, examined more brain regions with ^{1}H-MRS and used the MoCA as a more general screening instrument for mild cognitive impairment in older adults (44). Furthermore, we took into account the effect of age, fat % and sex and excluded subjects with neurological and psychiatric disorders. Finally, quantification of neurometabolites such as myoinositol and Glx may not be consistent across vendors and algorithms (64). Therefore, we used LCModel and Tarquin, two linear-combination modeling algorithms commonly implemented for the quantification of ^{1}H-MRS spectra, to examine the robustness of our neurometabolic estimates and the predictability of their associations with the other biomarkers. Ultimately, this paper may lead to a better understanding of the expected detrimental effect of inflammation on brain health and cognitive function.

2. Material and methods

2.1 Participants and setting

A total of 74 apparently healthy adults aged 60 years and older were recruited in Kaunas, Lithuania. Recruitment strategies included presentations in local community organizations, contacting subjects from a list of patients provided by general practitioners and volunteers from previous studies. Candidates were invited for an interview in the primary care center Saulės Family Medical Center (Kaunas) before inclusion. Exclusion criteria were a diagnosis of a neurological disorder, including stroke, epilepsy, multiple sclerosis, traumatic brain injury, brain tumor or neurodegenerative diseases like dementia. Furthermore, we excluded subjects with alcohol or drug abuse, diabetes, psychiatric disorders like depression, or usage of psychopharmacological drugs in the last five years and oncologic disorders or a history of chemotherapy use. Finally, we applied the exclusion criteria for magnetic resonance imaging (MRI) studies as formulated by the checklist of the department of radiology at the Lithuanian University of Health Science in Kaunas, including metal or MR-incompatible implants and claustrophobia. Participants indicated that they did not participate in any regular exercise program in the last 6 months, but were able to perform 10 sit-ups. Participants could voluntarily withdraw from the study at any time. The study was approved by the Kaunas Regional Biomedical Research Ethics Committee (No. BE-10-7) and a written informed consent was obtained from all participants prior to their inclusion in the study.

2.2 Demographic and clinical characteristics

All participants were asked to report age, sex, smoking status and educational level (i.e. basic education, secondary education or higher education). Furthermore, the Montreal Cognitive Assessment (MoCA) test was conducted by a qualified mental health care specialist (co-author SK). The MoCA test is a brief cognitive screening instrument developed by Nasreddine et al. (2005) (60). It contains 12 items measuring seven cognitive domains: executive functioning; visuospatial abilities; language; attention, concentration and working memory; abstract reasoning; memory and orientation. All items add up to a total score with a maximum of 30 points, where a higher score indicates better cognitive functioning. This screening tool is widely used and is especially considered as a reliable and sensitive tool to evaluate the risk of mild cognitive impairment in the geriatric population (60)(61). At last, we assessed the subjects' body mass index (BMI) and measured their body fat percentage (fat %) using leg-to-leg bio-impedance analysis (Tanita TBF-300-A).

2.3 Blood analysis

Venous blood samples were drawn by a qualified medical professional at the antecubital vein. All blood samples were collected between 9 a.m. and 1 p.m. in 5 ml serum separation gel tubes. After blood collection, the tubes were gently inverted 8-10 times and kept at room temperature for 30 min until centrifugation for 15 minutes at 4000 g centrifugal force. After centrifugation, serum was aliquoted into 1.5 mL polypropylene tubes and immediately frozen and stored at -80 °C in the refrigerator compartment of the laboratory of the Lithuanian Sports University Institute of Sports Science and Innovation until further analysis. Enzyme linked immunosorbent assay (ELISA) tests for the assessment of the circulating levels of IL-6 and kynurenine were analyzed with spectrophotometry (Spark 10M, Tecan Group Ltd., Zürich, Switzerland) by an experienced researcher (co-author MK). *IL-6 measurement*. IL-6 concentrations were measured using a commercially available ELISA kit purchased from DIAsource ImmunoAssays S.A., Belgium (KAP1216). It was a sandwich immunometric assay utilizing recombinant human cytokines and antibodies raised against recombinant human cytokines. Lower limit of detection being 2 pg/ml. Absorbance was measured using a spectrophotometer at 450 nm absorbance.

Kynurenine measurement. Kynurenine concentrations were measured using a commercially available ELISA kit purchased from MyBiosource, Inc., USA. Lower limit of detection being 45.7 ng/ml.

2.4 Brain imaging and ^{1}H-MRS

Brain scanning consisted of whole brain MRI and ^{1}H-MRS with voxels in five brain regions with a total length of about 90 min per subject. MRI was performed using a Siemens 3T Skyra scanner (Siemens Healthineers, Erlangen, Germany) with a 32-channel receiver head

coil. A high-resolution T1-weighted (T1W) structural MR image (repetition time (TR) = 2200 ms, echo time (TE) = 2.48 ms, 0.9 × 0.9 × 1.0 mm^3 voxels, field of view: 230 × 256 mm, number of sagittal slices = 176) was used to acquire a 3D magnetization prepared gradient echo (MPRAGE). T2-weighted (T2W) turbo-spin echo scan, fluid-attenuated inversion recovery (FLAIR) and susceptibility weighted imaging (SWI) were reviewed for brain lesions by an experienced radiologist with more than 10 years of experience (co-author KV).

Total gray matter volume (GMV) and GMVs of dorsal posterior cingulate cortex (DPCC), left and right hippocampal cortex (HPC), left middle temporal cortex (MTC), left primary sensorimotor cortex (SM1) and right dorsolateral prefrontal cortex (DLPFC) were analyzed using FreeSurfer v7.1.1 (Harvard, MA, USA, http://surfer.nmr.mgh.harvard.edu/) on isotropic 3D T1W images of 0.9 mm slice thickness. The DPCC GMV was calculated by combining the Freesurfer region volumes right and left posterior cingulate. For left and right HPC GMV the Freesurfer regions left and right whole hippocampus were used. The left MTC GMV was calculated from middle temporal Freesurfer site of the left hemisphere. Left SM1 GMV was the combined volume of Freesurfer precentral area of frontal lobe and postcentral area of parietal lobe of the left hemisphere. Finally, the right DLPFC GMV was calculated by combining the Freesurfer region volumes of left rostral and caudal middle frontal regions.

^{1}H-MRS spectra were acquired in five voxels using a Point RESolved Spectroscopy (PRESS) sequence (TR = 2000 ms, TE = 30 ms, number of averages = 128, spectral bandwidth = 2000 Hz, data size = 1024 points) with excitation water suppression (sequence svs_se_30). The unsuppressed water signal was also acquired to measure absolute metabolite concentrations using the same acquisition parameters. The regions of interest where voxels were placed included DPCC, left HPC, left MTC, left SM1 and right DLPFC and corresponded to the regions used to calculate GMVs. The DPCC voxel was placed in the midline of both posterior parts of cingulate cortex in axial plane anterior to precuneus and parieto-occipital sulcus, corresponding to the Freesurfer sites used to calculate DPCC GMV. The hippocampal voxel was centered over the whole hippocampus in left hemisphere in the medial part of the temporal lobe anterior to lateral ventricle, corresponding to the Freesurfer region used to calculate left HPC GMV. The MTC voxel was placed in the left middle part of temporal lobe over the middle temporal gyrus caudal to superior temporal gyrus and cranial to inferior temporal gyrus, corresponding to the Freesurfer site used to measure left MTC GMV. The SM1 voxel was centered over the left hand-knob, corresponding to the Freesurfer sites used to calculate left SM1 GMV. The DLPFC voxel was placed over the right middle frontal gyrus inferior to the superior frontal sulcus and anterior to the precentral sulcus, corresponding to the Freesurfer sites used to calculate right DLPFC GMV. The voxel sizes were: (i) 1.6 × 1.6 × 1.6 cm^3 in the DPCC, left SM1 and right DLPFC voxels, (ii) 20 × 12 × 16 cm^3 in the left MTC and (iii) 26 × 12 × 12 cm^3 in the left HPC. MR spectra were processed using the totally automatic robust quantification in nuclear MR (TARQUIN, version 4.3.10) and the linear combination of model spectra (LCModel, version

6.3.1-R). These two post-processing software packages are widely used (64). However, LCModel may offer a more accurate quantification of Glx and macromolecular constituents (65). We presented Tarquin results after lipid signal extraction using the lipid-filtering preprocessing option prior to quantification, as recommended by Near et al. (2021) (66). However, LCModel has no lipid filter option at the preprocessing phase. Therefore, we present right DLPFC Tarquin results with no filtering of lipids compared to LCModel results in Supplementary table 1. This table confirms that the same conclusion can be drawn as when lipids were filtered before processing with Tarquin, as shown in Table 1, see results section. Only spectra with linewidths less than 12 Hz and signal-to-noise ratio greater than 5 were included in the statistical analyses, other values were considered missing. For spectra that were processed with LCModel, all included neurometabolites were quantified with a Cramér-Rao lower bound < 20%. ^{1}H-MRS spectra were visually checked to ensure the absence of artefacts prior to quantification. Quantifiable neurometabolites were (1) total NAA (tNAA) composed of N-acetyl aspartate and N-acetyl glutamate, (2) total creatine (tCr) composed of creatine and phosphocreatine, (3), total choline (tCho) composed of phosphorylcholine and glycerophosphocholine, (4) myoinositol (mIns), and (5) the glutamate-glutamine complex (Glx).

Water-referenced levels of tNAA, tCr, tCho, mIns, and Glx were quantified for each voxel location and ratios relative to tCr were calculated. For tNAA also the ratio relative to mIns was calculated. Neuroinflammation and gliosis were reported in association with elevated levels of mIns, tCr and tCho (45)(67)(68). The concentrations of these neurometabolites are significantly higher in glial cells than in neurons and the concomitant increase in mIns and tCho is considered to indicate glial proliferation (69). In addition, decreased concentrations of tNAA are considered a robust marker of neurodegeneration (70). The ratio of tNAA relative to mIns is an interesting marker for neurodegenerative processes that involve inflammation, as it indicates a combined loss of neural integrity (tNAA) and increase in neuroinflammation (mIns) (71).

2.5 Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics version 27 (SPSS Inc, Chicago USA). We used parametric tests where possible. In case this was not appropriate due to not meeting normality assumptions, data was log transformed, as was the case for IL-6 measurements. After data inspection and cleaning bad quality measurements (see criteria in 4. Brain imaging and ^{1}H-MRS), the agreement between LCModel and Tarquin ^{1}H-MRS post-processing software packages was evaluated using intraclass correlation coefficients (ICC, single measure two-way random effects model for absolute agreement). Subject demographics were correlated to MoCA test scores using linear regression analysis for continuous markers and two-sided independent t-tests to compare MoCA test results between two groups. Linear regression was used to correlate age with levels of serum inflammatory markers, brain GMV and

neurometabolite levels. Multiple linear regression analysis was used to investigate the association between MoCA score, adjusted for age and educational level, or fat %, adjusted for age and sex and peripheral inflammatory marker levels, brain GMV and neurometabolite levels. Finally, multiple regression analysis was used to investigate the association between peripheral inflammatory marker levels, adjusted for age, sex and fat % and brain GMV and neurometabolite levels. For the purpose of this exploratory study, p-values below 0.05 were considered statistically significant. In addition, we presented which of these p-values survived correction for multiple testing with false discovery rate (FDR) analysis (Benjamini and Hochberg adjustment) (72). In the FDR procedure, every p-value is compared against a sequentially weighted threshold on all p-values. The FRD procedure was done multiple times for each of the independent variables age, MoCA, fat %, IL-6, IL-6 after exclusion of an outlier, and kynurenine; and separately for LCModel results, Tarquin results, and the combination of all other dependent variables for all p-values presented in Supplementary tables 3-10. It should be noted that the results surviving the multiple testing adjustment are strong, whereas interpretation of the remaining results should be made with caution.

3. Results

3.1 Magnetic resonance data quality and analysis

Of all included subjects, 68 (91.9%) underwent complete brain scanning sessions with MRI and ^{1}H-MRS. Reasons for missing data were inclusion in a pilot ^{1}H-MRS scanning session using stimulated echo acquisition mode (STEAM) sequence (n = 2) or scanning cessation due to claustrophobia, unrest, discomfort, or pain during scanning (n = 4). After excluding data of bad quality, a complete dataset of all MRI and MRS values was attained for 33 subjects. Further analysis was done each time with all the data available, which differed depending on the measurement. In order to know specifically from how many subjects the data was used for each of the associations assessed below, Supplementary table 2 contains the number of subjects with good quality MRI and ^{1}H-MRS values, with ^{1}H-MRS values presented per region and compared between LCModel and Tarquin, see also Figure 1.

Overall, the agreement between tNAA, tCho, mIns and tCr measurements postprocessed with LCModel compared to measurements after processing with Tarquin were strong (R or ICC > 0.750) or moderate (0.500 < R or ICC < 0.750), with exception of weak correlations (0.250 < R < 0.500) for tNAA levels in the right DLPFC, mIns levels in the left HPC and tCr levels in the left MTC; or low ICC values (0.250 < ICC < 0.500) for tNAA levels in the left HPC, left MTC and right DLPFC, mIns levels in the DPCC, left HPC, left MTC and tCr levels in the DPCC, left MTC, left SM1 and right DLPFC. The agreement of Glx levels between the two models was very weak (R or ICC < 0.250) in all regions of interest, except for a weak correlation in the left SM1 (R = 0.376); for details see Table 1.

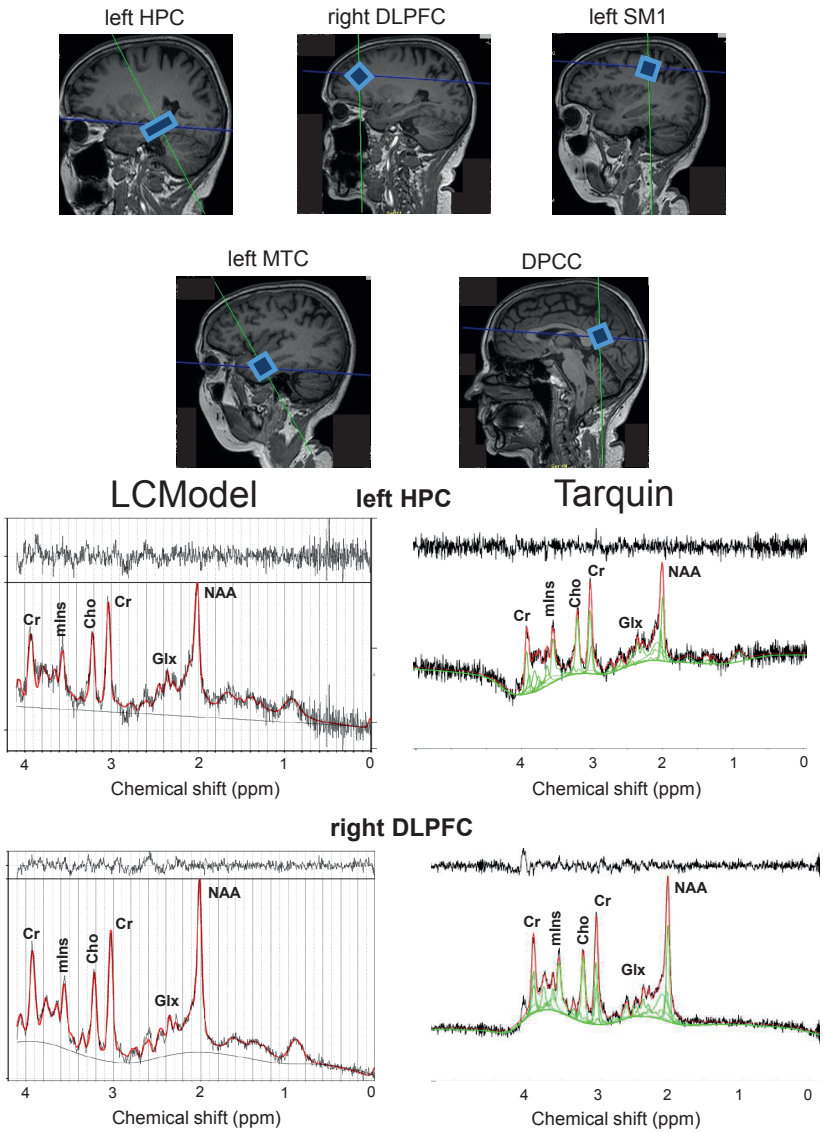


Figure 1. (A) Example voxel positions and spectra from a representative subject. (B) Raw (black curve) and fitted (red curve) spectra from LCModel (left hand side panel) and Tarquin (right hand side panel) are illustrated for the left HPC and right DLPFC.

Abbreviations: Cho, total choline; Cr, creatine + phosphocreatine; DLPFC, dorsolateral prefrontal cortex; DPCC, dorsal posterior cingulate cortex; Glx, glutamate-glutamine complex; HPC, hippocampal cortex; mIns, myoinositol; MTC, medial temporal cortex; NAA, N-acetyl aspartate; SM1, primary sensorimotor cortex.

Table 1. Agreement between LCModel and Tarquin ^{1}H-MRS post-processing software packages

		tNAA	tCho	Glx	mIns	tCr
DPCC	R-value	0.699***	0.597***	0.097	0.687***	0.789***
	ICC	0.666***	0.514***	0.030	0.466***	0.408***
Left HPC	R-value	0.574***	0.619***	0.228	0.358**	0.641***
	ICC	0.354***	0.516***	0.074	0.314**	0.606***
Left MTC	R-value	0.515***	0.692***	-0.011	0.726***	0.485***
	ICC	0.407***	0.655***	-0.003	0.474***	0.411***
Left SM1	R-value	0.579***	0.790***	0.376**	0.817***	0.742***
	ICC	0.541***	0.661	0.161**	0.663***	0.213***
Right DLPFC	R-value	0.486***	0.852***	0.187	0.751***	0.678***
	ICC	0.472***	0.795***	0.061	0.559***	0.227***

Pearson R and ICC values are presented for the correlation between Tarquin and LCModel measurements. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; Abbreviations: DLPFC, dorsolateral prefrontal cortex; DPCC, dorsal posterior cingulate cortex; Glx, glutamate-glutamine complex; HPC, hippocampal cortex; ICC, intraclass correlation coefficient; mIns, myoinositol; MTC, medial temporal cortex; SM1, primary sensorimotor cortex; tCho, total choline; tCr, total creatine, tNAA, total N-acetyl aspartate

3.2 Subject characteristics

Subject characteristics are described in Table 2. The mean (SD) MoCA score was 25.2 (2.8), ranging from 19-30. The age of the included subjects ranged from 60-85 years old. Older aged adults scored significantly lower on the MoCA test ($R^2 = 0.175$, $p < 0.001$). Fat % ranged from 3.4-51.6% and BMI from 19.2-47.9. As expected, fat % was highly dependent on sex ($p < 0.001$), with mean (SD) fat % in men 26.3% (8.7) and in women 35.8% (7.8). BMI did not significantly differ between sexes ($p = 0.361$), with mean (SD) BMI in men 28.6 (8.7) and in women 27.5 (4.5). Fat % and BMI, adjusted for age and sex, were not significantly correlated with MoCA scores ($p = 0.202$, $p = 0.127$ respectively). Older age was significantly correlated with lower fat % ($R^2 = 0.143$, $p = 0.001$), but not with BMI ($R^2 = 0.019$, $p = 0.128$).

3.3 The relation between age, body fat percentage and cognition, and brain gray matter volume and peripheral inflammation

Older age was significantly correlated with lower brain volumes in all regions of interest (all $p < 0.05$). Multiple linear regression, adjusted for age and educational level, showed that higher MoCA scores significantly correlated with higher levels of kynurenine ($p = 0.027$), which is contradictory to what we hypothesized. After adjusting for age and sex, higher fat % was correlated with lower total GMV ($p = 0.029$) and lower left MTC GMV ($p = 0.031$), see Table 3. The correlations with age all survived multiple testing correction with FDR, while the correlations with MoCA and fat % did not remain statistically significant. No significant correlations were found between age or fat % and IL-6 or kynurenine levels, see Supplementary table 3.

Table 2. Subject characteristics and their relation to MoCA score

	Total (n = 74)	β	Regression p-value	T-test p-value	Missing (n)
Age	69.4 (6.2)	-0.418	0.0001		0
Fat %[a]	31.4 (9.4)	0.174	0.202		3
BMI[a]	28.0 (4.9)	0.172	0.127		3
Sex				0.747	0
Male	34 (45.9%)				
Female	40 (54.1%)				
Smoking status				0.767	
Non smoker	71 (95.9%)				
Smoker	3 (4.1%)				
Education				All > 0.259	1
Higher education	57 (78.1%)				
Secondary education	14 (19.2%)				
Basic education	2 (2.7%)				

[a] adjusted for age and sex. Continuous parameters are expressed as mean values (SD); categorical parameters are expressed as n (%).
Abbreviations: BMI, body mass index; MoCA, Montreal Cognitive Assessment.

Table 3. The effect of age, MoCA and fat % on brain gray matter volume and peripheral inflammation

		β	p-value
Age	Total GMV	-0.372	0.005
	DPCC GMV	-0.426	0.001
	Left HPC GMV	-0.450	0.0002
	Right HPC GMV	-0.534	0.000005
	Left MTC GMV	-0.320	0.017
	Left SM1 GMV	-0.431	0.0008
	Right DLPFC GMV	-0.377	0.004
MoCA[a]	Kynurenine	0.312	0.027
Fat %[b]	Total GMV	-0.292	0.029
	Left MTC GMV	-0.320	0.031

Only statistically significant correlations are presented. See Supplementary table 3 for all results. [a] adjusted for age and educational level, [b] adjusted for age and sex. Abbreviations: DLPFC, dorsolateral prefrontal cortex; DPCC, dorsal posterior cingulate cortex; GMV, gray matter volume; HPC, hippocampal cortex; mIns, myoinositol; MoCA, Montreal Cognitive Assessment; MTC, medial temporal cortex; o.e., outlier excluded; SM1, primary sensorimotor cortex; tCho, total choline; tCr, total creatine, tNAA, total N-acetyl aspartate

3.4 The relation between age, body fat percentage and cognition, and neurometabolites

Correlations obtained from regression analyses between age, fat % and cognition and the neurometabolites are depicted in Table 4 and Supplementary tables 4-6. To assess potential effects of MRS data analysis software, we have compared LCModel and Tarquin for the quantification of neurometabolites.

A common finding after processing ^{1}H-MRS data with LCModel and Tarquin was the significant age-related decrease in tCr DPCC levels. Furthermore, we expect tNAA levels to be lower as a function of age. Even though we did not compare to young adults, we were able to find a decrease of tNAA DPCC (Tarquin) and tNAA left SM1 (LCModel) in association with older age. The age-related decrease in tNAA DPCC levels was also close to significance level for LCModel ($p = 0.053$). No age-related changes in neuroinflammation were discovered. After adjusting for age and educational level, MoCA test results were not related to signs of neurodegeneration or neuroinflammation. Contradictory, higher fat %, adjusted for age and sex, was associated with increased tNAA/tCr levels in left MTC, but decreased tNAA/tCr levels in left SM1. In addition, a negative association was found between fat % and tNAA/tCr DPCC (Tarquin) and tNAA/tCr right DLPFC (LCModel). Also Glx level changes were inconsistent. Results after processing with LCModel showed higher fat % correlates with lower Glx/tCr in left HPC, while after processing with Tarquin higher fat % correlated with higher levels of Glx/tCr in left MTC and Glx in DPCC. Here, the discordance may be explained by the very weak agreement of Glx concentrations between LCModel and Tarquin results. None of the significant p-values survived FDR correction for multiple testing.

3.5 The relation between peripheral inflammatory markers, and brain gray matter volume and neurometabolites adjusted for age, sex and body fat percentage

Multiple regression analysis adjusted for age, sex and fat % and after exclusion of an outlier in IL-6 found no associations between the inflammatory serum markers and brain gray matter volume, see Supplementary table 7 for all results. Correlations obtained from multiple regression analysis between the inflammatory serum markers and neurometabolites are depicted in Table 5 and Supplementary tables 8-10.

The only concordant significant association between peripheral inflammatory markers and neurometabolites for results after processing ^{1}H-MRS data with LCModel compared to Tarquin, was the positive association between serum kynurenine levels and mIns levels in the left SM1 and tCho levels in the right DLPFC. Serum kynurenine was associated with signs of neuroinflammation (i.e. the concomitant increase in tCho and mIns) in both left SM1 and right DLPFC when considering LCModel results. After processing ^{1}H-MRS data with Tarquin, the positive association between serum kynurenine and tCho left SM1 ($p = 0.204$) and mIns right DLPFC ($p = 0.063$) was nonsignificant. The increase in tCho in the right DLPFC (LCModel and Tarquin) and the increase in mIns left SM1 (Tarquin) were the only significant associations between neurometabolites and kynurenine that survived the FDR procedure to correct for

multiple testing. In addition, although not surviving multiple testing correction, we found a significant positive association between serum kynurenine and mIns concentrations in the DPCC (Tarquin), which was accompanied with a nonsignificant positive association with tCho DPCC (p = 0.162). For LCModel results both the association between serum kynurenine and mIns DPCC (p = 0.849) and tCho DPCC (p = 0.286) was positive but nonsignificant. Of interest was also the negative association between serum kynurenine levels and tNAA/mIns concentrations in left SM1 (Tarquin), tNAA left HPC (Tarquin) and tNAA left MTC (LCModel), which may indicate neurodegeneration. When looking at the same results from the other ^{1}H-MRS post-processing method were these findings were not significant, we found similar trends for serum kynurenine and tNAA/mIns in left SM1 (with LCModel p = 0.128), tNAA left HPC (with LCModel p = 0.076), and tNAA left MTC (with Tarquin p = 0.053).

Table 4. Effect of age, MoCA and fat% on neurometabolites

		LCModel		Tarquin	
		β	p-value	β	p-value
Age	tCho/tCr DPCC o.e.	0.252	0.044		
	tNAA DPCC			-0.276	0.027
	tCr DPCC	-0.315	0.011	-0.305	0.015
	tCr l HPC			-0.279	0.037
	tNAA l SM1	-0.304	0.013		
	tCr l MTC	0.332	0.013		
MoCA[a]	Glx/tCr l MTC			0.299	0.040
	tCho DPCC			0.322	0.020
Fat % [b]	tNAA/tCr DPCC			-0.314	0.041
	Glx/tCr l HPC	-0.384	0.019		
	tNAA/tCr l MTC	0.330	0.044	0.348	0.037
	Glx/tCr l MTC			0.388	0.021
	tNAA/tCr l SM1	-0.362	0.016	-0.315	0.041
	tNAA/tCr r DLPFC	-0.338	0.026		
	Glx DPCC			0.353	0.029

Only statistically significant correlations are presented. See Supplementary tables 4-6 for all results. [a] adjusted for age and educational level, [b] adjusted for age and sex.
Abbreviations: DLPFC, dorsolateral prefrontal cortex; DPCC, dorsal posterior cingulate cortex; GMV, gray matter volume; HPC, hippocampal cortex; l, left; mIns, myoinositol; MoCA, Montreal Cognitive Assessment; MTC, medial temporal cortex; o.e., after exclusion of an influential outlier; r, right; SM1, primary sensorimotor cortex; tCho, total choline; tCr, total creatine, tNAA, total N-acetyl aspartate.

Table 5. The effect of inflammatory blood markers adjusted for age, sex and fat% on neurometabolites

		LCModel		Tarquin	
		β	p-value	β	p-value
Log IL-6 o.e.	Glx/tCr l HPC			0.317	0.033
	tNAA/tCr l MTC	-0.407	0.011		
	tCho/tCr l MTC	0.442	0.007		
	Glx/tCr l MTC			0.425	0.006
	Glx l HPC			0.306	0.043
	tNAA l MTC	-0.486	0.002		
	Glx l MTC			0.433	0.005
	tNAA l SM1	0.403	0.002		
	Glx rDLPFC			0.339	0.022
Kynurenine	tNAA/tCr l MTC	-0.312	0.026		
	tCho/tCr l MTC	0.418	0.003		
	mIns/tCr l SM1			0.317	0.012
	tNAA/mIns l SM1			-0.255	0.039
	tCho/tCr rDLPFC	0.297	0.020		
	mIns DPCC			0.314	0.016
	tNAA l HPC			-0.317	0.022
	tCr l HPC			-0.379	0.005
	tNAA l MTC	-0.364	0.007		
	tCho l MTC	0.275	0.049		
	tCho l SM1	0.319	0.015		
	mIns l SM1	0.278	0.020	0.375	0.005
	tCho rDLPFC	0.436	0.001	0.386	0.002
	mIns rDLPFC	0.302	0.014		

Only statistically significant correlations are presented. See Supplementary tables 8-10 for all results, including results before exclusion of the outlier in Log IL-6. Multiple linear regression analysis was used adjusted for age, sex and fat%. Abbreviations: DLPFC, dorsolateral prefrontal cortex; DPCC, dorsal posterior cingulate cortex; Glx, glutamate-glutamine complex; GMV, gray matter volume; HPC, hippocampal cortex; IL-6, interleukin-6; l, left; mIns, myoinositol; MoCA, Montreal Cognitive Assessment; MTC, medial temporal cortex; o.e., outlier excluded; r, right; SM1, primary sensorimotor cortex; tCho, total choline; tCr, total creatine, tNAA, total N-acetyl aspartate

Associations between serum IL-6 levels and the neurometabolites were discordant between and within post-processing methods LCModel and Tarquin. While tNAA levels in the left MTC were decreased, tNAA levels in left SM1 were increased in association with high IL-6 levels after processing with LCModel, while results from Tarquin showed the same trends but not reaching significance. These LCModel results were also the only two associations between IL-6 and neurometabolites that survived the FDR multiple testing correction. After processing with Tarquin, we found only an increase in Glx levels related to high IL-6 levels. This was significant for left HPC, left MTC and right DLPFC, and close to significant for DPCC ($p = 0.062$), but none of them survived correction for multiple testing.

4. Discussion

We examined the association between serum inflammatory factors and neurometabolic, brain volume and cognitive changes, taking into account the effect of age, sex and fat %. Our results showed that elevated levels of serum kynurenine were associated with signs of neuroinflammation (defined as a concomitant increase in tCho and mIns levels (69)) in the left SM1 and right DLPFC after ^{1}H-MRS post-analysis with the LCModel. After analysis with Tarquin, only a significant increase in mIns left SM1 and tCho right DLPFC was found. After correction for multiple testing, the positive association between serum kynurenine and tCho right DLPFC (LCModel and Tarquin) and mIns left SM1 (Tarquin) remained statistically significant. Furthermore, we found an association between higher kynurenine levels and increases in mIns in the DPCC (Tarquin). This elevation of mIns associated with only a trend of increased tCho levels, as seen in DPCC, most likely also represents neuroinflammation, as increased levels of mIns are believed to represent glial proliferation or an increase in glial cell size (73). Moreover, it has been suggested that elevated levels of mIns or mIns/tCr are an early event in the course towards Alzheimer's dementia and can precede tNAA reduction (74). Previous studies have also reported a positive correlation between pro-inflammatory factors, IL-8 (44) or C-reactive protein (75) and hippocampal mIns levels after processing the ^{1}H-MRS data with the LCModel (44) or in a study where the ^{1}H-MRS post-processing analytical method was not defined (75). In our study, we did not find a significant relationship between one of the measured pro-inflammatory factors and mIns levels in the hippocampus with either of the post-processing software packages. An interesting finding was the association between peripheral inflammation, as marked by high serum levels of kynurenine, and decreases in tNAA/mIns levels in left SM1 (Tarquin). As tNAA/mIns is considered to reflect the combination of decreased neural integrity and increased neuroinflammation, which may indicate neurodegeneration and was previously found to be related to an increased risk of developing clinical Alzheimer's disease (71). Furthermore, an increase in tCho or

tCho/tCr was found in the left MTC in association with elevated levels of serum IL-6 and kynurenine in LCModel results. In this case, there was no associated trend of increased mIns. However, the left MTC did present significant decreases in tNAA levels in association with increased levels of serum IL-6 or kynurenine (LCModel). This elevation of tCho, which not coincides with an elevation in mIns, is difficult to interpret, as tCho is a marker of increased synthesis and degradation of cellular membranes, which is not only seen in local inflammation, but also in several other neurological disorders, including cerebral infarctions, multiple sclerosis and malignant tumors (76).

An interesting finding that was only seen in the results obtained after processing the ^{1}H-MRS data with Tarquin was the elevation of Glx and Glx/tCr levels in left HPC and left MTC in association with higher levels of serum IL-6, adjusted for age, sex and fat %. Notably, also in the DPCC and right DLPFC, Glx and Glx/tCr levels were close to being significantly positively associated with IL-6 levels, see Supplementary table 9. These findings are in line with observations in a recent study by Ho and colleagues who used the LCModel post-processing software and reported an association between increases in circulating IL-6 and increased concentrations of glutamate in the anterior cingulate cortex in adolescents with depression (77). Elevated IL-6 levels have previously also been linked to depression in older adults (78) and recent studies also found increases in Glx in the anterior or posterior cingulate cortex with LCModel in adolescents with suicidal ideation or in cognitively impaired adults (79)(78)(80). It has been suggested that inflammatory cytokines can induce an excessive release of glutamate, which results in oligodendrocyte excitotoxicity. Over time, oligodendrocyte excitotoxicity leads to white matter damage and cell apoptosis (81)(82)(77). In contrast, a meta-analysis showed that Glx levels were significantly decreased in the medial frontal cortex in depressed subjects receiving antidepressant medication (83). Also in mild cognitive impairment and Alzheimer's disease, most studies showed decreases of brain glutamate and Glx (74)(84). Furthermore, most studies have reported a decrease in brain glutamate levels with older age, while glutamine was found to increase and Glx to change inconsistently across studies (85)(86).

The discordance between studies and absence of an association between Glx and serum IL-6 levels in the LCModel in our study can partly be explained by the very low agreement between Tarquin and LCModel results concerning Glx. For all other neurometabolites we found an overall moderate to high reliability between the two post-processing software packages. This is in line with other studies who also reported that neurometabolites with prominent singlets (like tNAA and tCho) show better agreement between linear-combination modeling algorithms than signals of lower intensity (like mIns and Glx) (64)(87)(88)(89)(90). Based on these findings, it is advised to be careful when interpreting results from studies who use only one software package, especially when sample sizes are small and specifically when findings concern Glx. It has been advocated that reporting results of more than one algorithm may improve the power of ^{1}H-MRS studies (64).

In this study, serum IL-6 and kynurenine levels were not associated with older age, fat %, or cognitive decline. We even found a positive association between serum kynurenine and cognitive function. These findings are in contrast to findings from previous studies. Considering IL-6, chronic elevations were reported in association with older age (47) and obesity (91)(92). In addition, high levels of peripheral IL-6 negatively affected memory and learning (93) and correlated with poor overall cognitive performance (94)(95). Moreover, high basal IL-6 levels were also associated with an increased risk of future age-related cognitive decline (48)(96). In our study, IL-6 levels inversely correlated with hippocampal gray matter volume (93)(97), which could only be found in the right HPC before exclusion of an outlier in IL-6, see Supplementary table 7. Based on the findings from existing literature, it has been suggested that high levels of IL-6 may increase the risk of developing neurodegenerative diseases (98). However, our results suggest that serum kynurenine, despite its contradictory positive association with cognitive functioning, would be a better predictor of neuroinflammation and neurodegeneration than IL-6. Of note, IL-6, though widely used as a marker of inflammation, has been suggested to be not only involved in pro-inflammatory processes, but to act as a regulator of both pro- and anti-inflammatory activity (49). For example, a study in healthy humans showed that the administration of IL-6 before an infusion with endotoxin abolished the increase in plasma levels of the pro-inflammatory factor TNF-α (99). In contrast, kynurenine is considered a more robust marker of inflammation, as several pro-inflammatory cytokines, like CRP, IL-1β, IL-6, IL-8, TNF-α and Interferon-gamma (IFN-γ), were shown to enhance the activation of indoleamine 2,3-dioxygenase, increasing kynurenine levels, while the anti-inflammatory cytokines IL-4 and IL-10 counteracted this increase in kynurenine levels (50)(51)(52)(53)(54). Furthermore, kynurenine has previously been linked to obesity (57), cognitive aging (100), psychiatric disorders (54)(55)(58), brain volume loss (58), neurodegenerative disorders (59) and central nervous system injury (50). Taking into account the vast amount of evidence in the recent literature and considering our own results, we propose that kynurenine can be used as a generic peripheral inflammatory marker that is associated with neuroinflammation and neurodegeneration. However, still more research needs to confirm the role of elevated kynurenine levels in age-related cognitive decline and neurodegenerative diseases.

In conclusion, serum kynurenine levels were positively associated with signs of neuroinflammation, specifically in the DPCC, left SM1 and right DLPFC, and signs of neurodegeneration, specifically in the left HPC, left MTC and left SM1, after adjusting for age, sex and fat %. Interestingly, higher levels of IL-6 were associated with an elevation of Glx levels in left HPC, left MTC, and right DLPFC, but only following ^{1}H-MRS post-processing analysis with Tarquin. Of note, the agreement between Tarquin and LCModel results was moderate to strong for tNAA, tCho, mIns and tCr, but weak to very weak for Glx in all regions of interest. At last, our results did not find a link between the peripheral inflammatory markers kynurenine or IL-6

and older age, fat %, or cognitive decline. It should be noted that our results are exploratory and should be interpreted with caution due to multiple testing. After correcting for multiple testing with FDR analysis, the positive association between serum kynurenine and tCho right DLPFC (LCModel and Tarquin) and mIns left SM1 (Tarquin) remained statistically significant. Furthermore, a discordant finding of an association between higher IL-6 levels and lower tNAA levels in the left MTC, but higher tNAA levels in left SM1 survived the correction for multiple testing. At last, the negative associations between age and MoCA score and between age and GMV in all regions of interest remained statistically significant after the FDR procedure. Further analyses are needed to specifically assess the relationship between increases of peripheral inflammatory factors and markers of neuroinflammation in older adults and obesity and investigate their role in cognitive decline.

Acknowledgements

This work was supported by the Research Council of Lithuania (grant number S-SEN-20-5). The sponsor was not involved in study design; collection, analysis and interpretation of the data; in writing of the report; nor in the decision to submit the article for publication.

Conflicts of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

1. Dall TM, Gallo PD, Chakrabarti R, West T, Semilla AP, Storm M V. An Aging Population And Growing Disease Burden Will Require A Large And Specialized Health Care Workforce By 2025. *Health Aff* (2013) **32**:2013–2020. doi:10.1377/hlthaff.2013.0714
2. Mattiuzzi C, Lippi G. Worldwide disease epidemiology in the older persons. *Eur Geriatr Med* (2020) **11**:147–153. doi:10.1007/S41999-019-00265-2/TABLES/4
3. Mayeux R, Stern Y. Epidemiology of Alzheimer disease. *Cold Spring Harb Perspect Med* (2012) **2**: doi:10.1101/CSHPERSPECT.A006239
4. Haththotuwa RN, Wijeyaratne CN, Senarath U. Worldwide epidemic of obesity. *Obes Obstet* (2020)3–8. doi:10.1016/B978-0-12-817921-5.00001-1
5. Cournot M, Marquié JC, Ansiau D, Martinaud C, Fonds H, Ferrières J, Ruidavets JB. Relation between body mass index and cognitive function in healthy middle-aged men and women. *Neurology* (2006) **67**:1208–1214. doi:10.1212/01.WNL.0000238082.13860.50
6. Mrak RE. Alzheimer-type neuropathological changes in morbidly obese elderly individuals. *Clin Neuropathol* (2009) **28**:40–45. doi:10.5414/NPP28040
7. Rakesh G, Szabo ST, Alexopoulos GS, Zannas AS. Strategies for dementia prevention: latest evidence and implications. *Ther Adv Chronic Dis* (2017) **8**:121–136. doi:10.1177/2040622317712442
8. Sartori AC, Vance DE, Slater LZ, Crowe M. The impact of inflammation on cognitive function in older adults: Implications for healthcare practice and research. *J Neurosci Nurs* (2012) **44**:206–217. doi:10.1097/JNN.0b013e3182527690
9. Tang Y, Purkayastha S, Cai D. Hypothalamic microinflammation: a common basis of metabolic syndrome and aging. *Trends Neurosci* (2015) **38**:36–44. doi:10.1016/J.TINS.2014.10.002
10. Beilharz JE, Maniam J, Morris MJ. Short-term exposure to a diet high in fat and sugar, or liquid sugar, selectively impairs hippocampal-dependent memory, with differential impacts on inflammation. *Behav Brain Res* (2016) **306**:1–7. doi:10.1016/J.BBR.2016.03.018
11. Franceschi C, Bonafè M, Valensin S, Olivieri F, De Luca M, Ottaviani E, De Benedictis G. Inflamm-aging. An evolutionary perspective on immunosenescence. in *Annals of the New York Academy of Sciences* (New York Academy of Sciences), 244–254. doi:10.1111/j.1749-6632.2000.tb06651.x
12. Borodkina A V, Deryabin PI, Giukova AA, Nikolsky NN. "Social Life" of Senescent Cells: What Is SASP and Why Study It? *Acta Naturae* (2018) **10**:4–14. Available at: http://www.ncbi.nlm.nih.gov/pubmed/29713514 [Accessed October 25, 2020]
13. Dimri GP, Lee X, Basile G, Acosta M, Scott G, Roskelley C, Medrano EE, Linskens M, Rubelj I, Pereira-Smith O, et al. A biomarker that identifies senescent human cells in culture and in aging skin in vivo. *Proc Natl Acad Sci U S A* (1995) **92**:9363–9367. doi:10.1073/pnas.92.20.9363
14. Campisi J, D'Adda Di Fagagna F. Cellular senescence: When bad things happen to good cells. *Nat Rev Mol Cell Biol* (2007) **8**:729–740. doi:10.1038/nrm2233
15. Yudkin JS. Inflammation, obesity, and the metabolic syndrome. in *Hormone and Metabolic Research* (© Georg Thieme Verlag KG Stuttgart · New York), 707–709. doi:10.1055/s-2007-985898
16. Woods JA, Wilund KR, Martin SA, Kistler BM. Exercise, inflammation and aging. *Aging Dis* (2012) **3**:130–140. Available at: /pmc/articles/PMC3320801/?report=abstract [Accessed October 20, 2020]
17. Scheiblich H, Trombly M, Ramirez A, Heneka MT. Neuroimmune Connections in Aging and Neurodegenerative Diseases. *Trends Immunol* (2020) **41**:300–312. doi:10.1016/j.it.2020.02.002
18. Guillemot-Legris O, Muccioli GG. Obesity-Induced Neuroinflammation: Beyond the Hypothalamus. *Trends Neurosci* (2017) **40**:237–253. doi:10.1016/J.TINS.2017.02.005
19. Terry RD, DeTeresa R, Hansen LA. Neocortical cell counts in normal human adult aging. *Ann Neurol* (1987) **21**:530–539. doi:10.1002/ana.410210603

20. Perry VH, Cunningham C, Holmes C. Systemic infections and inflammation affect chronic neurodegeneration. *Nat Rev Immunol* (2007) **7**:161–167. doi:10.1038/nri2015
21. Cohen J, Torres C. Astrocyte senescence: Evidence and significance. *Aging Cell* (2019) **18**: doi:10.1111/acel.12937
22. Lu J, Wu D mei, Zheng Y lin, Hu B, Cheng W, Zhang Z feng, Shan Q. Ursolic acid improves high fat diet-induced cognitive impairments by blocking endoplasmic reticulum stress and IκB kinase β/nuclear factor-κB-mediated inflammatory pathways in mice. *Brain Behav Immun* (2011) **25**:1658–1667. doi:10.1016/J.BBI.2011.06.009
23. Tapia-González S, García-Segura LM, Tena-Sempere M, Frago LM, Castellano JM, Fuente-Martín E, García-Cáceres C, Argente J, Chowen JA. Activation of Microglia in Specific Hypothalamic Nuclei and the Cerebellum of Adult Rats Exposed to Neonatal Overnutrition. *J Neuroendocrinol* (2011) **23**:365–370. doi:10.1111/J.1365-2826.2011.02113.X
24. Guillemot-Legris O, Masquelier J, Everard A, Cani PD, Alhouayek M, Muccioli GG. High-fat diet feeding differentially affects the development of inflammation in the central nervous system. *J Neuroinflammation* (2016) **13**:1–11. doi:10.1186/S12974-016-0666-8/FIGURES/5
25. van Dam AM, Bauer J, Tilders FJH, Berkenbosch F. Endotoxin-induced appearance of immunoreactive interleukin-1 beta in ramified microglia in rat brain: a light and electron microscopic study. *Neuroscience* (1995) **65**:815–826. doi:10.1016/0306-4522(94)00549-K
26. Layé S, Goujon E, Combe C, VanHoy R, Kelley KW, Parnet P, Dantzer R. Effects of lipopolysaccharide and glucocorticoids on expression of interleukin-1β converting enzyme in the pituitary and brain of mice. *J Neuroimmunol* (1996) **68**:61–66. doi:10.1016/0165-5728(96)00066-5
27. Nguyen KT, Deak T, Owens SM, Kohno T, Fleshner M, Watkins LR, Maier SF. Exposure to Acute Stress Induces Brain Interleukin-1β Protein in the Rat. *J Neurosci* (1998) **18**:2246. doi:10.1523/JNEUROSCI.18-06-02239.1998
28. Turrin NP, Gayle D, Ilyin SE, Flynn MC, Langhans W, Schwartz GJ, Plata-Salamán CR. Pro-inflammatory and anti-inflammatory cytokine mRNA induction in the periphery and brain following intraperitoneal administration of bacterial lipopolysaccharide. *Brain Res Bull* (2001) **54**:443–453. doi:10.1016/S0361-9230(01)00445-2
29. Bourgognon J-M, Cavanagh J. The role of cytokines in modulating learning and memory and brain plasticity. *Brain Neurosci Adv* (2020) **4**:239821282097980. doi:10.1177/2398212820979802
30. Dinarello CA, Cannon JG, Wolff SM. New Concepts on the Pathogenesis of Fever. *Rev Infect Dis* (1988) **10**:168–189. doi:10.1093/CLINIDS/10.1.168
31. Ericsson A, Kovács KJ, Sawchenko PE. A functional anatomical analysis of central pathways subserving the effects of interleukin-1 on stress-related neuroendocrine neurons. *J Neurosci* (1994) **14**:897–913. doi:10.1523/JNEUROSCI.14-02-00897.1994
32. Goehler LE, Relton JK, Dripps D, Kiechle R, Tartaglia N, Maier SF, Watkins LR. Vagal paraganglia bind biotinylated interleukin-1 receptor antagonist: a possible mechanism for immune-to-brain communication. *Brain Res Bull* (1997) **43**:357–364. doi:10.1016/S0361-9230(97)00020-8
33. Gaykema RPA, Goehler LE, Tilders FJH, Bol JGJM, McGorry M, Fleshner M, Maier SF, Watkins LR. Bacterial endotoxin induces fos immunoreactivity in primary afferent neurons of the vagus nerve. *Neuroimmunomodulation* (1998) **5**:234–240. doi:10.1159/000026343
34. Banks WA, Kastin AJ, Brennan JM, Vallance KL. Adsorptive endocytosis of HIV-1gp120 by blood-brain barrier is enhanced by lipopolysaccharide. *Exp Neurol* (1999) **156**:165–171. doi:10.1006/EXNR.1998.7011
35. Maier SF. Bi-directional immune-brain communication: Implications for understanding stress, pain, and cognition. *Brain Behav Immun* (2003) **17**:69–85. doi:10.1016/S0889-1591(03)00032-1
36. Bachstetter AD, Morganti JM, Jernberg J, Schlunk A, Mitchell SH, Brewster KW, Hudson CE, Cole MJ, Harrison JK, Bickford PC, et al. Fractalkine and CX 3 CR1 regulate hippocampal neurogenesis in adult and aged rats. *Neurobiol Aging* (2011) **32**:2030–2044. doi:10.1016/J.NEUROBIOLAGING.2009.11.022

37. Barrientos RM, Kitt MM, Watkins LR, Maier SF. Neuroinflammation in the normal aging hippocampus. *Neuroscience* (2015) **309**:84–99. doi:10.1016/j.neuroscience.2015.03.007
38. Maier SF, Watkins LR. Cytokines for psychologists: implications of bidirectional immune-to-brain communication for understanding behavior, mood, and cognition. *Psychol Rev* (1998) **105**:83–107. doi:10.1037/0033-295X.105.1.83
39. Campbell SJ, Jiang Y, Davis AEM, Farrands R, Holbrook J, Leppert D, Anthony DC. Immunomodulatory effects of etanercept in a model of brain injury act through attenuation of the acute-phase response. *J Neurochem* (2007) **103**:2245–2255. doi:10.1111/J.1471-4159.2007.04928.X
40. Jiang Y, Deacon R, Anthony DC, Campbell SJ. Inhibition of peripheral TNF can block the malaise associated with CNS inflammatory diseases. *Neurobiol Dis* (2008) **32**:125–132. doi:10.1016/J.NBD.2008.06.017
41. Campbell SJ, Hughes PM, Iredale JP, Wilcockson DC, Waters S, Docagne F, Perry VH, Anthony DC. CINC-1 is an acute-phase protein induced by focal brain injury causing leukocyte mobilization and liver injury. *FASEB J* (2003) **17**:1168–1170. doi:10.1096/FJ.02-0757FJE
42. Campbell SJ, Perry VH, Pitossi FJ, Butchart AG, Chertoff M, Waters S, Dempster R, Anthony DC. Central nervous system injury triggers hepatic CC and CXC chemokine expression that is associated with leukocyte mobilization and recruitment to both the central nervous system and the liver. *Am J Pathol* (2005) **166**:1487–1497. doi:10.1016/S0002-9440(10)62365-6
43. Campbell SJ, Deacon RMJ, Jiang Y, Ferrari C, Pitossi FJ, Anthony DC. Overexpression of IL-1beta by adenoviral-mediated gene transfer in the rat brain causes a prolonged hepatic chemokine response, axonal injury and the suppression of spontaneous behaviour. *Neurobiol Dis* (2007) **27**:151–163. doi:10.1016/J.NBD.2007.04.013
44. Lind A, Boraxbekk CJ, Petersen ET, Paulson OB, Andersen O, Siebner HR, Marsman A. Do glia provide the link between low-grade systemic inflammation and normal cognitive ageing? A 1H magnetic resonance spectroscopy study at 7 tesla. *J Neurochem* (2021) **159**:185–196. doi:10.1111/JNC.15456
45. Lind A, Boraxbekk C-J, Petersen ET, Paulson OB, Siebner HR, Marsman A. Regional Myo-Inositol, Creatine, and Choline Levels Are Higher at Older Age and Scale Negatively with Visuospatial Working Memory: A Cross-Sectional Proton MR Spectroscopy Study at 7 Tesla on Normal Cognitive Ageing. *J Neurosci* (2020) **40**:8149–8159. doi:10.1523/JNEUROSCI.2883-19.2020
46. Ershler WB. Interleukin-6: A Cytokine for Gerontolgists. *J Am Geriatr Soc* (1993) **41**:176–181. doi:10.1111/J.1532-5415.1993.TB02054.X
47. Maggio M, Guralnik JM, Longo DL, Ferrucci L. Interleukin-6 in aging and chronic disease: A magnificent pathway. *Journals Gerontol - Ser A Biol Sci Med Sci* (2006) **61**:575–584. doi:10.1093/gerona/61.6.575
48. Bradburn S, Sarginson J, Murgatroyd CA. Association of peripheral interleukin-6 with global cognitive decline in non-demented adults: A meta-analysis of prospective studies. *Front Aging Neurosci* (2018) **9**: doi:10.3389/fnagi.2017.00438
49. Smith LL, Miles MP. "Exercise-Induced Muscle Injury and Inflammation," in *Exercise and Sport Science*, eds. W. E. Garret, D. T. Kirkendall (Philadelphia: Lippincott Williams & Wilkins), 401–412. Available at: https://books.google.nl/books?hl=nl&lr=&id=Cx22TcXodrwC&oi=fnd&pg=PA401&dq=exercise-induced+inflammation&ots=9L98ewUuuC&sig=WGkSl6M8qmkH6K4lsSoIVRInkTo#v=onepage&q=exercise-induced inflammation&f=false [Accessed October 20, 2020]
50. Allison DJ, Josse AR, Gabriel DA, Klentrou P, Ditor DS. Targeting inflammation to influence cognitive function following spinal cord injury: A randomized clinical trial. *Spinal Cord* (2017) **55**:26–32. doi:10.1038/sc.2016.96
51. Chiarugi A, Calvani M, Meli E, Traggiai E, Moroni F. Synthesis and release of neurotoxic kynurenine metabolites by human monocyte-derived macrophages. *J Neuroimmunol* (2001) **120**:190–198. doi:10.1016/S0165-5728(01)00418-0

52. Lustgarten MS, Fielding RA. Metabolites Associated With Circulating Interleukin-6 in Older Adults. *Journals Gerontol Ser A* (2017) **72**:1277–1283. doi:10.1093/GERONA/GLW039
53. Kindler J, Lim CK, Weickert CS, Boerrigter D, Galletly C, Liu D, Jacobs KR, Balzan R, Bruggemann J, O'Donnell M, et al. Dysregulation of kynurenine metabolism is related to proinflammatory cytokines, attention, and prefrontal cortex volume in schizophrenia. *Mol Psychiatry 2019 2511* (2019) **25**:2860–2872. doi:10.1038/s41380-019-0401-9
54. Pedraz-Petrozzi B, Elyamany O, Rummel C, Mulert C. Effects of inflammation on the kynurenine pathway in schizophrenia - A systematic review. *J Neuroinflammation* (2020) **17**:1–17. doi:10.1186/S12974-020-1721-Z/TABLES/4
55. Wichers MC, Maes M. The role of indoleamine 2,3-dioxygenase (IDO) in the pathophysiology of interferon-α-induced depression. *J Psychiatry Neurosci* (2004) **29**:11. Available at: /pmc/articles/PMC305266/ [Accessed December 16, 2021]
56. Vécsei L, Szalárdy L, Fülöp F, Toldi J. Kynurenines in the CNS: Recent advances and new questions. *Nat Rev Drug Discov* (2013) **12**:64–82. doi:10.1038/nrd3793
57. Mangge H, Summers KL, Meinitzer A, Zelzer S, Almer G, Prassl R, Schnedl WJ, Reininghaus E, Paulmichl K, Weghuber D, et al. Obesity-related dysregulation of the Tryptophan–Kynurenine metabolism: Role of age and parameters of the metabolic syndrome. *Obesity* (2014) **22**:195–201. doi:10.1002/OBY.20491
58. Skorobogatov K, De Picker L, Verkerk R, Coppens V, Leboyer M, Müller N, Morrens M. Brain Versus Blood: A Systematic Review on the Concordance Between Peripheral and Central Kynurenine Pathway Measures in Psychiatric Disorders. *Front Immunol* (2021) **12**: doi:10.3389/FIMMU.2021.716980
59. Zwilling D, Huang SY, Sathyasaikumar K V., Notarangelo FM, Guidetti P, Wu HQ, Lee J, Truong J, Andrews-Zwilling Y, Hsieh EW, et al. Kynurenine 3-monooxygenase inhibition in blood ameliorates neurodegeneration. *Cell* (2011) **145**:863–874. doi:10.1016/J.CELL.2011.05.020
60. Nasreddine ZS, Phillips NA, Bédirian V, Charbonneau S, Whitehead V, Collin I, Cummings JL, Chertkow H. The Montreal Cognitive Assessment, MoCA: A brief screening tool for mild cognitive impairment. *J Am Geriatr Soc* (2005) **53**:695–699. doi:10.1111/j.1532-5415.2005.53221.x
61. Bruijnen CJWH, Dijkstra BAG, Walvoort SJW, Budy MJJ, Beurmanjer H, De Jong CAJ, Kessels RPC. Psychometric properties of the Montreal Cognitive Assessment (MoCA) in healthy participants aged 18-70. *Int J Psychiatry Clin Pract* (2020) **24**:293–300. doi:10.1080/13651501.2020.1746348
62. Malek-Ahmadi M, Powell JJ, Belden CM, Oconnor K, Evans L, Coon DW, Nieri W. Age- and education-adjusted normative data for the Montreal Cognitive Assessment (MoCA) in older adults age 70-99. *Neuropsychol Dev Cogn B Aging Neuropsychol Cogn* (2015) **22**:755–761. doi:10.1080/13825585.2015.1041449
63. He X, Li Z, Tang X, Zhang L, Wang L, He Y, Jin T, Yuan D. Age- and sex-related differences in body composition in healthy subjects aged 18 to 82 years. *Medicine (Baltimore)* (2018) **97**: doi:10.1097/MD.0000000000011152
64. Zöllner HJ, Považan M, Hui SCN, Tapper S, Edden RAE, Oeltzschner G. Comparison of different linear-combination modeling algorithms for short-TE proton spectra. *NMR Biomed* (2021) **34**: doi:10.1002/NBM.4482
65. Kanowski M, Kaufmann J, Braun J, Bernarding J, Tempelmann C. Quantitation of simulated short echo time 1H human brain spectra by LCModel and AMARES. *Magn Reson Med* (2004) **51**:904–912. doi:10.1002/MRM.20063
66. Near J, Harris AD, Juchem C, Kreis R, Marjańska M, Öz G, Slotboom J, Wilson M, Gasparovic C. Preprocessing, analysis and quantification in single-voxel magnetic resonance spectroscopy: experts' consensus recommendations. *NMR Biomed* (2021) **34**: doi:10.1002/NBM.4257

67. Glanville NT, Byers DM, Cook HW, Spence MW, Palmer FBSC. Differences in the metabolism of inositol and phosphoinositides by cultured cells of neuronal and glial origin. *Biochim Biophys Acta (BBA)/Lipids Lipid Metab* (1989) **1004**:169–179. doi:10.1016/0005-2760(89)90265-8
68. Urenjak J, Williams SR, Gadian DG, Noble M. Proton nuclear magnetic resonance spectroscopy unambiguously identifies different neural cell types. *J Neurosci* (1993) **13**:981–989. doi:10.1523/jneurosci.13-03-00981.1993
69. Bitsch A, Bruhn H, Vougioukas V, Stringaris A, Lassmann H, Frahm J, Brück W. Inflammatory CNS Demyelination: Histopathologic Correlation with In Vivo Quantitative Proton MR Spectroscopy. (1999).
70. Cichocka M, Bereś A. From fetus to older age: A review of brain metabolic changes across the lifespan. *Ageing Res Rev* (2018) **46**:60–73. doi:10.1016/j.arr.2018.05.005
71. Waragai M, Moriya M, Nojo T. Decreased N-Acetyl Aspartate/Myo-Inositol Ratio in the Posterior Cingulate Cortex Shown by Magnetic Resonance Spectroscopy May Be One of the Risk Markers of Preclinical Alzheimer's Disease: A 7-Year Follow-Up Study. *J Alzheimer's Dis* (2017) **60**:1411–1427. doi:10.3233/JAD-170450
72. Benjamini Y, Hochberg Y. Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. *J R Stat Soc Ser B* (1995) **57**:289–300. doi:10.1111/J.2517-6161.1995.TB02031.X
73. Rosen Y, Lenkinski RE. Recent Advances in Magnetic Resonance Neurospectroscopy. *Neurotherapeutics* (2007) **4**:330–345. doi:10.1016/j.nurt.2007.04.009
74. Graff-Radford J, Kantarci K. Magnetic resonance spectroscopy in Alzheimer's disease. *Neuropsychiatr Dis Treat* (2013) **9**:687–96. doi:10.2147/NDT.S35440
75. Ge X, Xu X-Y, Feng C-H, Wang Y, Li Y-L, Feng B. Relationships among serum C-reactive protein, receptor for advanced glycation products, metabolic dysfunction, and cognitive impairments. *BMC Neurol* (2013) **13**:110. doi:10.1186/1471-2377-13-110
76. Danielsen ER, Ross B eds. *Magnetic Resonance Spectroscopy Diagnosis of Neurological Diseases*. 1st ed. New York: Marcel Dekker Inc (1999). Available at: https://www.amazon.com/Magnetic-Resonance-Spectroscopy-Diagnosis-Neurological/dp/0824702387 [Accessed October 24, 2020]
77. Ho TC, Teresi GI, Segarra JR, Ojha A, Walker JC, Gu M, Spielman DM, Sacchet MD, Jiang F, Rosenberg-Hasson Y, et al. Higher Levels of Pro-inflammatory Cytokines Are Associated With Higher Levels of Glutamate in the Anterior Cingulate Cortex in Depressed Adolescents. *Front Psychiatry* (2021) **12**:346. doi:10.3389/FPSYT.2021.642976/BIBTEX
78. Ali NS, Hashem AHH, Hassan AM, Saleh AA, El-Baz HN. Serum interleukin-6 is related to lower cognitive functioning in elderly patients with major depression. *Aging Ment Health* (2018) **22**:655–661. doi:10.1080/13607863.2017.1293005
79. Fayed N, Andrés E, Viguera L, Modrego PJ, Garcia-Campayo J. Higher glutamate+glutamine and reduction of N-acetylaspartate in posterior cingulate according to age range in patients with cognitive impairment and/or pain. *Acad Radiol* (2014) **21**:1211–1217. doi:10.1016/J.ACRA.2014.04.009
80. Lewis CP, Port JD, Blacker CJ, Sonmez AI, Seewoo BJ, Leffler JM, Frye MA, Croarkin PE. Altered anterior cingulate glutamatergic metabolism in depressed adolescents with current suicidal ideation. *Transl Psychiatry* (2020) **10**:119. doi:10.1038/S41398-020-0792-Z
81. Káradóttir R, Attwell D. Neurotransmitter receptors in the life and death of oligodendrocytes. *Neuroscience* (2007) **145**:1426–1438. doi:10.1016/J.NEUROSCIENCE.2006.08.070
82. Matute C. Glutamate and ATP signalling in white matter pathology. *J Anat* (2011) **219**:53–64. doi:10.1111/J.1469-7580.2010.01339.X
83. Moriguchi S, Takamiya A, Noda Y, Horita N, Wada M, Tsugawa S, Plitman E, Sano Y, Tarumi R, ElSalhy M, et al. Glutamatergic neurometabolite levels in major depressive disorder: a systematic review and meta-analysis of proton magnetic resonance spectroscopy studies. *Mol Psychiatry 2018 247* (2018) **24**:952–964. doi:10.1038/s41380-018-0252-9

84. Oeltzschner G, Wijtenburg SA, Mikkelsen M, Edden RAE, Barker PB, Joo JH, Leoutsakos JMS, Rowland LM, Workman CI, Smith GS. Neurometabolites and associations with cognitive deficits in mild cognitive impairment: a magnetic resonance spectroscopy study at 7 Tesla. *Neurobiol Aging* (2019) **73**:211–218. doi:10.1016/J.NEUROBIOLAGING.2018.09.027
85. Harris JL, Yeh H-W, Swerdlow RH, Choi I-Y, Lee P, Brooks WM. High-field proton magnetic resonance spectroscopy reveals metabolic effects of normal brain aging. *Neurobiol Aging* (2014) **35**:1686–1694. doi:10.1016/j.neurobiolaging.2014.01.018
86. Cleeland C, Pipingas A, Scholey A, White D. Neurochemical changes in the aging brain: A systematic review. *Neurosci Biobehav Rev* (2019) **98**:306–319. doi:10.1016/j.neubiorev.2019.01.003
87. Wilson M, Reynolds G, Kauppinen RA, Arvanitis TN, Peet AC. A constrained least-squares approach to the automated quantitation of in vivo ^{1}H magnetic resonance spectroscopy data. *Magn Reson Med* (2011) **65**:1–12. doi:10.1002/MRM.22579
88. Mosconi E, Sima DM, Osorio Garcia MI, Fontanella M, Fiorini S, Van Huffel S, Marzola P. Different quantification algorithms may lead to different results : a comparison using proton MRS lipid signals. *NMR Biomed* (2014) **27**:431–443. doi:10.1002/NBM.3079
89. Scott J, Underwood J, Garvey LJ, Mora-Peris B, Winston A. A comparison of two post-processing analysis methods to quantify cerebral metabolites measured via proton magnetic resonance spectroscopy in HIV disease. *Br J Radiol* (2016) **89**:20150979. doi:10.1259/BJR.20150979
90. Kossowski B, Orzeł J, Bogorodzki P, Wilson M, Setkowicz Z, P. Gazdzinski S. Follow-up analyses on the effects of long-term use of high fat diet on hippocampal metabolite concentrations in Wistar rats: Comparing Tarquin quantification of 7.0T rat metabolites to LCModel. *Biol Eng Med* (2017) **2**:1–7. doi:10.15761/BEM.1000129
91. Pedersen BK, Febbraio MA. Muscles, exercise and obesity: Skeletal muscle as a secretory organ. *Nat Rev Endocrinol* (2012) **8**:457–465. doi:10.1038/nrendo.2012.49
92. Carey AL, Bruce CR, Sacchetti M, Anderson MJ, Olsen DB, Saltin B, Hawley JA, Febbraio MA. Interleukin-6 and tumor necrosis factor-α are not increased in patients with Type 2 diabetes: Evidence that plasma interleukin-6 is related to fat mass and not insulin responsiveness. *Diabetologia* (2004) **47**:1029–1037. doi:10.1007/s00125-004-1403-x
93. Kline R, Wong E, Haile M, Didehvar S. Peri-Operative Inflammatory Cytokines in Plasma of the Elderly Correlate in Prospective Study with Postoperative Changes in Cognitive Test Scores. *Int J Anesthesiol Res* (2016) **4**:313–321. doi:10.19070/2332-2780-1600065
94. Puzianowska-Kuźnicka M, Owczarz M, Wieczorowska-Tobis K, Nadrowski P, Chudek J, Slusarczyk P, Skalska A, Jonas M, Franek E, Mossakowska M. Interleukin-6 and C-reactive protein, successful aging, and mortality: The PolSenior study. *Immun Ageing* (2016) **13**: doi:10.1186/s12979-016-0076-x
95. Paine NJ, Bosch JA, Ring C, Drayson MT, Veldhuijzen van Zanten JJCS. Induced mild systemic inflammation is associated with impaired ability to improve cognitive task performance by practice. *Psychophysiology* (2015) **52**:333–341. doi:10.1111/psyp.12360
96. Shivakumar V, Debnath M, Venugopal D, Rajasekaran A, Kalmady S V., Subbanna M, Narayanaswamy JC, Amaresha AC, Venkatasubramanian G. Influence of correlation between HLA-G polymorphism and Interleukin-6 (IL6) gene expression on the risk of schizophrenia. *Cytokine* (2018) **107**:59–64. doi:10.1016/j.cyto.2017.11.016
97. Marsland AL, Gianaros PJ, Kuan DCH, Sheu LK, Krajina K, Manuck SB. Brain morphology links systemic inflammation to cognitive function in midlife adults. *Brain Behav Immun* (2015) **48**:195–204. doi:10.1016/j.bbi.2015.03.015
98. Thielen JW, Kärgel C, Müller BW, Rasche I, Genius J, Bus B, Maderwald S, Norris DG, Wiltfang J, Tendolkar I. Aerobic activity in the healthy elderly is associated with larger plasticity in memory related brain structures and lower systemic inflammation. *Front Aging Neurosci* (2016) **8**: doi:10.3389/fnagi.2016.00319

99. Starkie R, Ostrowski SR, Jauffred S, Febbraio M, Pedersen BK. Exercise and IL-6 infusion inhibit endotoxin-induced TNF-α production in humans. *FASEB J* (2003) **17**:1–10. doi:10.1096/fj.02-0670fje
100. Solvang SEH, Nordrehaug JE, Tell GS, Nygård O, McCann A, Ueland PM, Midttun Ø, Meyer K, Vedeler CA, Aarsland D, et al. The kynurenine pathway and cognitive performance in community-dwelling older adults. The Hordaland Health Study. *Brain Behav Immun* (2019) **75**:155–162. doi:10.1016/j.bbi.2018.10.003

CHAPTER 4

Body fat and components of sarcopenia relate to inflammation, brain volume, and neurometabolism in older adults

Authors: Wouter A.J. Vints, Simona Kušleikienė, Samrat Sheoran, Kristina Valatkevičienė, Rymantė Gleiznienė, Uwe Himmelreich, Mati Pääsuke, Vida J. Česnaitienė, Oron Levin, Jeanine Verbunt, Nerijus Masiulis

Published in Neurobiology of Aging, 2023, Vol. 127. p. 1-11
https://doi-org.mu.idm.oclc.org/10.1016/j.neurobiolaging.2023.02.011

Abstract

Obesity and sarcopenia are associated with cognitive impairments at older age. Current research suggests that blood biomarkers may mediate this body-brain crosstalk, altering neurometabolism and brain structure eventually resulting in cognitive performance changes. Seventy-four older adults (60-85 years old) underwent bio-impedance body composition analysis, handgrip strength measurements, 8-Foot Up-and-Go (8UG) test, Montreal Cognitive Assessment (MoCA), blood analysis of interleukin-6 (IL-6), kynurenine, and insulin-like growth factor-1 (IGF-1), as well as brain magnetic resonance imaging (MRI) and proton magnetic resonance spectroscopy (^{1}H-MRS), estimating neurodegeneration and neuroinflammation. Normal fat% or overweight was associated with larger total gray matter volume compared to underweight or obesity in older adults and obesity was associated with higher N-acetylaspartate/Creatine levels in the sensorimotor and dorsolateral prefrontal cortex. Muscle strength, not muscle mass/physical performance, corresponded to lower kynurenine and higher N-acetylaspartate/Creatine levels in the dorsal posterior cingulate and dorsolateral prefrontal cortex. The inflammatory and neurotrophic blood biomarkers did not significantly mediate these body-brain associations. This study used a multimodal approach to comprehensively assess the proposed mechanism of body-brain crosstalk.

Abbreviations

^{1}H-MRS, proton magnetic resonance spectroscopy; BMI, body mass index; CRP, C-reactive protein; DLPFC, dorsolateral prefrontal cortex; DPCC, dorsal posterior cingulate cortex; ELISA, enzyme-linked immunosorbent assay; EWGSOP, European Working Group on Sarcopenia in Older People; FDR, false discovery rate; fat %, body fat percentage; FFM, fat free mass; FWHM, full width at half maximum; Glx, glutamate-glutamine complex; GMV, gray matter volume; HPC, hippocampal cortex; ICC, intraclass correlation coefficient; IFN-γ, interferon-γ; IGF-1, insulin-like growth factor-1; IL-1β, interleukin-1β; IL-4, interleukin-4; IL-6, interleukin-6; IL-8, interleukin-8; IL-10, interleukin-10; LCModel, linear combination of model spectra; mIns, myoinositol; MoCA, Montreal Cognitive Assessment; MPRAGE, magnetization prepared gradient echo; MTC, medial temporal cortex; PRESS, Point RESolved Spectroscopy; SM1, primary sensorimotor cortex; SMI, skeletal muscle mass index; tCho, total choline; tCr, total creatine; tNAA, total N-acetyl aspartate; TNF-α, tumor necrosis factor-α; 8UG, 8-Foot Up-and-Go.

1. Introduction

Age-related cognitive decline (i.e. cognitive aging) is one of the major concerns of the presently aging society (Kronschnabl et al., 2021). Poor cognitive function is associated with a loss of independence (Tucker-Drob, 2019; Zhu et al., 2008), social withdrawal (Sartori et al., 2012), and decreased quality of life (Stites et al., 2018). Even mild declines in cognitive abilities can impel an individual to alter his/her activities of daily living, and may lead to frustrations (Sartori et al., 2012). It seems inevitable that the big majority of our population will someday experience functional deficits due to cognitive aging (Petersen, 2011). However, there is a large interindividual variability in the age at which people start to experience these functional deficits (Nyberg et al., 2020; Tucker-Drob, 2019). This interindividual variability can be explained by genetic and environmental risk factors. Importantly, it has been reported that around one in three cases of Alzheimer's disease worldwide could be prevented if we would optimize modifiable risk factors (Livingston et al., 2020; Norton et al., 2014). Two well-known modifiable risk factors of cognitive decline are obesity and sarcopenia. Both are important contributors to functional deficits at older age, as they are not only risk factors of cognitive decline, but also directly contribute to physical disabilities (Baumgartner, 2000; Chang et al., 2016; Cipolli et al., 2019; Cournot et al., 2006; Mrak, 2009; Peng et al., 2020; Sartori et al., 2012; Tolea and Galvin, 2015). A better understanding of the link between cognitive aging and modifiable risk factors, such as obesity and sarcopenia, may direct researchers to specific targets for preventing cognitive aging.

Obesity is a major health problem in the current society. The prevalence of overweight and obesity is increasing worldwide (Blüher, 2019). Especially in Europe (Peralta et al., 2018) and the USA (Ogden et al., 2014), obesity prevalence in older adults has reached epidemic proportions. Of importance, a longitudinal study discovered that middle-aged obese adults suffer accelerated cognitive aging when compared with non-obese counterparts over a 10 year follow-up period (Singh-Manoux et al., 2012). It has been suggested that the link between obesity and cognitive decline is mediated by a chronic state of inflammation (Beilharz et al., 2016; Tang et al., 2015). Indeed, adipose tissue is found to be one of the largest sources of inflammatory markers in the human body (Woods et al., 2012; Yudkin, 2007). These inflammatory markers readily cross the blood brain barrier and may result in neuroinflammation (Agudelo et al., 2014; Banks et al., 1995; Fukui et al., 1991). Neuroinflammatory processes can subsequently damage neurons in the central nervous system, resulting in a loss of neural integrity or density (i.e. neurodegeneration), eventually leading to cognitive decline (Bourgognon and Cavanagh, 2020; Sartori et al., 2012; Scheiblich et al., 2020). To estimate the level of neuroinflammation and neurodegeneration in vivo, proton magnetic resonance spectroscopy (^{1}H-MRS) can be adopted. However, only few studies have used this technique in the context of obesity. For example, Gonzales et al. (2012), reported that a higher body mass index (BMI) was associated with higher levels of neuroinflammation, as expressed by an elevation of the ratio of myo-inositol (mIns) to creatine (Cr), in the occipitoparietal cortex, which was indirectly related to decreased memory performance (Gonzales et al., 2012). Another ^{1}H-MRS study example was published by Coplan et al. (2014), who discovered that a higher BMI was associated with decreased levels of neural integrity, as expressed by reduced levels of N-acetylaspartate (NAA) in the hippocampus (Coplan et al., 2014). Neuroinflammation is also considered to explain the increased rate of age-related brain volume loss in the context of obesity, as reviewed by García-García et al. (2022). However, the authors of this review only refer to animal studies and not to ^{1}H-MRS research when it comes to neuroinflammation. Anyway, the negative effects of obesity on brain volume have been described by multiple longitudinal magnetic resonance imaging (MRI) studies (Bobb et al., 2014; Shaw et al., 2017). Of interest, a meta-analysis of ten cross-sectional studies reported lower brain grey matter volume (GMV) of the left, middle and right inferior frontal gyrus, the left precentral gyrus, the left middle temporal cortex and the cerebellum, while there was a larger GMV of the left cuneus, left middle frontal gyrus, left inferior occipital gyrus and corpus callosum in obese young and older adults (Herrmann et al., 2019). Another longitudinal study using whole-body MRI reported that specifically liver fat, muscle fat infiltration and weight-to-muscle ratio were predictors of accelerated brain aging (Beck et al., 2022).

Sarcopenia is another health concern with increasing societal prevalence (Ethgen et al., 2017). Importantly, older adults with sarcopenia are six times more likely to have a combined cognitive and physical impairment compared to healthy controls (Tolea and Galvin, 2015). Tolea and Galvin (2015) discovered that lower muscle strength, and not

muscle mass, was related to cognitive impairment (Tolea and Galvin, 2015). Other studies have also recognized muscle strength as a better predictor of other adverse outcomes, which has led to the decision to revise the 2010 European Working Group on Sarcopenia in Older People (EWGSOP) definition of sarcopenia. In the new definition that was published in 2019, muscle strength is now the principal determinant of sarcopenia instead of muscle mass (Cruz-Jentoft et al., 2019). There is abundant evidence that muscle strength and muscle mass should be considered separate health markers that are regulated differently (Clark and Manini, 2008). This warrants the need to investigate each of the components of sarcopenia separately. Similar to obesity, sarcopenia has also been linked to chronic inflammation (Beyer et al., 2012; Tuttle et al., 2020). However, to our knowledge, no human ^{1}H-MRS studies exist that have examined the link between (components of) sarcopenia and neuroinflammation. Concerning the link between (components of) sarcopenia and brain volume, the literature is also limited. However, some studies have shown a positive correlation between brain volume and muscle mass (Burns et al., 2010; Kilgour et al., 2014) or physical performance assessed as gait speed (Rosano et al., 2005; Silbert et al., 2008). We also found one longitudinal study with a four year follow-up that showed associations between sarcopenia at baseline and increased parietal GMV atrophy, lower muscle strength at baseline and larger age-related decreases in total brain GMV, and lower muscle mass at baseline and larger age-related decreases in total GMV, frontal GMV and occipital GMV (Yu et al., 2021). In addition to its link with inflammation, sarcopenia has also been associated with lower levels of insulin-like growth factor-1 (IGF-1) (Bian et al., 2020). This hormone is considered to be critical for neuroplastic processes in the adult brain (Frater et al., 2018; Vints et al., 2022b). Like the age-related increases in inflammation in our blood circulation, the age-related reduction in circulating IGF-1 levels is also suggested to play a role in cognitive aging (Frater et al., 2018).

The aim of the current study was to provide a comprehensive overview on the proposed mechanisms underlying body-brain crosstalk in the context of obesity and sarcopenia (Figure 1) by assessing how (1) body fat percentage (fat%), and (2) muscle strength, muscle mass, and physical performance are associated with serum inflammatory and neurotrophic factor levels, markers of neuroinflammation and neural integrity, total and regional brain GMV and general cognitive function. In accordance with the literature provided above, we hypothesized that high fat%, low muscle strength, low muscle mass and poor physical performance would be associated with high serum inflammatory and low neurotrophic factor levels, high markers of neuroinflammation and low neural integrity, low total and regional brain volumes, and poor general cognitive function (Gonzales et al., 2012; Herrmann et al., 2019; Scheiblich et al., 2020; Tang et al., 2015; Tolea and Galvin, 2015; Yudkin, 2007). Finally, we predicted that the blood serum biomarkers would be possible mediators of the relationship between body and brain/cognitive function. We hypothesized that the neurotrophic marker insulin-like growth factor-1 (IGF-1) would play a role in mediating the relationship between strong older adults

or older adults with large muscular volume and markers of brain health or high cognitive test scores (Bian et al., 2020; Frater et al., 2018; Vints et al., 2022b), while we hypothesized that the inflammatory blood biomarkers interleukin-6 (IL-6) and kynurenine would play a role in mediating the relationship of high fat%, poor strength, low muscle mass or poor physical performance with poor brain health measures and poor cognitive performance (Beyer et al., 2012; Sartori et al., 2012; Tang et al., 2015; Vints et al., 2022a).

To the best of our knowledge, we are the first to profoundly explore the mediating effect of blood biomarkers on brain health and general cognition in the concept of the link between fat% and the brain and the link between components of sarcopenia and the brain in older adults. This paper may lead to a better understanding of the influential effect of different aspects of body composition and muscular fitness on brain health and cognitive function. Ultimately, this knowledge may serve in designing treatment strategies aiming to prevent age-related cognitive decline.

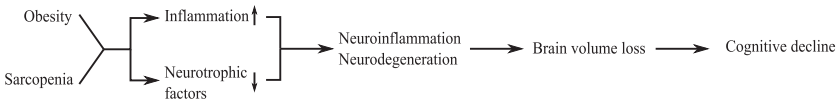


Figure 1. Schematic hypothetical framework of the relationship between obesity or sarcopenia and cognitive aging via alterations in blood biomarkers, neurometabolites and brain volume

2. Material and methods

2.1 Participants and setting

Participants were 74 apparently healthy male and female adults aged from 60 to 85 years old that were from the same pool of participants as in the study of Vints et al. (2022a) recruited as described previously (Vints et al., 2022a). The exclusion criteria included a diagnosis of a psychiatric or neurological disorder or the use of centrally acting medication in the last five years. Participants were physically healthy and able to perform 10 sit-ups, but they did not participate in any regular exercise program in the last six months. We excluded participants with current alcohol or drug abuse. Additionally, excluded participants were those with diabetes mellitus, or with oncologic disorders or a history of chemotherapy treatment. At last, we followed the exclusion criteria for MRI scanning derived from a checklist provided by the department of radiology at the Lithuanian University of Health Science. This list excluded participants with MRI-incompatible implants, claustrophobia, or a weight over 130kg. Participants could voluntarily withdraw from the study at any time. The study was approved by the Kaunas Regional Biomedical Research Ethics Committee (No. BE-10-7) and a written informed consent was obtained from all participants prior to their inclusion in the study.

2.2 Demographic characteristics

All participants were asked to report age, sex, smoking status and educational level (i.e. basic education, secondary education or higher education). Furthermore, the Montreal Cognitive Assessment (MoCA) test was conducted by a qualified health care specialist in psychiatry (co-author SK) to evaluate the cognitive status of the participants. The MoCA test is reliable and sensitive screening tool to evaluate the risk of mild cognitive impairment in the geriatric population (Bruijnen et al., 2020; Nasreddine et al., 2005). It consists of an assessment of seven cognitive domains: executive functioning/ visuospatial abilities; naming; memory; attention, language; abstract reasoning; and orientation. The maximum total score is 30 points, where a higher score indicates better cognitive functioning. As indicated on the MoCA test instructions, one point was added to the score if a participant did not complete a higher education. Nasreddine et al. 2005 proposed that total MoCA scores of 25 or below indicate a high risk for mild cognitive impairment (Nasreddine et al., 2005). Based on this cutoff, 52.1% of the included participants had mild cognitive impairment. The lowest MoCA test score in our study was 19. None of the included participants were considered to have Alzheimer's dementia, based on MoCA test cutoffs and clinical evaluation by a psychiatrist (co-author SK).

At last, we assessed the participants' body mass index (BMI) and measured their body fat percentage (fat%) and fat free mass using leg-to-leg bio-impedance analysis (BIA, Tanita TBF-300-A). Standing leg-to-leg BIA, also called bipolar or foot-to-foot BIA, measures impedance through an electronic pathway of the lower extremities, and is widely used as an easy, noninvasive and inexpensive method to estimate whole-body composition (Wu et al., 2015). It is considered a valid method for the estimation of total body fat free mass (Cable et al., 2001) and total body fat% (Ritchie et al., 2005). Analysis with fat% is considered an improved phenotypic characteristic over BMI when assessing participants' health (Gallagher et al., 2000). For non-Asian adults aged 60 years and over, a healthy fat% lies between 24 and 36% for women and 13 and 25% for men. Any values below correspond to underweight, and above correspond to overweight. Obesity as a measure of fat% starts from 42% for women and 30% for men (Gallagher et al., 2000).

2.3 Functional assessment

Based on the revised European Working Group on Sarcopenia in Older People 2019 (EWGSOP2) criteria (Cruz-Jentoft et al., 2019), participants were categorized in three groups, (1) probable sarcopenia (i.e. low muscle strength), (2) confirmed sarcopenia (i.e. low muscle strength + low muscle quality or quantity) or (3) severe sarcopenia (i.e. low muscle strength + low muscle mass + low physical performance). We used handgrip strength of the right hand as a measure for muscle strength, BIA-estimated skeletal muscle mass index (SMI, see formula below) as a measure of muscle quantity, and the 8UG test as a measure of physical performance, using cut-off values presented in Table 1 (Cruz-Jentoft et al., 2019).

Measures of muscle mass:
- Skeletal muscle mass index (kg/m²): leg-to-leg BIA was used to estimate the SMI. First, absolute skeletal muscle mass was calculated using the BIA equation from a previous study (Janssen et al., 2000): Absolute skeletal muscle mass (kg) = [0.401 × (height²/ resistance) + (3.825 × sex) - (0.071 × age) + 5.102], where height is in cm; resistance is in ohms; for sex, men = 1 and women = 0; and age is in years. Absolute skeletal muscle mass measured with leg-to-leg BIA was shown to provide accurate results on a group level (Bosy-Westphal et al., 2008). Second, SMI was calculated by dividing absolute skeletal muscle mass by height in meters squared (kg/m²) (Chien et al., 2008). Third, we also reported the fat free mass (kg) as given by the BIA.

Measures of muscle strength:
- Handgrip strength (kg): Participants' handgrip strength of the right hand was measured using a dynamometer (JAMAR 11940248 Adjustable Hand Grip Strength Testing System) in standing position. The grip size was adjusted so that the second joint of the index finger was at a 90 degree angle on the handle. After a first try at submaximal effort, participants were instructed to squeeze the handle as hard as they can. The test was performed two times with 1 min rest period between trials, and the highest value was used for analysis.

Measures of physical performance:
- 8-Foot Up-and-Go test (s): The 8UG test was performed as described for the Fullerton Fitness Test and assesses a person's agility and dynamic balance (Kirschke et al., 2006). The test result is the time required for a person to rise from an armless chair, walk 8 feet (2.44 m), turn around a cone and return to the sitting position as fast as possible. Participants were given two trials with 1 min rest period in seated position, and the trial with shortest time was used for analysis.

In addition, we described self-reported physical activity level based on self-reported light/moderate/high intense exercise time per week. Based on the time participants described to perform a specific type of physical activity, the total calories burned during exercise per week were estimated. The following equation was used based on previous research from Sjostrom et al. (2005): Total kcal/week burned during exercise = the sum of days performing light/moderate/vigorous intense exercise × average time/day performing light/moderate/vigorous intense exercise × F, where F is 8.0 for vigorous intense exercise, 4.0 for moderate intense exercise and 3.3 for light intense exercise. Total weekly kcal burned with exercise was used to estimate physical activity level of the participants, with participants burning < 600 kcal/week defined as sedentary, 600-3000 kcal/week defined as moderately physically active, and participants burning > 3000 kcal/week defined as highly physically active (Sjostrom et al., 2005).

2.4 Blood serum analysis

Blood samples were drawn between 9 a.m. and 1 p.m. by a qualified medical professional. Blood was collected at the antecubital vein in 5 mL serum separator tubes. Immediately after collection, tubes were gently inverted 8-10 times and allowed to clot for 30 min at room temperature. Then, the tubes were centrifugated at 4000 g for 15 min. Finally, blood serum was aliquoted into 1.5 mL polypropylene tubes and stored at -80°C in the refrigerator compartment of the laboratory of the Lithuanian Sports University until further analysis with enzyme-linked immunosorbent assay (ELISA). After completion of the study, ELISA tests for IL-6, kynurenine and IGF-1 were analyzed with a spectrophotometer (Spark 10M, Tecan Group Ltd., Zürich, Switzerland) by an experienced lab technician.

Table 1. Variables and cut-off values for diagnosis of sarcopenia

Criterion	Measurement method	Cut-off points by sex
Muscle strength	Handgrip strength	• Men: < 27.0 kg • Women: < 16.0 kg
Muscle mass	BIA-predicted SMI	• Men: ≤ 8.9 kg/m² • Women: ≤ 6.4 kg/m²
Physical performance	8-foot Up-and-Go test (2.44m)	• Men: > 9.2 s • Women: > 10.0 s

Cut-off values were based on EWGSOP2 consensus recommendations (Cruz-Jentoft et al., 2019). Cut-off values for handgrip strength were derived from the study of Dodds et al. (2014). The EWGSOP2 describes a cut-off for SMI derived from a dual-energy X-ray absorptiometry (DXA) study, while in our study BIA was used to estimate SMI. They also suggest the 3m Timed Up-and-Go test, while we used the American alternative, the 8-foot Up-and-Go test. Therefore, we calculated the cut-off values for the BIA-predicted SMI and 8-foot Up-and-Go test from the studies of Janssen et al. (2000) and Rikli and Jones (1999) respectively, according to the EWGSOP2 recommendation to place cut-off points at two standard deviations from the mean reference value.
Abbreviations: BIA, bio-impedance analysis; EWGSOP2, European Working Group on Sarcopenia in Older People; SMI, skeletal muscle mass index

IL-6 levels were analyzed using a commercially available ELISA kit purchased from DIAsource ImmunoAssays S.A., Belgium (KAP1216). The lower limit of detection was 2 pg/mL. Kynurenine levels were analyzed using a commercially available ELISA kit purchased from MyBiosource, Inc., USA. The lower limit of detection was 45.7 ng/mL. IGF-1 levels were measured using a commercially available ELISA kit purchased from IBL International, GMBH, Germany (MD58011). The lower limit of detection was 0.03 ng/ml.

2.5 Brain imaging and ^{1}H-MRS

Whole brain MRI and ^{1}H-MRS scanning was performed using a 3 Tesla Skyra scanner (Siemens Healthineers, Erlangen, Germany) with a 32-channel receiver head coil. Total scanning duration was 90 min per participant.

Total and regional gray matter volumes (GMV) were calculated from 3D magnetization prepared gradient echo (MPRAGE) images acquired from high-resolution T1-weighted (T1W) structural MRI (repetition time (TR) = 2200 ms, echo time (TE) = 2.48 ms, 0.9 × 0.9 × 1.0 mm^3 voxels, field of view: 230 × 256 mm, number of sagittal slices = 176) using the FreeSurfer software v7.1.1 (Harvard, MA, USA, http://surfer.nmr.mgh.harvard.edu/). Regions of interest were the dorsal posterior cingulate cortex (DPCC), left and right hippocampal cortex (HPC), left middle temporal cortex (MTC), left primary sensorimotor cortex (SM1) and right dorsolateral prefrontal cortex (DLPFC). A detailed description of the FreeSurfer regions was presented previously in Vints et al. (2022a).

Regional voxel-based neurometabolite levels were calculated from ^{1}H-MRS spectra using a Point RESolved Spectroscopy (PRESS) sequence (TR = 2000 ms, TE = 30 ms, number of averages = 128, spectral bandwidth = 2000 Hz, data size = 1024 points) with chemical shift selective water suppression (sequence svs_se_30). A detailed description of the ^{1}H-MRS methods is presented in Appendix B based on the minimum reporting standards of the MRSinMRS experts' consensus recommendations (Lin et al., 2021). Voxels were placed on the DPCC, left HPC, left MTC, left SM1 and right DLPFC; see Figure 2. A detailed description of the voxel position was described previously in Vints et al. (2022a). Voxel sizes were: (i) 1.6 × 1.6 × 1.6 cm^3 in the DPCC, left SM1 and right DLPFC voxels, (ii) 20 × 12 × 16 cm3 in the left MTC and (iii) 26 × 12 × 12 cm3 in the left HPC. Voxel-specific shimming was performed using automated B0-field mapping followed by manual adjustment to reduce the water signal full width at half maximum (FWHM) below 15 Hz. The MR spectra were processed using the linear combination of model spectra (LCModel, version 6.3.1-R). In total 340 spectra (i.e. 68 participants × 5 voxels of interest) were acquired. Only spectra with FWHM < 15 Hz, signal-to-noise ratio > 5 were considered of sufficient quality to be included. All included neurometabolites were quantified with a Cramér-Rao lower bound <20%. ^{1}H-MRS spectra were visually checked to ensure the absence of artifacts prior to quantification. This resulted in the elimination of 57 spectra (8.4%). The number of participants (n = 74) from whom good quality measurements could be attained ranged from 56 (75.7%) for the left MTC to 67 (90.5%) for the right DLPFC. The number of retained spectra per voxel of interest are presented in Supplementary table A.1. ^{1}H-MRS quantifiable neurometabolites were (1) total NAA (tNAA) composed of N-acetylaspartate and N-acetylaspartylglutamate, (2) total creatine (tCr) composed of creatine and phosphocreatine, (3), total choline (tCho) composed of phosphorylcholine and glycerophosphocholine, (4) mIns, and (5) Glx composed of glutamate and glutamine. Both water-referenced levels of tNAA, tCr, tCho, mIns, and Glx and ratios relative to tCr were calculated for each voxel location. For tNAA

also the ratio relative to mIns was calculated. The main outcome measures were tNAA/tCr, as a marker of neural integrity or neural density, mIns/tCr, as a marker of glial cell proliferation or neuroinflammation, and the ratio of tNAA/mIns. Results from analyses with the other neurometabolites (namely tCho and Glx) are only presented in Appendix A. We noted that in general the same conclusions could be drawn from the relative and absolute, water-referenced, neurometabolite levels. Therefore, observations including the water-referenced neurometabolite levels are also only presented in Appendix A.

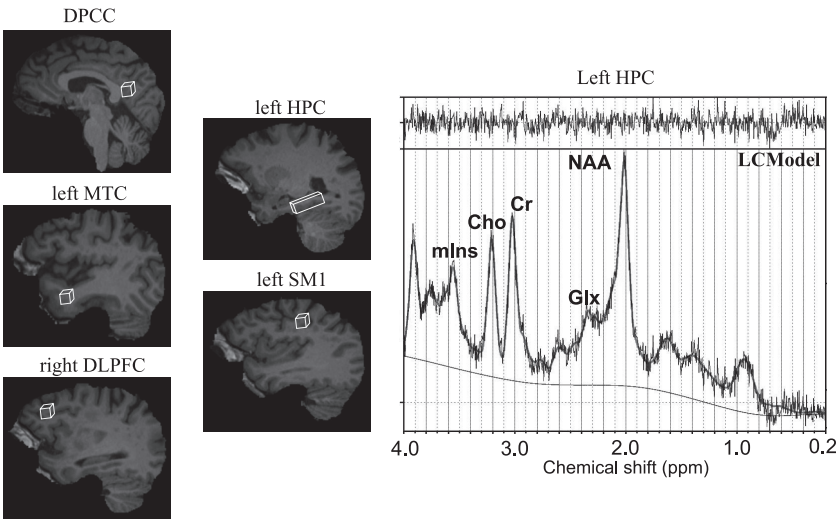


Figure 2. Example voxel positions and spectra from a representative participant

Voxel positions are presented on the left side of the figure. Raw (black curve) and fitted (red curve) spectra from LCModel are illustrated for the left HPC on the right side of the figure.

Abbreviations: Cho, total choline; Cr, creatine + phosphocreatine; DLPFC, dorsolateral prefrontal cortex; DPCC, dorsal posterior cingulate cortex; Glx, glutamate-glutamine complex; HPC, hippocampal cortex; mIns, myoinositol; MTC, medial temporal cortex; NAA, N-acetyl aspartate; SM1, primary sensorimotor cortex.

2.6 Statistical analysis

Statistical analysis was performed using SPSS Statistics version 27 (IBM Inc, Chicago USA). The data was first checked for extreme outliers (defined as lying more than three times interquartile range from the mean) and normal distribution (defined as kurtosis and skewness between -2 and +2 and checked graphically using PP plots and histograms) (George and Mallery, 2010). IL-6 levels were log transformed, as they did not meet the normality assumption.

2.6.1 Investigation of sex differences

To evaluate the effect of sex differences on nominal variables, we used $\chi 2$ or Fisher Exact statistics. To compare continuous variables between sexes, a two-sided independent t-test was performed.

2.6.2 Investigations of body-brain or body-blood biomarker associations

Multiple linear regression adjusted for age and sex was used to assess how fat% was related to serum levels of IL-6, kynurenine and IGF-1, brain total and regional GMV measures and cognition. In addition, each association with brain volume measurements in this paper was adjusted for intracranial volume. Assessments of relationships with SMI were not adjusted for age and sex, as the formula used to calculate absolute skeletal muscle mass already contains these factors. Associations with right handgrip strength as well as with performance on the 8UG test were adjusted for age, sex, and body fat%. Appendix A also includes these associations adjusted for age, sex and BMI.

2.6.3 Mediation analysis

We investigated if differences in blood biomarker levels could be part of the underlying mechanism responsible for relationships between body and brain outcome measures by using mediation analysis. Blood biomarkers that showed significant associations with fat%, handgrip strength, SMI or the 8UG test result (significant body-blood biomarker associations) were considered possible mediators of the significant associations between fat%, handgrip strength, SMI or 8UG test results and brain volume or neurometabolite levels. Mediation analysis was performed using model 4 of the SPSS macro named PROCESS, developed by Hayes (2022), including age, sex and fat% as covariates. This macro was used to calculate the indirect effect of the blood biomarker on the body-brain interaction. The indirect effect was presented as a 95% confidence interval determined using 5000 stratified bootstrap samples. Effect sizes were R^2_{med} values, representing the proportion of variance attributed to the indirect effect by the mediator, which is calculated by subtracting the R^2 of the model including the mediator by the R^2 of the model without the mediator (Fairchild et al., 2009). The direct effects between the variables included in the mediation model were derived from the multiple regression analysis described in section 2.6.2.

2.6.4 Interpretation of statistical significance

For the purpose of this exploratory study, p-values below 0.05 were considered statistically significant. In addition, we tested if these p-values survived correction for multiple testing with false discovery rate (FDR) analysis (Benjamini and Hochberg adjustment) (Benjamini and Hochberg, 1995). The FRD procedure was done multiple times for each of the independent variables (fat%, right handgrip strength, SMI, and 8UG test) for all p-values presented in Supplementary Tables A.2-A.17. It should be noted that the results surviving the multiple

testing adjustment are strong, whereas interpretation of the remaining results should be made with caution. The authors confirm that the data supporting the findings of this study are available within the article and/or its supplementary materials, see Appendix C.

3. Results

3.1 Participant characteristics

Participant characteristics are described in Table 2. The difference between sexes found for fat%, SMI, absolute skeletal muscle mass, fat free mass and handgrip strength survived FDR correction for multiple testing. Of all 74 participants, only one man and two women were diagnosed with probable sarcopenia according to the EWGSOP2 criteria, based on a low muscle strength. None of the participants was diagnosed with confirmed sarcopenia. In total, three participants scored below the sarcopenia cut-off level for muscle quantity measured as SMI with BIA and one scored above the sarcopenia cut-off time for physical performance based on the 8UG test. Of note, multiple linear regression analysis showed that higher fat% was correlated with a higher right handgrip strength ($p = 0.022$) after adjusting for age and sex, but with a lower SMI ($p = 0.017$).

3.2 Higher fat% correlates with decreases in brain volume and ^{1}H-MRS markers of neural integrity

Multiple linear regression analysis did not reveal any significant correlations between fat% and serum IGF-1, IL-6 or kynurenine levels, between fat% and brain volume, nor between fat% and MoCA scores, see Table 3 and Supplementary Table A.2. However, total GMV seemed to be non-linearly related to fat%. Graphical representation showed that participants with underweight based on measures of fat% (Gallagher et al., 2000) in our study (n=2) have lower total GMV, indicating there is most probably a hyperbolic relationship between fat% and total GMV, see Figure 3. Therefore, we repeated linear analysis after excluding the two participants that fell into the underweight category. After exclusion of the two underweight participants, higher levels of fat% were associated with lower total GMV ($p = 0.021$), right HPC GMV ($p = 0.005$), and left MTC GMV ($p = 0.040$). The negative association between fat% after exclusion of the two underweight participants and total GMV, right HPC GMV and left MTC GMV survived the FDR correction for multiple testing.

Some neurometabolite levels were associated with fat% in specific brain regions. The most notable was the significant association between higher fat% and lower tNAA/tCr levels in three of the five regions of interest, a negative association for the left SM1 and right DLPFC, but a positive association for the left MTC, see Table 4 and Supplementary Table A.3. None of these significant results survived correction for multiple testing with the FDR procedure.

Table 2. Participant characteristics and sex differences

	Men (n = 34)	Women (n = 40)	**Total (n = 74)**	p-value
Age	70.8 (6.0)	68.2 (6.1)	69.4 (6.2)	0.069
Montreal Cognitive Assessment	25.0 (3.1)	25.3 (2.7)	25.2 (2.9)	0.747
Education:				0.953
• Higher	26 (35.6%)	31 (42.5%)	57 (78.1%)	
• Secondary	7 (9.6%)	7 (9.6%)	14 (19.2%)	
• Basic	1 (1.4%)	1 (1.4%)	2 (2.7%)	
Smoker	1 (1.4%)	2 (2.7%)	3 (4.1%)	0.562
Body mass index (kg/m²)	28.6 (5.4)	27.5 (4.5)	28.0 (4.9)	0.361
Fat %	26.3 (8.7)	35.8 (7.8)	31.4 (9.4)	<0.001***
Sarcopenia:	0 (0%)	0 (0%)	0 (0%)	NA
• possible sarcopenia	1 (1.4%)	2 (2.9%)	3 (3.3%)	
• confirmed sarcopenia	0 (0%)	0 (0%)	0 (0%)	
• severe sarcopenia	0 (0%)	0 (0%)	0 (0%)	
Skeletal muscle mass index (kg/m²)	10.9 (1.3)	8.5 (1.0)	9.6 (1.7)	< 0.001***
Absolute skeletal muscle mass (kg)	32.9 (4.6)	22.2 (2.8)	27.1 (6.6)	< 0.001***
Fat free mass (kg)	62.5 (7.0)	44.8 (3.6)	52.9 (10.4)	< 0.001***
Handgrip strength (kg)	42.4 (6.1)	25.0 (4.5)	31.8 (10.0)	< 0.001***
8-Foot Up-and-Go test (s)	4.9 (1.4)	4.7 (0.9)	4.8 (1.1)	0.273
Self-reported kcal/week burned with physical activity	5600 (4025)	3534 (2512)	4353 (3322)	0.273
Estimated physical activity level:				0.754
• sedentary	2 (2.8%)	3 (4.2%)	5 (6.9%)	
• moderately active	12 (16.7%)	17 (23.6%)	29 (39.2%)	
• highly active	19 (26.4%)	19 (26.4%)	38 (51.4%)	

Continuous parameters are expressed as mean values (standard deviation); categorical parameters are expressed as n (% of total).* p < 0.05, ** p < 0.01, *** p < 0.001 (not FDR corrected). Abbreviations: NA, not applicable

Table 3. How fat %, skeletal muscle mass index, right handgrip strength and physical performance on the 8-Foot Up-and-Go test relate to brain gray matter volume and peripheral inflammation

		β	p-value
Fat %[a]	-	-	-
Fat % after exclusion of underweight participants[a]	Total GMV	-0.190	0.021
	Right HPC GMV	-0.374	0.005
	Left MTC GMV	-0.421	0.040
SMI	-	-	-
Right handgrip strength[b]	Kynurenine	-0.498	0.045
	Total GMV	0.334	0.018
8UG time o.e.[b]	-	-	-

Only statistically significant correlations (not FDR corrected) are presented. See Supplementary Tables A.2, A.4, A.8, and A.16 for all results. β represents the standardized regression coefficient. a adjusted for age and sex, b adjusted for age, sex and fat %. Additionally, associations with GMV were adjusted for total intracranial volume. Abbreviations: DLPFC, dorsolateral prefrontal cortex; DPCC, dorsal posterior cingulate cortex; GMV, gray matter volume; HPC, hippocampal cortex; mIns, myoinositol; MTC, medial temporal cortex; o.e., outlier excluded; SMI, skeletal muscle mass index; SM1, primary sensorimotor cortex; tCho, total choline; tCr, total creatine, tNAA, total N-acetyl aspartate; 8UG, 8-Foot Up-and-Go test.

3.3 Higher skeletal muscle mass index is associated with decreased levels of neural integrity markers

Linear regression analysis found no correlation between SMI and IGF-1, IL-6 or kynurenine levels, nor between SMI and MoCA test scores. Multiple linear regression analysis did also not result in any significant associations between SMI and brain volume in any of the regions of interest, see Table 3 and Supplementary Table A.4. In contrast to what we expected, SMI was negatively associated with tNAA/tCr in the left SM1 ($p = 0.011$), see Table 4; for all results see Supplementary Table A.5. However, this result did not remain significant after correction for multiple testing with the FDR procedure.

3.4 Increased handgrip strength is associated with higher markers of neural integrity, and lower levels of peripheral and neural inflammatory markers

Multiple regression analysis showed that higher right handgrip strength was associated with lower kynurenine levels (p = 0.045) and larger total GMV (p = 0.018). Additionally, a positive association was found between right handgrip strength and tNAA/tCr levels in the DPCC (p = 0.006) and right DLPFC (p = 0.022) and between right handgrip strength and mIns/tCr levels in the left MTC (p = 0.012), see Tables 3 and 4, and Supplementary Tables A.8 and A.9. None of the results remained significant after correction for multiple testing with the FDR procedure.

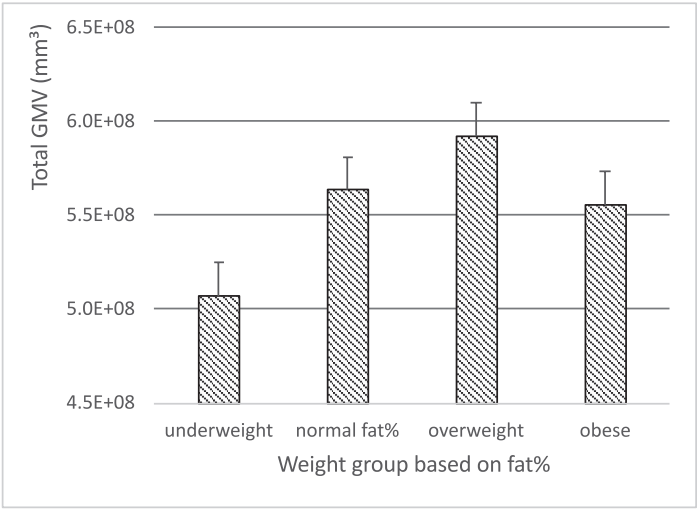


Figure 3. How body fat percentage relates to total gray matter volume
The bars display the mean and the whiskers the standard errors of total gray matter volume (mm^3) for underweight (n = 2), normal fat % (n = 29), overweight (n = 23), and obese (n = 17) participants, based on fat % based weight categories defined by Gallagher et al. (2000) (Gallagher et al., 2000). The figure shows that underweight and obese participants have lowest gray matter volumes.

3.5 Better physical performance on the 8UG test was associated with lower markers of neural integrity and higher levels of neuroinflammation

Before analysis of associations with 8UG test results, we excluded an extreme outlier. Results from multiple linear regression analysis before exclusion of the outlier are described in Supplementary Tables A.10-A.13. Multiple linear regression analysis did not show any significant associations between 8UG test time and serum biomarker levels or scores on the MoCA test, nor with any of the brain volumes after exclusion of this outlier. Associations with neurometabolic biomarkers showed that a longer time needed to complete the 8UG test was associated with higher levels of tNAA/mIns in the left MTC ($p = 0.039$), see Table 4 and Supplementary Tables A.16 and A.17. However, this result did not remain significant after correction for multiple testing with the FDR procedure.

Table 4. How fat %, skeletal muscle mass index, right handgrip strength and physical performance on the 8-Foot Up-and-Go test relate to neurometabolite levels

		β	p-value
Fat %[a]	tNAA/tCr l SM1	-0.362	0.016
	tNAA/tCr l MTC	0.330	0.044
	tNAA/tCr r DLPFC	-0.338	0.026
SMI	tNAA/tCr l SM1	-0.316	0.011
Right handgrip strength[b]	tNAA/tCr DPCC	0.647	0.006
	mIns/tCr l MTC	0.667	0.012
	tNAA/tCr r DLPFC	0.524	0.022
8UG time o.e.[b]	tNAA/mIns l MTC	0.305	0.039

Only statistically significant correlations (not FDR corrected) are presented. See Supplementary Tables A.3, A.5, A.9, and A.17 for all nonsignificant results. β represents the standardized regression coefficient. a adjusted for age and sex, b adjusted for age, sex and fat %. Abbreviations: DLPFC, dorsolateral prefrontal cortex; DPCC, dorsal posterior cingulate cortex; GMV, gray matter volume; HPC, hippocampal cortex; l, left; mIns, myoinositol; MoCA, Montreal Cognitive Assessment; MTC, medial temporal cortex; o.e., after exclusion of an influential outlier; r, right; SMI, skeletal muscle mass index; SM1, primary sensorimotor cortex; tCho, total choline; tCr, total creatine, tNAA, total N-acetyl aspartate; 8UG, 8-Foot Up-and-Go test.

3.6 The mediating effect of blood biomarkers on the body-brain crosstalk

Based on our hypotheses and the associations presented above, kynurenine was considered a possible mediator of the relationships between right handgrip strength and the following brain outcome measures: (1) total GMV (direct effect: b = 1577.053, p = 0.018) , (2) tNAA/tCr levels in the DPCC (b = 0.007, p = 0.006) and right DLPFC (b = 0.006, p = 0.022), and (3) mIns/tCr levels in the left MTC (b = 0.010, p = 0.012). Our data showed that the indirect effect of the mediation analysis was not significant for all aforementioned outcome measures (all R^2_{med} values ≤ 0.011). Thus, we found no evidence for a mediating effect of kynurenine on any of the above-mentioned associations. The indirect and direct effects are presented in Figure 4.

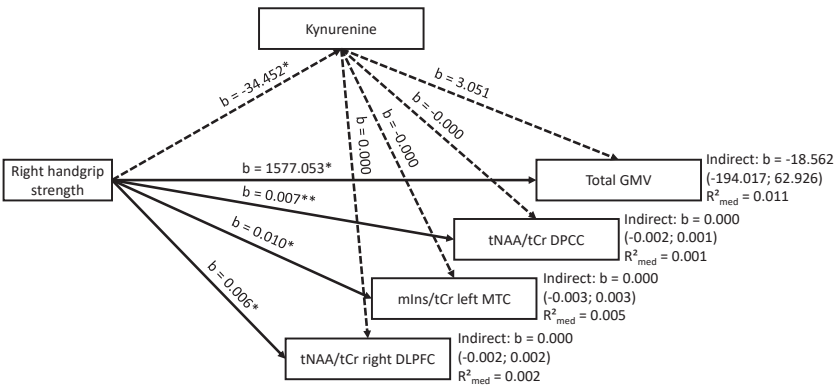


Figure 4. Mediation model

Effect of right handgrip strength on total GMV, tNAA/tCr DPCC, mIns/tCr left MTC and tNAA/tCr right DLPFC with kynurenine as a potential mediator. Solid arrows indicate direct pathways, the dashed arrows indicate the indirect pathways. Correlation coefficients with the respective p-values are presented, * p < 0.05, ** p < 0.01, *** p < 0.001 (not FDR corrected). The correlation coefficient of the indirect effect is presented with a 95% confidence interval and R²med effect sizes, representing the proportion of variance attributed to the indirect effect by the mediator. Abbreviations: DLPFC, dorsolateral prefrontal cortex; DPCC, dorsal posterior cingulate cortex; GMV, gray matter volume; mIns, myoinositol; MTC, medial temporal cortex; tCr, total creatine, tNAA, total N-acetyl aspartate.

4. Discussion

This paper is original in its holistic view on examining body fat% and components of sarcopenia (muscle strength, muscle mass and physical performance) and the relationship with brain volume, neurometabolite levels and general cognition. The investigation was complemented with the assessment of inflammatory biomarkers (IL-6 and kynurenine) and the neurotrophic blood biomarker IGF-1 as possible mediators of the link between body and brain. Mediation analysis did not confirm a significant indirect effect of the blood biomarkers on any of the outcomes and the nonsignificant findings had low effect sizes.

Our results suggest a hyperbolic relationship between fat % and total GMV, with lowest levels of total GMV in the two underweight participants included in our study. This is in line with a study who reported that participants with anorexia nervosa have lower GMV. Following weight gain, these participants showed a significant increase in their GMV in this study (Roberto et al., 2011). After excluding the two underweight participants from our study, higher fat % was significantly associated with lower total GMV, right HPC GMV and left MTC GMV. These findings remained significant after correction for multiple testing. Similar results have also been reported in several other studies (Dekkers et al., 2019; Hamer and Batty, 2019; Opel et al., 2020). One of these studies reported that higher fat % is also associated with decreased subcortical GMV (Dekkers et al., 2019).

Our analysis revealed only weak associations between body composition, muscular fitness, and physical performance measures and the ^{1}H-MRS measures. None of our results survived the FDR procedure to correct for multiple testing. This may be due to the exploratory nature of this study, including a large amount of statistical tests. Taken together, our observations provide evidence for plausible underlying effects of high body fat% and components of sarcopenia on brain health. However, our results not surviving correction for multiple testing, should be interpreted with caution, especially if they are not supported by further research, as will be discussed below.

An interesting finding from our study was that higher fat %, adjusted for age and sex, was associated with lower levels of neural integrity biomarker tNAA/tCr in the left SM1, and right DLPFC, but an increase in tNAA/tCr levels in the left MTC. Lower levels of neural integrity in overweight adults has also been described in another study, but this study only investigated tNAA levels in the HPC (Coplan et al., 2014).

At this point, we cannot explain why the opposite relationship was found concerning the fat% to tNAA/tCr relationship in the left MTC compared to the left SM1 and right DLPFC. Furthermore, we could not find the link we expected between fat % and peripheral or neural inflammation, and we did not discover an association with MoCA scores. In previous studies, obesity has been advocated as a possible cause of chronic low-grade inflammation (Mangge et al., 2014; Woods et al., 2012; Yudkin, 2007), associated with neuroinflammation in the hypothalamus, hippocampus, amygdala, cerebral cortex and

cerebellum in diet-induced obesity animal models (Beilharz et al., 2016; Guillemot-Legris et al., 2016; Lu et al., 2011; Tapia-González et al., 2011) and in the occipitoparietal cortex in a human ^{1}H-MRS study (Gonzales et al., 2012). Furthermore, obesity has been associated with cognitive decline after adjusting for age and educational level (Cournot et al., 2006), and with an increased risk of Alzheimer's disease (Mrak, 2009). Taken together, our observations suggest that while higher fat% has been linked to lower neuronal density, estimated by tNAA/tCr in the left SM1 and right DLPFC, the association with cognition and (neuro)inflammatory processes were less visible in our sample of older adults.

Muscle strength, as measured with right handgrip dynamometry, was associated with higher levels of tNAA/tCr in the right DLPFC, and the DPCC. This indicates that muscle strength is associated with higher levels of neural integrity in these brain regions (Castillo et al., 1998). Contrary to what we expected, mIns/tCr levels in the left MTC were positively related to handgrip strength, which may indicate a link between muscle strength and neuroinflammation (Castillo et al., 1998). To our knowledge, no other studies have previously reported a link between muscle strength and brain ^{1}H-MRS results, making it impossible to compare our results with existing literature.

Contradictory to the association with neuroinflammation in the left MTC, muscle strength was associated with lower serum IL-6 levels (adjusted for BMI, see Supplementary table A.6.) and kynurenine levels (both when adjusted for BMI or fat%). IL-6 is considered an important mediator of inflammatory processes, being involved both in pro- and anti-inflammatory processes (Smith and Miles, 2000). It is typically elevated in a state of chronic inflammation, which is often seen at older age and is called 'inflammaging' (Ershler, 1993; Franceschi et al., 2000; Maggio et al., 2006). The negative relationship between handgrip strength and serum IL-6 is in line with previous studies (Visser et al., 2002). Furthermore, we reported previously that serum kynurenine is associated with ^{1}H-MRS signs of neurodegeneration and neuroinflammation (Vints et al., 2022a). Serum kynurenine levels are increased in a state of elevated inflammation, as is commonly found at older age (Allison et al., 2017). It is considered a robust marker of inflammation, as the enzyme indolamine-2,3-dioxygenase, which converts tryptophan into kynurenine, is upregulated by a wide array of pro-inflammatory cytokines, including C-reactive protein (CRP), IL-1β, IL-6, IL-8, tumor necrosis factor-alpha (TNF-α) and interferon-gamma (IFN-γ) and downregulated by anti-inflammatory cytokines like IL-4 and IL-10 (Allison et al., 2017; Chiarugi et al., 2001; Kindler et al., 2019; Lustgarten and Fielding, 2017; Pedraz-Petrozzi et al., 2020). Notably, aging-associated elevations of serum kynurenine have previously been suggested to play a role in the development of sarcopenia in mice, possibly by increasing oxidative stress markers. In this study, administration of kynurenine caused a reduction in muscle protein synthesis, leading to a reduction in muscle size and mass (Kaiser et al., 2019). Furthermore, in adults with heart failure, plasma kynurenine levels were found to be elevated, and this elevation was negatively associated with handgrip strength (Konishi et al., 2016). The latter is in line with the findings from our study.

In contrast to muscle strength, we discovered that higher SMI and better performance on the 8UG test were associated with signs of neurodegeneration, as expressed by the negative association between SMI and levels of tNAA/tCr in SM1 and the positive association between time on the 8UG test and tNAA/mIns in the left MTC. This would indicate that having more muscle mass or being able to perform better on physical tasks would be detrimental for brain metabolism, which was contradictory to our hypothesis.

Overall, our results suggests that muscle strength rather than muscle mass or physical performance is positively associated with a healthy brain neurometabolism, even though we did not find an association between muscle strength and cognitive function as measured with the MoCA scale. Other studies reported that muscle strength in older adults is a good predictor of cognitive function, physical performance and risk of falls. These studies also indicated that muscle strength is a better predictor of mental and physical health than muscle mass (Menant et al., 2017; Sui et al., 2020; Tolea and Galvin, 2015), which is in line with our findings. Menant et al. (2017), who measured muscle strength as a measure of maximal isometric knee extension force, even proposed that a simple muscle strength measurement is to be preferred as a predictor of health-related outcomes in older people instead of assessing if a person has sarcopenia (defined as the combination of low muscle strength and muscle mass by the EWGSOP2 (Cruz-Jentoft et al., 2019)).

Limitations of this study include the exploratory nature of our design. As a consequence, our results needed to be corrected for multiple testing with FDR analysis. Findings that did not survive FDR correction should be interpreted as possible trends. We should note that our sample size was relatively small for the elaborate statistical analysis performed, including mediation analysis. Furthermore, we only included older adults (60-85 years old) and excluded participants weighing more than 130kg because this was an exclusion criterium for MRI scanning. This limits the interpretation of the results related to age and fat%. Moreover, we cannot make causal inferences about the effects of a change in age, fat%, or sarcopenia-related measures in this study. Finally, cognitive function was assessed only with the MoCA test, which should be considered a global cognition screening tool and cannot accurately be used to capture variations in cognitive subdomains.

In conclusion, our results in older adults suggest that a healthy fat% and muscle strength are associated with correlates of brain health, measured as larger brain volumes or neural integrity. Based on these findings, researchers may consider longitudinal studies to investigate if maintaining a healthy fat% and muscle strength throughout life may be part of preventive measures to combat cognitive decline at older age. We invite other researchers to investigate fat % and muscle strength in their relationship to neural integrity in older adults. From a mechanistical point of view, the role of serum kynurenine and other inflammatory blood biomarkers as possible mediators of both a decrease in muscle strength and neurodegeneration in older adults would be a relevant topic to investigate again in larger study cohorts.

Acknowledgments

None

Declarations of interest

None

References

Agudelo, L.Z., Femenía, T., Orhan, F., Porsmyr-Palmertz, M., Goiny, M., Martinez-Redondo, V., Correia, J.C., Izadi, M., Bhat, M., Schuppe-Koistinen, I., Pettersson, A.T., Ferreira, D.M.S., Krook, A., Barres, R., Zierath, J.R., Erhardt, S., Lindskog, M., Ruas, J.L., 2014. Skeletal muscle PGC-1α1 modulates kynurenine metabolism and mediates resilience to stress-induced depression. Cell 159, 33–45. https://doi.org/10.1016/j.cell.2014.07.051

Allison, D.J., Josse, A.R., Gabriel, D.A., Klentrou, P., Ditor, D.S., 2017. Targeting inflammation to influence cognitive function following spinal cord injury: A randomized clinical trial. Spinal Cord 55, 26–32. https://doi.org/10.1038/sc.2016.96

Banks, W.A., Kastin, A.J., Broadwell, R.D., 1995. Passage of cytokines across the blood-brain barrier. Neuroimmunomodulation 2, 241–248. https://doi.org/10.1159/000097202

Baumgartner, R.N., 2000. Body Composition in Healthy Aging. Ann. N. Y. Acad. Sci. 904, 437–448. https://doi.org/10.1111/J.1749-6632.2000.TB06498.X

Beck, D., de Lange, A.M.G., Alnæs, D., Maximov, I.I., Pedersen, M.L., Leinhard, O.D., Linge, J., Simon, R., Richard, G., Ulrichsen, K.M., Dørum, E.S., Kolskår, K.K., Sanders, A.M., Winterton, A., Gurholt, T.P., Kaufmann, T., Steen, N.E., Nordvik, J.E., Andreassen, O.A., Westlye, L.T., 2022. Adipose tissue distribution from body MRI is associated with cross-sectional and longitudinal brain age in adults. NeuroImage. Clin. 33, 102949. https://doi.org/10.1016/J.NICL.2022.102949

Beilharz, J.E., Maniam, J., Morris, M.J., 2016. Short-term exposure to a diet high in fat and sugar, or liquid sugar, selectively impairs hippocampal-dependent memory, with differential impacts on inflammation. Behav. Brain Res. 306, 1–7. https://doi.org/10.1016/J.BBR.2016.03.018

Benjamini, Y., Hochberg, Y., 1995. Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. J. R. Stat. Soc. Ser. B 57, 289–300. https://doi.org/10.1111/J.2517-6161.1995.TB02031.X

Beyer, I., Mets, T., Bautmans, I., 2012. Chronic low-grade inflammation and age-related sarcopenia. Curr. Opin. Clin. Nutr. Metab. Care 15, 12–22. https://doi.org/10.1097/MCO.0b013e32834dd297

Bian, A., Ma, Y., Zhou, X., Guo, Y., Wang, W., Zhang, Y., Wang, X., 2020. Association between sarcopenia and levels of growth hormone and insulin-like growth factor-1 in the elderly. BMC Musculoskelet. Disord. 21, 214. https://doi.org/10.1186/S12891-020-03236-Y

Blüher, M., 2019. Obesity: global epidemiology and pathogenesis. Nat. Rev. Endocrinol. 2019 155 15, 288–298. https://doi.org/10.1038/S41574-019-0176-8

Bobb, J.F., Schwartz, B.S., Davatzikos, C., Caffo, B., 2014. Cross-sectional and longitudinal association of body mass index and brain volume. Hum. Brain Mapp. 35, 75–88. https://doi.org/10.1002/HBM.22159

Bosy-Westphal, A., Later, W., Hitze, B., Sato, T., Kossel, E., Glüer, C.C., Heller, M., Müller, M.J., 2008. Accuracy of Bioelectrical Impedance Consumer Devices for Measurement of Body Composition in Comparison to Whole Body Magnetic Resonance Imaging and Dual X-Ray Absorptiometry. Obes. Facts 1, 319–324. https://doi.org/10.1159/000176061

Bourgognon, J.-M., Cavanagh, J., 2020. The role of cytokines in modulating learning and memory and brain plasticity. Brain Neurosci. Adv. 4, 239821282097980. https://doi.org/10.1177/2398212820979802

Bruijnen, C.J.W.H., Dijkstra, B.A.G., Walvoort, S.J.W., Budy, M.J.J., Beurmanjer, H., De Jong, C.A.J., Kessels, R.P.C., 2020. Psychometric properties of the Montreal Cognitive Assessment (MoCA) in healthy participants aged 18-70. Int. J. Psychiatry Clin. Pract. 24, 293–300. https://doi.org/10.1080/13651501.2020.1746348

Burns, J.M., Johnson, D.K., Watts, A., Swerdlow, R.H., Brooks, W.M., 2010. Reduced lean mass in early Alzheimer disease and its association with brain atrophy. Arch. Neurol. 67, 428–433. https://doi.org/10.1001/archneurol.2010.38

Cable, A., Nieman, D.C., Austin, M., Hogen, E., Utter, A.C., 2001. Validity of leg-to-leg bioelectrical impedance measurement in males. J Sport. Med Phys Fit. 41, 411–414.

Castillo, M., Kwock, L., Scatliff, J., Mukherji, S.K., 1998. Proton MR spectroscopy in neoplastic and non-neoplastic brain disorders. Magn. Reson. Imaging Clin. N. Am.

Chang, K.V., Hsu, T.H., Wu, W.T., Huang, K.C., Han, D.S., 2016. Association Between Sarcopenia and Cognitive Impairment: A Systematic Review and Meta-Analysis. J. Am. Med. Dir. Assoc. 17, 1164.e7-1164.e15. https://doi.org/10.1016/j.jamda.2016.09.013

Chiarugi, A., Calvani, M., Meli, E., Traggiai, E., Moroni, F., 2001. Synthesis and release of neurotoxic kynurenine metabolites by human monocyte-derived macrophages. J. Neuroimmunol. 120, 190–198. https://doi.org/10.1016/S0165-5728(01)00418-0

Chien, M.Y., Huang, T.Y., Wu, Y.T., 2008. Prevalence of sarcopenia estimated using a bioelectrical impedance analysis prediction equation in community-dwelling elderly people in Taiwan. J. Am. Geriatr. Soc. 56, 1710–1715. https://doi.org/10.1111/J.1532-5415.2008.01854.X

Cipolli, G.C., Sanches, M.Y., Aprahamian, I., 2019. Sarcopenia Is Associated with Cognitive Impairment in Older Adults: A Systematic Review and Meta-Analysis. J. Nutr. Health Aging 23, 525–531.

Clark, B.C., Manini, T.M., 2008. Sarcopenia ≠ Dynapenia. Journals Gerontol. Ser. A 63, 829–834. https://doi.org/10.1093/GERONA/63.8.829

Coplan, J.D., Fathy, H.M., Abdallah, C.G., Ragab, S.A., Kral, J.G., Mao, X., Shungu, D.C., Mathew, S.J., 2014. Reduced hippocampal N-acetyl-aspartate (NAA) as a biomarker for overweight. NeuroImage Clin. 4, 326–335. https://doi.org/10.1016/J.NICL.2013.12.014

Cournot, M., Marquié, J.C., Ansiau, D., Martinaud, C., Fonds, H., Ferrières, J., Ruidavets, J.B., 2006. Relation between body mass index and cognitive function in healthy middle-aged men and women. Neurology 67, 1208–1214. https://doi.org/10.1212/01.WNL.0000238082.13860.50

Cruz-Jentoft, A.J., Bahat, G., Bauer, J., Boirie, Y., Bruyère, O., Cederholm, T., Cooper, C., Landi, F., Rolland, Y., Sayer, A.A., Schneider, S.M., Sieber, C.C., Topinkova, E., Vandewoude, M., Visser, M., Zamboni, M., Bautmans, I., Baeyens, J.P., Cesari, M., Cherubini, A., Kanis, J., Maggio, M., Martin, F., Michel, J.P., Pitkala, K., Reginster, J.Y., Rizzoli, R., Sánchez-Rodríguez, D., Schols, J., 2019. Sarcopenia: revised European consensus on definition and diagnosis. Age Ageing 48, 16–31. https://doi.org/10.1093/AGEING/AFY169

Dekkers, I.A., Jansen, P.R., Lamb, H.J., 2019. Obesity, Brain Volume, and White Matter Microstructure at MRI: A Cross-sectional UK Biobank Study. Radiology 291, 763–771. https://doi.org/10.1148/RADIOL.2019181012

Dodds, R.M., Syddall, H.E., Cooper, R., Benzeval, M., Deary, I.J., Dennison, E.M., Der, G., Gale, C.R., Inskip, H.M., Jagger, C., Kirkwood, T.B., Lawlor, D.A., Robinson, S.M., Starr, J.M., Steptoe, A., Tilling, K., Kuh, D., Cooper, C., Sayer, A.A., 2014. Grip Strength across the Life Course: Normative Data from Twelve British Studies. PLoS One 9, e113637. https://doi.org/10.1371/JOURNAL.PONE.0113637

Ershler, W.B., 1993. Interleukin-6: A Cytokine for Gerontolgists. J. Am. Geriatr. Soc. 41, 176–181. https://doi.org/10.1111/J.1532-5415.1993.TB02054.X

Ethgen, O., Beaudart, C., Buckinx, F., Bruyère, O., Reginster, J.Y., 2017. The Future Prevalence of Sarcopenia in Europe: A Claim for Public Health Action. Calcif. Tissue Int. 100, 229. https://doi.org/10.1007/S00223-016-0220-9

Fairchild, A.J., MacKinnon, D.P., Taborga, M.P., Taylor, A.B., 2009. R^2 effect-size measures for mediation analysis. Behav. Res. Methods 2009 412 41, 486–498. https://doi.org/10.3758/BRM.41.2.486

Franceschi, C., Bonafè, M., Valensin, S., Olivieri, F., De Luca, M., Ottaviani, E., De Benedictis, G., 2000. Inflamm-aging. An evolutionary perspective on immunosenescence, in: Annals of the New York Academy of Sciences. New York Academy of Sciences, pp. 244–254. https://doi.org/10.1111/j.1749-6632.2000.tb06651.x

Frater, J., Lie, D., Bartlett, P., McGrath, J.J., 2018. Insulin-like Growth Factor 1 (IGF-1) as a marker of cognitive decline in normal ageing: A review. Ageing Res. Rev. 42, 14–27. https://doi.org/10.1016/J.ARR.2017.12.002

Fukui, S., Schwarcz, R., Rapoport, S.I., Takada, Y., Smith, Q.R., 1991. Blood-brain barrier transport of kynurenines: implications for brain synthesis and metabolism. J. Neurochem. 56, 2007–2017. https://doi.org/10.1111/J.1471-4159.1991.TB03460.X

Gallagher, D., Heymsfield, S.B., Heo, M., Jebb, S.A., Murgatroyd, P.R., Sakamoto, Y., 2000. Healthy percentage body fat ranges: an approach for developing guidelines based on body mass index. Am. J. Clin. Nutr. 72, 694–701. https://doi.org/10.1093/AJCN/72.3.694

García-García, I., Michaud, A., Jurado, M.Á., Dagher, A., Morys, F., 2022. Mechanisms linking obesity and its metabolic comorbidities with cerebral grey and white matter changes. Rev. Endocr. Metab. Disord. 23, 833–843. https://doi.org/10.1007/S11154-021-09706-5/FIGURES/2

George, D., Mallery, P., 2010. SPSS for Windows Step by Step: A Simple Guide and Reference, 17.0 update, 10th ed. Allyn & Bacon, Boston.

Gonzales, M.M., Tarumi, T., Eagan, D.E., Tanaka, H., Vaghasia, M., Haley, A.P., 2012. Indirect effects of elevated body mass index on memory performance through altered cerebral metabolite concentrations. Psychosom. Med. 74, 691–698. https://doi.org/10.1097/PSY.0b013e31825ff1de

Guillemot-Legris, O., Masquelier, J., Everard, A., Cani, P.D., Alhouayek, M., Muccioli, G.G., 2016. High-fat diet feeding differentially affects the development of inflammation in the central nervous system. J. Neuroinflammation 13, 1–11. https://doi.org/10.1186/S12974-016-0666-8/FIGURES/5

Hamer, M., Batty, G.D., 2019. Association of body mass index and waist-to-hip ratio with brain structure. Neurology 92, e594–e600. https://doi.org/10.1212/WNL.0000000000006879

Hayes, A.F., 2022. Introduction to mediation, moderation, and conditional process analysis: A regression-based approach, 3rd ed. The Guilford Press, New York.

Herrmann, M.J., Tesar, A.K., Beier, J., Berg, M., Warrings, B., 2019. Grey matter alterations in obesity: A meta-analysis of whole-brain studies. Obes. Rev. 20, 464–471. https://doi.org/10.1111/OBR.12799

Janssen, I., Heymsfield, S.B., Baumgartner, R.N., Ross, R., 2000. Estimation of skeletal muscle mass by bioelectrical impedance analysis. J. Appl. Physiol. 89, 465–471. https://doi.org/10.1152/JAPPL.2000.89.2.465/ASSET/IMAGES/LARGE/DG0800064005.JPEG

Kaiser, H., Yu, K., Pandya, C., Mendhe, B., Isales, C.M., McGee-Lawrence, M.E., Johnson, M., Fulzele, S., Hamrick, M.W., 2019. Kynurenine, a Tryptophan Metabolite That Increases with Age, Induces Muscle Atrophy and Lipid Peroxidation. Oxid. Med. Cell. Longev. 2019. https://doi.org/10.1155/2019/9894238

Kilgour, A.H., Todd, O.M., Starr, J.M., 2014. A systematic review of the evidence that brain structure is related to muscle structure and their relationship to brain and muscle function in humans over the lifecourse. BMC Geriatr. 14, 85. https://doi.org/10.1186/1471-2318-14-85

Kindler, J., Lim, C.K., Weickert, C.S., Boerrigter, D., Galletly, C., Liu, D., Jacobs, K.R., Balzan, R., Bruggemann, J., O'Donnell, M., Lenroot, R., Guillemin, G.J., Weickert, T.W., 2019. Dysregulation of kynurenine metabolism is related to proinflammatory cytokines, attention, and prefrontal cortex volume in schizophrenia. Mol. Psychiatry 2019 2511 25, 2860–2872. https://doi.org/10.1038/s41380-019-0401-9

Kirschke, A.R., Kocur, P., Wilk, M., Dylewicz, P., 2006. The Fullerton Fitness Test as an index of fitness in the elderly. Med. Rehabil. 10, 9–16.

Konishi, M., Ebner, N., Springer, J., Schefold, J.C., Doehner, W., Dschietzig, T.B., Anker, S.D., von Haehling, S., 2016. Impact of Plasma Kynurenine Level on Functional Capacity and Outcome in Heart Failure – Results From Studies Investigating Co-morbidities Aggravating Heart Failure (SICA-HF). Circ. J. 81, 52–61. https://doi.org/10.1253/CIRCJ.CJ-16-0791

Kronschnabl, J.M., Kneip, T., Weiss, L.M., Bergmann, M., 2021. Bodyweight change and cognitive performance in the older population. PLoS One 16, e0249651. https://doi.org/10.1371/JOURNAL.PONE.0249651

Lin, A., Andronesi, O., Bogner, W., Choi, I.Y., Coello, E., Cudalbu, C., Juchem, C., Kemp, G.J., Kreis, R., Kršňák, M., Lee, P., Maudsley, A.A., Meyerspeer, M., Mlynarik, V., Near, J., Öz, G., Peek, A.L., Puts, N.A., Ratai, E.M., Tkáč, I., Mullins, P.G., 2021. Minimum Reporting Standards for in vivo Magnetic Resonance Spectroscopy (MRSinMRS): Experts' consensus recommendations. NMR Biomed. 34, e4484. https://doi.org/10.1002/NBM.4484

Livingston, G., Huntley, J., Sommerlad, A., Ames, D., Ballard, C., Banerjee, S., Brayne, C., Burns, A., Cohen-Mansfield, J., Cooper, C., Costafreda, S.G., Dias, A., Fox, N., Gitlin, L.N., Howard, R., Kales, H.C., Kivimäki, M., Larson, E.B., Ogunniyi, A., Orgeta, V., Ritchie, K., Rockwood, K., Sampson, E.L., Samus, Q., Schneider, L.S., Selbæk, G., Teri, L., Mukadam, N., 2020. Dementia prevention, intervention, and care: 2020 report of the Lancet Commission. Lancet Comm. 396, 413–446. https://doi.org/10.1016/S0140-6736(20)30367-6/ATTACHMENT/CEE43A30-904B-4A45-A4E5-AFE48804398D/MMC1.PDF

Lu, J., Wu, D. mei, Zheng, Y. lin, Hu, B., Cheng, W., Zhang, Z. feng, Shan, Q., 2011. Ursolic acid improves high fat diet-induced cognitive impairments by blocking endoplasmic reticulum stress and IκB kinase β/nuclear factor-κB-mediated inflammatory pathways in mice. Brain. Behav. Immun. 25, 1658–1667. https://doi.org/10.1016/J.BBI.2011.06.009

Lustgarten, M.S., Fielding, R.A., 2017. Metabolites Associated With Circulating Interleukin-6 in Older Adults. Journals Gerontol. Ser. A 72, 1277–1283. https://doi.org/10.1093/GERONA/GLW039

Maggio, M., Guralnik, J.M., Longo, D.L., Ferrucci, L., 2006. Interleukin-6 in aging and chronic disease: A magnificent pathway. Journals Gerontol. - Ser. A Biol. Sci. Med. Sci. https://doi.org/10.1093/gerona/61.6.575

Mangge, H., Summers, K.L., Meinitzer, A., Zelzer, S., Almer, G., Prassl, R., Schnedl, W.J., Reininghaus, E., Paulmichl, K., Weghuber, D., Fuchs, D., 2014. Obesity-related dysregulation of the Tryptophan–Kynurenine metabolism: Role of age and parameters of the metabolic syndrome. Obesity 22, 195–201. https://doi.org/10.1002/OBY.20491

Menant, J.C., Weber, F., Lo, J., Sturnieks, D.L., Close, J.C., Sachdev, P.S., Brodaty, H., Lord, S.R., 2017. Strength measures are better than muscle mass measures in predicting health-related outcomes in older people: time to abandon the term sarcopenia? Osteoporos. Int. 28, 59–70. https://doi.org/10.1007/S00198-016-3691-7/TABLES/4

Mrak, R.E., 2009. Alzheimer-type neuropathological changes in morbidly obese elderly individuals. Clin. Neuropathol. 28, 40–45. https://doi.org/10.5414/NPP28040

Nasreddine, Z.S., Phillips, N.A., Bédirian, V., Charbonneau, S., Whitehead, V., Collin, I., Cummings, J.L., Chertkow, H., 2005. The Montreal Cognitive Assessment, MoCA: A brief screening tool for mild cognitive impairment. J. Am. Geriatr. Soc. 53, 695–699. https://doi.org/10.1111/j.1532-5415.2005.53221.x

Norton, S., Matthews, F.E., Barnes, D.E., Yaffe, K., Brayne, C., 2014. Potential for primary prevention of Alzheimer's disease: an analysis of population-based data. Lancet Neurol. 13, 788–794. https://doi.org/10.1016/S1474-4422(14)70136-X

Nyberg, L., Boraxbekk, C.J., Sörman, D.E., Hansson, P., Herlitz, A., Kauppi, K., Ljungberg, J.K., Lövheim, H., Lundquist, A., Adolfsson, A.N., Oudin, A., Pudas, S., Rönnlund, M., Stiernstedt, M., Sundström, A., Adolfsson, R., 2020. Biological and environmental predictors of heterogeneity in neurocognitive ageing: Evidence from Betula and other longitudinal studies. Ageing Res. Rev. 64, 101184. https://doi.org/10.1016/J.ARR.2020.101184

Ogden, C.L., Carroll, M.D., Kit, B.K., Flegal, K.M., 2014. Prevalence of childhood and adult obesity in the United States, 2011-2012. JAMA 311, 806–814. https://doi.org/10.1001/JAMA.2014.732

Opel, N., Thalamuthu, A., Milaneschi, Y., Grotegerd, D., Flint, C., Leenings, R., Goltermann, J., Richter, M., Hahn, T., Woditsch, G., Berger, K., Hermesdorf, M., McIntosh, A., Whalley, H.C., Harris, M.A., MacMaster, F.P., Walter, H., Veer, I.M., Frodl, T., Carballedo, A., Krug, A., Nenadic, I., Kircher, T., Aleman, A., Groenewold, N.A., Stein, D.J., Soares, J.C., Zunta-Soares, G.B., Mwangi, B., Wu, M.J., Walter, M., Li, M., Harrison, B.J., Davey, C.G., Cullen, K.R., Klimes-Dougan, B., Mueller, B.A., Sämann, P.G., Penninx, B., Nawijn, L., Veltman, D.J., Aftanas, L., Brak, I. V., Filimonova, E.A., Osipov, E.A., Reneman, L., Schrantee, A., Grabe, H.J., Van der Auwera, S., Wittfeld, K., Hosten, N., Völzke, H., Sim, K., Gotlib, I.H., Sacchet, M.D., Lagopoulos, J., Hatton, S.N., Hickie, I., Pozzi, E., Thompson, P.M., Jahanshad, N., Schmaal, L., Baune, B.T., Dannlowski, U., 2020. Brain structural abnormalities in obesity: relation to age, genetic risk, and common psychiatric disorders. Mol. Psychiatry 2020 269 26, 4839–4852. https://doi.org/10.1038/s41380-020-0774-9

Pedraz-Petrozzi, B., Elyamany, O., Rummel, C., Mulert, C., 2020. Effects of inflammation on the kynurenine pathway in schizophrenia - A systematic review. J. Neuroinflammation 17, 1–17. https://doi.org/10.1186/S12974-020-1721-Z/TABLES/4

Peng, T.C., Chen, W.L., Wu, L.W., Chang, Y.W., Kao, T.W., 2020. Sarcopenia and cognitive impairment: A systematic review and meta-analysis. Clin. Nutr. 39, 2695–2701. https://doi.org/10.1016/J.CLNU.2019.12.014

Peralta, M., Ramos, M., Lipert, A., Martins, J., Marques, A., 2018. Prevalence and trends of overweight and obesity in older adults from 10 European countries from 2005 to 2013. https://doi.org/10.1177/1403494818764810 46, 522–529. https://doi.org/10.1177/1403494818764810

Petersen, R.C., 2011. Mild Cognitive Impairment. N. Engl. J. Med. 364, 2227–2261.

Rikli, R.E., Jones, C.J., 1999. Functional Fitness Normative Scores for Community-Residing Older Adults, Ages 60-94. J. Aging Phys. Act. 7, 162–181. https://doi.org/10.1123/JAPA.7.2.162

Ritchie, J.D., Miller, C.K., Smiciklas-Wright, H., 2005. Tanita Foot-to-Foot Bioelectrical Impedance Analysis System Validated in Older Adults. J. Am. Diet. Assoc. 105, 1617–1619. https://doi.org/10.1016/J.JADA.2005.07.011

Roberto, C.A., Mayer, L.E.S., Brickman, A.M., Barnes, A., Muraskin, J., Yeung, L.K., Steffener, J., Sy, M., Hirsch, J., Stern, Y., Walsh, B.T., 2011. Brain Tissue Volume Changes Following Weight Gain in Adults with Anorexia Nervosa. Int. J. Eat. Disord. 44, 406–411. https://doi.org/10.1002/EAT.20840

Rosano, C., Kuller, L.H., Chung, H., Arnold, A.M., Longstreth, W.T., Newman, A.B., 2005. Subclinical brain magnetic resonance imaging abnormalities predict physical functional decline in high-functioning older adults. J. Am. Geriatr. Soc. 53, 649–654. https://doi.org/10.1111/J.1532-5415.2005.53214.X

Sartori, A.C., Vance, D.E., Slater, L.Z., Crowe, M., 2012. The impact of inflammation on cognitive function in older adults: Implications for healthcare practice and research. J. Neurosci. Nurs. https://doi.org/10.1097/JNN.0b013e3182527690

Scheiblich, H., Trombly, M., Ramirez, A., Heneka, M.T., 2020. Neuroimmune Connections in Aging and Neurodegenerative Diseases. Trends Immunol. https://doi.org/10.1016/j.it.2020.02.002

Shaw, M.E., Sachdev, P.S., Abhayaratna, W., Anstey, K.J., Cherbuin, N., 2017. Body mass index is associated with cortical thinning with different patterns in mid- and late-life. Int. J. Obes. 2018 423 42, 455–461. https://doi.org/10.1038/ijo.2017.254

Silbert, L.C., Nelson, C., Howieson, D.B., Moore, M.M., Kaye, J.A., 2008. Impact of white matter hyperintensity volume progression on rate of cognitive and motor decline. Neurology 71, 108. https://doi.org/10.1212/01.WNL.0000316799.86917.37

Singh-Manoux, A., Czernichow, S., Elbaz, A., Dugravot, A., Sabia, S., Hagger-Johnson, G., Kaffashian, S., Zins, M., Brunner, E.J., Nabi, H., Kivimäki, M., 2012. Obesity phenotypes in midlife and cognition in early old age: The Whitehall II cohort study. Neurology 79, 762. https://doi.org/10.1212/WNL.0B013E3182661F63

Sjostrom, M., Ainsworth, B., Bauman, A., Bull, F., Hamilton-Craig, C., Sallis, J., 2005. Guidelines for Data Processing and Analysis of the International Physical Activity Questionnaire (IPAQ) – Short and Long Forms, Medicine.

Smith, L.L., Miles, M.P., 2000. Exercise-Induced Muscle Injury and Inflammation, in: Garret, W.E., Kirkendall, D.T. (Eds.), Exercise and Sport Science. Lippincott Williams & Wilkins, Philadelphia, pp. 401–412.

Stites, S.D., Harkins, K., Rubright, J.D., Karlawish, J., 2018. Relationships between Cognitive Complaints and Quality of Life in Older Adults with Mild Cognitive Impairment, Mild Alzheimer's Disease Dementia, and Normal Cognition. Alzheimer Dis. Assoc. Disord. 32, 283. https://doi.org/10.1097/WAD.0000000000000262

Sui, S.X., Holloway-Kew, K.L., Hyde, N.K., Williams, L.J., Leach, S., Pasco, J.A., 2020. Muscle strength and gait speed rather than lean mass are better indicators for poor cognitive function in older men. Sci. Rep. 10. https://doi.org/10.1038/S41598-020-67251-8

Tang, Y., Purkayastha, S., Cai, D., 2015. Hypothalamic microinflammation: a common basis of metabolic syndrome and aging. Trends Neurosci. 38, 36–44. https://doi.org/10.1016/J.TINS.2014.10.002

Tapia-González, S., García-Segura, L.M., Tena-Sempere, M., Frago, L.M., Castellano, J.M., Fuente-Martín, E., García-Cáceres, C., Argente, J., Chowen, J.A., 2011. Activation of Microglia in Specific Hypothalamic Nuclei and the Cerebellum of Adult Rats Exposed to Neonatal Overnutrition. J. Neuroendocrinol. 23, 365–370. https://doi.org/10.1111/J.1365-2826.2011.02113.X

Tolea, M.I., Galvin, J.E., 2015. Sarcopenia and impairment in cognitive and physical performance. Clin. Interv. Aging 10, 663–671. https://doi.org/10.2147/CIA.S76275

Tucker-Drob, E.M., 2019. Cognitive Aging and Dementia: A Life-Span Perspective. Annu. Rev. Dev. Psychol. 1, 177–196. https://doi.org/10.1146/ANNUREV-DEVPSYCH-121318-085204

Tuttle, C.S.L., Thang, L.A.N., Maier, A.B., 2020. Markers of inflammation and their association with muscle strength and mass: A systematic review and meta-analysis. Ageing Res. Rev. 64, 101185. https://doi.org/10.1016/J.ARR.2020.101185

Vints, W.A.J., Kušleikienė, S., Sheoran, S., Šarkinaitė, M., Valatkevičienė, K., Gleiznienė, R., Kvedaras, M., Pukėnas, K., Himmelreich, U., Česnaitienė, V.J., Levin, O., Verbunt, J., Masiulis, N., 2022a. Inflammatory blood biomarker kynurenine is linked with elevated neuroinflammation and neurodegeneration in older adults: evidence from two 1H-MRS post-processing analysis methods. Front. Psychiatry. https://doi.org/10.3389/FPSYT.2022.859772

Vints, W.A.J., Levin, O., Fujiyama, H., Verbunt, J., Masiulis, N., 2022b. Exerkines and long-term synaptic potentiation: Mechanisms of exercise-induced neuroplasticity. Front. Neuroendocrinol. 66, 100993. https://doi.org/10.1016/J.YFRNE.2022.100993

Visser, M., Pahor, M., Taaffe, D.R., Goodpaster, B.H., Simonsick, E.M., Newman, A.B., Nevitt, M., Harris, T.B., 2002. Relationship of interleukin-6 and tumor necrosis factor-alpha with muscle mass and muscle strength in elderly men and women: the Health ABC Study. J. Gerontol. A. Biol. Sci. Med. Sci. 57. https://doi.org/10.1093/GERONA/57.5.M326

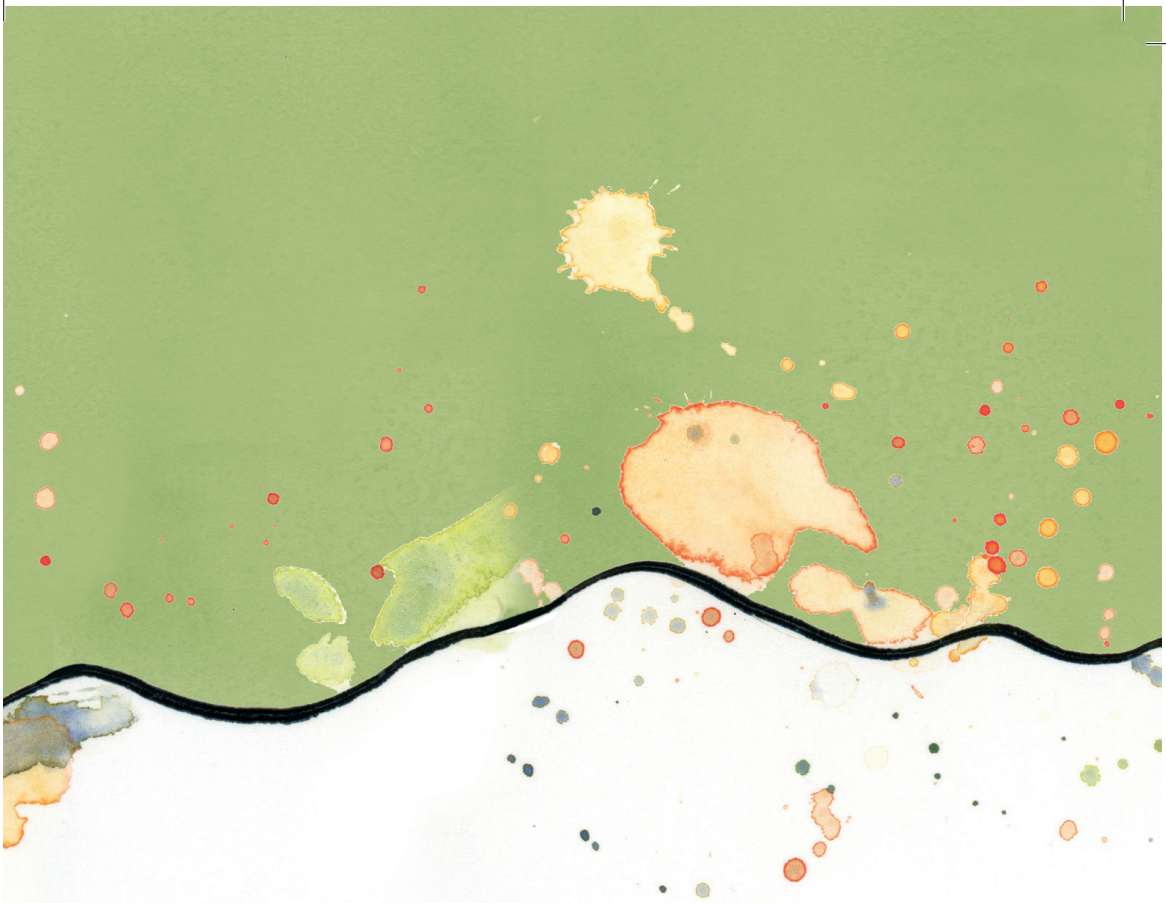
Woods, J.A., Wilund, K.R., Martin, S.A., Kistler, B.M., 2012. Exercise, inflammation and aging. Aging Dis.

Wu, C.S., Chen, Y.Y., Chuang, C.L., Chiang, L.M., Dwyer, G.B., Hsu, Y.L., Huang, A.C., Lai, C.L., Hsieh, K.C., 2015. Predicting body composition using foot-to-foot bioelectrical impedance analysis in healthy Asian individuals. Nutr. J. 14, 1–9. https://doi.org/10.1186/S12937-015-0041-0/TABLES/5

Yu, J.H., Kim, R.E.Y., Jung, J.M., Park, S.Y., Lee, D.Y., Cho, H.J., Kim, Nam Hoon, Yoo, H.J., Seo, J.A., Kim, S.G., Choi, K.M., Baik, S.H., Shin, C., Kim, Nan Hee, 2021. Sarcopenia is associated with decreased gray matter volume in the parietal lobe: a longitudinal cohort study. BMC Geriatr. 21, 622. https://doi.org/10.1186/S12877-021-02581-4

Yudkin, J.S., 2007. Inflammation, obesity, and the metabolic syndrome, in: Hormone and Metabolic Research. © Georg Thieme Verlag KG Stuttgart · New York, pp. 707–709. https://doi.org/10.1055/s-2007-985898

Zhu, C.W., Leibman, C., McLaughlin, T., Zbrozek, A.S., Scarmeas, N., Albert, M., Brandt, J., Blacker, D., Sano, M., Stern, Y., 2008. Patient Dependence and Longitudinal Changes in Costs of Care in Alzheimer's Disease. Dement. Geriatr. Cogn. Disord. 26, 423. https://doi.org/10.1159/000164797



PART III

The effect of resistance exercise on brain and cognitive outcomes in older adults with and without mild cognitive impairment

CHAPTER 5

The effects of a single bout of high intense strength exercise on cognitive function and postural dual-task control in older adults

Authors: Wouter A.J. Vints, Feryal Ghafelzadeh Ahwaz, Charlotte Westhof-Jacobs, Margarita Drozdova-Statkevičienė, Vida J. Česnaitienė, Gal Ziv, Lisa Pauwels, Oron Levin, Jeanine Verbunt, Nerijus Masiulis

Published in International Journal of Sport and Exercise Psychology, 2023
https://doi.org/10.1080/1612197X.2023.2255604

Abstract

In this study we aimed to test if acute strength exercise would induce cognitive improvements. Secondarily, we examined the relationship between exercise-induced changes in cognitive function and postural dual-task control. Thirty-seven cognitively intact, non-faller older adults (≥60 years) were nonrandomly allocated to strength exercise or control. Strength exercise consisted of Smith machine squats (one session, 3×3reps at 90%, 95%, and 100% one-repetition maximum). Control participants held seated rest for 45 min. Cognitive functions, recognition (memory search), working memory (mathematical processing), processing speed (2-choice reaction time), and postural dual-task control were tested before and immediately after exercise or control using the Automated Neuropsychological Assessment Metrics-4 (ANAM4) battery and a mathematical counting task while maintaining a tandem Romberg stance with eyes open on a force plate. Outcome measures were response time and performance index (100×[accuracy/response time]) on the ANAM4 tests and sway activity and entropy during the postural dual-task. We found a non-significant improvement with moderate effect size in performance index on the mathematical processing task of experimental participants compared to control participants ($p = 0.145$, $\eta_p^2 = 0.060$). Improvements in the mathematical processing task over time in the control group were associated with increased sway activity during the postural dual-task. No significant associations were found between changes in cognitive function and changes in postural control in the experimental group. Ultimately, our results may direct researchers and healthcare professionals in designing the optimal exercise treatment to improve cognitive function and postural control in older adults.

Abbreviations

ANAM4, Automated Neuropsychological Assessment Metrics-4; BMI, body mass index; BDNF, brain-derived neurotrophic factor; IGF-1, insulin-like growth factor-1; IL-6, interleukin-6; IPAQ, International Physical Activity Questionnaire; MMSE, Mini-Mental State Examination; PI, performance index; RT, response time; 1RM, one-repetition maximum.

1. Introduction

Healthy aging is defined in the World Report on Ageing And Health by the World Health Organization as "the process of developing and maintaining the functional ability that enables wellbeing in older age" (World Health Organization, 2015, p. 28). Maintaining functional ability in older age is a challenge, as cognitive and motor functions including postural control start to decline already from the age of 20 (Fjell & Walhovd, 2010; Seidler et al., 2010; McPhee et al., 2016; Degani et al., 2017). As individuals age beyond a certain point, they experience significant grey matter volume reductions, interruption of white matter microstructural organization, and age-related decline in functional network segregation and integration leading to cognitive and motor deficits (e.g., Seidler et al., 2010; Di et al., 2014; King et al., 2018). With the cohort of older adults being the fastest-growing subpopulation in current society (Dall et al., 2013), there is an increasing interest in non-pharmacological, low-risk interventions to prevent motor and cognitive declines and improve functional independence and mobility in aging (Netz, 2017), especially as cognitive dysfunction and falls are currently the two most rapidly increasing causes of death in older adults (Mattiuzzi & Lippi, 2020).

Physical exercise is considered a promising candidate to function as a treatment strategy for mitigating age-related cognitive and motor decline (Kirk-Sanchez & McGough, 2013; Hunter et al., 2016). For example, extensive evidence supports the cognitive benefits of moderate to high-intensity cardiovascular or resistance exercise programs among older adults (Erickson et al., 2011; C. L. Tsai et al., 2015; Mandolesi et al., 2018; Landrigan et al., 2019; C.-L. Tsai et al., 2019; Northey et al., 2018). Improved cognition through chronic cardiovascular exercise has been related to the up-regulation of brain-derived neurotrophic factor (BDNF) (C.-L. Tsai et al., 2019). However, the beneficial effect of chronic cardiovascular exercise on the brain may depend less on the upregulation of neurotrophic factors and to a greater extent to improvements in cardiovascular function or changes in energy metabolism, such as increased delivery of nutrients and oxygen (S. Kim et al., 2019).

The abovementioned benefits of cardiovascular exercise may differ from those related to resistance exercise. However, there is a limited body of work exploring the impact of resistance exercise on cognitive function and exercise-induced neuroplasticity

(Huang et al., 2022; Vints et al., 2022). This gap in the current literature could be critical as Huang et al. (2022) reported that chronic resistance exercise has a greater potential than cardiovascular exercise in effectively slowing cognitive decline in older adults with cognitive impairment. According to Huang et al. (2022), chronic resistance training is likely to have more pronounced effects on cognition than cardiovascular exercise through a larger activation of multiple signalling pathways activated by both cytokines and growth factors released from muscle tissue. The enhanced cognitive function resulting from chronic resistance exercise could be mediated through the elevation of brain/circulating levels of insulin-like growth factor-1 (IGF-1) and irisin, which are essential for neurogenesis and synaptogenesis, as well as through the down-regulation of circulating levels of inflammatory cytokines (e.g., interleukin 6 and interleukin 1β) who may inhibit neuroplastic processes (Gruol, 2015; H. J. Kim et al., 2015; Nielsen & Pedersen, 2007; C.-L. Tsai et al., 2019; for reviews see: Gajewski & Falkenstein, 2016 and Vints et al., 2022) .

To further elucidate the exercise-effect mechanism, researchers have investigated the effect of acute (i.e., a single bout of) exercise on cognitive function. It was discovered that even a single bout of physical exercise may transiently enhance cognitive and motor functioning (Y.K. Chang et al., 2012; Hsieh, Chang, Fang, et al., 2016; Ludyga et al., 2016; Netz et al., 2016; Thomas et al., 2016; McSween et al., 2019; Wanner et al., 2020; Park et al., 2021; Netz et al., 2023). Again, there are fewer studies examining the acute effects of resistance exercise compared with studies examining the acute effects of cardiovascular exercise (Vints et al., 2022). Therefore, we could not make a firm prediction on differences between cardiovascular and resistance exercise effects nor on mechanisms which may underlie the exercise-induced improvement in cognition following a single bout of resistance exercise. In addition, the optimal design of a resistance exercise protocol for maximizing cognitive benefits in older adults remains unclear (Huang et al., 2022; Vints et al., 2022).

Strength training, a specific type of resistance training typically consisting of lifting relatively heavy weights for a low number of repetitions (Schoenfeld et al., 2021), could act as a promising treatment strategy against cognitive and motor decline at older age. Both acute and chronic strength training can improve muscle strength, balance, and mobility (Hess & Woollacott, 2005; Cassilhas et al., 2007; van het Reve & De Bruin, 2014), cognitive functioning (e.g., Y.-K. Chang et al., 2012; T. Liu-Ambrose et al., 2012; Hsieh, Chang, Fang, et al., 2016; Mavros et al., 2017), and postural control (after a single bout; Drozdova-Statkevičienė et al., 2021). Furthermore, acute strength training can transiently improve cognition across multiple domains including attention, working memory, executive control and balance control (Y.-K. Chang et al., 2014; Hsieh, Chang, Hung, et al., 2016; Hsieh, Chang, Fang, et al., 2016; Dunsky et al., 2017; Drozdova-Statkevičienė et al., 2021). This is of particular interest, as cognitive decline was reported to be associated with fall risk in older adults (T. Y. Liu-Ambrose et al., 2008; Muir et al., 2012). Moreover, falls and dementias were respectively the first (+212.1%) and second (+149.6%) fastest increasing cause of death between 2000

and 2016 in older adults, and displayed respectively the third (+59.9%) and first (+108.8%) largest increment as a cause of disability at older age (Mattiuzzi & Lippi, 2020). Notably, falls and dementias were the only two causes of death to show an increment of more than 100% in 16 years' time (Mattiuzzi & Lippi, 2020).

The similarity of the trends in falling and cognitive decline may be explained by the finding that older adults require a higher level of attention to conserve gait/stance stability, as vestibular and proprioceptive sensitivity decreases with age. Consequently, postural control necessitates increased attentional input (Woollacott & Shumway-Cook, 2002; Rogers et al., 2013). Indeed, an increased risk of falling can already be noticed in older adults with only subtle cognitive impairment (T. Y. Liu-Ambrose et al., 2008; Muir et al., 2012). Particularly in more demanding circumstances, such as activities that require both motor and cognitive activity (i.e. dual-tasks), the performance of older adults on either of the postural or cognitive task or on both may be lower compared to younger adults (Woollacott & Shumway-Cook, 2002; Rogers et al., 2013). Therefore, dual-tasks are considered a sensitive tool for identifying cognitive and balance impairments in older adults (Muir-Hunter & Wittwer, 2016; Bayot et al., 2018). Given the possible facilitatory effects of strength exercise on attentional control and balance (Dunsky et al, 2017; Drozdova-Statkeviciene et al, 2021) and given the importance of these abilities in older age, the present study had two purposes: (1) to study the effect of a single bout of strength exercise with high relative loads and long rest periods on cognitive function in older adults, and (2) to examine whether changes in postural control performance from before to after a single bout of strength exercise with high relative loads and long rest periods would be associated with cognitive function.

In line with the abovementioned goals, our primary objective is to determine the specific cognitive areas impacted by the intervention and whether enhanced performance in these areas leads to overall improvements in maintaining balance. Based on existing research indicating that acute exercise can result in improvements in processing speed, attention, working memory, and basic cognitive abilities (e.g., Y.-K. Chang et al., 2014; Hsieh, Chang, Hung, et al., 2016; Hsieh, Chang, Fang, et al., 2016; Dunsky et al., 2017), and considering that immediate strength training positively influences attentional focus on the task of maintaining balance (Drozdova-Statkevičienė et al., 2021), we hypothesize the following: (1) our strength exercise protocol would induce improvements in cognitive function, as was previously reported with different resistance exercise protocols (Y.-K. Chang et al., 2014; Dunsky et al., 2017). Here, we expected that improvement in cognition following acute strength training would not be limited to a specific single cognitive domain, but this improvement would be seen across multiple aspects of cognition as well as in attentional control of balance; and (2) improvements in these cognitive domains will be related to improvement in balance control during dual-task.

2. Material and methods

2.1 Participants and setting

Thirty-seven (19 experimental and 18 control) healthy males above the age of 60 years were recruited in Kaunas, Lithuania. Based on a power analysis using G*Power 3.1.9.7 to calculate the sample size needed to find an interaction in a repeated measures ANOVA implemented on outcome measures obtained from cognitive (section 2.4.2) and postural dual-task (section 2.4.3) assessment tests in this study with a moderate effect size (Cohen's f = 0.25), an alpha of 0.05, and a power of 0.80, we needed at least 34 participants in total. Recruitment was done via presentations in local community organizations and via personal contacts with the experimenters (co-authors D-S.M., V.J.C. and N.M.). The same cohort was included in the study of Drozdova-Statkevičienė et al. (2021). Participants were interviewed prior to their inclusion by a public health specialist (co-author V.J.C.) and were introduced to the intervention by a certified trainer and exercise physiology expert (co-author N.M.). All participants and the experimenters who communicated with participants during the test/training sessions (co-authors D-S.M., V.J.C. and N.M.) were Lithuanian native speakers. All participants were free of chronic pain, diabetes mellitus and physical or neurological disorders. Participants were screened for cognitive impairment with the validated Lithuanian version of the Mini-Mental State Examination (MMSE) test by co-author V.J.C., excluding participants scoring below 24/30 (Pangman et al., 2000). We excluded participants with alcohol or drug abuse, psychopharmacological drug use, and those participating in a resistance training program in the last six months.

A randomization process was implemented at first to allocate subjects to one of the two groups (i.e., exercise and passive controls). Specifically, we intended to allocate participants eligible for inclusion to experimental or control group in a random order based on day of birth (odd days – control group; even days – experimental group) and participant entry to the research (the latter was used to compensate for unequal sample size). However, when we explained the nature of the strength exercise protocol to the participants allocated to the experimental group, some of the recruited participants were less keen to undergo the high-intense strength exercise intervention. Given the difficulty to find older adults willing to participate in our experimental group, about the last half of the recruited participants were allowed to be reallocated to the opposite group when they refused to start in the experimental group. Note that reallocation of participants to the opposite group was applied only on participants that did not agree to join the experimental (training group) whereas individuals that were allocated to the experimental group were selected randomly from the overall pool of subjects. Participants in both groups could voluntarily withdraw from the study at any time. The study was approved by the Kaunas Regional Biomedical Research Ethical Committee (No. BE-2-46) and a written informed consent was obtained from all participants prior to their inclusion in the study.

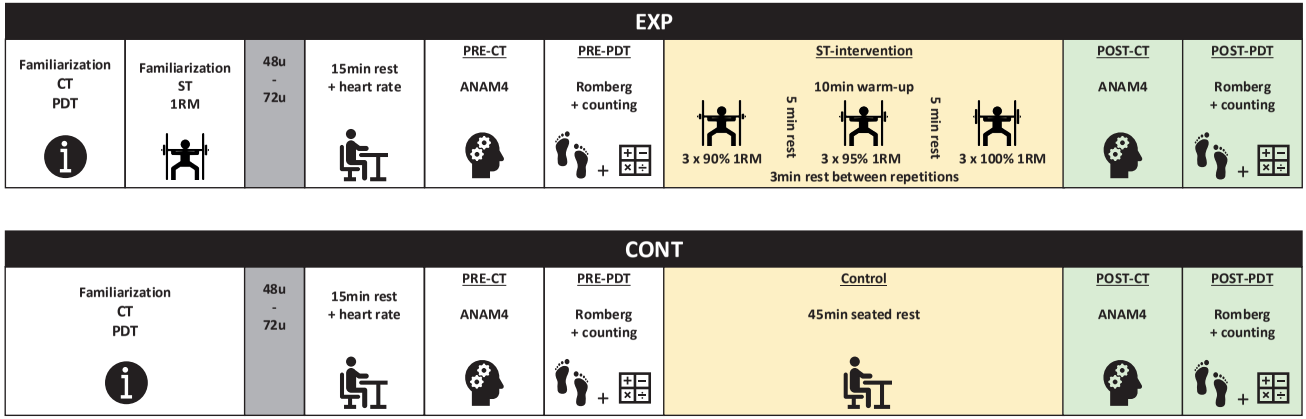


Figure 1. Study design overview

Abbreviations: 1RM, one-repetition maximum test; ANAM4, Automated Neuropsychological Assessment Metrics 4; CONT, control group; CT, cognitive task; EXP, experimental group; PDT, postural dual task; ST, strength training.

2.2 Procedure

An overview of the study design has already been presented in (Drozdova-Statkevičienė et al., 2021) and is illustrated in Figure 1. Participants were invited to visit the Lithuanian Sports University, Institute of Sport Science and Innovation in Kaunas on two separate days, with 2-3 days between visits. Testing took place between 9 and 11 am. On the first day, after recording demographic and clinical characteristics, participants in the experimental group underwent one-repetition maximum (1RM) testing. On the second visit, testing took place in the following order: 15 min rest with heart rate monitoring using a pulse meter (Sigma Sport PC 15.11) in a sitting position, cognitive testing, postural dual-task testing, strength exercise (see section 2.3.1), 5 min rest, cognitive testing and (about 15-20 min after exercise) postural dual-task testing. Cognitive testing consisted of three tasks that were selected from the Automated Neuropsychological Assessment Metrics-4 (ANAM4) battery (see section 2.4.2). Postural dual-task testing consisted each time of three trials where participants were requested to maintain a tandem Romberg stance while performing a mathematical counting task (see section 2.4.3) and was described in more detail in Drozdova-Statkevičienė et al. (2021)

Control participants underwent the same familiarization procedure on the first visit but were not required to undergo 1RM testing. On the second visit, testing was performed in the same order as in the experimental group, but strength exercise was replaced by 45min seated rest (see section 2.3.2). During both visits, the whole procedure was led by the same researcher (co-author D-S.M.), who was not blinded for the group allocation.

2.3 Interventions

Experimental condition: acute strength exercise intervention

Participants in the experimental group underwent a single session of strength exercise consisting of barbell squats on a Smith machine (Drozdova-Statkevičienė et al., 2021). The strength exercise session was preceded by a 10 min warm-up on a cycle ergometer (Monark 834E) at a power output of 60-80 W and a cadence of 50-60 rpm. Next, participants performed three sets of three repetitions of barbell squats with increasing intensity, i.e., 90%, 95%, and 100% of 1RM. Specifically, participants completed nine repetitions in total: three repetitions with 90% 1RM loading, three repetitions with 95% 1RM loading, and three repetitions with 100% 1RM loading. A 3-minute rest was given between repetitions at each loading condition, and 5-minute rest was given at the end of every 3rd repetition loading while weight was increased by the trainer. Participants were instructed to reach a squat depth with a knee angle of approximately 90°. During the squats, verbal encouragement was provided by the trainer. All participants were able to complete the whole protocol. The total duration of the intervention was 30min. During the whole duration of the intervention, heart rate was monitored for safety reasons. 1RM testing was done 2-3 days before the intervention. A standard 1RM testing protocol was used as outlined by

the National Strength and Conditioning Association (Baechle & Earle, 2000; Macht et al., 2016). Starting weight was decided individually so that the 1RM could be achieved in as few attempts as possible. In each trial, weight was gradually increased by 10-20% until the participant was no longer able to lift it three times while maintaining a squat dept so that the knees of the participant were at about a 90° angle at the lowest point. This was visually checked by the trainer. Participants were verbally encouraged during each repetition. Rest periods between sets during the 1RM testing protocol were approximately 3 min. The predicted 1RM was calculated using an online 1RM calculator https://exrx.net/Calculators/OneRepMax (*ExRx.Net : Predicting One-Rep Max*, n.d.).

Control condition: 45 min seated rest

Participants in the control condition were instructed to remain seated in the waiting room for 45min and were allowed to read magazines or to interact with the researchers.

2.4 Assessments

Demographic and exercise-related characteristics

All participants were asked to report their age, smoking status, and medical history. Participants also completed the International Physical Activity Questionnaire (IPAQ) Short Form, which was used to assess participants' physical activity levels (Sjostrom et al., 2005). We also calculated the participants' body mass index (BMI).

Cognitive tasks

To assess cognitive performance, we used a reliable computerized test, namely the ANAM4 battery (Vista Life Sciences, USA) (Vincent et al., 2017). The ANAM4 is a library containing 28 cognitive tests and behavioral questionnaires, which can be configured for various clinical or experimental applications. A selection of tests from this library was used, including (a) the 2-choice reaction time test, (b) the memory search task, and (c) the mathematical processing task, in this order (Center for the Study of Human Operator Performance, 2007). The three tasks included in our study were selected based on previous evidence showing they are valid for assessing executive functions and global cognition in aging but also for age-unrelated neurological conditions (e.g., Vincent et al., 2012; Tayer-Shifman et al., 2020). Specifically, these tasks incorporate several cognitive processes, including visual working memory, attention, and executive control, which are key cognitive domains that can be alleviated by exercise (Engeroff et al., 2018; Herold et al., 2019). All participants were familiarized with the three cognitive tasks during their first visit to the lab, 48-72h before the testing day. On the testing day, participants performed the tasks before and about 5 min after the strength exercise intervention (experimental group) or after 45 min of rest (control group); see Figure 1. It took the participants approximately 10-12 min to complete these three tests. They were instructed that speed and accuracy were equally important.

For all three cognitive tasks, we assessed accuracy (% correct answers), response time (RT in ms), and performance index (PI, in arbitrary units). The PI (computed as PI = 100 × [accuracy/response time]) is an outcome parameter used to compensate for the fact that some participants may be faster despite lower accuracy, while others may be more accurate despite slower speeds, e.g. (Netz et al., 2016). Accuracy measures were used to exclude trials with more than 50% incorrect responses, as this may indicate that the participant did not understand the task. For all trials, response accuracy was never below 50%.

(a) The *memory search task* is used as an index of verbal working memory, immediate recognition, and attention. For this task, six characters (the positive memory set) are displayed for memorization. Next, individual characteristics are presented, and the participant needs to press designated buttons to indicate whether or not the character is a member of the memorized set.
(b) The *mathematical processing task* examines basic computing skills, concentration, and working memory. During this task, an arithmetic problem involving three single-digit numbers and two operators is displayed on a computer screen (e.g., "5 - 2 + 3 ="). The participant needs to press the left or right mouse button, left button if the answer to the problem is less than five, and right button if the answer is higher than five.
(c) The *2-choice reaction time test* examines processing speed and alternating attention with a motor speed component. It is a psychomotor reaction time task where the participant is presented with a "*" or "o" on the computer display. The participant needs to press the left or right mouse button depending on which stimulus appears.

Postural dual-task

The protocol for postural dual-task testing consisted of maintaining a tandem Romberg stance position on a single piezoelectric force plate (KISTLER, Slimline System 9286) with their eyes open, while performing a mathematical counting task, as has been presented before (Drozdova-Statkevičienė et al., 2021). On the testing day, all participants performed the postural dual-task after the ANAM4 tests before and about 15-20 min after the intervention or a 45min rest period. In contrast to the results presented in this previous study, we reported the total component of sway activity and entropy instead of presenting the anteroposterior and mediolateral components separately. The total vectors were calculated from the anteroposterior and mediolateral ground reaction forces, using a custom-written MATLAB script (MathWorks, Natick, MA) (Drozdova-Statkevičienė et al., 2018). High sway activity reflects poor postural control. Sway entropy is a measure of statical sway regularity. Higher sway entropy indicates more irregular sway activity, which is linked with automatic postural control and decreased deployment of attention to the postural task. In contrast, lower sway entropy indicates more regular sway activity which is hypothesized to be associated with the deployment of more attention to the postural task (Donker et al., 2007).

2.5 Statistical analysis

Statistical analysis was performed using SPSS Statistics version 27 (IBM, USA). None of the data contained extreme outliers, defined as a value lying further than three times the interquartile range away from the median. All data were normally distributed, as was decided based on visual interpretation of histograms and measurements of skewness and kurtosis (normality assumed when values were between -2 and +2) (George & Mallery, 2010).

To test our primary research question, we used a two-way repeated measures ANOVA (Group*Time) with repeated measures on the Time factor. Additionally, as part of an exploratory analysis, we used two-way ANCOVA with pre-test results and age as covariates and with post-test cognitive and postural task results as the dependent variables because it has been suggested to provide higher statistical power and accuracy when assessing groups changed from pre-test to post-test (Rausch et al., 2003). In case the ANOVA or the ANCOVA results were of moderate to high effect size (partial eta squared, $\eta_p^2 > 0.06$), they were further explored using paired samples t-tests to report the change over time in experimental or control groups separately (Cohen, 1988; Netz et al., 2023). To test our secondary research question, we used multiple linear regression analysis adjusted for age to search for an association between the pre-to-post difference on RT or PI on each one of the ANAM4 cognitive tests and pre-to-post difference in sway activity or entropy during the postural dual-task.

3. Results

3.1 Participants' characteristics

Participants' age ranged between 60 and 77 years old, BMI ranged between 21.0 and 29.0, and the MMSE scores ranged between 27 and 30. Mean values and differences between groups are presented in Supplementary table A.1. Three of the included participants were smokers (8.1%), and physical activity level of all participants was moderate based on the IPAQ short form questionnaire. In the experimental group, the mean increase in heart rate during exercise compared to before exercise was +59.2%. No significant group differences in age, BMI and MMSE scores were found (all $p \geq 0.455$; see Supplementary Table A.1), suggesting that the two groups were demographically balanced.

3.2 The effect of acute strength exercise on ANAM4 cognitive performance and postural MPcontrol

Mean values of our outcome measures at baseline and pre-to-post change (in % changes from baseline) within control (n = 18) and experimental (n = 19) subjects are illustrated in Figure 2. There were no significant Time*Group interaction effects for any of the cognitive tests or postural control tasks (see Table 1). This indicates that strength training did not

significantly influence the change in performance. However, the Time*Group interaction effect for PI on the mathematical processing test had a moderate effect size ($p = 0.145$, $\eta_p^2 = 0.060$). Further exploration with paired t-tests showed that the experimental group and not the control group improved significantly over time ($p < 0.001$), see Figure 2 and Supplementary Table A.2.

Table 1. Repeated measures ANOVA test results

		p-value	Partial Eta Squared
RT Memory Search	Time	0.292	0.032
	Group	0.586	0.009
	Time*Group	0.910	0.000
RT Mathematical Processing	Time	0.056	0.101
	Group	0.061	0.097
	Time*Group	0.313	0.029
RT 2 choice RT test	Time	0.003*	0.228
	Group	0.055	0.101
	Time*Group	0.987	0.000
PI Memory Search	Time	0.285	0.033
	Group	0.441	0.017
	Time*Group	0.488	0.014
PI Mathematical Processing	Time	0.00006*	0.374
	Group	0.031*	0.126
	Time*Group	0.145	0.060
PI 2 choice RT test	Time	0.003*	0.230
	Group	0.040*	0.115
	Time*Group	0.704	0.004
Postural sway DT	Time	0.005*	0.201
	Group	0.583	0.009
	Time*Group	0.576	0.009
Entropy DT	Time	0.0003*	0.312
	Group	0.065	0.094
	Time*Group	0.778	0.002

* $p < 0.05$ Abbreviations: DT, dual task; PI, performance index; RT, response time.

3.3 Exploratory analysis

Exploratory analysis on the effect of acute strength exercise on ANAM4 cognitive performance and postural control using ANCOVA

ANCOVA results showed that RT on the mathematical processing post-test was significantly faster in the experimental group compared to the control group after adjusting for the pre-test results and for age ($p = 0.042$, $\eta_p^2 = 0.119$). None of the other ANCOVA results for RTs or PIs of the ANAM4 cognitive tests were significantly different between groups. After adjusting for pre-test and age, both post-test postural sway activity and entropy during the postural dual-task did not differ between the experimental and control group, see Table 2.

Table 2. ANCOVA test results adjusted for pre-test values and age

	Group	Mean (SE)	p-value	Partial Eta Squared
RT Memory Search	Control	1223.06 (45.64)	0.851	0.001
	Experimental	1210.95 (44.42)		
RT Mathematical Processing	Control	3584.71 (140.30)	0.042*	0.119
	Experimental	3166.76 (136.47)		
RT 2 choice RT test	Control	592.35 (13.20)	0.203	0.049
	Experimental	568.02 (12.83)		
PI Memory Search	Control	7.89 (0.33)	0.306	0.032
	Experimental	8.38 (0.32)		
PI Mathematical Processing	Control	2.88 (0.13)	0.091	0.084
	Experimental	3.20 (0.13)		
PI 2 choice RT test	Control	16.92 (0.41)	0.143	0.064
	Experimental	17.80 (0.40)		
Postural sway DT	Control	28.79 (1.64)	0.493	0.014
	Experimental	27.20 (1.60)		
Entropy DT	Control	0.45 (0.01)	0.238	0.042
	Experimental	0.47 (0.01)		

* $p < 0.05$ Note that the mean (standard error) values presented here are those used for ANCOVA testing. They are evaluated at the mean age (67.9) and mean pre-test results. The mean values before adjusting for age and pre-test results are illustrated in Figure 2. Abbreviations: DT, dual task; PI, performance index; RT, response time; SE, standard error.

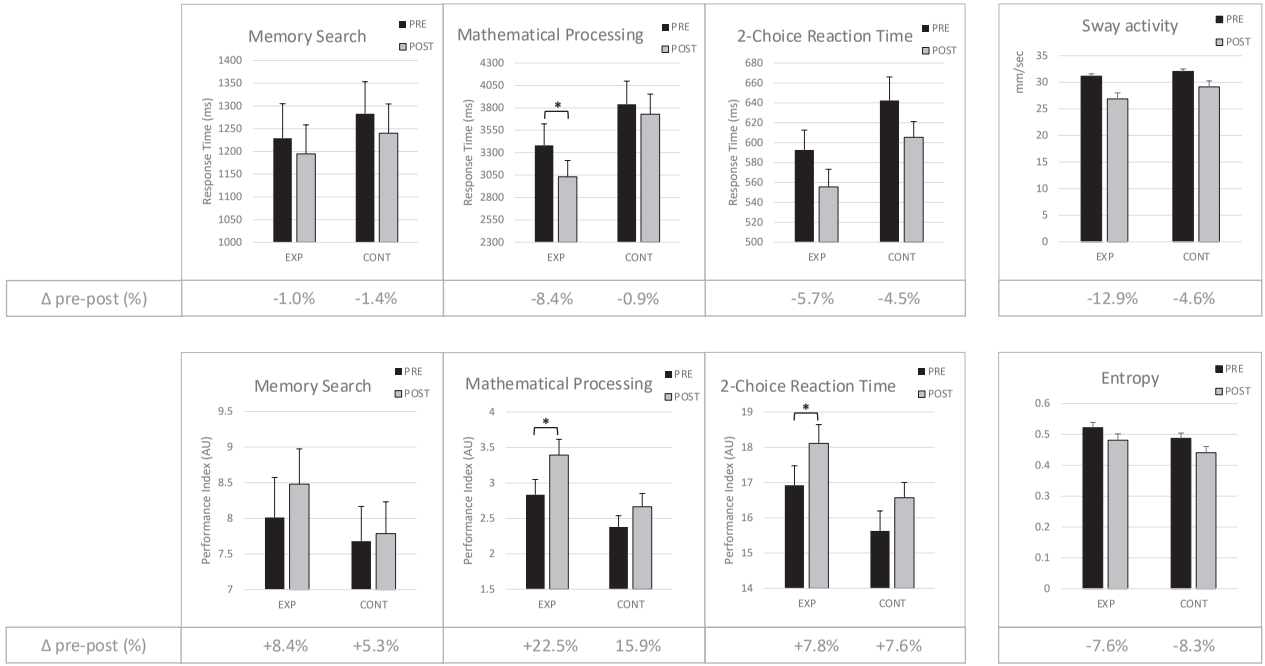


Figure 2. ANAM4 and postural dual task test results

Mean and standard error values of pre- and post-test cognitive test and postural dual task results in experimental and control group. The first row illustrates response time values, the second row illustrates performance index values. The right column illustrates the postural results.

* $p < 0.05$ Abbreviations: AU, arbitrary units; CONT, control; EXP, experimental.

Exploratory analysis of results with moderate to strong effect sizes

Non-significant results with at least moderate effect sizes ($\eta_p^2 > 0.06$) were found for PI on the mathematical processing task ($p = 0.091$, $\eta_p^2 = 0.084$), and PI on the 2-choice reaction time test ($p = 0.143$, $\eta_p^2 = 0.064$). In each case, it was the experimental group that improved significantly over time, $p < 0.001$ and $p = 0.010$ for mathematical processing PI and 2-choice reaction time PI, respectively, see Figure 2 and Supplementary Table A.2.

3.4 The relationship between ANAM4 cognitive test results and postural dual-task control

Multiple linear regression analysis with age and pre-to-post difference in RT on the mathematical processing task for the dependent pre-to-post difference in sway activity showed that a decrease in mathematical processing RT from pre to post was associated with an increase in sway activity on the postural dual-task in the control group ($\beta = -0.556$, $p = 0.020$). This model, including age and pre-to-post differences in RT on the mathematical processing task, had a statistically insignificant ($p = 0.059$) R^2 of 0.314. Multiple linear regression analysis with age and pre-to-post difference in PI on the mathematical processing task for the dependent pre-to-post difference in sway activity showed that an increase in mathematical processing PI from pre-to-post was associated with an increase in sway activity on the postural dual-task in the control group ($\beta = 0.675$, $p = 0.001$). This model, including age and pre-to-post difference in RT on the mathematical processing task, had a statistically significant ($p = 0.004$) R^2 of 0.515. No significant relationships were found for the other ANAM4 cognitive tests nor for changes in the experimental group, see Table 3.

Table 3. Multiple linear regression results with independents age and pre-to-post change on one of the ANAM4 cognitive performance outcome measures, with dependents pre-to-post change in sway activity or entropy for control and experimental groups separately

	PRE-TO-POST DIFFERENCE			
	Sway activity			
	Control		Experimental	
	Model summary including age	Coefficient Δ adjusted for age	Model summary including age	Coefficient Δ adjusted for age
RT Memory Search	$R^2 = 0.069$	$\beta = 0.257$	$R^2 = 0.148$	$\beta = -0.328$
	$p = 0.587$	$p = 0.319$	$p = 0.277$	$p = 0.195$
RT Mathematical Processing	$R^2 = 0.314$	$\beta = -0.556$	$R^2 = 0.115$	$\beta = 0.242$
	$p = 0.059$	$p = 0.020$*	$p = 0.378$	$p = 0.299$
RT 2-Choice Reaction Time	$R^2 = 0.130$	$\beta = 0.352$	$R^2 = 0.086$	$\beta = 0.192$
	$p = 0.353$	$p = 0.160$	$p = 0.489$	$p = 0.447$

Table 3. Continued

PI Memory Search	R^2 = 0.060 p = 0.630	β = -0.239 p = 0.356	R^2 = 0.057 p = 0.623	β = 0.084 p = 0.744
PI Mathematical Processing	R^2 = 0.515 p = 0.004*	β = 0.672 p = 0.001*	R^2 = 0.124 p = 0.346	β = -0.283 p = 0.264
PI 2-Choice Reaction Time	R^2 = 0.132 p = 0.345	β = -0.351 p = 0.155	R^2 = 0.066 p = 0.581	β = -0.124 p = 0.623

	Entropy			
	Control		Experimental	
	Model summary including age	Coefficient Δ adjusted for age	Model summary including age	Coefficient Δ adjusted for age
RT Memory Search	R^2 = 0.165 p = 0.259	β = 0.345 p = 0.189	R^2 = 0.122 p = 0.354	β = -0.093 p = 0.730
RT Mathematical Processing	R^2 = 0.068 p = 0.590	β = 0.099 p = 0.713	R^2 = 0.134 p = 0.318	β = 0.140 p = 0.566
RT 2-Choice Reaction Time	R^2 = 0.061 p = 0.622	β = -0.048 p = 0.857	R^2 = 0.188 p = 0.189	β = 0.299 p = 0.247

	Entropy			
	Control		Experimental	
	Model summary including age	Coefficient Δ adjusted for age	Model summary including age	Coefficient Δ adjusted for age
PI Memory Search	R^2 = 0.115 p = 0.401	β = -0.250 p = 0.347	R^2 = 0.115 p = 0.375	β = 0.020 p = 0.939
PI Mathematical Processing	R^2 = 0.065 p = 0.606	β = 0.073 p = 0.772	R^2 = 0.122 p = 0.353	β = -0.093 p = 0.727
PI 2-Choice Reaction Time	R^2 = 0.064 p = 0.607	β = -0.075 p = 0.775	R^2 = 0.144 p = 0.290	β = -0.186 p = 0.476

Pre-to-post difference (Δ) was calculated by subtracting the values from second testing from those of the first testing. The model summary with independents age and one of the ANAM4 cognitive test outcome measures and with dependents one of the postural dual task outcomes are presented. In addition, the table includes the relationship (β and p-value) between each one of the ANAM4 cognitive test outcome measures and sway activity or entropy on the postural dual task adjustedg for age. * $p < 0.05$. Abbreviations: DT, dual task; PI, performance index; RT, response time.

4. Discussion

We hypothesized that a single bout of high-intensity strength exercise would induce beneficial effects on cognition in older adults and that this cognitive performance improvement would be associated with improved postural control during a dual-task. Cognitive performance was examined for recognition (memory search), working memory (mathematical processing), processing speed (2-choice reaction time), and attention (all three tests). To our knowledge, this is the first study to investigate the effects of high-intensity strength exercise on cognitive function versus attentional control of balance in challenging dual-task conditions in older adults. Our data partially support the first hypothesis. Specifically, we found pre-to-post improvement in performance on the mathematical processing task and reduction in sway activity and sway entropy. However, our data did not support the second hypothesis. We observed that pre-to-post improvements in these cognitive domains were not associated with improvements in balance control.

The abovementioned observations suggest that exercise-related pre-to-post gains in attentional (conscious) control of balance (as expressed by decreased entropy) and working memory may be mediated by separate neuronal substrates. However, in the control group, the multiple linear regression analysis revealed significant associations between pre-to-post changes in sway activity (or entropy) and pre-to-post improvements in the performance of the mathematical processing task. Furthermore, the control group also showed a significant pre-to-post decline of sway entropy, indicating an increase in regularity of balance (e.g., Drozdova-Statkeviciene et al, 2021). In line with these findings, one should not exclude the possibility that pre-to-post changes observed in our study were partly influenced by test-retest learning effects. However, a careful examination of the findings showed that: (1) gains on entropy in the control group were not associated with pre-to-post changes in cognition, and (2) improvement in balance stability (i.e., pre-to-post decrease in sway activity) was associated primarily with a decrease in performance of the mathematical processing task. Therefore, it is possible that test–retest learning gains in balance stability were more prominent in individuals with poorer working memory and processing speed.

Results from a two-way repeated measures ANOVA showed that a single bout of high-intensity strength exercise induced a non-significant improvement with moderate effect size in PI on the mathematical processing task compared to the control group. Further exploratory analysis with a two-way ANCOVA with pre-test results and age as covariates showed a significantly larger improvement in RT on the mathematical processing task compared to control. None of the other cognitive tests changed significantly.

However, within this exploratory analysis, we discovered moderate effect sizes for an improvement in PI on the mathematical processing and 2-choice reaction time task in the experimental group compared to the control group. Our findings partly support those

of previous literature, albeit tasks used in our study were not similar to those used by others. For example, Netz et al. (2016) reported that a single bout of moderate-intensity cardiovascular exercise in middle-aged adults induced improvements in response inhibition on the Go/No-Go task, where participants need to press a button when a visual stimulus is being presented on a computer screen and inhibit pressing the button when another stimulus is being presented (Netz et al., 2016). Hsieh et al. (2016) discovered that response time on the Go/No-Go task also improved after a single bout of eight resistance exercises (two sets of ten repetitions at 70% 10RM) in older men. They reported that compared to young adults, older adults improved more on tasks with higher working memory demands (Hsieh, Chang, Hung, et al., 2016).

Furthermore, Chang and Etnier (2009) showed that a single session of six resistance exercises, each performed for two sets of ten repetitions at 75% 1RM, induced improvements in processing speed and tended to improve executive function (Yu Kai Chang & Etnier, 2009). In contrast, Pontifex et al. (2009) indicated that only acute cardiovascular exercise and not resistance exercise resulted in working memory task improvements (Pontifex et al., 2009). However, Dunsky et al. (2017) refuted their finding by reporting similar changes following acute cardiovascular and resistance exercise on executive functions in middle-aged physically active adults.

Our findings revealed no significant associations between the pre-to-post change in cognitive performance on the ANAM4 cognitive test battery and the pre-to-post change in performance on the postural balance task in the experimental group. Therefore, we could not confirm our hypothesis that improvements in one or more cognitive domains following a single bout of high-intensity strength exercise would coincide with the improvement of balance control. In contrast, our results indicated that improvements in the performance of the mathematical processing task over time were associated with increased sway activity during the postural dual-task in the control group. It should be noted that both the ANAM4 mathematical processing task and the mathematical counting task performed during the postural dual-task are generally working memory tests. Therefore, it was surprising that those performing better on the working memory test following the control session were also those who performed worse on the postural dual-task. Since pre-to-post gains observed in the control group could be attributed in part to a learning effect, it is reasonable to assume that improvements would be also evident in one or more cognitive domains in this group. Nonetheless, no significant pre-to-post performance gains on cognitive outcome measures were visible in the controls (see Supplementary Table A.2). An alternative explanation is that test-retest learning effect in the control group was associated with increased prioritization of this task under dual-task condition which in turn may have a detrimental effect on balance. Another reason why our results are surprising is that previous studies reported that impaired executive function (which includes working memory) is the cognitive deficit

most consistently reported to be associated with an increased risk of falls in older adults (Horak, 2006; Yogev-Seligmann et al., 2008). Therefore, one would expect that postural stability in older adults would be more affected in those with more difficulty on the cognitive component of the dual-task. To know if worse performance on postural dual-task studies is linked to the type of cognitive deficit in the older adult or the type of task being used in combination with the balance task, we advise other researchers to follow our example and report findings from both postural dual-task and cognitive tests.

From a mechanistic point of view, we used a strength exercise protocol with high relative loads and long rest periods. This protocol is optimal to improving muscle strength (Schoenfeld et al., 2021). However, its effects on cognitive performance and brain structure and function in apparently healthy older individuals have remained unclear. Previous studies with cardiovascular exercise like Thomas et al. (2016) and Opie and Semmler (2019), indicated that high-intensity exercise has a larger beneficial effect on motor skill learning than low-intensity exercise (Thomas et al., 2016; Opie & Semmler, 2019). Importantly, pooled data from the meta-analysis of Wanner et al. (2020) suggested that only high-intensity exercise can significantly improve motor task performance when compared to rest at both short-term and long-term retention testing.

Furthermore, high-intensity exercise was reported to induce higher levels of IGF-1, irisin and BDNF, which are suggested to mediate (part of) the beneficial effect of exercise on the brain through enhancing neuroplasticity (Schwarz et al., 1996; Daskalopoulou et al., 2014; Huh et al., 2014; Müller et al., 2020; Vints et al., 2022). Possibly, the augmented increase in circulating IGF-1, irisin and BDNF with high-intensity cardiovascular exercise is caused in part by pathways activated by lactate, as was suggested by others (Schiffer et al., 2011; Salgueiro et al., 2014; El Hayek et al., 2019; Kujach et al., 2020; Müller et al., 2020). In contrast to these studies, we adopted strength exercise with high relative loads and long rest intervals, which was probably not associated with elevated lactate levels. Thus, although we used a high-intensity protocol, the significant difference in the improvement on the mathematical processing task between experimental and control groups was probably not mediated by lactate-induced pathways. In addition, high-intensity resistance exercise has been shown to induce elevated inflammatory markers, such as interleukin-6 (IL-6), associated with exercise-induced muscle damage (Mendham et al., 2011). High inflammatory markers exert detrimental effects on neurotrophic signalling pathways and may thus counteract the effect of elevated neurotrophic factors (Sun et al., 2017; Bourgognon & Cavanagh, 2020). Although we did not measure blood biomarkers in this study, our exercise protocol most likely induced muscle damage to some extent in our cohort of older adults not participating regularly in any resistance training program, and thus resulted most likely in elevated inflammatory markers. Taken together, we found an effect of high intensity strength exercise on RT performance on the mathematical processing task even though proposed lactate- and inflammation-

mediated pathways were most likely not in our favor. Studies including blood analysis are needed to test if strength exercise can induce high levels of neurotrophic factors in the absence of lactate elevations, which may explain the beneficial effects of strength exercise on cognitive function.

Some notes and limitations should be mentioned concerning our study design. First, we failed to randomly allocate participants to the experiment because of the difficulty finding older adults willing to participate in high-intensity resistance exercise. This may have caused fitter or healthier participants to be allocated to the exercise condition. This may be linked with better cognitive and postural performance at baseline (Nemmers & Miller, 2008; Erickson et al., 2019). However, baseline performance was not significantly different in our study. Second, we included only healthy older adults with preserved physical and cognitive abilities and no history of falls, as was also the case in the study of Degani et al. (2017), who found increased sway activity in old compared to young adults (Degani et al., 2017). Of note, in the study of Fino et al. (2016), older adults with regular fall incidents showed different behaviors in postural control compared to older adults without regular fall incidents (Fino et al., 2016). Therefore, our results indicating no significant changes in postural control on the dual-task following acute strength exercise cannot be extrapolated to older adults with frequent fall incidents. It is arguable that the effects of acute strength exercise on postural control, which were not significant in our study, could have been more prominent in older adults an elevated fall risk.

In conclusion, our findings indicated that a single bout of high-intensity strength exercise induced a marginal improvement in working memory, measured as PI on the mathematical processing task. Further exploratory analysis also discovered a significant improvement in RT speed on the same task, which was not seen in the control group. In contrast to the hypothesis that improvements in one or more cognitive domains will be related to improvement in balance control in dual-task, improvements in RT and PI on the mathematical processing task were associated with increases rather than decrease in sway activity during the postural dual-task. The current findings call for future research into the effects of acute strength training on the interplay between cognitive and motor domains in older adults towards the discovery of tailored exercise programs for this population that could be beneficial for both cognitive and functional outcomes. Specifically, researchers should investigate the combined effects of exercise on the interplay between cognitive processes and motor functions related to postural control and locomotion. Future studies may also consider investigating the impact of a chronic intervention with high-intensity strength training on cognitive and postural performance.

Declarations of interest

The authors report there are no competing interests to declare.

Author's contributions

Margarita Drozdova-Statkevičienė, Vida J. Česnaitienė, Oron Levin and Nerijus Masiulis contributed to conception and design of the study. Margarita Drozdova-Statkevičienė and Vida J. Česnaitienė were involved in data collection and/or analysis. Wouter Vints performed the statistical analysis. Wouter Vints wrote the first draft of the manuscript. Feryal Ghafelzadeh Ahwaz, Charlotte Westhof-Jacobs and Lisa Pauwels wrote sections of the manuscript. Wouter Vints prepared the figures. Gal Ziv, Lisa Pauwels, Oron Levin, Jeanine Verbunt, and Nerijus Masiulis had a role in supervision. All authors contributed to manuscript revision, read, and approved the submitted version.

Funding

This work was supported by the Research Council of Lithuania under Grant number S-MIP-21-37. The sponsor was not involved in study design; collection, analysis and interpretation of the data; in writing of the report; nor in the decision to submit the article for publication.

Acknowledgements

Not applicable

Data availability statement

The authors confirm that the data supporting the findings of this study are available within the article and/or its supplementary materials.

References

Baechle, T. R., & Earle, R. W. (Eds.). (2000). *Essentials of Strength and Conditioning* (2nd ed.). Human Kinetics. https://books.google.com/books/about/Essentials_of_Strength_Training_and_Cond.html?hl=nl&id=zw_-9QgKTeYC

Bayot, M., Dujardin, K., Tard, C., Defebvre, L., Bonnet, C. T., Allart, E., & Delval, A. (2018). The interaction between cognition and motor control: A theoretical framework for dual-task interference effects on posture, gait initiation, gait and turning. *Neurophysiologie Clinique*, *48*(6), 361–375. https://doi.org/10.1016/J.NEUCLI.2018.10.003

Bourgognon, J.-M., & Cavanagh, J. (2020). The role of cytokines in modulating learning and memory and brain plasticity. *Brain and Neuroscience Advances*, *4*, 239821282097980. https://doi.org/10.1177/2398212820979802

Cassilhas, R. C., Viana, V. A. R., Grassmann, V., Santos, R. T., Santos, R. F., Tufik, S., & Mello, M. T. (2007). The Impact of Resistance Exercise on the Cognitive Function of the Elderly. *Medicine & Science in Sports & Exercise*, *39*(8), 1401–1407. https://doi.org/10.1249/mss.0b013e318060111f

Center for the Study of Human Operator Performance. (2007). *C-SHOP ANAM4: User Manual*. Center for the Study of Human Operator Performance (C-SHOP).

Chang, Y.-K., Pan, C.-Y., Chen, F.-T., Tsai, C.-L., & Huang, C.-C. (2012). Effect of Resistance-Exercise Training on Cognitive Function in Healthy Older Adults: A Review. *Journal of Aging and Physical Activity*, *20*(4), 497–517. https://doi.org/10.1123/japa.20.4.497

Chang, Y.-K., Tsai, C.-L., Huang, C.-C., Wang, C.-C., & Chu, I.-H. (2014). Effects of acute resistance exercise on cognition in late middle-aged adults: General or specific cognitive improvement? *Journal of Science and Medicine in Sport*, *17*(1), 51–55. https://www.sciencedirect.com/science/article/pii/S144024401300039X

Chang, Y.K., Labban, J. D., Gapin, J. I., & Etnier, J. L. (2012). The effects of acute exercise on cognitive performance: A meta-analysis. *Brain Research*, *1453*, 87–101. https://doi.org/10.1016/J.BRAINRES.2012.02.068

Chang, Yu Kai, & Etnier, J. L. (2009). Effects of an acute bout of localized resistance exercise on cognitive performance in middle-aged adults: A randomized controlled trial study. *Psychology of Sport and Exercise*, *10*(1), 19–24. https://doi.org/10.1016/J.PSYCHSPORT.2008.05.004

Cohen, J. (1988). Statistical Power Analysis for the Behavioral Sciences. In *Statistical Power Analysis for the Behavioral Sciences*. Routledge. https://doi.org/10.4324/9780203771587

Dall, T. M., Gallo, P. D., Chakrabarti, R., West, T., Semilla, A. P., & Storm, M. V. (2013). An Aging Population And Growing Disease Burden Will Require A Large And Specialized Health Care Workforce By 2025. *Health Affairs*, *32*(11), 2013–2020. https://doi.org/10.1377/hlthaff.2013.0714

Daskalopoulou, S. S., Cooke, A. B., Gomez, Y. H., Mutter, A. F., Filippaios, A., Mesfum, E. T., & Mantzoros, C. S. (2014). Plasma irisin levels progressively increase in response to increasing exercise workloads in young, healthy, active subjects. *European Journal of Endocrinology*, *171*(3), 343–352. https://doi.org/10.1530/EJE-14-0204

Degani, A. M., Leonard, C. T., & Danna-dos-Santos, A. (2017). The effects of early stages of aging on postural sway: A multiple domain balance assessment using a force platform. *Journal of Biomechanics*, *64*, 8–15. https://doi.org/10.1016/J.JBIOMECH.2017.08.029

Di, X., Rypma, B., & Biswal, B. B. (2014). Correspondence of Executive Function Related Functional and Anatomical Alterations in Aging Brain. *Progress in Neuro-Psychopharmacology & Biological Psychiatry*, *48*, 41–50. https://doi.org/10.1016/J.PNPBP.2013.09.001

Donker, S. F., Roerdink, M., Greven, A. J., & Beek, P. J. (2007). Regularity of center-of-pressure trajectories depends on the amount of attention invested in postural control. *Experimental Brain Research*, *181*(1), 1–11. https://doi.org/10.1007/S00221-007-0905-4

Drozdova-Statkevičienė, M., Česnaitienė, V. J., Levin, O., Pauwels, L., Pukėnas, K., Helsen, W. F., Staes, F., & Masiulis, N. (2021). The beneficial effects of acute strength training on sway activity and sway regularity in healthy older men: Evidence from a posturography study. *Neuroscience Letters*, *749*, 135718. https://doi.org/10.1016/J.NEULET.2021.135718

Drozdova-Statkevičienė, M., Česnaitienė, V. J., Pukėnas, K., Levin, O., & Masiulis, N. (2018). Sway regularity and sway activity in older adults' upright stance are differentially affected by dual task. *Neuroscience Letters*, *666*, 153–157. https://doi.org/10.1016/J.NEULET.2017.12.054

Dunsky, A., Abu-Rukun, M., Tsuk, S., Dwolatzky, T., Carasso, R., & Netz, Y. (2017). The effects of a resistance vs. an aerobic single session on attention and executive functioning in adults. *PloS One*, *12*(4), e0176092. https://doi.org/10.1371/journal.pone.0176092

El Hayek, L., Khalifeh, M., Zibara, V., Abi Assaad, R., Emmanuel, N., Karnib, N., El-Ghandour, R., Nasrallah, P., Bilen, M., Ibrahim, P., Younes, J., Abou Haidar, E., Barmo, N., Jabre, V., Stephan, J. S., & Sleiman, S. F. (2019). Lactate mediates the effects of exercise on learning and memory through sirt1-dependent activation of hippocampal brain-derived neurotrophic factor (BDNF). *Journal of Neuroscience*, *39*(13), 2369–2382. https://doi.org/10.1523/JNEUROSCI.1661-18.2019

Engeroff, T., Ingmann, T., & Banzer, W. (2018). Physical Activity Throughout the Adult Life Span and Domain-Specific Cognitive Function in Old Age: A Systematic Review of Cross-Sectional and Longitudinal Data. *Sports Medicine*, *48*(6), 1405–1436. https://doi.org/10.1007/S40279-018-0920-6

Erickson, K. I., Hillman, C., Stillman, C. M., Ballard, R. M., Bloodgood, B., Conroy, D. E., Macko, R., Marquez, D. X., Petruzzello, S. J., & Powell, K. E. (2019). Physical Activity, Cognition, and Brain Outcomes: A Review of the 2018 Physical Activity Guidelines. In *Medicine and Science in Sports and Exercise* (Vol. 51, Issue 6, pp. 1242–1251). Lippincott Williams and Wilkins. https://doi.org/10.1249/MSS.0000000000001936

Erickson, K. I., Voss, M. W., Prakash, R. S., Basak, C., Szabo, A., Chaddock, L., Kim, J. S., Heo, S., Alves, H., White, S. M., Wojcicki, T. R., Mailey, E., Vieira, V. J., Martin, S. A., Pence, B. D., Woods, J. A., McAuley, E., & Kramer, A. F. (2011). Exercise training increases size of hippocampus and improves memory. *Proceedings of the National Academy of Sciences of the United States of America*, *108*(7), 3017–3022. https://doi.org/10.1073/pnas.1015950108

ExRx.net : Predicting One-rep Max. (n.d.). Retrieved March 29, 2022, from https://exrx.net/Calculators/OneRepMax

Fino, P. C., Mojdehi, A. R., Adjerid, K., Habibi, M., Lockhart, T. E., & Ross, S. D. (2016). Comparing Postural Stability Entropy Analyses to Differentiate Fallers and Non-Fallers. *Annals of Biomedical Engineering*, *44*(5), 1636–1645. https://doi.org/10.1007/S10439-015-1479-0

Fjell, A. M., & Walhovd, K. B. (2010). Structural brain changes in aging: Courses, causes and cognitive consequences. In *Reviews in the Neurosciences* (Vol. 21, Issue 3, pp. 187–221). Freund Publishing House Ltd. https://doi.org/10.1515/REVNEURO.2010.21.3.187

Gajewski, P. D., & Falkenstein, M. (2016). Physical activity and neurocognitive functioning in aging - a condensed updated review. *European Review of Aging and Physical Activity : Official Journal of the European Group for Research into Elderly and Physical Activity*, *13*, 1. https://doi.org/10.1186/s11556-016-0161-3

George, D., & Mallery, P. (2010). *SPSS for Windows Step by Step: A Simple Guide and Reference, 17.0 update* (10th ed.). Allyn & Bacon.

Gruol, D. L. (2015). IL-6 regulation of synaptic function in the CNS. *Neuropharmacology*, *96*(PA), 42–54. https://doi.org/10.1016/j.neuropharm.2014.10.023

Herold, F., Törpel, A., Schega, L., & Müller, N. G. (2019). Functional and/or structural brain changes in response to resistance exercises and resistance training lead to cognitive improvements - A systematic review. In *European Review of Aging and Physical Activity* (Vol. 16, Issue 1). BioMed Central Ltd. https://doi.org/10.1186/s11556-019-0217-2

Hess, J. A., & Woollacott, M. (2005). Effect of High-Intensity Strength-Training on Functional Measures of Balance Ability in Balance-Impaired Older Adults. *Journal of Manipulative and Physiological Therapeutics*, *28*(8), 582–590. https://doi.org/10.1016/J.JMPT.2005.08.013

Horak, F. B. (2006). Postural orientation and equilibrium: what do we need to know about neural control of balance to prevent falls? *Age and Ageing*, *35*(SUPPL 2), ii7–ii11. https://doi.org/10.1093/AGEING/AFL077

Hsieh, S.-S., Chang, Y.-K., Fang, C.-L., & Hung, T.-M. (2016). Acute Resistance Exercise Facilitates Attention Control in Adult Males without an Age-Moderating Effect. *Journal of Sport and Exercise Psychology*, *38*(3), 247–254. https://doi.org/10.1123/jsep.2015-0282

Hsieh, S.-S., Chang, Y.-K., Hung, T.-M., & Fang, C.-L. (2016). The effects of acute resistance exercise on young and older males' working memory. *Psychology of Sport and Exercise*, *22*, 286–293. https://doi.org/10.1016/J.PSYCHSPORT.2015.09.004

Huang, X., Zhao, X., Li, B., Cai, Y., Zhang, S., Wan, Q., & Yu, F. (2022). Comparative efficacy of various exercise interventions on cognitive function in patients with mild cognitive impairment or dementia: A systematic review and network meta-analysis. *Journal of Sport and Health Science*, *11*(2), 212–223. https://doi.org/10.1016/J.JSHS.2021.05.003

Huh, J. Y., Mougios, V., Kabasakalis, A., Fatouros, I., Siopi, A., Douroudos, I. I., Filippaios, A., Panagiotou, G., Park, K. H., & Mantzoros, C. S. (2014). Exercise-induced irisin secretion is independent of age or fitness level and increased irisin may directly modulate muscle metabolism through AMPK activation. *Journal of Clinical Endocrinology and Metabolism*, *99*(11), E2154–E2161. https://doi.org/10.1210/jc.2014-1437

Hunter, S. K., Pereira, X. H. M., & Keenan, K. G. (2016). The aging neuromuscular system and motor performance. *Journal of Applied Physiology*, *121*(4), 982–995. https://doi.org/10.1152/JAPPLPHYSIOL.00475.2016/ASSET/IMAGES/LARGE/ZDG99915194300004.JPEG

Kim, H. J., So, B., Choi, M., Kang, D., & Song, W. (2015). Resistance exercise training increases the expression of irisin concomitant with improvement of muscle function in aging mice and humans. *Experimental Gerontology*, *70*, 11–17. https://doi.org/10.1016/j.exger.2015.07.006

Kim, S., Choi, J. Y., Moon, S., Park, D. H., Kwak, H. B., & Kang, J. H. (2019). Roles of myokines in exercise-induced improvement of neuropsychiatric function. In *Pflugers Archiv European Journal of Physiology* (Vol. 471, Issue 3, pp. 491–505). Springer Verlag. https://doi.org/10.1007/s00424-019-02253-8

King, B. R., Van Ruitenbeek, P., Leunissen, I., Cuypers, K., Heise, K. F., Santos Monteiro, T., Hermans, L., Levin, O., Albouy, G., Mantini, D., & Swinnen, S. P. (2018). Age-Related Declines in Motor Performance are Associated With Decreased Segregation of Large-Scale Resting State Brain Networks. *Cerebral Cortex (New York, NY)*, *28*(12), 4390–4402. https://doi.org/10.1093/CERCOR/BHX297

Kirk-Sanchez, N. J., & McGough, E. L. (2013). Physical exercise and cognitive performance in the elderly: Current perspectives. *Clinical Interventions in Aging*, *9*, 51–62. https://doi.org/10.2147/CIA.S39506

Kujach, S., Olek, R. A., Byun, K., Suwabe, K., Sitek, E. J., Ziemann, E., Laskowski, R., & Soya, H. (2020). Acute Sprint Interval Exercise Increases Both Cognitive Functions and Peripheral Neurotrophic Factors in Humans: The Possible Involvement of Lactate. *Frontiers in Neuroscience*, *13*. https://doi.org/10.3389/fnins.2019.01455

Landrigan, J.-F., Bell, T., Crowe, M., Clay, O. J., & Mirman, D. (2019). Lifting cognition: a meta-analysis of effects of resistance exercise on cognition. *Psychological Research 2019 84:5*, *84*(5), 1167–1183. https://doi.org/10.1007/S00426-019-01145-X

Liu-Ambrose, T., Nagamatsu, L. S., Voss, M. W., Khan, K. M., & Handy, T. C. (2012). Resistance training and functional plasticity of the aging brain: a 12-month randomized controlled trial. *Neurobiology of Aging*, *33*(8), 1690–1698. https://doi.org/10.1016/J.NEUROBIOLAGING.2011.05.010

Liu-Ambrose, T. Y., Ashe, M. C., Graf, P., Beattie, B. L., & Khan, K. M. (2008). Increased risk of falling in older community-dwelling women with mild cognitive impairment. *Physical Therapy*, *88*(12), 1482–1491. https://doi.org/10.2522/PTJ.20080117

Ludyga, S., Gerber, M., Brand, S., Holsboer-Trachsler, E., & Pühse, U. (2016). Acute effects of moderate aerobic exercise on specific aspects of executive function in different age and fitness groups: A meta-analysis. *Psychophysiology*, *53*(11), 1611–1626. https://doi.org/10.1111/psyp.12736

Macht, J. W., Abel, M. G., Mullineaux, D. R., & Yates, J. W. (2016). Development of 1RM Prediction Equations for Bench Press in Moderately Trained Men. *Journal of Strength and Conditioning Research*, *30*(10), 2901–2906. https://doi.org/10.1519/JSC.0000000000001385

Mandolesi, L., Polverino, A., Montuori, S., Foti, F., Ferraioli, G., Sorrentino, P., & Sorrentino, G. (2018). Effects of physical exercise on cognitive functioning and wellbeing: Biological and psychological benefits. In *Frontiers in Psychology* (Vol. 9, Issue APR). Frontiers Media S.A. https://doi.org/10.3389/fpsyg.2018.00509

Mattiuzzi, C., & Lippi, G. (2020). Worldwide disease epidemiology in the older persons. *European Geriatric Medicine*, *11*(1), 147–153. https://doi.org/10.1007/S41999-019-00265-2/TABLES/4

Mavros, Y., Gates, N., Wilson, G. C., Jain, N., Meiklejohn, J., Brodaty, H., Wen, W., Singh, N., Baune, B. T., Suo, C., Baker, M. K., Foroughi, N., Wang, Y., Sachdev, P. S., Valenzuela, M., & Fiatarone Singh, M. A. (2017). Mediation of Cognitive Function Improvements by Strength Gains After Resistance Training in Older Adults with Mild Cognitive Impairment: Outcomes of the Study of Mental and Resistance Training. *Journal of the American Geriatrics Society*, *65*(3), 550–559. https://doi.org/10.1111/JGS.14542

McPhee, J. S., French, D. P., Jackson, D., Nazroo, J., Pendleton, N., & Degens, H. (2016). Physical activity in older age: perspectives for healthy ageing and frailty. *Biogerontology*, *17*(3), 567. https://doi.org/10.1007/S10522-016-9641-0

McSween, M.-P., Coombes, J. S., Christopher, P. M., Rodriguez, A. D., Erickson, I. K., Copland, D. A., & McMahon, K. L. (2019). The Immediate Effects of Acute Aerobic Exercise on Cognition in Healthy Older Adults: A Systematic Review. *Sports Medicine*, *49*, 67–82. https://doi.org/10.1007/s40279-018-01039-9

Mendham, A. E., Donges, C. E., Liberts, E. A., & Duffield, R. (2011). Effects of mode and intensity on the acute exercise-induced IL-6 and CRP responses in a sedentary, overweight population. *European Journal of Applied Physiology*, *111*(6), 1035–1045. https://doi.org/10.1007/s00421-010-1724-z

Muir-Hunter, S. W., & Wittwer, J. E. (2016). Dual-task testing to predict falls in community-dwelling older adults: a systematic review. *Physiotherapy*, *102*(1), 29–40. https://doi.org/10.1016/J.PHYSIO.2015.04.011

Muir, S. W., Gopaul, K., & Montero Odasso, M. M. (2012). The role of cognitive impairment in fall risk among older adults: a systematic review and meta-analysis. *Age and Ageing*, *41*(3), 299–308. https://doi.org/10.1093/AGEING/AFS012

Müller, P., Duderstadt, Y., Lessmann, V., & Müller, N. G. (2020). Lactate and BDNF: Key Mediators of Exercise Induced Neuroplasticity? *Journal of Clinical Medicine*, *9*(4), 1136. https://doi.org/10.3390/jcm9041136

Nemmers, T. M., & Miller, J. W. (2008). Factors influencing balance in healthy community-dwelling women age 60 and older. *Journal of Geriatric Physical Therapy (2001)*, *31*(3), 93–100. https://doi.org/10.1519/00139143-200831030-00003

Netz, Y. (2017). Is the Comparison between exercise and pharmacologic treatment of depression in the clinical practice guideline of the American college of physicians evidence-based? *Frontiers in Pharmacology*, *8*(MAY), 1–9. https://doi.org/10.3389/fphar.2017.00257

Netz, Y., Abu-Rukun, M., Tsuk, S., Dwolatzky, T., Carasso, R., Levin, O., & Dunsky, A. (2016). Acute aerobic activity enhances response inhibition for less than 30 min. *Brain and Cognition*, *109*, 59–65. https://doi.org/10.1016/j.bandc.2016.08.002

Netz, Y., Herschkovitz, S. F., Levin, O., & Ziv, G. (2023). The effect of acute exercise on cognitive and motor inhibition – Does fitness moderate this effect? *Psychology of Sport and Exercise*, *65*, 102344. https://doi.org/10.1016/J.PSYCHSPORT.2022.102344

Nielsen, A. R., & Pedersen, B. K. (2007). The biological roles of exercise-induced cytokines: IL-6, IL-8, and IL-15. In *Applied Physiology, Nutrition and Metabolism* (Vol. 32, Issue 5, pp. 833–839). https://doi.org/10.1139/H07-054

Northey, J. M., Cherbuin, N., Pumpa, K. L., Smee, D. J., & Rattray, B. (2018). Exercise interventions for cognitive function in adults older than 50: A systematic review with meta-Analysis. In *British Journal of Sports Medicine* (Vol. 52, Issue 3, pp. 154–160). BMJ Publishing Group. https://doi.org/10.1136/bjsports-2016-096587

Opie, G. M., & Semmler, J. G. (2019). Acute Exercise at Different Intensities Influences Corticomotor Excitability and Performance of a Ballistic Thumb Training Task. *Neuroscience*, *412*, 29–39. https://doi.org/10.1016/j.neuroscience.2019.05.049

Pangman, V. C., Sloan, J., & Guse, L. (2000). An examination of psychometric properties of the mini-mental state examination and the standardized mini-mental state examination: implications for clinical practice. *Applied Nursing Research : ANR*, *13*(4), 209–213. https://doi.org/10.1053/APNR.2000.9231

Park, S. Y., Reinl, M., & Schott, N. (2021). Effects of acute exercise at different intensities on fine motor-cognitive dual-task performance while walking: A functional near-infrared spectroscopy study. *European Journal of Neuroscience*, 1–24. https://doi.org/10.1111/EJN.15241

Pontifex, M. B., Hillman, C. H., Fernhall, B., Thompson, K. M., & Valentini, T. A. (2009). The effect of acute aerobic and resistance exercise on working memory. *Medicine and Science in Sports and Exercise*, *41*(4), 927–934. https://doi.org/10.1249/MSS.0B013E3181907D69

Rausch, J. R., Maxwell, S. E., & Kelley, K. (2003). Analytic methods for questions pertaining to a randomized pretest, posttest, follow-up design. *Journal of Clinical Child and Adolescent Psychology*, *32*(3), 467–486. https://doi.org/10.1207/S15374424JCCP3203_15

Rogers, M., Page, P., Takeshima, N., & Rogers, M. E. (2013). Balance training for the older athlete. *International Journal of Sports Physical Therapy*, *8*(4), 530. /pmc/articles/PMC3812830/

Salgueiro, R. B., Peliciari-Garcia, R. A., do Carmo Buonfiglio, D., Peroni, C. N., & Nunes, M. T. (2014). Lactate activates the somatotropic axis in rats. *Growth Hormone and IGF Research*, *24*(6), 268–270. https://doi.org/10.1016/j.ghir.2014.09.003

Schiffer, T., Schulte, S., Sperlich, B., Achtzehn, S., Fricke, H., & Strüder, H. K. (2011). Lactate infusion at rest increases BDNF blood concentration in humans. *Neuroscience Letters*, *488*(3), 234–237. https://doi.org/10.1016/J.NEULET.2010.11.035

Schoenfeld, B. J., Grgic, J., Van Every, D. W., & Plotkin, D. L. (2021). Loading Recommendations for Muscle Strength, Hypertrophy, and Local Endurance: A Re-Examination of the Repetition Continuum. *Sports 2021, Vol. 9, Page 32*, *9*(2), 32. https://doi.org/10.3390/SPORTS9020032

Schwarz, A. J., Brasel, J. A., Hintz, R. L., Mohan, S., & Cooper, D. M. (1996). Acute effect of brief low- and high-intensity exercise on circulating insulin-like growth factor (IGF) I, II, and IGF-binding protein-3 and its proteolysis in young healthy men. *The Journal of Clinical Endocrinology & Metabolism*, *81*(10), 3492–3497. https://doi.org/10.1210/jcem.81.10.8855791

Seidler, R. D., Bernard, J. A., Burutolu, T. B., Fling, B. W., Gordon, M. T., Gwin, J. T., Kwak, Y., & Lipps, D. B. (2010). Motor control and aging: links to age-related brain structural, functional, and biochemical effects. *Neuroscience and Biobehavioral Reviews*, *34*(5), 721–733. https://doi.org/10.1016/j.neubiorev.2009.10.005

Sjostrom, M., Ainsworth, B., Bauman, A., Bull, F., Hamilton-Craig, C., & Sallis, J. (2005). Guidelines for Data Processing and Analysis of the International Physical Activity Questionnaire (IPAQ) – Short and Long Forms. In *Medicine* (Issue November).

Sun, L., Li, X., Wang, F., Zhang, J., Wang, D., Yuan, L., Wu, M., Wang, Z., & Qi, J. (2017). High-intensity treadmill running impairs cognitive behavior and hippocampal synaptic plasticity of rats via activation of inflammatory response. *Journal of Neuroscience Research*, *95*(8), 1611–1620. https://doi.org/10.1002/JNR.23996

Tayer-Shifman, O. E., Green, R., Beaton, D. E., Ruttan, L., Wither, J. E., Tartaglia, M. C., Kakvan, M., Lombardi, S., Anderson, N., Su, J., Bonilla, D., Zandy, M., Choi, M. Y., Fritzler, M. J., & Touma, Z. (2020). Validity Evidence for the Use of Automated Neuropsychologic Assessment Metrics As a Screening Tool for Cognitive Impairment in Systemic Lupus Erythematosus. *Arthritis Care & Research*, *72*(12), 1809–1819. https://doi.org/10.1002/ACR.24096

Thomas, R., Beck, M. M., Lind, R. R., Johnsen, L. K., Geertsen, S. S., Christiansen, L., Ritz, C., Roig, M., & Lundbye-Jensen, J. (2016). Acute exercise and motor memory consolidation: The role of exercise intensity. *PLoS ONE*, *11*(7), 6205452. https://doi.org/10.1371/journal.pone.0159589

Tsai, C.-L., Pai, M.-C., Ukropec, J., & Ukropcová, B. (2019). Distinctive Effects of Aerobic and Resistance Exercise Modes on Neurocognitive and Biochemical Changes in Individuals with Mild Cognitive Impairment. *Current Alzheimer Research*, *16*(4), 316–332. https://doi.org/10.2174/1567205016666190228125429

Tsai, C. L., Wang, C. H., Pan, C. Y., & Chen, F. C. (2015). The effects of long-term resistance exercise on the relationship between neurocognitive performance and GH, IGF-1, and homocysteine levels in the elderly. *Frontiers in Behavioral Neuroscience*, *9*(FEB). https://doi.org/10.3389/fnbeh.2015.00023

van het Reve, E., & De Bruin, E. D. (2014). Strength-balance supplemented with computerized cognitive training to improve dual task gait and divided attention in older adults: A multicenter randomized-controlled trial. *BMC Geriatrics*, *14*(1), 1–15. https://doi.org/10.1186/1471-2318-14-134/FIGURES/4

Vincent, A. S., Roebuck-Spencer, T., Gilliland, K., & Schlegel, R. (2012). Automated Neuropsychological Assessment Metrics (v4) Traumatic Brain Injury Battery: Military Normative Data. *Military Medicine*, *177*(3), 256–269. https://doi.org/10.7205/MILMED-D-11-00289

Vincent, A. S., Roebuck-Spencer, T. M., Fuenzalida, E., & Gilliland, K. (2017). Test–retest reliability and practice effects for the ANAM General Neuropsychological Screening battery. *The Clinical Neuropsychologist*, *32*(3), 479–494. https://doi.org/10.1080/13854046.2017.1368716

Vints, W. A. J., Levin, O., Fujiyama, H., Verbunt, J., & Masiulis, N. (2022). Exerkines and long-term synaptic potentiation: Mechanisms of exercise-induced neuroplasticity. *Frontiers in Neuroendocrinology*, *66*, 100993. https://doi.org/10.1016/J.YFRNE.2022.100993

Wanner, P., Cheng, F. H., & Steib, S. (2020). Effects of acute cardiovascular exercise on motor memory encoding and consolidation: A systematic review with meta-analysis. *Neuroscience and Biobehavioral Reviews*, *116*(June), 365–381. https://doi.org/10.1016/j.neubiorev.2020.06.018

Woollacott, M., & Shumway-Cook, A. (2002). Attention and the control of posture and gait: a review of an emerging area of research. *Gait & Posture*, *16*(1), 1–14. https://doi.org/10.1016/S0966-6362(01)00156-4

World Health Organization. (2015). World report on Ageing And Health. *World Health Organization*, 28. www.who.int

Yogev-Seligmann, G., Hausdorff, J. M., & Giladi, N. (2008). The Role of Executive Function and Attention in Gait. *Movement Disorders*, *23*(3), 472. https://doi.org/10.1002/MDS.21720

CHAPTER 6

Resistance exercise effects on hippocampus subfield volumes and biomarkers of neuroplasticity and neuroinflammation in older adults with low and high risk of mild cognitive impairment : a randomized controlled trial

Authors: Wouter A.J. Vints, Julija Šeikinaitė, Evrim Gökçe, Simona Kušleikienė, Milda Šarkinaite, Kristina Valatkeviciene, Vida J. Česnaitienė, Jeanine Verbunt, Oron Levin, Nerijus Masiulis

Published in Geroscience, 2024, Vol. 46(4), p. 3971-3991
https://doi-org.mu.idm.oclc.org/10.1007/s11357-024-01110-6

Abstract

Background: Physical exercise is suggested to promote hippocampal neuroplasticity by increasing circulating neurotrophic and anti-inflammatory factors. Our aim was to explore the interplay between the effect of progressive resistance exercise on blood biomarker levels, hippocampal neurometabolite levels and hippocampal volume in older adults with a low compared to a high risk of mild cognitive impairment (MCI).

Methods: Seventy apparently healthy male/female older adults (aged 60-85 years old) were randomly allocated to a 12 week lower limb progressive resistance or no intervention, stratified for low (<26/30) or high (≥26/30) Montreal Cognitive Assessment (MoCA) score, indicating MCI risk. Outcome measures were blood levels of insulin-like growth factor-1 (IGF-1), interleukin-6 (IL-6) or kynurenine (KYN); hippocampal total and subfield volumes of the cornu ammonis 1 (CA1) and 4 (CA4), subiculum, presubiculum, and dentate gyrus measured with magnetic resonance imaging (MRI); and hippocampus neurometabolites including total N-acetylaspartate (NAA), myo-inositol (mIns), and total creatine (Cr) measured with proton magnetic resonance spectroscopy (^{1}H-MRS). We evaluated the intervention effect, cognitive status effect, their interaction and the bivariate relationship between exercise-induced changes between the outcome measures.

Results: Higher kynurenine levels (p=0.015) and lower subiculum volumes (p=0.043) were found in older adults with high MCI risk compared to older adults with low MCI risk. Exercise-induced CA1 volume changes were negatively correlated with hippocampal tNAA/mIns level changes (r=-0.605, p=0.006).

Conclusion: This study provides valuable insight in the multifactorial processes related to resistance training in older adults with low or high MCI risk.

Abbreviations

^{1}H-MRS, proton magnetic resonance spectroscopy; 1 RM, one repetition maximum; BMI, body mass index; CA, cornu ammonis; DG, dentate gyrus; ELISA, enzyme-linked immunosorbent assay; fat%, body fat percentage; GMV, grey matter volume; HPC, hippocampal cortex; IFN-γ, interferon-γ; IGF-1, insulin-like growth factor-1; IL-6, interleukin-6; IPAQ-SF, International Physical Activity Questionnaire-Short Form; KYN, kynurenine; LTP, long-term synaptic potentiation; MCI, mild cognitive impairment; mIns, myo-inositol; MoCA, Montreal Cognitive Assessment; tCr, total creatine; tNAA, total N-acetylaspartate; TNF-α, tumor necrosis factor-α.

1. Introduction

The hippocampus is a brain region with a substantial capacity for structural reorganization, or neuroplasticity. It can rapidly modify existing neural circuits and even create entirely novel neural connections through the process of neurogenesis (Leuner and Gould, 2010). Specifically, the dentate gyrus (DG) of the hippocampus is known for its continued ability to generate new neurons throughout life (Eriksson et al., 1998). Importantly, the neurogenetic potential of the hippocampus seems to be highly responsive to external stimuli. For example, hippocampal neurogenesis and neuroplastic processes are facilitated in response to physical activity (Erickson et al., 2011), while they are impaired by stress, alcohol, and sleep deprivation (Shors et al., 2012; Jurkowski et al., 2020). Furthermore, studies in older adults show significant age-related decreases in hippocampal neuroplasticity and hippocampal volume, associated with age-related cognitive decline (Dawe et al., 2020; Wu et al., 2023). Hippocampal volume loss can precede cognitive impairment by several years (Jack et al., 2000), and in older adults with mild cognitive impairment (MCI) severe losses in the cornu ammonis subfield 1 (CA1) and subiculum hippocampal subfields predict progression towards Alzheimer's dementia (West et al., 1994; Rössler et al., 2002; Apostolova et al., 2006; Apostolova et al., 2010; Costafreda et al., 2011).

It has been suggested that the hippocampal neurogenetic and neuroplastic potential is modulated by several neurotrophic and inflammatory markers (Bourgognon and Cavanagh, 2020). In older adults, a state of low-grade inflammation, referred to as 'inflamm-aging' (Franceschi et al., 2000), is thought to impair hippocampal plasticity (Bourgognon and Cavanagh, 2020; Vints et al., 2022b). With inflamm-aging, old and damaged cells throughout the body start to release inflammatory cytokines, such as interleukin-6 (IL-6), into the blood stream. The number of these senescent cells gradually increases with aging (Dimri et al., 1995) resulting in an increase in pro-inflammatory factors that can

cross the blood-brain barrier. This, in turn, increases the numbers and pro-inflammatory activity of microglia in the hippocampus and other brain regions, leading to impairments in memory and executive control functions (Barrientos et al., 2015). Increased microglial activity and its effect on brain's structural and neurochemical properties can be estimated in-vivo with various neuroimaging techniques. For example, proton magnetic resonance spectroscopy (^{1}H-MRS) studies overall report that older adults, especially cases with MCI who are at high risk of converting into Alzheimer's disease (AD), have increased levels of the neurometabolites myo-inositol (mIns), a marker for microglial cell density, and later on also decreased levels of N-acetylaspartate (NAA), a marker for neural density (Tumati et al., 2013; Cleeland et al., 2019; Vints et al., 2022a). Specifically, decreased levels of NAA and/or decreased ratio of NAA to creatine (Cr) have been proposed as markers of brain atrophy, increased levels of myo-inositol (mIns) and/or mIns/Cr are proposed to mark neuroinflammation, whereas decreased ratio of NAA/mIns has been proposed as a combined marker of neurodegeneration and neuroinflammation (Waragai et al., 2017). Increased brain mIns levels may be found in preclinical Alzheimer's disease, and even precede detectability of amyloid β in cerebrospinal fluid (Graff-Radford and Kantarci, 2013; Voevodskaya et al., 2016), which had been reported before as the first biomarker to become abnormal in Alzheimer's disease (Jack and Holtzman, 2013). Interestingly, we previously showed that circulating levels of the inflammatory marker kynurenine (KYN) were associated with levels of neuroinflammation and neurodegeneration markers measured with ^{1}H-MRS in older adults (Vints et al., 2022a). Specifically, elevated levels of serum KYN were associated with signs of neurodegeneration (i.e., decreased levels of NAA) in hippocampus and medial temporal cortex and signs of neuroinflammation (i.e., increased levels of mIns) in the posterior cingulate cortex and dorsolateral prefrontal cortex. KYN is of particular interest in the context of hippocampal neuroinflammation, since elevated circulatory KYN levels were associated with reduced memory function in older adults (Solvang et al., 2019). Moreover, the activity of the enzyme responsible for the upregulation of KYN levels is increased by pro-inflammatory cytokines, making KYN a generic marker of pro-inflammatory cytokine activity (Allison et al., 2017).

The use of physical activity as a nonpharmacological treatment to mitigate pro-inflammatory processes and increase the neuroplastic capacity of the hippocampus present an exciting avenue for healthy aging interventions (Jurkowski et al., 2020). As we mentioned before, physical exercise was reported to enhance hippocampal neurogenetic potential (Van Praag, 2008), but also facilitate other neuroplastic processes such as long-term synaptic potentiation (LTP) (Vints et al., 2022b), increase hippocampal volume, and improve memory function (Erickson et al., 2011). For example, cytokines and peptides released from muscle tissue during exercise, such as IL-6 which was introduced by Pedersen and colleagues as the first "myokine" (Pedersen et al., 2003), are argued to play a role as mediators of exercise-induced beneficial effects on neurogenesis and

cognition (Pedersen, 2019; Vints et al., 2022b). Furthermore, it was discovered that exercise increases muscle expression of kynurenine aminotransferase, an enzyme that converts KYN into kynurenic acid. In contrast to KYN, kynurenic acid is no longer capable of crossing the blood-brain barrier and provoking pro-inflammatory processes in the brain (Agudelo et al., 2014). Instead, kynurenic acid has anti-inflammatory and neuroprotective effects (Vécsei et al., 2013). In general, however, a bout of physical exercise transiently increases inflammatory markers, like IL-6, and neurotrophic factors, such as insulin-like growth factor-1 (IGF-1), while chronic exercise interventions result in decreased levels of inflammatory markers and increased levels of neurotrophic factors (Vints et al., 2022b). This exercise-effect has been shown for various exercise modes and doses. However, some authors suggest that resistance exercise may cause larger changes in neurotrophic and inflammatory blood biomarkers (Tsuchiya et al., 2015; Kim et al., 2019; Marinus et al., 2019; de Alcantara Borba et al., 2020; Gulick et al., 2020; Jiang et al., 2020). It should be noted, however, that more research has been done with endurance exercise compared to resistance exercise, and the variability in reported findings between studies may as well depend on other study characteristics than exercise mode. For example, IGF-1 was particularly increased by resistance exercise in older adults, but to a lesser extent in younger adults (Jiang et al., 2020; Ye et al., 2020; Amiri et al., 2021). To our knowledge, the interrelationship between resistance exercise, blood biomarkers, hippocampal neurometabolites, and hippocampal subfield volumes has never been explored in detail, with the hippocampus being considered as primary brain area for neurogenesis but also a brain structure vulnerable to pro-inflammatory conditions leading to cognitive declines (Leuner and Gould, 2010; Shors et al., 2012; Jurkowski et al., 2020). Additionally, the comparison between cognitively healthy older adults with low risk of MCI and older adults with near-normal cognitive performance who are at high risk of MCI would provide valuable novel information for the research field.

2. Objectives and hypotheses

The primary objective of this randomized controlled trial was to explore the effect of chronic resistance training on the hippocampus in a population of older adults with normal cognitive function or with probable MCI. Specifically, to examine the changes induced by 12 weeks of resistance exercise on blood biomarker levels, hippocampal neurometabolite levels and hippocampal volume and the interplay between those changes. Resistance exercise effects were compared to time effects seen in a waiting list control condition. The outcome measures encompassed blood circulating markers (IL-6, KYN, and IGF-1) and brain neurometabolic markers (tNAA/tCr, mIns/tCr, and tNAA/mIns) that have been identified as biomarkers related to neuroplasticity and neuroinflammation

(Waragai et al., 2017; Bourgognon and Cavanagh, 2020; Vints et al., 2022b), as well as total and subfield hippocampus volumes. Our second objective was to explore the influence of cognitive status at baseline on these biomarkers and the resistance exercise effect. In consideration of our objectives, we formulated a hypothesis that (1) resistance exercise would induce increases in the neurotrophic blood biomarker IGF-1 and neurometabolites tNAA/tCr and tNAA/mIns in the hippocampus and decreases in the inflammatory blood biomarkers IL-6 and KYN and brain biomarker mIns/tCr in the hippocampus. Furthermore, we hypothesized that (2) resistance exercise would induce an increase in total and subfield hippocampus volumes. Moreover, we hypothesized that (3) the resistance exercise-induced changes in these biomarkers and hippocampus volumes would be interrelated. Finally, we hypothesized that (4) the changes induced by resistance exercise would be larger in older adults with high risk of MCI compared to their low-risk peers. This knowledge may serve to design interventions aimed at preventing the age-related deterioration of brain health.

3. Methods

3.1 Participants and setting

Seventy older adults (male/female, 32/38) aged 60 to 85 years participated in a randomized controlled trial. Participants were recruited between July 2020 and July 2021 from volunteers from previous studies, a list of patients provided by general practitioners, and presentations in local community organizations in Kaunas, Lithuania. The exercise-induced changes in muscular strength from a subgroup of these participants and how they relate to neurometabolic changes has been published previously (Sheoran et al., 2023). In addition, the baseline balance, physical fitness, and body composition measurements have previously been evaluated in relation to baseline blood biomarker levels, brain health markers, cognitive function tests and balance control (Vints et al., 2022a; Levin et al., 2023; Valatkevičienė et al., 2023; Vints et al., 2023). In comparison to the studies published earlier, four participants were deleted. Two of these participants underwent baseline assessments, but they participated in a pilot version of the current intervention. After their participation, the final version of the resistance exercise protocol was decided. Two other participants did not continue after baseline assessments, either because of a claustrophobic attack during magnetic resonance imaging (MRI) or because of a pathological finding on brain MRI scanning.

Exclusion criteria were alcohol or drug abuse, neurologic, oncologic or psychiatric diagnosis, use of psychopharmacological drugs in the last five years, or a history of chemotherapy. Participants were excluded if they had signs or symptoms of cardiovascular, pulmonary or metabolic diseases, with the only exception that some of the participants

had hypertension. The mean blood pressure and heart rate of the included participants before intervention were respectively 139/78 (range systolic blood pressure: 108-182; range diastolic blood pressure: 55-119) and 70bpm (range: 52-96bpm). These measurements were done in a seated position after 30 minutes of seated rest. Participants had to be physically healthy and able to perform ten sit-ups. They had to be allowed to undergo MRI according to the checklist provided by the Department of Radiology at the Lithuanian University of Health Science. Finally, we excluded participants that participated in an exercise program on a regular basis in the last six months. Participants were allowed to withdraw from the study at any time. The study methods were approved by the Kaunas Regional Biomedical Research Ethics Committee (No. BE-10-7) and a written informed consent was obtained from all participants prior to their inclusion in the study.

3.2 Study design

This study was a parallel group randomized controlled trial with a 12-week resistance exercise intervention. Randomization was done using a stratified permuted block procedure. The stratification factor was the participants score on the Montreal Cognitive Assessment (MoCA) test, subdividing participants into high and low risk of MCI. MoCA tests were conducted by a qualified mental-health care specialist (co-author SK). While the MoCA test is not a diagnostic tool, a score of 18-25 is considered a reliable and sensitive marker of MCI, whereas a score of 26 to 30 is considered to indicate normal cognitive ability and low risk for MCI (Nasreddine et al., 2005; Bruijnen et al., 2020). The MoCA test scores from the participants ranged from 19-30. In every block of four participants with low or high risk of MCI, two were randomly allocated to the experimental and two to the control condition. This allocation was done in an Excel spreadsheet, using a random number generator set to indicate either 1 or 2 for exercise or control group respectively. If a block of four participants (N) with the same cognitive status contained two participants of the same group, the third participant was allocated to block N+1 with this cognitive status. In the last block of eight participants, only high MCI risk participants were included and seven out of eight participants were allocated to the control group. The reason for this decision was to correct for a higher number of drop-outs in the high MCI risk control groups at that time during the project. A participant flow diagram is presented in Appendix B, Supplementary Figure 1. The resistance training protocol was supervised by qualified fitness coaches, who were not involved in pre-and post-intervention data collection. The investigators involved in data collection were blinded to the group allocation.

3.3 Assessments

An overview of the timing of all assessments and summary of the interventional protocol is presented in Figure 1.

3.3.1 Demographic and clinical characteristics

Participants were asked to complete a demographics questionnaire assessing their age, sex, educational level (categorized as basic education, secondary education or higher education), and smoking status. Self-reported physical activity level was estimated using the International Physical Activity Questionnaire – Short Form (IPAQ-SF). Physical activity level is calculated based on the total kcal burned per week during exercise of light, moderate or vigorous intensity, using the formula: total kcal/week = the sum of days performing light/moderate/vigorous activity × average time/day performing these activities × F, where F equals 3.3 for light intense exercise, 4.0 for moderate intense exercise and 8.0 for vigorous intense exercise. Participants burning less than 600 kcal per week were categorized as sedentary, 600-3000 kcal per week as moderately active and > 3000 kcal per week as highly active (Sjostrom et al., 2005).

All participants' body mass index (BMI) and body fat percentage (fat%) were measured on a leg-to-leg bio-impedance analyser (Tanita TBF-300-A). After a 5 min warm-up by pedalling at an intensity of 60-90 Watts on a veloergometer, and 3 min of dynamic activation exercises including lunges, butt kicks, side step lunges, half-squats, and front and side cross swings, isometric knee extension torque on maximum voluntary contraction (MVC, in Newton meters) of the dominant leg was measured with a Biodex System 3 dynamometer (Biodex Medical Systems, NY, USA). The highest value out of two trials was used for analysis. The MVC was only measured in a subgroup of the participants (45 participants pre-intervention and 20 post-intervention; 8 from the control and 12 from the exercise group). This subgroup was analysed in study of Sheoran et al. (2023). Handgrip strength (in kg) was measured using a JAMAR 11940248 adjustable hand grip strength testing system in standing position. The grip size was adjusted so that there was a 90° angle in the second joint of the index finger. The test was preceded by a first try at submaximal effort, followed by 2 tries at maximal effort with 1 min interval between trials and the highest value was used for analysis.

3.3.2 Blood serum analysis

All blood samples were drawn between 9 a.m. and 1 p.m. by a qualified medical professional. For participants in the experimental group, the second blood collection took place at least 48 hours or more after the last exercise bout. Blood was collected in 5mL serum separator tubes at the antecubital vein. The samples were gently inverted 8-10 times and stored for 30min at room temperature to allow clotting. Subsequently, the tubes were centrifugated for 15min at 4000g. From these samples, blood serum was aliquoted into 1.5 mL polypropylene tubes and stored until further analysis in a -80°C refrigerator compartment at the laboratory of the Lithuanian Sports University. After full completion of the study, the samples were analysed with enzyme-linked immunosorbent assays (ELISA) using a spectrophotometer (Spark 10M, Tecan Group Ltd., Zürich, Switzerland) by

an experienced lab technician. The following blood biomarkers were measured using commercially available ELISA kits: IGF-1 (IBL International, GMBH, Germany, MD58011, with a lower limit of detection 0.03 ng/mL), IL-6 (DIAsource ImmunoAssays S.A., Belgium, KAP1216, with a lower limit of detection 2 pg/mL), KYN (MyBiosource, Inc., USA, with lower limit of detection 45.7 ng/mL).

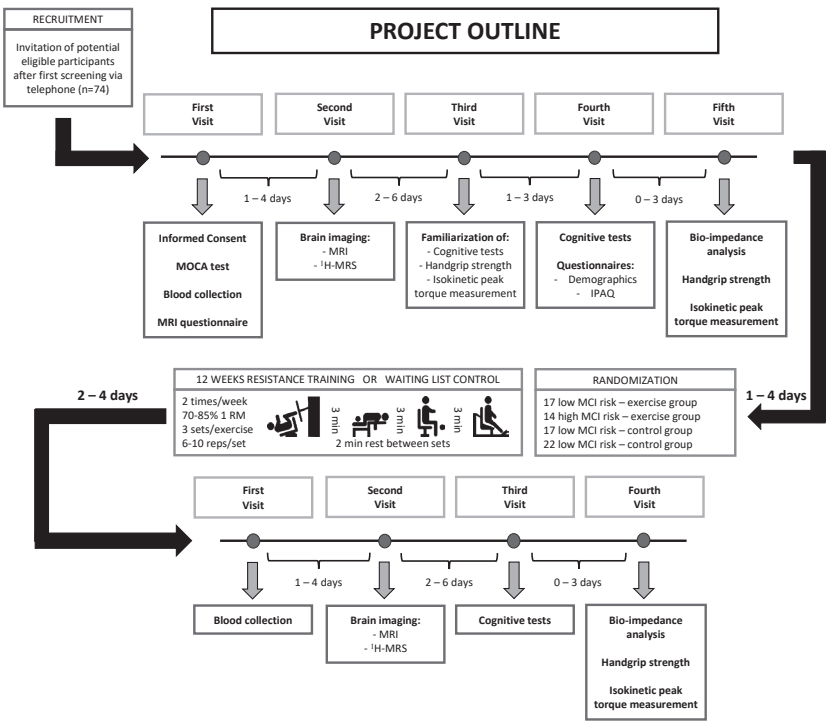


Figure 1. Project outline

Abbreviations: ^{1}H-MRS, proton magnetic resonance spectroscopy; 1 RM, one repetition maximum; IPAQ, International Physical Activity Questionnaire – Short Form; MCI, mild cognitive impairment; MOCA, Montreal Cognitive Assessment; MRI, magnetic resonance imaging

3.3.3 Hippocampus volume and neurometabolites

Participants underwent whole brain MRI and ^{1}H-MRS scanning in a 3 Tesla Skyra scanner (Siemens Healthineers, Erlangen, Germany) with a 32-channel receiver head coil. Total scanning duration was 90 min per participant. Only hippocampus measurements are presented in this paper.

Pre- and post-intervention hippocampus total and subfield grey matter volumes (GMV) were calculated using 3D magnetization prepared gradient echo (MPRAGE) images from high resolution T1-weighted structural MRI (repetition time (TR) = 2,200 ms, echo time (TE) = 2,48 ms, voxel size 0.9 × 0.9 × 1.0 mm^3, field of view 230 × 256 mm, number of sagittal slices = 176). A longitudinal analysis pipeline of FreeSurfer v7.1.1 (Harvard, MA, USA, http://surfer.nmr.mgh.harvard.edu/) was used to measure the volumes of the left hippocampal subfields. The following subfields were examined: left whole hippocampus, left CA1 body and head (combined the left CA1), left subiculum body and head (combined the left subiculum), left presubiculum body and head (combined the left presubiculum), the left CA4 body and head (combined the left CA4), and the left granule cell (GC) and molecular layer (ML) of the dentate gyrus (DG) (combined the DG).

Hippocampus ^{1}H-MRS spectra were acquired using a Point RESolved Spectroscopy (PRESS) sequence (TR = 2,000 ms, TE = 30 ms, number of averages = 128, spectral bandwidth = 2,000 Hz, and data size = 1,024 points) with chemical shift selective (CHESS) water suppression (bandwidth = 50 Hz) and a voxel size of 26 × 12 × 12 cm^3. The hippocampal voxel was centered over the whole hippocampus in left hemisphere in the medial part of the temporal lobe anterior to lateral ventricle, corresponding to the left whole hippocampus Freesurfer region. Voxel position and an example of a post-processed spectrum are presented in Appendix A, Supplementary Figure 2. The acquired spectra were processed with linear combination of model spectra (LCModel, version 6.3.1-R) postprocessing software. Spectra with Cramér-Rao lower bound ≥20%, linewidths ≥12 Hz and signal-to-noise ratio <5 were excluded. All ^{1}H-MRS spectra were visually checked to ensure the absence of artifacts prior to quantification. In this paper, we reported results from total NAA (tNAA) composed of N-acetyl aspartate and N-acetyl glutamate, mIns, and their ratio to total Cr (tCr) composed of creatine and phosphocreatine. In addition, the ratio of tNAA relative to mIns was calculated. A detailed overview of the ^{1}H-MRS methods according to the minimum reporting standards of the MRSinMRS experts' consensus recommendations can be found in Appendix B (Lin et al., 2021).

3.4 Intervention: Resistance exercise intervention

Participants in the exercise group took part in a 12-week progressive resistance exercise program for the lower limbs, while participants in the control group did not receive any treatment and were asked not to change their physical activity levels until post-intervention outcome measurements. The resistance exercise protocol was in line with latest position statement by the National Strength and Conditioning Association (USA) for resistance training for older adults (Fragala et al., 2019). Older adults in the exercise group trained two times per week under direct supervision of professional fitness instructors, with maximum two participants per fitness instructor at the same time. The training sessions took place in the gym at the Lithuanian Sports University using resistance training equipment from Technogym

(Italy). Warm-up consisted of 5-min cycling on a cycloergometer, at an intensity (in Watts) approximately equal to the participants' body weight in kilograms, followed by a few dynamic stretching and activation exercises including lunges, butt kicks, side step lunges, half-squats, and front and side cross swings. The main resistance exercise program consisted of three sets of four lower limb exercises, (1) leg press, (2) leg curl, (3) leg extension, and (4) calf raises. The order of these exercises was not controlled, but in general the participants were instructed to start with the leg press as this is a multi-joint exercise and end with a single-joint exercise such as the calf raises. Lower limb strength and power are critical determinants of physical functioning in older adults, and correlate with their overall well-being (Marsh et al., 2009). Moreover, age-related muscle atrophy was observed to mainly affect the lower limbs, while upper limb muscles remain relatively unaffected (Aagaard et al., 2010). In the first week of training, the older adults were familiarized with the exercise movements and underwent a1-repetition maximum (1-RM) test for all four exercises. The 1-RM assessment was based on a standard protocol recommended by the National Strength and Conditioning Association (Haff and Triplett, 2015). It involved a prediction calculation based on the number of repetitions performed at submaximal loads which was performed using the ExRx.net calculator (https://exrx.net/Calculators/OneRepMax) (Swain and Brawner, 2013). In the remaining weeks, participants in the exercise group did one warm-up set and three working sets for all four lower limb exercises, with a rest of 2 min between sets and 3 min between exercises. From week 1 till 3, participants did 8-10 repetitions at 70-75% 1-RM, from week 4 till 9 they worked at 6-8 repetitions at 75-80% 1-RM, and from week 10 till 12 they did 6 repetitions at 80-85% 1-RM. The weight was adjusted during the three training blocks according to the participants' rate of perceived exertion (RPE) on a 10-point Borg scale (Morishita et al., 2019). The weight was increased when the older adult indicated a score below 7 on 10. The RPE was logged in a notebook by the fitness instructors, along with the number of repetitions and the weight lifted.

3.5 Statistical analysis

IBM SPSS Statistics version 27 (IBM Inc., Chicago, USA) was used to perform all analyses. First, the data was inspected for outliers and normality. Extreme outliers, defined as values lying more than 3× the interquartile range away from the median were excluded. Normality was defined as a kurtosis and skewness measure between -2 and +2. Additionally, normality was checked visually using PP-plots and histograms. Data not meeting one of the normality assumptions was log transformed. Homoskedasticity was tested with the Levene's test.

Group differences in baseline descriptives were analysed using independent t-tests and Chi² tests (or Fisher Exact tests, if the expected count in any of the cells was below 5) for continuous and categorical variable respectively. Our primary research question, the evaluation of the relationship between changes in outcome measurements from baseline to 12 weeks later, was measured using bivariate correlations. R-values based

on Spearman's rho were chosen, given the non-normal distribution of some of the outcome measures. Our secondary objectives, to test the effect of exercise compared to control, the influence of cognitive status and their interaction, were evaluated using two-way ANCOVA with either blood biomarker levels, hippocampal neurometabolites or hippocampal subfield volume results at the 12 week measurement point as the dependent variable. Group (experimental versus control) and cognitive status (low MCI risk versus high MCI risk) were entered as fixed factors, and age and pretest values of the dependent variable as covariates. For analysis with IL-6 or KYN levels as dependent variables, fat% was entered as an additional covariate given that inflammatory markers are known to be moderated by individuals' fat% (Maggio et al., 2006; Mangge et al., 2014). For analysis with hippocampus (subfield) volumes, intracranial volume was entered as an additional covariate in order to adjust the values for head size. The intracranial volumes measured at baseline and after 12 weeks were identical for all participants. We chose for a two-way ANCOVA instead of a three-way repeated measures ANOVA, because it was shown to reduce the population error variance and increase the power and preciseness of the test. The conclusion of these two test is generally the same when the ANCOVA tests takes into account the pretest value of the dependent variable by entering it as a covariate in the model, compared to adding it as a level of the time factor in repeated measures ANOVA. For further reading, see Rausch et al. (2003).

4. Results

4.1 Participants characteristics

52 participants (74.3%) completed the intervention. Reasons for dropping out were COVID-19 or fear of catching a SARS-COV-2 infection, lack of motivation, and intervention related trauma or fear of injury. The descriptive values of the baseline characteristics for exercise group, control group and total sample are presented in Appendix A, Supplementary Table 1. There was a baseline difference between the two groups for the amount of kilocalories burned with physical activity per week ($p = 0.024$) and educational level ($p = 0.038$). The mean group change from baseline to 12 weeks later in knee extension MVC in the control group (n=8) was -1.7 Nm (SD = 8.7 Nm), compared to +31.7 Nm (SD = 41.3 Nm) in the experimental group (n=12) ($p = 0.038$). There were no significant differences between groups for blood or left hippocampus (subfield) volumes and neurometabolite levels at baseline. The values are presented in Table 1, 3 and 4. Finally, there were no significant differences between older adults with low MCI risk and high MCI risk for any of the baseline measurements (see Supplementary Table 2).

Table 1. Descriptive values of baseline/12weeks blood biomarker levels and percentage change

		BASELINE	12 WEEKS	Δ (%)
IGF-1	EXP (n=24)	119.0 (57.6)	139.2 (85.4)	+17.0
	• hrMCI (n=12)	118.0 (55.2)	143.2 (81.2)	+21.4
	• lrMCI (n=12)	120.1 (62.4)	135.3 (92.8)	+12.7
	CON (n=18)	112.5 (50.2)	136.3 (66.4)	+21.2
	• hrMCI (n=8)	117.7 (58.3)	141.3 (58.3)	+20.1
	• lrMCI (n=10)	108.3 (45.5)	132.4 (75.1)	+22.2
	Total (n=42)	116.2 (54.0)	138.0 (77.0)	+18.8
	• hrMCI (n=20)	117.9 (54.9)	142.4 (71.2)	+20.8
	• lrMCI (n=22)	114.7 (54.4)	133.9 (83.3)	+16.7
IL-6	EXP (n=23)	8.5 (9.2)	12.2 (12.2)	+43.5
	• hrMCI (n=12)	6.7 (5.5)	10.3 (11.2)	+53.7
	• lrMCI (n=11)	10.5 (12.0)	14.4 (13.5)	+37.1
	CON (n=20)	7.7 (8.2)	7.2 (8.9)	-6.5
	• hrMCI (n=10)	4.5 (4.2)	4.1 (3.1)	-8.9
	• lrMCI (n=10)	11.2 (10.2)	10.7 (11.8)	-4.5
	Total (n=43)	8.1 (8.6)	9.9 (11.0)	+22.2
	• hrMCI (n=22)	5.6 (4.9)	7.3 (8.8)	+30.4
	• lrMCI (n=21)	10.8 (10.9)	12.7 (12.6)	+17.6
KYN	EXP (n=23)	1582.3 (755.7)	1301.3 (606.4)	-17.8
	• hrMCI (n=11)	1434.8 (650.6)	1442.3 (579.5)	+0.5
	• lrMCI (n=12)	1717.4 (846.1)	1172.2 (626.2)	-46.5
	CON (n=22)	1616.4 (709.1)	1507.1 (578.2)	-6.8
	• hrMCI (n=12)	1308.2 (441.0)	1722.2 (593.8)	+31.6
	• lrMCI (n=10)	2017.2 (808.8)	1227.5 (439.8)	-39.1
	Total (n=45)	1599.3 (724.8)	1404.2 (595.0)	-12.2
	• hrMCI (n=23)	1366.2 (538.2)	1593.9 (591.8)	+16.7
	• lrMCI (n=22)	1853.7 (823.8)	1197.3 (537.7)	-35.4

Only the values of the participants with measurements at baseline and after 12weeks (number of participants, n) were used for analysis.
Abbreviations: CON, control; EXP, experimental; hrMCI, high risk for mild cognitive impairment; IGF-1, insulin-like growth factor-1; IL-6, interleukin-6; KYN, kynurenine; lrMCI, low risk for mild cognitive impairment.

4.2 Exercise-induced blood biomarker changes in cases with low versus high MCI risk

ANCOVA results did not indicate a significant group effect on the levels of IGF-1, IL-6, or KYN. However, the effect size for the IL-6 group effect was of moderate magnitude ($_p\eta^2$ = 0.078, p = 0.089). IL-6 levels in the exercise group increased by 43.5%, while they decreased by 6.5% in the control group. This change was most distinct in the older adults with high MCI risk, who showed IL-6 changes of +53.7% and -8.9% in the exercise versus control group, respectively. Cognitive status significantly influenced KYN level (p = 0.015), with higher KYN levels found in high MCI risk individuals. There was no significant group x cognitive status interaction effect on IGF-1, IL-6, KYN levels. Table 1 presents the descriptive values, and Table 2 contains the two-way ANCOVA results for the blood biomarkers.

Table 2. ANCOVA results

	Group		Cognitive status		Group*Cognitive status	
	p-value	$_p\eta^2$	p-value	$_p\eta^2$	p-value	$_p\eta^2$
IGF-1	0.439	0.016	0.612	0.007	0.717	0.004
IL-6	0.089	0.078	0.401	0.020	0.476	0.014
KYN	0.372	0.021	0.015*	0.147	0.328	0.025
Whole hippocampus	0.722	0.004	0.074	0.093	0.343	0.027
CA1	0.977	0.000	0.218	0.046	0.259	0.038
Subiculum	0.190	0.052	0.043*	0.119	0.471	0.016
Presubiculum	0.728	0.004	0.483	0.015	0.991	0.000
CA4	0.171	0.056	0.227	0.044	0.677	0.005
DG	0.119	0.072	0.252	0.040	0.320	0.030
tNAA/tCr	0.072	0.107	0.835	0.002	0.808	0.002
mIns/tCr	0.942	0.000	0.538	0.013	0.894	0.001
tNAA/mIns	0.537	0.013	0.785	0.003	0.859	0.001

The dependent variables are presented in the first row. P-values and effect sizes (partial eta squared) are given for the group effect (exercise versus control), the cognitive status effect (high versus low MCI risk) and their interaction (Group*Cognitive status). Significant p-values are marked *. The ANCOVA was adjusted for the dependent variable's baseline value and for age. The ANCOVA for IL-6 and KYN were additionally adjusted for fat%. Of note, pre-intervention KYN, post-intervention IGF-1 and IL-6, and pre- and post-intervention tNAA/mIns left hippocampal cortex were log-transformed due to a non-normal distribution, and in pre-intervention IL-6 and post-intervention IL-6 and KYN an extreme outlier was removed before analysis.

Abbreviations: CA, cornu ammonis; DG, dentate gyrus; IGF-1, insulin-like growth factor-1; IL-6, interleukin-6; KYN, kynurenine; mIns, myo-inositol; tCr, total creatine; tNAA, total N-acetylaspartate; $_p\eta^2$ = partial eta squared

4.3 Exercise-induced hippocampus total and subfield volume changes in cases with low versus high MCI risk

Hippocampus (subfield) volumes did not significantly differ between exercise and control group. However, the effect size for the DG group effect was of moderate magnitude ($_p\eta^2$ = 0.072, p = 0.119). DG volume in the exercise group increased by 0.3%, while it decreased by 1.2% in the control group. Overall, hippocampus (subfield) volume decreased over time, except for subiculum volume in the control group and the volumes of the presubiculum, CA4, and DG in the exercise group. Cognitive status had a significant influence on subiculum volume ($_p\eta^2$ = 0.119, p=0.043) and an effect size of moderate magnitude was found for the total hippocampus ($_p\eta^2$ = 0.093, p = 0.074). In both cases, individuals with high MCI risk had lower (subfield) volumes than individuals with low MCI risk. No group x cognitive status interaction effect was observed for hippocampal volume. Table 3 contains the descriptive values and Table 2 presents the two-way ANCOVA results for (subfield) hippocampus volume.

4.5 Correlations between blood and hippocampal changes

Correlations between changes in blood biomarkers, neurometabolites, and hippocampal volume in the exercise group are reported in Table 5. Control group and total group correlations are presented in Supplementary Table 3 and 4, respectively. In the exercise group participants, CA1 volume changes showed a negative correlation with changes in hippocampal tNAA/mIns ratio (r = -0.605, p = 0.006; see also Appendix A, Figure 2).

4.4 Exercise-induced hippocampal neurometabolite changes in cases with low and high MCI risk

There was no group or cognitive status effect nor a group x cognitive status effect for hippocampal neurometabolite changes. The effect size for the tNAA/tCr ratio group effect was of moderate magnitude ($_p\eta^2$ = 0.107, p = 0.072), with tNAA/tCr increasing more in the resistance exercise group than controls. Table 4 contains the descriptive values and Table 2 presents the two-way ANCOVA results for the hippocampal neurometabolite ratios.

Table 3. Descriptive values of baseline/12weeks hippocampus (subfield) volumes and percentage change

		BASELINE	12 WEEKS	Δ (%)
Whole hippocampus	EXP (n=22)	3286.0 (329.0)	3281.6 (344.8)	-0.1%
	• hrMCI (n=11)	3181.4 (270.7)	3145.3 (296.5)	-1.1%
	• lrMCI (n=11)	3390.6 (360.5)	3417.9 (347.6)	+0.8%
	CON (n=18)	3288.3 (400.3)	3262.6 (395.2)	-0.8%
	• hrMCI (n=11)	3250.2 (497.9)	3216.8 (475.9)	-1.0%
	• lrMCI (n=7)	3348.2 (183.9)	3334.4 (234.8)	-0.4%
	Total (n=40)	3287.0 (358.0)	3273.0 (363.6)	-0.4%
	• hrMCI (n=22)	3215.8 (392.7)	3181.1 (388.7)	-1.1%
	• lrMCI (n=18)	3374.1 (298.1)	3385.5 (303.8)	+0.3
CA1	EXP (n=22)	623.2 (79.3)	620.3 (83.5)	-0.5%
	• hrMCI (n=11)	593.7 (54.6)	584.8 (60.6)	-1.5%
	• lrMCI (n=11)	652.7 (91.2)	655.8 (90.6)	+0.5%
	CON (n=18)	620.5 (92.8)	615.6 (92.5)	-0.8%
	• hrMCI (n=11)	623.6 (116.1)	618.5 (113.3)	-0.8%
	• lrMCI (n=7)	615.6 (43.3)	611.0 (53.2)	-0.7%
	Total (n=40)	622.0 (84.5)	618.2 (86.6)	-0.6%
	• hrMCI (n=22)	608.7 (89.8)	601.7 (90.3)	-1.1%
	• lrMCI (n=18)	638.3 (76.8)	638.4 (79.6)	+0.0%
Subiculum	EXP (n=22)	428.2 (49.7)	424.3 (54.3)	-0.9%
	• hrMCI (n=11)	416.7 (39.2)	407.7 (44.8)	-2.2%
	• lrMCI (n=11)	439.7 (58.0)	440.9 (59.8)	+0.2%
	CON (n=18)	412.9 (60.0)	413.5 (57.1)	+0.1%
	• hrMCI (n=11)	399.8 (63.8)	398.9 (58.5)	-0.2%
	• lrMCI (n=7)	433.4 (51.4)	436.4 (50.2)	+0.7%
	Total (n=40)	421.3 (54.4)	419.4 (55.1)	-0.5%
	• hrMCI (n=22)	408.3 (52.4)	403.3 (51.1)	-1.2%
	• lrMCI (n=18)	437.2 (54.0)	439.1 (54.8)	+0.4%
Presubiculum	EXP (n=22)	289.8 (34.0)	290.5 (38.6)	+0.2%
	• hrMCI (n=11)	278.0 (35.0)	276.7 (43.4)	-0.5%
	• lrMCI (n=11)	301.7 (29.9)	304.2 (28.6)	+0.8%
	CON (n=18)	305.0 (45.3)	304.4 (51.4)	-0.2%
	• hrMCI (n=11)	294.7 (47.0)	292.2 (50.4)	-0.8%
	• lrMCI (n=7)	321.2 (40.5)	323.5 (50.5)	+0.7%
	Total (n=40)	296.6 (39.7)	296.7 (44.7)	+0.0%
	• hrMCI (n=22)	286.3 (41.3)	284.5 (46.6)	-0.6%
	• lrMCI (n=18)	309.3 (34.7)	311.7 (38.4)	+0.8%

Table 3. Continued

		BASELINE	12 WEEKS	Δ (%)
CA4	EXP (n=22)	231.0 (26.6)	231.8 (26.5)	+0.3%
	• hrMCI (n=11)	222.6 (21.9)	222.0 (22.0)	-0.3%
	• lrMCI (n=11)	239.4 (29.1)	241.5 (28.0)	+0.9%
	CON (n=18)	230.1 (29.9)	227.8 (29.5)	-1.0%
	• hrMCI (n=11)	229.5 (36.7)	226.5 (35.4)	-1.3%
	• lrMCI (n=7)	230.9 (17.0)	229.7 (19.0)	-0.5%
	Total (n=40)	230.6 (27.7)	230.0 (27.6)	-0.3%
	• hrMCI (n=22)	226.1 (29.7)	224.3 (28.9)	-0.8%
	• lrMCI (n=18)	236.1 (24.9)	236.9 (25.0)	+0.3%
DG	EXP (n=22)	262.2 (32.3)	263.0 (31.8)	+0.3%
	• hrMCI (n=11)	252.7 (26.3)	251.6 (26.1)	-0.4%
	• lrMCI (n=11)	271.8 (36.0)	274.4 (33.9)	+1.0%
	CON (n=18)	263.1 (33.6)	260.0 (32.7)	-1.2%
	• hrMCI (n=11)	260.6 (41.4)	257.4 (39.4)	-1.2%
	• lrMCI (n=7)	267.0 (17.6)	264.1 (20.3)	-1.1%
	Total (n=40)	262.6 (32.4)	261.7 (31.8)	-0.3%
	• hrMCI (n=22)	256.7 (34.1)	254.5 (32.7)	-0.9%
	• lrMCI (n=18)	269.9 (29.6)	270.4 (29.1)	+0.2%

Only the values of the participants with measurements at baseline and after 12weeks (number of participants, n) were used for analysis.
Abbreviations: CA, cornu ammonis; CON, control; DG, dentate gyrus; EXP, experimental; hrMCI, high risk for mild cognitive impairment; lrMCI, low risk for mild cognitive impairment.

Table 4. Descriptive values of baseline/12weeks left hippocampus neurometabolite ratios and percentage change

		BASELINE	12 WEEKS	Δ (%)
tNAA/tCr	EXP (n=22)	1.18 (0.16)	1.25 (0.13)	+5.9%
	• hrMCI (n=11)	1.14 (0.11)	1.15 (0.18)	+0.9%
	• lrMCI (n=11)	1.18 (0.19)	1.25 (0.13)	+5.9%
	CON (n=13)	1.14 (0.11)	1.15 (0.18)	+0.9%
	• hrMCI (n=5)	1.15 (0.11)	1.12 (0.16)	-2.6%
	• lrMCI (n=8)	1.13 (0.11)	1.16 (0.20)	+2.7%
	Total (n=35)	1.17 (0.14)	1.21 (0.15)	+3.4%
	• hrMCI (n=16)	1.17 (0.12)	1.20 (0.15)	+2.6%
	• lrMCI (n=19)	1.16 (0.16)	1.22 (0.16)	+5.2%
mIns/tCr	EXP (n=22)	1.06 (0.18)	1.06 (0.15)	-0.0%
	• hrMCI (n=11)	1.07 (0.11)	1.03 (0.23)	-3.7%
	• lrMCI (n=11)	1.03 (0.14)	1.04 (0.15)	+1.0%
	CON (n=13)	1.07 (0.11)	1.03 (0.23)	-3.7%
	• hrMCI (n=5)	1.03 (0.11)	0.97 (0.25)	-5.8%
	• lrMCI (n=8)	1.10 (0.10)	1.07 (0.23)	-2.7%
	Total (n=35)	1.07 (0.15)	1.05 (0.18)	-1.9%
	• hrMCI (n=16)	1.07 (0.18)	1.04 (0.18)	-2.8%
	• lrMCI (n=19)	1.06 (0.13)	1.05 (0.18)	-0.9%
tNAA/mIns	EXP (n=22)	1.13 (0.15)	1.20 (0.21)	+6.2%
	• hrMCI (n=11)	1.07 (0.16)	1.20 (0.43)	+12.1%
	• lrMCI (n=11)	1.15 (0.13)	1.23 (0.24)	+7.0%
	CON (n=13)	1.07 (0.16)	1.20 (0.43)	+12.1%
	• hrMCI (n=5)	1.12 (0.18)	1.26 (0.53)	+12.5%
	• lrMCI (n=8)	1.03 (0.14)	1.15 (0.37)	+11.7%
	Total (n=35)	1.11 (0.15)	1.20 (0.31)	+8.1%
	• hrMCI (n=16)	1.11 (0.16)	1.20 (0.32)	+8.1%
	• lrMCI (n=19)	1.10 (0.14)	1.20 (0.30)	+9.1%

Only the values of the participants with measurements at baseline and after 12weeks (number of participants, n) were used for analysis.

Abbreviations: CON, control; EXP, experimental; hrMCI, high risk for mild cognitive impairment; lrMCI, low risk for mild cognitive impairment; mIns, myo-inositol; tCr, total creatine; tNAA, total N-acetylaspartate.

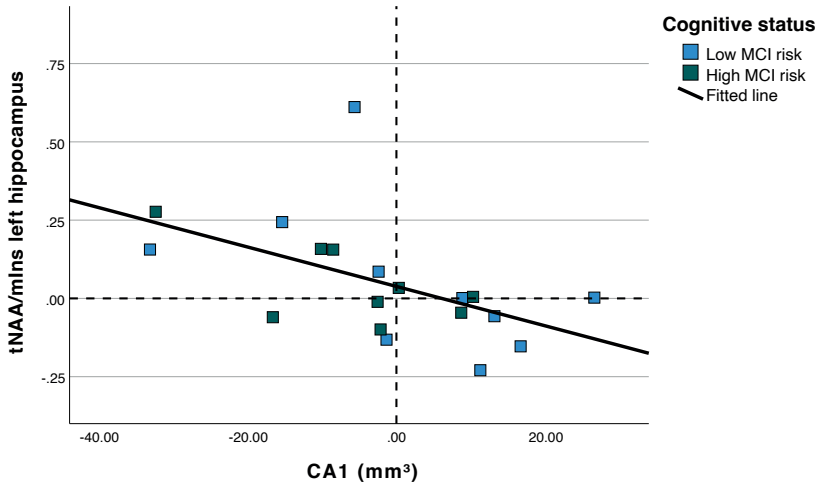


Figure 2. Bivariate relationship between pre-to-post changes in tNAA/mIns levels in the left hippocampus (ΔtNAA/mIns left HPC) and pre-to-post changes in CA1 volume (ΔCA1) for exercise group participants.

Positive values mark increases from pre- to post-intervention measurements. Abbreviations: CA1, cornu ammonis subfield 1; MCI, mild cognitive impairment; mIns, myo-inositol; tNAA, total N-acetylaspartate.

Table 5. Bivariate correlations between changes in blood biomarkers, hippocampus volume and neurometabolites for exercise group participants

		ΔIL-6	ΔKYN	ΔWhole hippocampus	ΔCA1	ΔSubiculum
ΔIGF-1	R	-0.271	0.022	-0.123	-0.131	-0.128
	p	0.210	0.922	0.605	0.582	0.591
ΔIL-6	R		0.155	-0.142	0.037	0.005
	p		0.492	0.561	0.881	0.983
ΔKYN	R			-0.393	-0.240	-0.298
	p			0.096	0.322	0.215
ΔWhole hippocampus	R				**0.666**	**0.633**
	p				**0.001**	**0.002**
ΔCA1	R					**0.450**
	p					**0.036**
ΔSubiculum	R					
	p					
ΔPresubiculum	R					
	p					
ΔCA4	R					
	p					
ΔDG	R					
	p					
ΔtNAA/tCr left hippocampus	R					
	p					
ΔmIns/tCr left hippocampus	R					
	p					

Significant correlations are marked in bold ($p < 0.05$). Correlations with a moderate effect size ($r>0.3$) are underlined. Δ values were calculated by substracting the post-intervention value from the pre-intervention value. Spearman's rho correlation values are presented.

	ΔPresubiculum	ΔCA4	ΔDG	ΔtNAA/tCr left hippocampus	ΔmIns/tCr left hippocampus	ΔtNAA/mIns left hippocampus
	-0.108	0.035	0.021	-0.192	-0.150	0.051
	0.650	0.885	0.930	0.416	0.527	0.830
	0.009	-0.402	-0.118	0.063	0.098	-0.144
	0.972	0.088	0.631	0.797	0.689	0.556
	-0.030	-0.181	-0.198	0.214	-0.288	0.391
	0.904	0.459	0.416	0.379	0.232	0.098
	0.488	**0.566**	**0.517**	-0.075	0.351	-0.401
	0.021	**0.006**	**0.014**	0.759	0.141	0.084
	0.404	0.120	**0.539**	-0.267	0.337	**-0.605**
	0.062	0.594	**0.010**	0.270	0.158	**0.006**
	0.158	0.081	0.074	0.072	0.447	-0.395
	0.484	0.721	0.744	0.770	0.055	0.094
		0.012	-0.001	-0.044	0.151	-0.193
		0.958	0.998	0.858	0.538	0.429
			0.412	-0.140	-0.089	0.077
			0.057	0.567	0.716	0.753
				-0.044	0.196	-0.284
				0.858	0.420	0.238
					0.558	0.278
					0.007	0.210
						-0.567
						0.006

Abbreviations: CA, cornu ammonis; DG, dentate gyrus; HPC, hippocampal cortex; IGF-1, insulin-like growth factor-1; IL-6, interleukin-6; KYN, kynurenine; mIns, myo-inositol; tCr, total creatine; tNAA, total N-acetylaspartate.

5. Discussion

Although there is rising interest in the effects of exercise-induced peripheral biomarkers on the brain, most studies have focused primarily on aerobic exercise investigations while the number of resistance exercise studies in this field is limited (Vints et al., 2022b). Another limitation of the literature regarding the resistance exercise effect on the brain is that the majority of mechanistic explanations are derived from animal experiments. In this randomized controlled trial, we examined the effect of 12 weeks of resistance exercise on blood biomarkers, hippocampus subfield volumes, and hippocampal neurometabolites in older adults with low or high risk of MCI. The effect of resistance exercise was contrasted to a waiting list control condition. To the best of our knowledge, only one study has previously reported resistance exercise-induced neurometabolic changes. In this study from our own research group, it was discovered that strength gains following resistance exercise corresponded with neurometabolic changes in the same cohort of older adults as the current study (Sheoran et al., 2023). So far, no studies have tested the relationship between these exercise-responsive blood and brain neurochemical biomarkers, while this could provide important insights into the mechanism behind the exercise-cognition relationship.

As our primary outcome, we discovered that pre-to-post exercise changes in volume of the CA1 were negatively associated with pre-to-post exercise changes in ratios of tNAA/mIns in the hippocampus. This indicates that the more the density of neurons (marked by tNAA) relative to the density of glial cells (marked by mIns) increases following exercise, the larger the CA1 volume loss following exercise. An interpretation could be that either exercise increases the density of hippocampal neurons at the expense of CA1 volume or an exercise-induced increase in glial cells is associated with CA1 volume gain. The former interpretation is not in line with previous studies showing that exercise can attenuate (subfield) hippocampal volume loss (e.g. Rosano et al., 2017; Broadhouse et al., 2020; Wilckens et al., 2021). However, from the neurometabolic perspective, it does fit our finding that hippocampal tNAA/tCr levels tended to increase in the resistance exercise group. The latter interpretation also does not fit our initial hypothesis that mIns would be associated with neuroinflammation, which at high levels is detrimental for neuroplasticity (Ekdahl et al., 2003; Bourgognon and Cavanagh, 2020). The relationship between inflammatory cells and neuroplasticity is however much more complex than initial studies had pointed out (Ekdahl et al., 2009). Research has shown that at least some inflammation is in fact essential for neuroplastic processes (Bourgognon and Cavanagh, 2020), and microglial cells can support neuroplasticity (Ekdahl et al., 2009). In our study, there was a general decrease in hippocampal mIns/tCr levels in the control group, while hippocampal mIns/tCr levels remained the same in the exercise group. This difference was not significant. Trends from our correlation analysis with moderate effect size ($r>0.3$) support a possible

role of mIns (glial cells) in hippocampus volume increases. Specifically, we found a triad relationship between lower KYN levels, lower tNAA/mIns or higher mIns/tCr levels, and total hippocampal volume. Additionally, we found a relationship between lower IL-6 levels and higher CA4 volume and higher mIns/tCr levels and higher CA4 volume. The inverse relationships between our inflammatory blood biomarkers and hippocampus (subfield) volumes is in line with our initial hypothesis. Our results suggest that mIns level changes (glial cells) may also play a role in the inverse relationship between blood inflammatory markers and hippocampal (subfield) volumes. To the best of our knowledge, there is no previous evidence of exercise-induced changes in mIns levels. Again, in our cohort of older adults, no significant group effect was found for mIns/tCr levels in the hippocampus, nor for the left sensorimotor cortex or right dorsolateral prefrontal cortex, as was previously reported by Sheoran et al. (2023). Furthermore, we did not find an effect of resistance exercise on tNAA/tCr or tNAA/mIns levels in the hippocampus, left sensorimotor cortex or right dorsolateral prefrontal cortex (Sheoran et al., 2023). However, hippocampal tNAA/tCr level group differences had a moderate effect size, suggesting that the resistance exercise group tended to increase tNAA/tCr in the hippocampus. Similar to our results, a study with a 12-week aerobic exercise program also reported no significant exercise-induced changes in tNAA in older adults (Matura et al., 2017). However, tNAA increases were reported in other aerobic exercise studies with the same exercise duration in middle-aged adults (Pajonk et al., 2010; Den Ouden et al., 2018). Notably, only few studies have examined neurometabolic changes caused by physical exercise and it remains unexplored if exercise-induced changes in brain neurometabolic properties precede, follow or coincide with volumetric changes. Studies with longer resistance exercise programs and repeated measurements are needed to discover if tNAA increases may follow at a later stage of physical exercise-induced neuroplastic changes in older adults, and if mIns may indeed play a role in the initial stages of exercise-induced hippocampal neurochemical and volumetric changes or if mIns levels may decrease at a later stage as we hypothesized.

Secondarily, we investigated resistance exercise-induced effects on blood and hippocampus biomarkers. ANCOVA test on the 12-week differences between groups, while taking into account the baseline values, did not indicate any significant effects. However, a nonsignificant difference with a moderate effect size was found for IL-6, with higher levels in the resistance exercise group compared to the control group, especially in older adults with high MCI risk. These findings were in contrast to our hypothesis, as a previous meta-analysis has reported that exercise, including also resistance exercise, can induce decreases in inflammatory markers such as IL-6 (Zhao et al., 2022). Notably, KYN levels did not show the same trend. A possible interpretation for the increase in IL-6 could be that our resistance exercise program was too intense for the participating older adults (with high MCI risk). The load lifted during our training protocol was constantly adjusted

to stay between 7 and 10/10 RPE, indicating that the training stimulus subjectively felt as a very difficult to maximal effort for the older adults (Morishita et al., 2019). Indeed, overtraining is known to be associated with elevated inflammation (da Rocha et al., 2019). Furthermore, the time-related decreases of IL-6 in the control group were also unexpected, as aging has been linked to increases in inflammatory marker levels (Franceschi et al., 2000). However, a meta-analysis has shown that elevated inflammatory markers are not always reported in older adults with MCI, while being found consistently in persons with Alzheimer's dementia, suggesting that systemic inflammation could be a marker for Alzheimer's dementia diagnosis (Su et al., 2019). Furthermore, a nonsignificant group effect with moderate effect size was found for DG volume changes, showing a slight increase in the resistance exercise group, but a decrease in the control group. We found only one previous randomized controlled trial that examined changes in subfield hippocampus volumes following resistance exercise (Broadhouse et al., 2020). In this study, 18 months of resistance exercise stopped atrophy of the left subiculum, and attenuated atrophy in the left CA1 and DG compared to sham physical training in older adults with MCI (Broadhouse et al., 2020). A 24-month multimodal exercise intervention consisting of walking at moderate intensity, lower extremity resistance exercises, balance exercises, stretching and behavioral counselling also increased total left hippocampus volume when adjusting for baseline volumes, while also the post-intervention total right hippocampus volume and volume of the left CA region differed significantly between intervention and control group when not adjusted for baseline volume (Rosano et al., 2017). The lack of total hippocampus volume changes in our study was in line other studies. For example, 6 months of resistance exercise did not change hippocampus volumes in older women with probable MCI, while these changes were found in the aerobic exercise group in this study (Ten Brinke et al., 2015). Moreover, a resistance exercise study with a longer duration of 12-months, comparing heavy or moderate intensity resistance exercise with a control group, reported age-related hippocampal volume losses in all groups without influence of strength training (Gylling et al., 2020).

Finally, when comparing older adults with low and high MCI risk, we found a significant difference in KYN levels and subiculum post-intervention volume. KYN can be considered a generic pro-inflammatory marker, as the enzyme that converts tryptophan to kynurenine in the liver, indoleamine 2,3 dioxygenase, is upregulated by pro-inflammatory cytokines, like IL-1β, tumour necrosis factor-α (TNF-α) and interferon-γ (IFN-γ) (Allison et al., 2017). In addition, we previously reported associations between KYN levels and neuroinflammation and neurodegeneration measured with ^{1}H-MRS (Vints et al., 2022a), suggesting KYN can serve as a surrogate marker of brain health. In addition to our results, higher KYN levels were associated with worse cognitive test scores in a previous study in older adults (Solvang et al., 2019). Concerning hippocampus subfield volume differences depending on cognitive status, a previous meta-analysis evaluating whole brain changes caused by

MCI has shown largest GMV decreases in the hippocampus, parahippocampal gyrus and amygdala in MCI compared to healthy older adults (Raine and Rao, 2022). Some authors have argued that CA1 (Pluta et al., 2012; La Joie et al., 2013) or subiculum (Hanseeuw et al., 2011; Devivo et al., 2019) volumes are even better markers of MCI than whole hippocampal volume. The latter subfield was also influenced by MCI risk in our study.

A limitation of this study is that it took place during the COVID-19 pandemic. This not only caused additional drop-outs from the study, COVID-19 may cause neurological symptoms, brain structural and neurochemical changes (Douaud et al., 2021; Rapalino et al., 2021). A separate pre-to-post analysis of the COVID-19 cases in our study (here these participants were excluded) is currently under review (unpublished).

6. Conclusion

In conclusion, this randomized controlled trial investigated the effects of 12 weeks resistance exercise compared to a waiting list control group on changes in blood biomarkers, hippocampus subfield volumes, hippocampal neurometabolites and their interrelationship in older adults with low or high risk of MCI. The study found a negative association between resistance exercise-induced changes in CA1 volume and hippocampal tNAA/mIns levels in the exercise group, possibly indicating a role of glial cells in exercise-induced neuroplasticity. Furthermore, we found a significant difference for KYN levels and subiculum volume between older adults with low compared to high MCI risk. These findings broaden the knowledge on the multifactorial effects of resistance exercise in older adults and the influence of cognitive status. Ultimately, this study may serve future work on designing individualized exercise interventions aiming to improve brain health in older adults with or without MCI or prevent age-related cognitive decline. Further investigation is warranted to assess the potential benefits that older adults may derive from a resistance exercise program with alternative intensity levels compared to the intensity used in the present protocol. Additionally, it would be of great value to explore the necessity of a longer duration of resistance exercise programs to elicit more pronounced changes.

Declaration of interest

None

Data availability

The coded data can be made available upon reasonable request and after approval by the Kaunas Regional Biomedical Research Ethics Committee.

Acknowledgements
None

Funding
This work was supported by the Research Council of Lithuania (grant number S-MIP-21-37). The sponsor was not involved in study design, collection, analysis or interpretation of the data, in writing the report, nor in the decision to submit the article for publication.

Author contributions
Wouter Vints, Simona Kušleikienė, Vida Česnaitienė, Oron Levin and Nerijus Masiulis contributed to conception, design and data collection of the study. Milda Šarkinaite and Kristina Valatkeviciene were involved in acquiring and analysing of MRI data. Wouter Vints and Julija Šeikinaitė performed the statistical analysis. Wouter Vints and Julija Šeikinaitė wrote the first draft of the manuscript. Oron Levin, Evrim Gökçe, Jeanine Verbunt and Nerijus Masiulis had a role in supervision. All authors contributed to manuscript revision, read, and approved the submitted version.

References

Aagaard, P., Suetta, C., Caserotti, P., Magnusson, S.P., Kjær, M., 2010. Role of the nervous system in sarcopenia and muscle atrophy with aging: strength training as a countermeasure. Scand. J. Med. Sci. Sports 20, 49–64. https://doi.org/10.1111/J.1600-0838.2009.01084.X

Agudelo, L.Z., Femenía, T., Orhan, F., Porsmyr-Palmertz, M., Goiny, M., Martinez-Redondo, V., Correia, J.C., Izadi, M., Bhat, M., Schuppe-Koistinen, I., Pettersson, A.T., Ferreira, D.M.S., Krook, A., Barres, R., Zierath, J.R., Erhardt, S., Lindskog, M., Ruas, J.L., 2014. Skeletal muscle PGC-1α1 modulates kynurenine metabolism and mediates resilience to stress-induced depression. Cell 159, 33–45. https://doi.org/10.1016/j.cell.2014.07.051

Allison, D.J., Josse, A.R., Gabriel, D.A., Klentrou, P., Ditor, D.S., 2017. Targeting inflammation to influence cognitive function following spinal cord injury: A randomized clinical trial. Spinal Cord 55, 26–32. https://doi.org/10.1038/sc.2016.96

Amiri, N., Fathei, M., Mosaferi Ziaaldini, M., 2021. Effects of resistance training on muscle strength, insulin-like growth factor-1, and insulin-like growth factor–binding protein-3 in healthy elderly subjects: a systematic review and meta-analysis of randomized controlled trials. Hormones. https://doi.org/10.1007/s42000-020-00250-6

Apostolova, L.G., Dutton, R.A., Dinov, I.D., Hayashi, K.M., Toga, A.W., Cummings, J.L., Thompson, P.M., 2006. Conversion of Mild Cognitive Impairment to Alzheimer Disease Predicted by Hippocampal Atrophy Maps. Arch. Neurol. 63, 693–699. https://doi.org/10.1001/ARCHNEUR.63.5.693

Apostolova, L.G., Mosconi, L., Thompson, P.M., Green, A.E., Hwang, K.S., Ramirez, A., Mistur, R., Tsui, W.H., de Leon, M.J., 2010. Subregional hippocampal atrophy predicts Alzheimer's dementia in the cognitively normal. Neurobiol. Aging 31, 1088. https://doi.org/10.1016/J.NEUROBIOLAGING.2008.08.008

Barrientos, R.M., Kitt, M.M., Watkins, L.R., Maier, S.F., 2015. Neuroinflammation in the normal aging hippocampus. Neuroscience. https://doi.org/10.1016/j.neuroscience.2015.03.007

Bourgognon, J.-M., Cavanagh, J., 2020. The role of cytokines in modulating learning and memory and brain plasticity. Brain Neurosci. Adv. 4, 239821282097980. https://doi.org/10.1177/2398212820979802

Broadhouse, K.M., Singh, M.F., Suo, C., Gates, N., Wen, W., Brodaty, H., Jain, N., Wilson, G.C., Meiklejohn, J., Singh, N., Baune, B.T., Baker, M., Foroughi, N., Wang, Y., Kochan, N., Ashton, K., Brown, M., Li, Z., Mavros, Y., Sachdev, P.S., Valenzuela, M.J., 2020. Hippocampal plasticity underpins long-term cognitive gains from resistance exercise in MCI. NeuroImage Clin. 25, 102182. https://doi.org/10.1016/J.NICL.2020.102182

Bruijnen, C.J.W.H., Dijkstra, B.A.G., Walvoort, S.J.W., Budy, M.J.J., Beurmanjer, H., De Jong, C.A.J., Kessels, R.P.C., 2020. Psychometric properties of the Montreal Cognitive Assessment (MoCA) in healthy participants aged 18-70. Int. J. Psychiatry Clin. Pract. 24, 293–300. https://doi.org/10.1080/13651501.2020.1746348

Cleeland, C., Pipingas, A., Scholey, A., White, D., 2019. Neurochemical changes in the aging brain: A systematic review. Neurosci. Biobehav. Rev. https://doi.org/10.1016/j.neubiorev.2019.01.003

Costafreda, S.G., Dinov, I.D., Tu, Z., Shi, Y., Liu, C.Y., Kloszewska, I., Mecocci, P., Soininen, H., Tsolaki, M., Vellas, B., Wahlund, L.O., Spenger, C., Toga, A.W., Lovestone, S., Simmons, A., 2011. Automated hippocampal shape analysis predicts the onset of dementia in mild cognitive impairment. Neuroimage 56, 212–219. https://doi.org/10.1016/J.NEUROIMAGE.2011.01.050

da Rocha, A.L., Pinto, A.P., Kohama, E.B., Pauli, J.R., de Moura, L.P., Cintra, D.E., Ropelle, E.R., da Silva, A.S.R., 2019. The proinflammatory effects of chronic excessive exercise. Cytokine. https://doi.org/10.1016/j.cyto.2019.02.016

Dawe, R.J., Yu, L., Arfanakis, K., Schneider, J.A., Bennett, D.A., Boyle, P.A., 2020. Late-life cognitive decline is associated with hippocampal volume, above and beyond its associations with traditional neuropathologic indices. Alzheimer's Dement. 16, 209–218. https://doi.org/10.1002/ALZ.12009

de Alcantara Borba, D., da Silva Alves, E., Rosa, J.P.P., Facundo, L.A., Costa, C.M.A., Silva, A.C., Narciso, F.V., Silva, A., de Mello, M.T., 2020. Can IGF-1 serum levels really be changed by acute physical exercise? A systematic review and meta-analysis. J. Phys. Act. Heal. 17, 575–584. https://doi.org/10.1123/jpah.2019-0453

Den Ouden, L., Kandola, A., Suo, C., Hendrikse, J., Costa, R.J.S., Watt, M.J., Lorenzetti, V., Chye, Y., Parkes, L., Sabaroedin, K., Yücel, M., 2018. The Influence of Aerobic Exercise on Hippocampal Integrity and Function: Preliminary Findings of a Multi-Modal Imaging Analysis. Brain Plast. 4, 211–216. https://doi.org/10.3233/bpl-170053

Devivo, R., Zajac, L., Mian, A., Cervantes-Arslanian, A., Steinberg, E., Alosco, M.L., Mez, J., Stern, R., Killany, R., 2019. Differentiating Between Healthy Control Participants and those with Mild Cognitive Impairment using Volumetric MRI Data. J. Int. Neuropsychol. Soc. 25, 810. https://doi.org/10.1017/S135561771900047X

Dimri, G.P., Lee, X., Basile, G., Acosta, M., Scott, G., Roskelley, C., Medrano, E.E., Linskens, M., Rubelj, I., Pereira-Smith, O., Peacocke, M., Campisi, J., 1995. A biomarker that identifies senescent human cells in culture and in aging skin in vivo. Proc. Natl. Acad. Sci. U. S. A. 92, 9363–9367. https://doi.org/10.1073/pnas.92.20.9363

Douaud, G., Lee, S., Alfaro-Almagro, F., Arthofer, C., Wang, C., Lange, F., Andersson, J.L.R., Griffanti, L., Duff, E., Jbabdi, S., Taschler, B., Winkler, A., Nichols, T.E., Collins, R., Matthews, P.M., Allen, N., Miller, K.L., Smith, S.M., 2021. Brain imaging before and after COVID-19 in UK Biobank. medRxiv (preprint server). https://doi.org/10.1101/2021.06.11.21258690

Ekdahl, C.T., Claasen, J.H., Bonde, S., Kokaia, Z., Lindvall, O., 2003. Inflammation is detrimental for neurogenesis in adult brain. Proc. Natl. Acad. Sci. U. S. A. 100, 13632–13637. https://doi.org/10.1073/pnas.2234031100

Ekdahl, C.T., Kokaia, Z., Lindvall, O., 2009. Brain inflammation and adult neurogenesis: The dual role of microglia. Neuroscience 158, 1021–1029. https://doi.org/10.1016/J.NEUROSCIENCE.2008.06.052

Erickson, K.I., Voss, M.W., Prakash, R.S., Basak, C., Szabo, A., Chaddock, L., Kim, J.S., Heo, S., Alves, H., White, S.M., Wojcicki, T.R., Mailey, E., Vieira, V.J., Martin, S.A., Pence, B.D., Woods, J.A., McAuley, E., Kramer, A.F., 2011. Exercise training increases size of hippocampus and improves memory. Proc. Natl. Acad. Sci. U. S. A. 108, 3017–22. https://doi.org/10.1073/pnas.1015950108

Eriksson, P.S., Perfilieva, E., Björk-Eriksson, T., Alborn, A.M., Nordborg, C., Peterson, D.A., Gage, F.H., 1998. Neurogenesis in the adult human hippocampus. Nat. Med. 4, 1313–1317. https://doi.org/10.1038/3305

Fragala, M.S., Cadore, E.L., Dorgo, S., Izquierdo, M., Kraemer, W.J., Peterson, M.D., Ryan, E.D., 2019. Resistance training for older adults: Position statement from the national strength and conditioning association. J. Strength Cond. Res. 33, 2019–2052. https://doi.org/10.1519/jsc.0000000000003230

Franceschi, C., Bonafè, M., Valensin, S., Olivieri, F., De Luca, M., Ottaviani, E., De Benedictis, G., 2000. Inflamm-aging. An evolutionary perspective on immunosenescence. Ann. N. Y. Acad. Sci. 908, 244–254. https://doi.org/10.1111/j.1749-6632.2000.tb06651.x

Graff-Radford, J., Kantarci, K., 2013. Magnetic resonance spectroscopy in Alzheimer's disease. Neuropsychiatr. Dis. Treat. 9, 687–96. https://doi.org/10.2147/NDT.S35440

Gulick, C.N., Peddie, M., Rehrer, N.J., 2020. Does Exercise Impact Insulin-like Growth Factor 1? : Systematic Review & Meta-Analysis. Open J. Heal. Sci. Med. 1, 104. https://doi.org/10.0000/OJHSM.1000104

Gylling, A.T., Eriksen, C.S., Garde, E., Wimmelmann, C.L., Reislev, N.L., Bieler, T., Ziegler, A.K., Andersen, K.W., Bauer, C., Dideriksen, K., Baekgaard, M., Mertz, K.H., Bayer, M.L., Bloch-Ibenfeldt, M., Boraxbekk, C.J., Siebner, H.R., Mortensen, E.L., Kjaer, M., 2020. The influence of prolonged strength training upon muscle and fat in healthy and chronically diseased older adults. Exp. Gerontol. 136, 110939. https://doi.org/10.1016/J.EXGER.2020.110939

Haff, G., Triplett, N., 2015. Essentials of strength training and conditioning, 4th ed. Human Kinetics Publishers Inc., Champaign, IL.

Hanseeuw, B.J., Van Leemput, K., Kavec, M., Grandin, C., Seron, X., Ivanoiu, A., 2011. Mild cognitive impairment: differential atrophy in the hippocampal subfields. AJNR. Am. J. Neuroradiol. 32, 1658–1661. https://doi.org/10.3174/AJNR.A2589

Jack, C.R., Holtzman, D.M., 2013. Biomarker Modeling of Alzheimer's Disease. Neuron 80, 1347–1358. https://doi.org/10.1016/J.NEURON.2013.12.003

Jack, C.R., Petersen, R.C., Xu, Y., O'Brien, P.C., Smith, G.E., Ivnik, R.J., Boeve, B.F., Tangalos, E.G., Kokmen, E., 2000. Rates of Hippocampal Atrophy Correlate with Change in Clinical Status in Aging and AD. Neurology 55, 489. https://doi.org/10.1212/WNL.55.4.484

Jiang, Q., Lou, K., Hou, L., Lu, Y., Sun, L., Tan, S.C., Low, T.Y., Kord-Varkaneh, H., Pang, S., 2020. The effect of resistance training on serum insulin-like growth factor 1(IGF-1): A systematic review and meta-analysis. Complement. Ther. Med. https://doi.org/10.1016/j.ctim.2020.102360

Jurkowski, M.P., Bettio, L., K. Woo, E., Patten, A., Yau, S.Y., Gil-Mohapel, J., 2020. Beyond the Hippocampus and the SVZ: Adult Neurogenesis Throughout the Brain. Front. Cell. Neurosci. https://doi.org/10.3389/fncel.2020.576444

Kim, S., Choi, J.Y., Moon, S., Park, D.H., Kwak, H.B., Kang, J.H., 2019. Roles of myokines in exercise-induced improvement of neuropsychiatric function. Pflugers Arch. Eur. J. Physiol. https://doi.org/10.1007/s00424-019-02253-8

La Joie, R., Perrotin, A., De La Sayette, V., Egret, S., Doeuvre, L., Belliard, S., Eustache, F., Desgranges, B., Chételat, G., 2013. Hippocampal subfield volumetry in mild cognitive impairment, Alzheimer's disease and semantic dementia. Neuroimage (Amst). 3, 155–162. https://doi.org/10.1016/J.NICL.2013.08.007

Leuner, B., Gould, E., 2010. Structural Plasticity and Hippocampal Function. Annu. Rev. Psychol. 61, C3. https://doi.org/10.1146/ANNUREV.PSYCH.093008.100359

Levin, O., Vints, W.A.J., Ziv, G., Katkutė, G., Kušleikienė, S., Valatkevičienė, K., Sheoran, S., Drozdova-Statkevičienė, M., Gleiznienė, R., Pääsuke, M., Dudonienė, V., Himmelreich, U., Česnaitienė, V.J., Masiulis, N., 2023. Neurometabolic correlates of posturography in normal aging and older adults with mild cognitive impairment: Evidence from a 1H-MRS study. NeuroImage Clin. 37, 103304. https://doi.org/10.1016/J.NICL.2022.103304

Lin, A., Andronesi, O., Bogner, W., Choi, I.Y., Coello, E., Cudalbu, C., Juchem, C., Kemp, G.J., Kreis, R., Krššák, M., Lee, P., Maudsley, A.A., Meyerspeer, M., Mlynarik, V., Near, J., Öz, G., Peek, A.L., Puts, N.A., Ratai, E.M., Tkáč, I., Mullins, P.G., 2021. Minimum Reporting Standards for in vivo Magnetic Resonance Spectroscopy (MRSinMRS): Experts' consensus recommendations. NMR Biomed. 34, e4484. https://doi.org/10.1002/NBM.4484

Maggio, M., Guralnik, J.M., Longo, D.L., Ferrucci, L., 2006. Interleukin-6 in aging and chronic disease: A magnificent pathway. Journals Gerontol. - Ser. A Biol. Sci. Med. Sci. https://doi.org/10.1093/gerona/61.6.575

Mangge, H., Summers, K.L., Meinitzer, A., Zelzer, S., Almer, G., Prassl, R., Schnedl, W.J., Reininghaus, E., Paulmichl, K., Weghuber, D., Fuchs, D., 2014. Obesity-related dysregulation of the Tryptophan–Kynurenine metabolism: Role of age and parameters of the metabolic syndrome. Obesity 22, 195–201. https://doi.org/10.1002/OBY.20491

Marinus, N., Hansen, D., Feys, P., Meesen, R., Timmermans, A., Spildooren, J., 2019. The Impact of Different Types of Exercise Training on Peripheral Blood Brain-Derived Neurotrophic Factor Concentrations in Older Adults: A Meta-Analysis. Sport. Med. https://doi.org/10.1007/s40279-019-01148-z

Marsh, A.P., Miller, M.E., Rejeski, W.J., Hutton, S.L., Kritchevsky, S.B., 2009. Lower Extremity Muscle Function after Strength or Power Training in Older Adults. J. Aging Phys. Act. 17, 416–443. https://doi.org/10.1123/JAPA.17.4.416

Matura, S., Fleckenstein, J., Deichmann, R., Engeroff, T., Füzéki, E., Hattingen, E., Hellweg, R., Lienerth, B., Pilatus, U., Schwarz, S., Tesky, V.A., Vogt, L., Banzer, W., Pantel, J., 2017. Effects of aerobic exercise on brain metabolism and grey matter volume in older adults: results of the randomised controlled SMART trial. Transl. Psychiatry 7, e1172. https://doi.org/10.1038/tp.2017.135

Morishita, S., Tsubaki, A., Nakamura, M., Nashimoto, S., Fu, J.B., Onishi, H., 2019. Rating of perceived exertion on resistance training in elderly subjects. Expert Rev Cardiovasc Ther. 17, 135–142. https://doi.org/10.1080/14779072.2019.1561278

Nasreddine, Z.S., Phillips, N.A., Bédirian, V., Charbonneau, S., Whitehead, V., Collin, I., Cummings, J.L., Chertkow, H., 2005. The Montreal Cognitive Assessment, MoCA: A brief screening tool for mild cognitive impairment. J. Am. Geriatr. Soc. 53, 695–699. https://doi.org/10.1111/j.1532-5415.2005.53221.x

Pajonk, F.G., Wobrock, T., Gruber, O., Scherk, H., Berner, D., Kaizl, I., Kierer, A., Müller, S., Oest, M., Meyer, T., Backens, M., Schneider-Axmann, T., Thornton, A.E., Honer, W.G., Falkai, P., 2010. Hippocampal plasticity in response to exercise in schizophrenia. Arch. Gen. Psychiatry 67, 133–143. https://doi.org/10.1001/archgenpsychiatry.2009.193

Pedersen, B.K., 2019. Physical activity and muscle–brain crosstalk. Nat. Rev. Endocrinol. https://doi.org/10.1038/s41574-019-0174-x

Pedersen, B.K., Steensberg, A., Fischer, C., Keller, C., Keller, P., Plomgaard, P., Febbraio, M., Saltin, B., 2003. Searching for the exercise factor: Is IL-6 a candidate?, in: Journal of Muscle Research and Cell Motility. J Muscle Res Cell Motil, pp. 113–119. https://doi.org/10.1023/A:1026070911202

Pluta, J., Yushkevich, P., Das, S., Wolk, D., 2012. In vivo analysis of hippocampal subfield atrophy in mild cognitive impairment via semi-automatic segmentation of T2-weighted MRI. J. Alzheimer's Dis. 31, 85–99. https://doi.org/10.3233/JAD-2012-111931

Raine, P.J., Rao, H., 2022. Volume, density, and thickness brain abnormalities in mild cognitive impairment: an ALE meta-analysis controlling for age and education. Brain Imaging Behav. 16, 2335–2352. https://doi.org/10.1007/S11682-022-00659-0

Rapalino, O., Weerasekera, A., Moum, S.J., Eikermann-Haerter, K., Edlow, B.L., Fischer, D., Torrado-Carvajal, A., Loggia, M.L., Mukerji, S.S., Schaefer, P.W., Gonzalez, R.G., Lev, M.H., Ratai, E.M., 2021. Brain MR spectroscopic findings in 3 consecutive patients with covid-19: Preliminary observations. Am. J. Neuroradiol. 42, 37–41. https://doi.org/10.3174/ajnr.A6877

Rausch, J.R., Maxwell, S.E., Kelley, K., 2003. Analytic methods for questions pertaining to a randomized pretest, posttest, follow-up design. J. Clin. Child Adolesc. Psychol. 32, 467–486. https://doi.org/10.1207/S15374424JCCP3203_15

Rosano, C., Guralnik, J., Pahor, M., Glynn, N.W., Newman, A.B., Ibrahim, T.S., Erickson, K., Cohen, R., Shaaban, C.E., MacCloud, R.L., Aizenstein, H.J., 2017. Hippocampal Response to a 24-Month Physical Activity Intervention in Sedentary Older Adults. Am. J. Geriatr. Psychiatry 25, 209–217. https://doi.org/10.1016/J.JAGP.2016.11.007

Rössler, M., Zarski, R., Bohl, J., Ohm, T.G., 2002. Stage-dependent and sector-specific neuronal loss in hippocampus during Alzheimer's disease. Acta Neuropathol. 103, 363–369. https://doi.org/10.1007/S00401-001-0475-7

Sheoran, S., Vints, W.A.J., Valatkevičienė, K., Kušleikienė, S., Gleiznienė, R., Česnaitienė, V.J., Himmelreich, U., Levin, O., Masiulis, N., 2023. Strength gains after 12 weeks of resistance training correlate with neurochemical markers of brain health in older adults: a randomized control 1H-MRS study. GeroScience 1–19. https://doi.org/10.1007/S11357-023-00732-6

Shors, T.J., Anderson, M.L., Curlik, D.M., Nokia, M.S., 2012. Use it or lose it: How neurogenesis keeps the brain fit for learning. Behav. Brain Res. https://doi.org/10.1016/j.bbr.2011.04.023

Sjostrom, M., Ainsworth, B., Bauman, A., Bull, F., Hamilton-Craig, C., Sallis, J., 2005. Guidelines for Data Processing and Analysis of the International Physical Activity Questionnaire (IPAQ) – Short and Long Forms, Medicine.

Solvang, S.E.H., Nordrehaug, J.E., Tell, G.S., Nygård, O., McCann, A., Ueland, P.M., Midttun, Ø., Meyer, K., Vedeler, C.A., Aarsland, D., Refsum, H., Smith, A.D., Giil, L.M., 2019. The kynurenine pathway and cognitive performance in community-dwelling older adults. The Hordaland Health Study. Brain. Behav. Immun. 75, 155–162. https://doi.org/10.1016/j.bbi.2018.10.003

Su, C., Zhao, K., Xia, H., Xu, Y., 2019. Peripheral inflammatory biomarkers in Alzheimer's disease and mild cognitive impairment: a systematic review and meta-analysis. Psychogeriatrics 19, 300–309. https://doi.org/10.1111/psyg.12403

Swain, D., Brawner, C., 2013. ACSM's resource manual for guidelines for exercise testing and prescription, 7th ed. Lippincott Williams & Wilkins, Philadelphia, Pennsylvania.

Ten Brinke, L.F., Bolandzadeh, N., Nagamatsu, L.S., Hsu, C.L., Davis, J.C., Miran-Khan, K., Liu-Ambrose, T., 2015. Aerobic exercise increases hippocampal volume in older women with probable mild cognitive impairment: a 6-month randomised controlled trial. Br. J. Sports Med. 49, 248–254. https://doi.org/10.1136/BJSPORTS-2013-093184

Tsuchiya, Y., Ando, D., Takamatsu, K., Goto, K., 2015. Resistance exercise induces a greater irisin response than endurance exercise. Metabolism. 64, 1042–1050. https://doi.org/10.1016/j.metabol.2015.05.010

Tumati, S., Martens, S., Aleman, A., 2013. Magnetic resonance spectroscopy in mild cognitive impairment: systematic review and meta-analysis. Neurosci. Biobehav. Rev. 37, 2571–2586. https://doi.org/10.1016/J.NEUBIOREV.2013.08.004

Valatkevičienė, K., Levin, O., Šarkinaitė, M., Vints, W.A.J., Kunickaitė, R., Danylė, G., Kušleikienė, S., Sheoran, S., Česnaitienė, V.J., Masiulis, N., Himmelreich, U., Gleiznienė, R., 2023. N-acetyl-aspartate and Myo-inositol as Markers of White Matter Microstructural Organization in Mild Cognitive Impairment: Evidence from a DTI-1H-MRS Pilot Study. Diagnostics 13, 654. https://doi.org/10.3390/DIAGNOSTICS13040654

Van Praag, H., 2008. Neurogenesis and exercise: past and future directions. Neuromolecular Med. 10, 128–140. https://doi.org/10.1007/S12017-008-8028-Z

Vécsei, L., Szalárdy, L., Fülöp, F., Toldi, J., 2013. Kynurenines in the CNS: Recent advances and new questions. Nat. Rev. Drug Discov. https://doi.org/10.1038/nrd3793

Vints, W.A.J., Kušleikienė, S., Sheoran, S., Šarkinaitė, M., Valatkevičienė, K., Gleiznienė, R., Kvedaras, M., Pukėnas, K., Himmelreich, U., Česnaitienė, V.J., Levin, O., Verbunt, J., Masiulis, N., 2022a. Inflammatory blood biomarker kynurenine is linked with elevated neuroinflammation and neurodegeneration in older adults: evidence from two 1H-MRS post-processing analysis methods. Front. Psychiatry 13, 859772. https://doi.org/10.3389/FPSYT.2022.859772

Vints, W.A.J., Kušleikienė, S., Sheoran, S., Valatkevičienė, K., Gleiznienė, R., Himmelreich, U., Pääsuke, M., Česnaitienė, V.J., Levin, O., Verbunt, J., Masiulis, N., 2023. Body fat and components of sarcopenia relate to inflammation, brain volume, and neurometabolism in older adults. Neurobiol. Aging 127, 1–11. https://doi.org/10.1016/j.neurobiolaging.2023.02.011

Vints, W.A.J., Levin, O., Fujiyama, H., Verbunt, J., Masiulis, N., 2022b. Exerkines and long-term synaptic potentiation: Mechanisms of exercise-induced neuroplasticity. Front. Neuroendocrinol. 66, 100993. https://doi.org/10.1016/J.YFRNE.2022.100993

Voevodskaya, O., Sundgren, P.C., Strandberg, O., Zetterberg, H., Minthon, L., Blennow, K., Wahlund, L.O., Westman, E., Hansson, O., 2016. Myo-inositol changes precede amyloid pathology and relate to APOE genotype in Alzheimer disease. Neurology 86, 1754–1761. https://doi.org/10.1212/WNL.0000000000002672

Waragai, M., Moriya, M., Nojo, T., 2017. Decreased N-Acetyl Aspartate/Myo-Inositol Ratio in the Posterior Cingulate Cortex Shown by Magnetic Resonance Spectroscopy May Be One of the Risk Markers of Preclinical Alzheimer's Disease: A 7-Year Follow-Up Study. J. Alzheimer's Dis. 60, 1411–1427. https://doi.org/10.3233/JAD-170450

West, M.J., Coleman, P.D., Flood, D.G., Troncoso, J.C., 1994. Differences in the pattern of hippocampal neuronal loss in normal ageing and Alzheimer's disease. Lancet 344, 769–772. https://doi.org/10.1016/S0140-6736(94)92338-8

Wilckens, K.A., Stillman, C.M., Waiwood, A.M., Kang, C., Leckie, R.L., Peven, J.C., Foust, J.E., Fraundorf, S.H., Erickson, K.I., 2021. Exercise interventions preserve hippocampal volume: A meta-analysis. Hippocampus 31, 335–347. https://doi.org/10.1002/hipo.23292

Wu, Y., Bottes, S., Fisch, R., Zehnder, C., Cole, J.D., Pilz, G.A., Helmchen, F., Simons, B.D., Jessberger, S., 2023. Chronic in vivo imaging defines age-dependent alterations of neurogenesis in the mouse hippocampus. Nat. Aging 1–11. https://doi.org/10.1038/s43587-023-00370-9

Ye, G., Xiao, Z., Luo, Z., Huang, X., Abdelrahim, M.E.A., Huang, W., 2020. Resistance training effect on serum insulin-like growth factor 1 in the serum: a meta-analysis. Aging Male. https://doi.org/10.1080/13685538.2020.1801622

Zhao, H., He, Z., Yun, H., Wang, R., Liu, C., 2022. A Meta-Analysis of the Effects of Different Exercise Modes on Inflammatory Response in the Elderly. Int. J. Environ. Res. Public Heal. 2022, Vol. 19, Page 10451 19, 10451. https://doi.org/10.3390/IJERPH191610451

CHAPTER 7

Resistance training's impact on blood biomarkers and cognitive function in older adults with low and high risk of mild cognitive impairment: a randomized controlled trial

Authors: Wouter A.J. Vints, Evrim Gökçe, Julija Šeikinaitė, Simona Kušleikienė, Vida J. Česnaitienė, Jeanine Verbunt, Oron Levin, Nerijus Masiulis

Published in European Review of Aging and Physical Activity, 2024, Vol. 21(1), p. 9
https://doi-org.mu.idm.oclc.org/10.1186/s11556-024-00344-9

Abstract

Background: The aging brain exhibits a neuroinflammatory state, driven partly by peripheral pro-inflammatory stimuli, that accelerates cognitive deterioration. A growing body of evidence clearly indicates that physical exercise partly alleviates neuroinflammation and positively affects the aging process and cognition. In this randomized controlled trial, we aimed to observe the effect of 12 weeks of resistance training (RT) on peripheral biomarker levels, cognitive function changes and their interrelationship, and explore differences in those exercise-induced changes in older adults with high risk of mild cognitive impairment (MCI) compared to older adults with low risk of MCI.

Methods: Fifty-two participants (aged 60-85 years old, 28 female) were randomly allocated to a 12 week lower limb RT program consisting of two training sessions per week or waiting list control group. The Montreal Cognitive Assessment (MoCA) was used to stratify participants screened as high (<26/30) or low risk (≥26/30) of MCI. We assessed serum Interleukin 6 (IL-6), Insulin-like Growth Factor-1 (IGF-1), and Kynurenine (KYN) levels. Cognitive measurement consisted of and four subtests of Automated Neuropsychological Assessment Metrics (ANAM), the two-choice reaction time, go/no-go, mathematical processing, and memory search test.

Results: 12 weeks of RT improved Go/No-go test results in older adults with high MCI risk. RT did not significantly affect blood biomarkers. However, IGF-1 level increases were associated with improvements in response time on the mathematical processing test in the exercise group, and IL-6 level increases were associated with improvements in response time on the memory search test in the total group of participants. Finally, KYN levels significantly differed between older adults with low and high MCI risk but no significant associations with performance were found.

Conclusion: Our study results suggest a different effect of RT on inhibitory control between older adults with low compared to high MCI risk. IGF-1 may play a role in the mechanism behind the cognitive benefit of RT and KYN may be a surrogate biomarker for neurodegeneration and cognitive decline.

Abbreviations

ANAM, Automated Neuropsychological Assessment Metrics; BMI, body mass index; ELISA, enzyme-linked immunosorbent assay; IGF-1, insulin-like growth factor-1; IL-6, interleukin-6; IPAQ-SF, International Physical Activity Questionnaire-Short Form; KYN, kynurenine; MCI, mild cognitive impairment; MoCA, Montreal Cognitive Assessment; MRI, magnetic resonance imaging; RPE, rate of perceived exertion; RT, resistance training

1. Introduction

Cognitive decline is a natural part of aging and can have a significant impact on an individual's quality of life and ability to live independently [1]. When objective evidence of cognitive impairment is present, the terminology employed distinguishes between mild cognitive impairment (MCI) and dementia, with the latter encompassing more than one cognitive domain and being characterized by a substantial interference with an individual's daily life [2]. MCI, considered a preclinical, but still reversible, stage between healthy aging and dementia, is viewed as a potential target for interventions aiming to delay progression towards dementia [3][4]. The worldwide prevalence of MCI is substantial, manifesting in 15.6% of community-dwelling adults aged 50 years and older [4]. Moreover, the worldwide prevalence of dementia (57.4 million in 2019) is continuously rising, even at a faster pace than can solely be explained by the gradual increase in older adults living in our society, and expected to almost triple by 2050 [5][6][7]. Therefore, it is argued that interventions targeting risk factors of dementia and factors that are known to affect reversal from MCI to healthy aging, such as physical exercise, need to be implemented [3][6]. However, the complexity of the underlying mechanisms and the heterogeneity of potential approaches makes that researchers are still unable to compose the optimal exercise treatment strategy.

Evidence from systematic reviews and meta-analyses consistently demonstrated that regular exercise improves cognitive function in older adults [8][9]. However, the effect of exercise on cognition is subject to variation based on the exercise modalities employed, the cognitive domains selected, as well as the participant's cognitive status. For instance, a network meta-analysis on the effect of exercise to improve cognition in older adults indicated that resistance training (RT) appears to have larger beneficial effects on cognitive and motor functioning than other exercise modalities, although more research has been done on aerobic exercise training [10]. In general, the beneficial effect of exercise was found for all subcognitive domains, with resistance exercise having the greatest benefits on executive function, according to a meta-analysis [11]. Another meta-analysis in individuals with MCI showed that RT improved cognition and alleviated MCI

[12]. Additionally, a meta-analysis on the effect of aerobic exercise indicated a larger effect size for improvements in cognition in participants with MCI compared to healthy and demented participants [13]. To the best of our knowledge, no similar analysis exists for RT interventions.

While the exact mechanism for this effect is not fully understood, some studies have suggested a role of exercise-induced anti-inflammatory and neurotrophic blood biomarkers which could serve as precursors for exercise-induced neuroplasticity [14][15]. On the one hand, older adults with higher levels of circulating inflammatory markers and lower levels of neurotrophic factors have been found to have a higher risk of cognitive decline and the development of neurodegenerative diseases [16][17][18]. On the other hand, there is a considerable body of literature showing that physical activity can reduce the expression of pro-inflammatory markers, such as interleukin-6 (IL-6) and kynurenine (KYN), and increase levels of neurotrophic factors, such as insulin-like growth factor-1 (IGF-1) in both cognitively intact older adults and older individuals with neurodegenerative disease conditions [19][20][21].

Based on these considerations, the primary aim of this exploratory study was to examine the relationship between RT-induced changes in blood levels of IL-6, KYN and IGF-1, and changes in cognitive function (specifically, processing speed and executive functions) in older adults and investigate whether this relationship was affected by the cognitive health status (high versus low MCI risk) of the older adult. First, we expected that resistance exercise would increase serum IGF-1 and would decrease serum IL-6 and KYN levels (hypothesis 1). Second, we expected that resistance exercise would improve cognitive performance (hypothesis 2). Based on the two previous hypotheses we further hypothesized that resistance exercise-induced changes in blood biomarkers and cognitive performance would be interrelated (hypothesis 3). Lastly, we expected that the changes induced by resistance exercise would be larger in older adults with a high MCI risk than in cognitively healthy ones (hypothesis 4).

2. Methods

2.1 Ethical approval and participants

Based on a priori sample size calculation done in G*Power 3.1, we needed to include 52 participants in order to find an interaction effect with medium effect size using a repeated measures ANOVA test with alpha 0.05 and power 0.80. Taking into account the possibility of drop-outs, seventy older adults were included in the study. Participants were eligible for the study if they: (1) were 60 years and older; (2) were not currently on psychopharmacological medication or had used these types of drugs in the last five years; (3) voluntarily participated in the study; (4) were fluent in Lithuanian language; (5) were not regularly participating in any exercise program during the previous six months.

Exclusion criteria were: (1) musculoskeletal disorders, especially of the lower extremity hindering participation in the exercise group; (2) neurological disorders such as previous brain injuries, stroke, multiple sclerosis, epilepsy, or neurodegenerative diseases, or a Montreal Cognitive Assessment (MoCA) score below 16/30 indicating possible undiagnosed dementia [22]; (3) psychiatric disorders such as depression or alcohol or drug abuse in the last five years; (4) diabetes mellitus; (5) deep vein thrombosis; (6) oncologic diseases or history of chemotherapy use; or participants that were not allowed or able to undergo magnetic resonance imaging (MRI) based on the exclusion checklist provided by the Department of Radiology, Lithuanian University of Health Science. MR data collected in the study are not presented in this article. For MRI results, see Vints et al., 2022, 2023 [23] [24]; Sheoran et al., 2023 [25]; Valatkeviciene et al., 2023 [26]; and Levin et al., 2023 [27].

Participants were recruited and continuously enrolled between July 2020 and July 2021 via presentations in local community organizations and contacting candidates from a list of patients provided by general practitioners. Interested individuals were invited to Saules Family Medical Centre, where the study's goals, objectives, and methodology were explained in detail. Participants gave written consent prior to study enrolment. The protocol was approved by the Kaunas Regional Biomedical Research Ethics Committee (No. BE-10-7). All participants signed an informed consent form prior to their inclusion in the study.

2.2 Study design

We conducted a single-blinded, two-arm randomized controlled trial with a 12 weeks intervention with lower body resistance exercises at the Institute of Sports Science and Innovation, Lithuanian Sports University. Randomization was performed using a stratified 8-blocked randomization process, stratifying by MoCA score (below 26/30 and 26-30/30), so that each block contained two participants with low MoCA score (i.e., MoCA < 26) and two participants with high MoCA score (i.e., MoCA ≥ 26) allocated to the control group, and two participants with low MoCA score and two participants with high MoCA score allocated to the experimental group. The random allocation was accomplished in an Excel spreadsheet using a random number generator set to indicate either 1 or 2 for the exercise or control group. If two participants from the same group were in a block (N) of four participants with the same cognitive status, a third participant with the same cognitive status and allocated to the same group was assigned to block N+1. The final block of eight participants included only participants at high risk of MCI, and seven were assigned to the control group. We took this decision to correct for the higher number of drop-outs in the control group participants with high risk of MCI at the beginning of the project. The reason for the difference in number of drop-outs most likely existed because participants allocated to the control group were less motivated to return for follow-up assessments. Assessors of the outcome measurements were blinded for the allocation of the participants to the experimental or control group. The participants were not blind to their group allocation.

The experimental group underwent 12 weeks of resistance exercise training, while the control group underwent no intervention. Participants from both groups were instructed to continue their daily life routines as usual. Following a cognitive screening with the MoCA test in the Saules Family Medical Center in Kaunas, Lithuania, participants were invited twice on separate days for additional testing at the Lithuanian Sports University in Kaunas, Lithuania. For each participant, the same test conditions were provided at the same time of day (8 am to 11 am) before and after the 12 weeks period. All participants were instructed to avoid unusual physical activity, alcohol, and caffeine intake the day before testing and to sleep at least 7 hours. They were asked to have breakfast at least 1-2 hours before the experiment. During the first testing day, participants reported their demographic and medical characteristics (see section 2.3). All assessments (see section 2.4-2.8) were performed before and after the 12 weeks intervention or control condition. See detailed description of the study procedure in Figure 1 and participant flow diagram in Figure 2.

2.3 Demographic and medical characteristics

All participants completed a questionnaire battery that assessed their demographic and medical characteristics, such as age, sex, educational level, and smoking status. Educational levels were categorized as primary, secondary, or higher education.

2.4 Physical activity assessment

Physical activity level was assessed using the IPAQ-SF. This self-report questionnaire comprises seven questions and four intensity levels of activity: 1) vigorous-intensity activity such as aerobics, 2) moderate-intensity activity such as leisure cycling, 3) walking, and 4) sitting [28]. Each activity type's frequency (days per week) and duration (minutes per day) in the last seven days are recorded. Each type of activity is characterized by METs (metabolic equivalent of task), and total IPAQ score is estimated by adding up the calculated MET-minutes within each physical activity intensity level (vigorous intensity = 8.0 MET, moderate intensity = 4.0, walking = 3.3 MET). Participants that indicated to burn less than 600 kcal/week are defined as sedentary, 600-3000 kcal/week as moderately physically active, and more than 3000 kcal/week as highly physically active.

2.5 Body composition analysis

Body weight (in kg), height (in cm), body fat percentage (fat %), and body mass index (BMI, in kg/m^2) were measured before and after the intervention. Weight and fat% were estimated using leg-to-leg bio-impedance analysis (BIA, Tanita TBF-300-A).

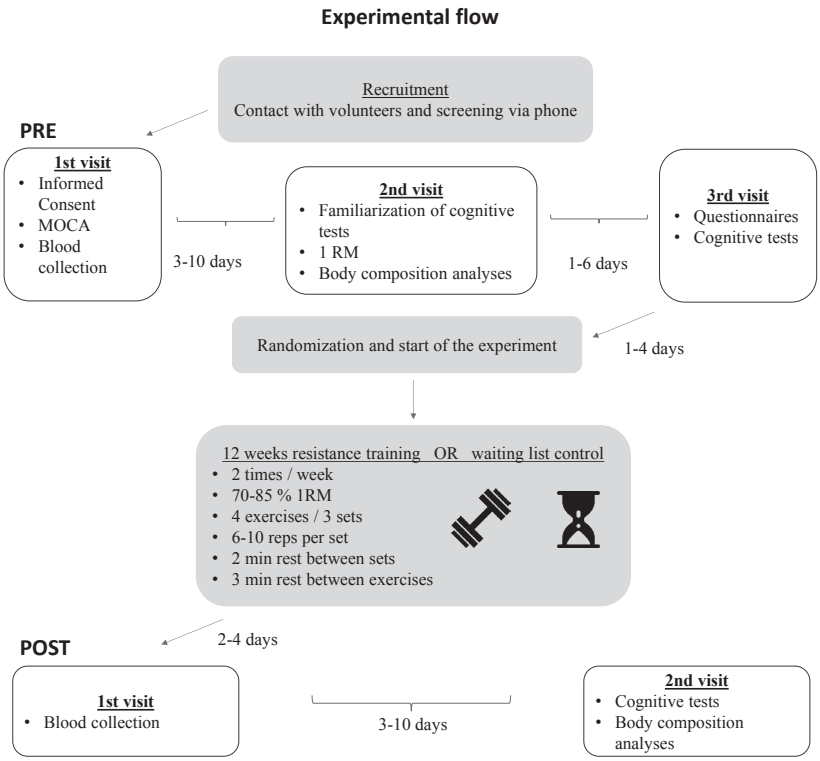


Figure 1. Experimental flow chart. Abbreviations: MOCA, Montreal Cognitive Assessment; RM, repetition maximum.

2.6 Maximum voluntary knee extension force

The maximum voluntary contraction (MVC, in Newton meters) of isometric knee extension torque of the dominant leg was measured with Biodex System 3 dynamometer (Biodex Medical Systems, NY, USA). The highest MVC value out of two trials was recorded.

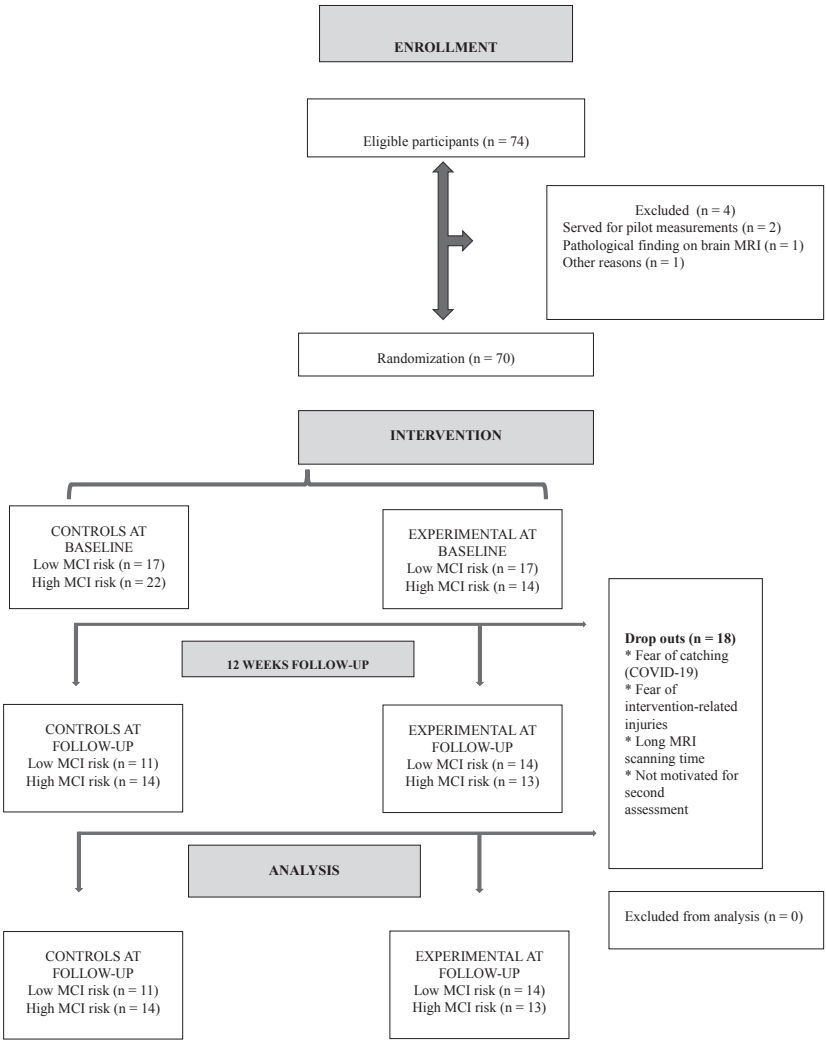


Figure 2. Participant flow diagram.
Please note that some participants enrolled in this project were excluded or dropped-out because of MRI scanning, while no MRI results are presented in this paper. Abbreviations: MCI, Mild cognitive impairment; MRI: Magnetic resonance imaging.

2.7 Neurocognitive assessments

MoCA test. A MoCA examination was conducted by a mental health care professional to evaluate cognitive abilities. This test is considered reliable and consists of 12 items that assess seven cognitive domains, including visuospatial ability and executive functioning, naming, memory, attention, language, abstract reasoning, and orientation. All items contribute to a total score of up to 30 points, with a higher score indicating stronger cognitive functioning. One point is added to the total score if the participant had less than 13 years of education. The participants were classified into different groups based on their MoCA test scores, with a cutoff score of 26 being used for stratification. It is worth noting that a MoCA test score of 25 or lower is generally considered indicative of a high risk of MCI in an otherwise healthy geriatric population [22].

ANAM test. Specific cognitive domains were tested with four selected tests of the ANAM4 (Automated Neuropsychological Assessment Metrics, version 4) test battery. The ANAM4 test system consists of a library of 28 computer-based self-administered tests that assess different aspects of neurocognition including executive functions and attentional processes. Since maintaining attention, inhibitory control, basic computational skills, and working memory are crucial for independent daily living in older adults, we have selected specific subtests to evaluate these functions before and after the intervention. These subtests comprised two-choice reaction time, Go/No-Go, mathematical processing, and memory search tasks. Subjects completed the cognitive tests using a Lithuanian Sports University (LSU) computer running the test suite software in a quiet environment. The software automatically provides the already averaged results of each test. Outcome measure was the response time (in milliseconds, ms). Accuracy measures were used to exclude trials with more than 50% incorrect responses, as this may indicate that the subject did not understand the task. We decided also to delete response times that were faster than the best percentile of young male college students, based on normative values presented in the ANAM4 user manual, considering that this likely indicates that the participants did not adequately perform or understand the task and attained the 50% correct responses by chance [29]. However, none of the participants‘ results had to be excluded based on this decision. A familiarization session took place 48-72h before the testing day and on the testing day the participants were allowed one practice trial before the results were being recorded.

2-choice reaction time test. We used this test to assess processing speed and alternating attention. It contains a motor speed component. The 2-choice reaction time test measures choice reaction time by presenting the participant with a “*” or “o” on the screen. The individual is instructed to respond as quickly as possible by pressing the left or right mouse button as soon as the stimulus appears.

Go/No-go test. It is used to assess response inhibition. The participant is presented with two characters, “o” and “x” and needs to respond as quickly as possible to the “x” character each time the stimulus appears. The subject is instructed to do nothing when the character “o” appears (inhibit response).

Memory search test. The results of the memory search test are used as an index of attention, immediate recognition, and verbal working memory. The program uses letters and symbols to assess verbal working memory as symbolic and non-verbal subparts. The user sees a positive memory set of four letters on the screen (e.g., “T B Q U”). Then, individual characters are displayed, and the participant needs to press mouse buttons to indicate if each character is or is not a member of the positive memory set.

Mathematical processing test. The mathematical processing test results are used as an index of concentration, working memory, and computational skills. During the test, the participant needs to solve an arithmetic problem (e.g., “4+8-5=”). The task involves only three single-digit numbers and two operators. The subject needs to indicate whether the answer is less or higher than five.

2.8 Blood sampling

Serum IL-6, KYN, and IGF-1 concentrations were measured using the ELISA method (ELISA, Biotek, model ELX 800) with spectrophotometry (Spark 10M, Tecan Group Ltd. Zürich, Switzerland) by an experienced technician. A nurse drew the venous blood samples from the antecubital vein into 5 ml EDTA-K3 vacuum tubes. All blood samples were collected between 9:00 a.m. and 1:00 p.m. The second blood collection was carried out 2 to 4 days after the last exercise session for participants in the experimental group. The tubes were gently inverted 8-10 times immediately after blood collection and kept at room temperature for no more than 30-35 minutes until centrifugation for 15 minutes at 4,000 g centrifugal force. Subsequently, serum was aliquoted into 1.5 mL polypropylene tubes. The serum samples were frozen immediately after serum separation and kept at -80 °C in the freezing room of the LSU Institute of Sports Science and Innovation laboratory until further examination.

The IL-6 ELISA kit was purchased from DIAsource ImmunoAssays S.A., Belgium (KAP1216). The lower detection limit is 2pg/mL. The KYN ELISA kit was purchased from MyBiosource, Inc., USA. The lower detection limit is 45.7ng/mL. The IGF-1 ELISA kit was purchased from IBL International, GMBH, Germany (MD58011). The lower detection limit is 0.03ng/mL.

2.9 Training intervention

RT intervention was conducted over 12 weeks in the Lithuanian Sport University gym in accordance with the National Strength and Conditioning Association (USA) position statement on resistance training for older adults [30]. Two to ten days prior to the exercise intervention, participants were familiarized with the RT procedure and underwent 1-repetition maximum (1RM) testing. Two resistance training sessions were scheduled per week with a minimum of two days apart. Warm-up consisted of 5-min cycling on a cycle ergometer at an intensity (in Watts) approximately equal to the participant's body weight in kilograms, followed by a few dynamic stretching and activation exercises including lunges, butt kicks, side step lunges, half-squats, and front and side cross swings. The training program comprised four exercises, namely knee extension (1), incline leg press(2), hamstring curls (3), and calf raise (4), using resistance training equipment from Technogym (Italy).

Each exercise was performed for 2-3 sets of 6-10 repetitions, at 70-85% of the baseline 1RM, with a 2-minute rest between sets and a 3-minute rest between exercises. From week 1 to 3, participants did 8-10 repetitions, starting at 70-75% 1-RM; from week 4 to 9, they worked 6-8 repetitions, starting at 75-80% 1-RM; and from week 10 to 12, they did six repetitions starting at 80-85% 1-RM. After the first session and during each of the three training blocks the weight was adjusted according to the participants' rate of perceived exertion (RPE) on a 10-point Borg scale. The weight was increased when the older adult indicated a score below seven on ten. The exercise sequence was periodically randomized. Qualified trainers supervised all training sessions.

2.10 Statistical analysis

IBM SPSS Statistics version 27 (IBM Corp., Armonk, NY) was used to perform all analyses. Data were initially inspected for outliers and normality. Extreme outliers were defined as values lying more than 3× the interquartile range away from the median and were excluded. Normality was checked graphically using PP-plots and histograms and numerically by a kurtosis and skewness measure between -2 and +2. If the normality assumptions were not met, data was log transformed. Homoscedasticity was tested with the Levene's test.

First, independent t-tests and Chi² tests (or Fisher Exact tests, if the expected count in any of the cells was below 5) were used to assess group differences in baseline variables for continuous and categorical variables respectively. Two-way ANCOVA was used with either post-intervention blood biomarker levels or ANAM test results as a dependent variable. Group (experimental versus control) and cognitive status (low MCI risk versus high MCI risk) were entered as fixed factors, and age and pretest values of the dependent variable as covariates. Body fat % was entered as an additional covariate for analysis with IL-6 or KYN levels. We chose this approach instead of a three-way repeated measures ANOVA, because it was demonstrated that ANCOVA tests taking into account the pretest value of the dependent variable by entering it as a covariate in the model, rather than as a level of the time factor

in repeated measures ANOVA, reduce the population error variance and are therefore more powerful and precise [31]. Bivariate correlation R-values were calculated between the pre- to post-intervention changes in blood biomarker levels and ANAM test results for total group and experimental group. Spearman's rho was chosen, because of the non-normal distribution of some of the outcome measures. Statistical significance was accepted at $\alpha = 0.05$.

3. Results

3.1 Participants characteristics

18 of 70 participants (25.7%) dropped out during the intervention reporting the following drop-out reasons: COVID-19 infection or fear of getting infected, lack of motivation, or intervention related trauma or fear of injury. Participants' ages ranged from 60 to 85 years (mean age: 69 ± 6.2 years) and over half (54.3%) were women. The descriptive values of the baseline characteristics after excluding the drop-outs are presented in Table 1. There was a significant difference at baseline between the experimental and control groups in educational levels and kilocalories burned per week. Missing values existed for IL-6 (n = 3), KYN (n = 1) and IGF-1 (n = 1). The MVC of knee extension torque was measured only in a subgroup of participants (n = 31), used for analysis in a study of Sheoran et al. (2023) [25].

3.2 Blood biomarkers following 12 weeks of RT

Between exercise and control group effects and group x cognitive status interaction effects derived from the ANCOVA test were nonsignificant for IGF-1, IL-6, and KYN. Cognitive status significantly affected KYN level ($p = 0.015$); individuals with higher MCI risk had higher KYN levels.

Of note, effect size of the group effect for IL-6 was of a moderate level (${}_p\eta^2 = 0.078$, $p = 0.089$). IL-6 levels decreased by 6.5% in the control group while they increased by 43.5% in the intervention group. This change was particularly evident among older persons with high MCI risk, with IL-6 change of +53.7% in the exercise group and -8.9% in the control group. Table 2 presents the absolute values, and Table 4 contains the two-way ANCOVA results for the blood biomarkers.

Table 1. Baseline participant characteristics and group differences

	Control (n = 25)	Experimental (n = 27)	Total (n=52)	*p*-value
Age	69.0 (5.9)	70.7 (5.6)	69.9 (5.8)	0.293
Sex:				
• Male	12 (48.0%)	12 (44.4%)	24 (46.2%)	0.797
• Female	13 (52.0%)	15 (55.6%)	28 (53.8%)	
Education:				0.038*
• Higher	22 (88.0%)	20 (74.1%)	42 (80.8%)	
• Secondary	1 (4.0%)	7 (25.9%)	8 (15.4%)	
• Basic	2 (8.0%)	0 (0%)	2 (3.8%)	
Smoking status				1.000
• Smoker	1 (4.0%)	2 (7.4%)	3 (5.8%)	
IPAQ-SF kcal/week	5759.8 (4542.3)	3296.7 (2711.3)	4480.9 (3873.1)	0.024*
IPAQ-SF PA level:				0.116
• sedentary	1 (4.0%)	4 (14.8%)	5 (9.6%)	
• moderately active	8 (32.0%)	13 (48.1%)	21 (40.4%)	
• highly active	16 (64.0%)	10 (37.0%)	26 (50.0%)	
Height (cm)	168.6 (7.8)	165.0 (8.8)	166.9 (8.4)	0.080
Weight (kg)	77.4 (21.8)	77.3 (14.7)	77.4 (18.8)	0.988
BMI (kg/m²)	27.4 (3.3)	28.4 (4.6)	28.1 (5.0)	0.385
Body fat (%)	31.1 (8.0)	32.2 (9.6)	31.5 (9.4)	0.656
MVC (Nm)	158.1 (32.4)	141.6 (38.5)	149.0 (36.3)	0.212
MoCA score	24.5 (3.4)	25.6 (2.5)	25.0 (3.0)	0.204
High MCI risk	14 (56.0%)	13 (48.1%)	27 (51.9%)	0.571
• 2-choice reaction time (ms)	604.4 (113.3)	637.1 (123.9)	621.4 (118.9)	0.326
• Go/No-go (ms)	475.5 (56.2)	471.8 (57.8)	473.6 (56.5)	0.818
• Memory search (ms)	1193.1 (298.7)	1306.8 (239.6)	1252.1 (273.0)	0.135
• Mathematical processing (ms)	3342.5 (1104.3)	3170.0 (716.3)	3253.0 (918.2)	0.511
• IL-6 (pg/mL)	7.5 (8.0)	7.8 (8.8)	7.6 (8.4)	0.888
• KYN (ng/mL)	1550.1 (724.3)	1686.1 (872.3)	1619.4 (798.1)	0.548
• IGF-1 (ng/mL)	124.7 (50.0)	123.4 (62.0)	124.0 (55.9)	0.934

Continuous parameters are expressed as mean values (SD), *p*-values are derived from independent t-tests; categorical parameters are expressed as n (% of total), *p*-values are derived from Chi² tests or Fisher Exact tests. Significant *p*-values are marked *.

Abbreviations: ANAM, Automated Neuropsychological Assessment Metrics; BMI, body mass index; ELISA, enzyme-linked immunosorbent assay; IGF-1, insulin-like growth factor-1; IL-6, interleukin-6; IPAQ-SF, International Physical Activity Questionnaire-Short Form; KYN, kynurenine; MCI, mild cognitive impairment; MoCA, Montreal Cognitive Assessment; MVC, maximum voluntary knee extension torque.

Table 2. Pre- and post-intervention blood biomarker absolute outcome values and percentage change

	Group	N	Pre-Intervention	Post-Intervention	Δ (%)
IGF-1 (ng/mL)	EXP	24	119.0 (57.6)	139.2 (85.4)	+17.0
	• hrMCI	12	118.0 (55.2)	143.2 (81.2)	+21.4
	• lrMCI	12	120.1 (62.4)	135.3 (92.8)	+12.7
	CON	18	112.5 (50.2)	136.3 (66.4)	+21.2
	• hrMCI	8	117.7 (58.3)	141.3 (58.3)	+20.1
	• lrMCI	10	108.3 (45.5)	132.4 (75.1)	+22.2
	Total	42	116.2 (54.0)	138.0 (77.0)	+18.8
	• hrMCI	20	117.9 (54.9)	142.4 (71.2)	+20.8
	• lrMCI	22	114.7 (54.4)	133.9 (83.3)	+16.7
IL-6 (pg/mL)	EXP	23	8.5 (9.2)	12.2 (12.2)	+43.5
	• hrMCI	12	6.7 (5.5)	10.3 (11.2)	+53.7
	• lrMCI	11	10.5 (12.0)	14.4 (13.5)	+37.1
	CON	20	7.7 (8.2)	7.2 (8.9)	-6.5
	• hrMCI	10	4.5 (4.2)	4.1 (3.1)	-8.9
	• lrMCI	10	11.2 (10.2)	10.7 (11.8)	-4.5
	Total	43	8.1 (8.6)	9.9 (11.0)	+22.2
	• hrMCI	22	5.6 (4.9)	7.3 (8.8)	+30.4
	• lrMCI	21	10.8 (10.9)	12.7 (12.6)	+17.6
KYN (ng/mL)	EXP	23	1582.3 (755.7)	1301.3 (606.4)	-17.8
	• hrMCI	11	1434.8 (650.6)	1442.3 (579.5)	+0.5
	• lrMCI	12	1717.4 (846.1)	1172.2 (626.2)	+46.5
	CON	22	1616.4 (709.1)	1507.1 (578.2	-6.8
	• hrMCI	12	1308.2 (441.0)	1722.2 (593.8)	+31.6
	• lrMCI	10	2017.2 (808.8)	1227.5 (439.8)	-39.1
	Total	45	1599.3 (724.8)	1404.2 (595.0)	-12.2
	• hrMCI	23	1366.2 (538.2)	1593.9 (591.8)	+16.7
	• lrMCI	22	1853.7 (823.8)	1197.3 (537.7)	-35.4

Only the values of the participants with pre-and post-intervention measurements were used for analysis.

Abbreviations: CON, control; EXP, experimental; hrMCI, high risk for mild cognitive impairment; IGF-1, insulin-like growth factor-1; IL-6, interleukin-6; KYN, kynurenine; lrMCI, low risk for mild cognitive impairment.

3.3 Cognitive performance changes

ANCOVA results did not indicate a significant group or cognitive status effect on neurocognitive performance. However, there was a significant interaction effect of group and cognitive status on Go/No-go test score (p = 0.010). The absolute values showed that resistance exercise improved reaction time more in older adults with high MCI risk (-4.3%) compared to healthy ones (-0.9%). In contrast, in the control group reaction times increased in older adults with high MCI risk (+2.1%), but decreased in healthy older adults (-6.8). Table 3 presents the absolute values and Table 4 contains the two-way ANCOVA results for cognitive tests.

3.4 Correlations between blood and cognitive changes

A significant negative correlation between changes in IGF-1 levels and changes in mathematical processing response time in the exercise group (r = -0.497, p = 0.014) was found (see Table 6, Figure 3). Furthermore, there was a significant negative correlation between changes in IL-6 level and changes in memory search score when combining experimental and control group (r = -0.313, p = 0.038) (see Additional File 1). Of note, after Bonferroni correction the needed significance levels was α = 0.002 (i.e. α = 0.05/21 significance tests) to which none of the significant results comply, suggesting we cannot state with certainty that these findings are robust.

Table 3. Pre- and post-intervention ANAM outcome values and percentage change

	Group	N	Pre-Intervention	Post-Intervention	Δ (%)
ANAM 2-choice reaction time (ms)	EXP	27	637.1 (123.9)	637.0 (133.1)	-0.0
	• hrMCI	13	662.7 (124.0)	645.0 (133.5)	-2.7
	• lrMCI	14	613.4 (123.4)	629.7 (137.3)	+2.7
	CON	24	599.3 (116.4)	593.2 (98.2)	-1.0
	• hrMCI	13	579.4 (123.0)	616.7 (113.9)	+6.4
	• lrMCI	11	621.0 (110.4)	567.6 (74.7)	-8.6
	Total	51	619.8 (120.8)	616.9 (119.3)	-0.5
	• hrMCI	26	622.7 (128.2)	631.4 (122.7)	+1.4
	• lrMCI	25	616.8 (115.5)	602.4 (116.3)	-2.3
ANAM Go/No-go (ms)	EXP	27	471.8 (57.8)	459.8 (62.6)	-2.5
	• hrMCI	13	480.2 (56.1)	459.6 (56.7)	-4.3
	• lrMCI	14	464.1 (60.3)	460.0 (69.8)	-0.9
	CON	23	472.3 (55.6)	463.3 (71.6)	-1.9
	• hrMCI	12	476.2 (53.0)	488.3 (76.0)	+2.1
	• lrMCI	11	468.0 (60.5)	436.0 (58.0)	-6.8
	Total	50	472.0 (56.2)	461.4 (66.2)	-2.2
	• hrMCI	25	478.2 (53.6)	473.3 (66.8)	-1.0
	• lrMCI	25	465.8 (59.1)	449.4 (64.7)	-3.5
ANAM Memory search (ms)	EXP	27	1306.8 (239.6)	1328.3 (316.3)	+1.6
	• hrMCI	13	1326.3 (212.7)	1288.8 (266.9)	-2.8
	• lrMCI	14	1288.7 (268.8)	1365.0 (362.4)	+5.9
	CON	23	1161.7 (271.5)	1204.9 (360.7)	+3.7
	• hrMCI	12	1145.9 (208.4)	1259.3 (408.3)	+9.9
	• lrMCI	11	1178.9 (337.3)	1145.5 (309.0)	-2.8
	Total	50	1240.0 (262.4)	1271.5 (339.7)	+2.5
	• hrMCI	25	1239.7 (225.8)	1274.6 (335.0)	+2.8
	• lrMCI	25	1240.4 (299.4)	1268.4 (351.1)	+2.3
ANAM Mathematical processing (ms)	EXP	27	3170.0 (716.3)	3187.6 (704.1)	+0.6
	• hrMCI	13	3286.8 (919.7)	3226.4 (609.3)	-5.7
	• lrMCI	14	3061.6 (467.9)	3151.6 (803.6)	+2.9
	CON	23	3267.5 (1109.8)	3068.3 (1110.9)	-6.1

Table 3. Continued

	Group	N	Pre-Intervention	Post-Intervention	Δ (%)
	• hrMCI	12	3521.2 (1355.6)	3320.2 (1376.6)	-5.7
	• lrMCI	11	2990.8 (725.7)	2793.5 (686.4)	-6.6
	Total	50	3214.9 (909.7)	3132.7 (905.9)	-2.6
	• hrMCI	25	3399.3 (1131.1)	3271.4 (1027.8)	-3.8
	• lrMCI	25	3030.5 (582.5)	2994.0 (760.9)	-1.2

Only the values of the participants with pre-and post-intervention measurements were used for analysis.
Abbreviations: ANAM, Automated Neuropsychological Assessment Metrics; CON, control; EXP, experimental; hrMCI, high risk for mild cognitive impairment; lrMCL, low risk for mild cognitive impairment.

Table 4. Two-way ANCOVA for blood biomarker and ANAM test results

	Group		Cognitive status		Group*Cognitive status	
	p-value	$_p\eta^2$	*p*-value	$_p\eta^2$	*p*-value	$_p\eta^2$
IGF-1	0.439	0.016	0.612	0.007	0.717	0.004
IL-6	0.089	0.078	0.401	0.020	0.476	0.014
KYN	0.372	0.021	0.015*	0.147	0.328	0.025
ANAM 2-choice reaction time	0.389	0.017	0.374	0.018	0.272	0.027
ANAM Go/No-Go	0.674	0.004	0.285	0.026	0.010*	0.141
ANAM Memory search	0.610	0.006	0.925	0.000	0.089	0.064
ANAM Mathematical Processing	0.175	0.041	0.736	0.003	0.752	0.002

The dependent variables are presented in the first row. *p*-values and effect sizes (partial eta squared) are given for the group effect, the cognitive status effect and their interaction (Group*Cognitive status). Significant *p*-values are marked *. The ANCOVA was adjusted for the dependent variable's baseline value and for age. The general linear model for IL-6 and KYN were additionally adjusted for fat%. Of note, pre-intervention KYN, post-intervention IGF-1, post-intervention IL-6, pre-intervention ANAM mathematical processing, post-intervention ANAM memory search and post-intervention ANAM mathematical processing were log-transformed due to a non-normal distribution, and in pre-intervention IL-6 and post-intervention IL-6 and KYN an extreme outlier was removed before analysis.
Abbreviations: ANAM, Automated Neuropsychological Assessment Metrics; IGF-1, insulin-like growth factor-1; IL-6, interleukin-6; KYN, kynurenine; MCI, mild cognitive impairment; $_p\eta^2$ = partial eta squared

Table 6. Bivariate correlations between changes in blood biomarkers and cognition in experimental group

		ΔIL-6	ΔKYN	ΔANAM 2-choice reaction time	ΔANAM Go/ No-go	ΔANAM Memory search	ΔANAM Mathematical processing
ΔIGF-1	R	-0.271	0.022	-0.025	0.276	-0.001	**-0.497***
	p	0.210	0.922	0.907	0.192	0.997	**0.014**
ΔIL-6	R		0.155	-0.121	0.125	-0.289	0.254
	p		0.492	0.581	0.569	0.181	0.242
ΔKYN	R			-0.004	0.004	-0.128	-0.167
	p			0.986	0.986	0.559	0.446
ΔANAM 2-choice reaction time	R				**0.432***	0.158	0.209
	p				**0.025**	0.431	0.296
ΔANAM Go/No-go	R					0.105	-0.206
	p					0.602	0.303
ΔANAM Memory search	R						-0.156
	p						0.436

Significant values are marked in bold, significance level * $p < 0.05$.
Δ values were calculated by subtracting the post-intervention value from the pre-intervention value. Spearman's rho correlation values are presented. Significant correlations are marked in bold.
Abbreviations: ANAM, Automated Neuropsychological Assessment Metrics; IGF-1, insulin-like growth factor-1; IL-6, interleukin-6; KYN, kynurenine

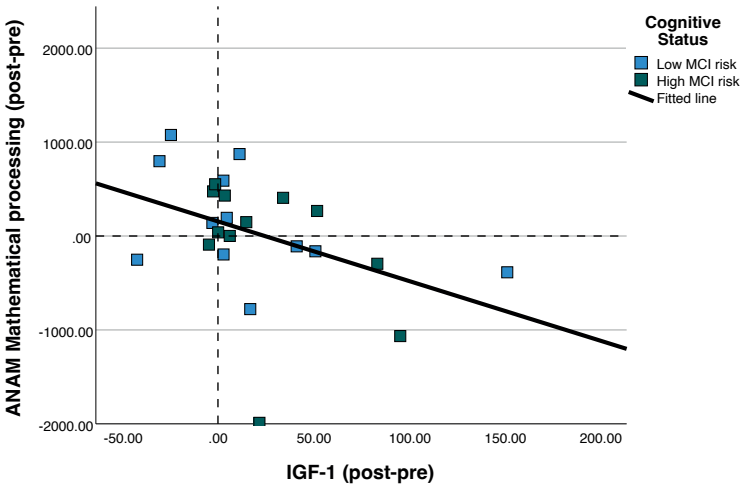


Figure 3. Bivariate relationship between pre-to-post changes in IGF-1 and changes in ANAM Mathematical processing response time (ms) in experimental group. Positive values mark increases from pre- to post test. Abbreviations: ANAM, Automated Neuropsychological Assessment Metrics; IL-6, interleukin-6; MCI, mild cognitive impairment.

4. Discussion

Our research provides additional insights into the effects of resistance exercise on inflammatory and neurotrophic blood biomarkers and cognitive performance in older adults with low or high risk of MCI. In addition, we obtained new findings on the relationship between exercise-induced changes in circulating biomarkers and cognitive performance.

Our primary finding was that larger increases in IGF-1 levels following RT were associated with larger improvements in response time on the mathematical processing task. Overall, IGF-1 increased both in the RT and control group, with no significant differences between groups. Notably, although the relationship between IGF-1 changes and cognitive performance changes is in line with findings from previous literature (see below), our result did not reach the Bonferroni corrected significance level. These results can be compared to those of Tsai and colleagues. In 2015 and 2019 respectively, they reported associations between changes in IGF-1 levels and changes in response time on a variant of the oddball task following a 12 month RT intervention in healthy older adults [32], but not with changes in performance and a switching task following a 16 week RT intervention in older adults with MCI [33]. Furthermore, a cross-sectional study by Al-Delaimy and colleagues showed that IGF-1 levels relate significantly to verbal

fluency and global cognition in older men [17]. IGF-1 is thought to have neuroprotective effects, promoting the growth and survival of neurons in the brain, as well as reducing inflammation and oxidative stress [34]. Furthermore, it has been reported that either injecting IGF-1 or exercise-induced IGF-1 increases can improve the transcription of hippocampal Brain-Derived Neurotrophic Factor (BDNF) [35][36], widely known as a mediator of exercise-induced cognitive improvement [37]. These findings support the hypothesis that IGF-1 may play a role in RT-induced cognitive benefits but cannot claim it acts as a mediator of the exercise-cognition effect. For mediation analysis, a larger sample size is needed.

Furthermore, we discovered that increases over time in IL-6 levels were associated with improvements in memory search scores when evaluating the total group of participants. This was in contrast to our hypothesis and previous studies that have demonstrated an inverse association between working memory and IL-6 levels in older adults [38][39]. However, in the RT group, the relationship between changes in IL-6 and memory search response time was no longer significant. At the molecular level, the link between IL-6 and memory function is previously explained by cytokines' involvement in synaptogenesis, neurogenesis, and memory consolidation [40]. Since the hippocampus has the highest expression of inflammatory cytokine receptors for IL-6 [41], peripheral IL-6 change may affect hippocampus-related memory score. It should be noted, however, that IL-6 has both pro- and anti-inflammatory actions [42] and pro-inflammatory cytokines can both be beneficial and detrimental for neuroplasticity depending on their cerebral concentration [43].

Another important finding from our study is the significant ANCOVA interaction effect for group x cognitive status on Go/No-go response time. This finding indicates that the post-intervention response time on the Go/No-go test differs between RT and control group taking into account the baseline Go/No-go results depending on the older adults cognitive status. In line with our hypothesis, RT enhanced the improvement over time on this inhibition task to a higher extent in older adults with high MCI risk compared to healthy older adults. In turn, it is likely to interpret that the supportive effect of exercise is more pronounced in older adults with more cognitive loss. This finding is conform that of a meta-analysis on the effect of aerobic exercise, that indicated a larger effect size for improvements in cognition in participants with MCI compared to healthy and demented participants [13]. Furthermore, the fact that this was only found for the response inhibition task, and not for the processing speed and two working memory tasks is in line with previous meta-analyses suggesting that executive functions are more likely to respond to resistance exercise, with the working memory component of executive functions being less responsive [11][44]. Finally, it is important to take into consideration that the changes in inhibitory control observed in the RT group for high risk MCI participants could be partly due to social interaction resulting from group activities. Studies have shown that

participating in social leisure activities can help older adults maintain their cognitive abilities [45]. Similarly, engaging in group activities has been linked to increased cognitive function by promoting an overall sense of well-being [46]. Therefore, future studies should recruit control groups engaged in social interaction to reduce the potential confounding factors related to social experiences.

Finally, we found that post-intervention KYN levels were significantly higher in older adults with high MCI risk compared to older adults with low MCI risk. Consistent with our finding, cognitive impairment has been associated with higher KYN levels in individuals with type 2 diabetes mellitus [47] and acute post COVID-19 individuals [48]. KYN levels increase in case of elevated pro-inflammatory cytokine concentrations, which stimulates its conversion from tryptophan by indoleamine-2,3-dioxygenase [49]. It has previously been related to neuroinflammation [23], neurodegeneration [23], cognitive decline [50] and increased dementia risk [51]. It should be noted that KYN can be metabolized within the brain to quinolinic acid and kynurenic acid. The former was found to have detrimental effects on neuroplasticity by inducing neuroinflammation and an overactivation of NMDA receptors, while the latter was found to be an antagonist of the NMDA receptor with beneficial effects in low concentrations, but detrimental effects in high concentrations [52][53][54]. All these findings suggest that KYN may be a marker of neurodegeneration and cognitive decline.

A limitation of the study is that it took place during the COVID-19 pandemic. Three participants dropped-out because of confirmed COVID-19, while others may have had subclinical infections. We did not exclude participants with a history of COVID-19 before inclusion in the study. As COVID-19 has been reported to induce molecular signatures of aging in the brain [55], we should not overlook that our blood biomarker and cognitive findings may have interfered with this condition.

To conclude, this randomized controlled trial indicated that 12 weeks of resistance exercise did not significantly affect peripheral biomarkers in older adults with low or high MCI risk. However, when taking into account the older adults cognitive status, RT positively affected inhibitory control, particularly in older adults with a high risk of MCI. Moreover, our study results suggest that RT-induced increases in the neurotrophic factor IGF-1 may play a role in RT-induced improvements in mathematical processing. Finally, KYN is put forward as a potential blood biomarker related to cognitive impairment.

Ethical approval and consent to participate

The protocol was approved by the Kaunas Regional Biomedical Research Ethics Committee (No. BE-10-7). All participants signed an informed consent form prior to their inclusion in the study.

Consent for publication
Not applicable

Availability of data and materials
The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests
The authors declare that they have no competing interests

Funding
This work was supported by the Research Council of Lithuania (Grant Number S-MIP-21–37). The sponsor was not involved in the conceptualization, design, data collection, analysis, decision to publish, or preparation of the manuscript.

Authors' contributions
Wouter Vints, Simona Kušleikienė, Vida Česnaitienė, Oron Levin and Nerijus Masiulis contributed to conception, design and data collection of the study. Wouter Vints and Julija Šeikinaitė performed the statistical analysis. Wouter Vints, Evrim Gökçe and Julija Šeikinaitė wrote the first draft of the manuscript. Oron Levin, Jeanine Verbunt and Nerijus Masiulis had a role in supervision. All authors contributed to manuscript revision, read, and approved the submitted version.

Acknowledgements
Not applicable

Additional material
Additional file 1.docx

This additional file includes:

- Supplementary Table 1: Bivariate correlations between changes in blood biomarkers and cognition in both experimental and control group
- Supplementary Figure 1: Bivariate relationship between pre-to-post changes in IL-6 and ANAM Memory search response time (ms) in both experimental and control group.

References

1. Cummings J, Aisen PS, Dubois B, Frölich L, Jack CR, Jones RW, et al. Drug development in Alzheimer's disease: The path to 2025. Alzheimer's Res Ther [Internet]. BioMed Central; 2016 [cited 2023 Sep 8];8:39. Available from: https://mayoclinic.elsevierpure.com/en/publications/drug-development-in-alzheimers-disease-the-path-to-2025
2. Knopman DS, Petersen RC. Mild Cognitive Impairment and Mild Dementia: A Clinical Perspective. Mayo Clin Proc [Internet]. NIH Public Access; 2014 [cited 2023 Sep 9];89:1452–9. Available from: /pmc/articles/PMC4185370/
3. Shimada H, Doi T, Lee S, Makizako H. Reversible predictors of reversion from mild cognitive impairment to normal cognition: a 4-year longitudinal study. Alzheimers Res Ther [Internet]. BMC; 2019 [cited 2023 Sep 9];11:24. Available from: /pmc/articles/PMC6416893/
4. Bai W, Chen P, Cai H, Zhang Q, Su Z, Cheung T, et al. Worldwide prevalence of mild cognitive impairment among community dwellers aged 50 years and older: a meta-analysis and systematic review of epidemiology studies. Age Ageing [Internet]. Oxford Academic; 2022 [cited 2023 Sep 9];51:1–14. Available from: https://dx.doi.org/10.1093/ageing/afac173
5. Mattiuzzi C, Lippi G. Worldwide disease epidemiology in the older persons. Eur Geriatr Med [Internet]. Springer; 2020 [cited 2022 Jan 3];11:147–53. Available from: https://link.springer.com/article/10.1007/s41999-019-00265-2
6. Livingston G, Huntley J, Sommerlad A, Ames D, Ballard C, Banerjee S, et al. Dementia prevention, intervention, and care: 2020 report of the Lancet Commission. Lancet Comm [Internet]. Lancet Publishing Group; 2020 [cited 2022 Aug 22];396:413–46. Available from: http://www.thelancet.com/article/S0140673620303676/fulltext
7. Nichols E, Steinmetz JD, Vollset SE, Fukutaki K, Chalek J, Abd-Allah F, et al. Estimation of the global prevalence of dementia in 2019 and forecasted prevalence in 2050: an analysis for the Global Burden of Disease Study 2019. Lancet Public Heal [Internet]. Elsevier Ltd; 2022 [cited 2022 Aug 22];7:e105–25. Available from: http://www.thelancet.com/article/S2468266721002498/fulltext
8. Xu L, Gu H, Cai X, Zhang Y, Hou X, Yu J, et al. The Effects of Exercise for Cognitive Function in Older Adults: A Systematic Review and Meta-Analysis of Randomized Controlled Trials. Int J Environ Res Public Health [Internet]. Int J Environ Res Public Health; 2023 [cited 2024 Mar 6];20:1088. Available from: https://pubmed.ncbi.nlm.nih.gov/36673844/
9. Coelho-Junior H, Marzetti E, Calvani R, Picca A, Arai H, Uchida M. Resistance training improves cognitive function in older adults with different cognitive status: a systematic review and Meta-analysis. Aging Ment Health [Internet]. Aging Ment Health; 2022 [cited 2023 Sep 8];26:213–24. Available from: https://pubmed.ncbi.nlm.nih.gov/33325273/
10. Gallardo-Gómez D, del Pozo-Cruz J, Noetel M, Álvarez-Barbosa F, Alfonso-Rosa RM, del Pozo Cruz B. Optimal dose and type of exercise to improve cognitive function in older adults: A systematic review and bayesian model-based network meta-analysis of RCTs. Ageing Res Rev. Elsevier; 2022;76:101591.
11. Zhang M, Jia J, Yang Y, Zhang L, Wang X. Effects of exercise interventions on cognitive functions in healthy populations: A systematic review and meta-analysis. Ageing Res Rev [Internet]. Ageing Res Rev; 2023 [cited 2024 Mar 8];92:102116. Available from: https://pubmed.ncbi.nlm.nih.gov/37924980/
12. Zhang L, Li B, Yang J, Wang F, Tang Q, Wang S. Meta-analysis: Resistance Training Improves Cognition in Mild Cognitive Impairment. Int J Sports Med [Internet]. Georg Thieme Verlag; 2020 [cited 2023 Sep 8];41:815–23. Available from: http://www.thieme-connect.com/products/ejournals/html/10.1055/a-1186-1272

13. Talar K, Vetrovsky T, van Haren M, Négyesi J, Granacher U, Váczi M, et al. The effects of aerobic exercise and transcranial direct current stimulation on cognitive function in older adults with and without cognitive impairment: A systematic review and meta-analysis. Ageing Res Rev. Elsevier; 2022;81:101738.
14. Vints WAJ, Levin O, Fujiyama H, Verbunt J, Masiulis N. Exerkines and long-term synaptic potentiation: Mechanisms of exercise-induced neuroplasticity. Front Neuroendocrinol [Internet]. Academic Press; 2022 [cited 2022 Mar 15];66:100993. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0091302222000164
15. Pedersen BK. Physical activity and muscle–brain crosstalk [Internet]. Nat. Rev. Endocrinol. Nature Publishing Group; 2019 [cited 2020 Sep 12]. p. 383–92. Available from: https://pubmed.ncbi.nlm.nih.gov/30837717/
16. Fang Y, Doyle MF, Chen J, Alosco ML, Mez J, Satizabal CL, et al. Association between inflammatory biomarkers and cognitive aging. PLoS One [Internet]. PLoS One; 2022 [cited 2023 Sep 8];17:e0274350. Available from: https://pubmed.ncbi.nlm.nih.gov/36083988/
17. Al-Delaimy WK, Von Muhlen D, Barrett-Connor E. IGF-1 and IGFBP-1 and Cognitive Function in Older Men and Women. J Am Geriatr Soc [Internet]. NIH Public Access; 2009 [cited 2023 Sep 9];57:1441–6. Available from: /pmc/articles/PMC2728156/
18. Westwood W, Beiser A, DeCarli C, Harris TB, Chen TC, He XM, et al. Insulin-like growth factor-1 and risk of Alzheimer dementia and brain atrophy. Neurology [Internet]. Neurology; 2014 [cited 2023 Sep 8];82:1613–9. Available from: https://pubmed.ncbi.nlm.nih.gov/24706014/
19. Khalafi M, Akbari A, Symonds ME, Pourvaghar MJ, Rosenkranz SK, Tabari E. Influence of different modes of exercise training on inflammatory markers in older adults with and without chronic diseases: A systematic review and meta-analysis. Cytokine [Internet]. Cytokine; 2023 [cited 2023 Sep 8];169:156303. Available from: https://pubmed.ncbi.nlm.nih.gov/37467710/
20. Kim D Il, Lee DH, Hong S, Jo S won, Won Y shin, Jeon JY. Six weeks of combined aerobic and resistance exercise using outdoor exercise machines improves fitness, insulin resistance, and chemerin in the Korean elderly: A pilot randomized controlled trial. Arch Gerontol Geriatr [Internet]. Arch Gerontol Geriatr; 2018 [cited 2023 Sep 8];75:59–64. Available from: https://pubmed.ncbi.nlm.nih.gov/29190545/
21. Jiang Q, Lou K, Hou L, Lu Y, Sun L, Tan SC, et al. The effect of resistance training on serum insulin-like growth factor 1(IGF-1): A systematic review and meta-analysis. Complement. Ther. Med. Churchill Livingstone; 2020. p. 102360.
22. Nasreddine ZS, Phillips NA, Bédirian V, Charbonneau S, Whitehead V, Collin I, et al. The Montreal Cognitive Assessment, MoCA: A brief screening tool for mild cognitive impairment. J Am Geriatr Soc [Internet]. Blackwell Publishing Inc.; 2005 [cited 2020 Nov 4];53:695–9. Available from: www.mocatest.
23. Vints WAJ, Kušleikienė S, Sheoran S, Šarkinaitė M, Valatkevičienė K, Gleiznienė R, et al. Inflammatory blood biomarker kynurenine is linked with elevated neuroinflammation and neurodegeneration in older adults: evidence from two 1H-MRS post-processing analysis methods. Front Psychiatry [Internet]. Frontiers; 2022 [cited 2022 Mar 17];13:859772. Available from: https://www.frontiersin.org/articles/10.3389/fpsyt.2022.859772/abstract
24. Vints WAJ, Kušleikienė S, Sheoran S, Valatkevičienė K, Gleiznienė R, Himmelreich U, et al. Body fat and components of sarcopenia relate to inflammation, brain volume, and neurometabolism in older adults. Neurobiol Aging [Internet]. 2023;127:1–11. Available from: https://doi.org/10.1016/j.neurobiolaging.2023.02.011
25. Sheoran S, Vints WAJ, Valatkevičienė K, Kušleikienė S, Gleiznienė R, Česnaitienė VJ, et al. Strength gains after 12 weeks of resistance training correlate with neurochemical markers of brain health in older adults: a randomized control 1H-MRS study. GeroScience [Internet]. Springer; 2023 [cited 2023 Jan 26];1–19. Available from: https://link.springer.com/article/10.1007/s11357-023-00732-6

26. Valatkevičienė K, Levin O, Šarkinaitė M, Vints WAJ, Kunickaitė R, Danylė G, et al. N-acetyl-aspartate and Myo-inositol as Markers of White Matter Microstructural Organization in Mild Cognitive Impairment: Evidence from a DTI-1H-MRS Pilot Study. Diagnostics [Internet]. Diagnostics (Basel); 2023 [cited 2023 Apr 12];13:654. Available from: https://pubmed.ncbi.nlm.nih.gov/36832141/
27. Levin O, Vints WAJ, Ziv G, Katkutė G, Kušleikienė S, Valatkevičienė K, et al. Neurometabolic correlates of posturography in normal aging and older adults with mild cognitive impairment: Evidence from a 1H-MRS study. NeuroImage Clin. Elsevier; 2023;37:103304.
28. Sjostrom M, Ainsworth B, Bauman A, Bull F, Hamilton-Craig C, Sallis J. Guidelines for Data Processing and Analysis of the International Physical Activity Questionnaire (IPAQ) – Short and Long Forms. Medicine (Baltimore). 2005.
29. Center for the Study of Human Operator Performance. C-SHOP ANAM4: User Manual. University of Oklahoma: Center for the Study of Human Operator Performance (C-SHOP); 2007.
30. Fragala MS, Cadore EL, Dorgo S, Izquierdo M, Kraemer WJ, Peterson MD, et al. Resistance training for older adults: Position statement from the national strength and conditioning association. J Strength Cond Res [Internet]. NSCA National Strength and Conditioning Association; 2019 [cited 2021 Mar 5];33:2019–52. Available from: https://pubmed.ncbi.nlm.nih.gov/31343601/
31. Rausch JR, Maxwell SE, Kelley K. Analytic methods for questions pertaining to a randomized pretest, posttest, follow-up design. J Clin Child Adolesc Psychol [Internet]. 2003;32:467–86. Available from: http://dx.doi.org/10.1207/S15374424JCCP3203_15
32. Tsai CL, Wang CH, Pan CY, Chen FC. The effects of long-term resistance exercise on the relationship between neurocognitive performance and GH, IGF-1, and homocysteine levels in the elderly. Front Behav Neurosci [Internet]. Frontiers Research Foundation; 2015 [cited 2021 Jan 8];9. Available from: /pmc/articles/PMC4322723/?report=abstract
33. Tsai C-L, Pai M-C, Ukropec J, Ukropcová B. Distinctive Effects of Aerobic and Resistance Exercise Modes on Neurocognitive and Biochemical Changes in Individuals with Mild Cognitive Impairment. Curr Alzheimer Res. Bentham Science Publishers Ltd.; 2019;16:316–32.
34. Ostrowski PP, Barszczyk A, Forstenpointner J, Zheng W, Feng ZP. Meta-Analysis of Serum Insulin-Like Growth Factor 1 in Alzheimer's Disease. PLoS One [Internet]. PLoS One; 2016 [cited 2023 Sep 8];11:e0155733. Available from: https://pubmed.ncbi.nlm.nih.gov/27227831/
35. Carro E, Nuñez A, Busiguina S, Torres-Aleman I. Circulating insulin-like growth factor I mediates effects of exercise on the brain. J Neurosci [Internet]. Society for Neuroscience; 2000 [cited 2021 Jan 17];20:2926–33. Available from: https://www.jneurosci.org/content/20/8/2926
36. Ding Q, Vaynman S, Akhavan M, Ying Z, Gomez-Pinilla F. Insulin-like growth factor I interfaces with brain-derived neurotrophic factor-mediated synaptic plasticity to modulate aspects of exercise-induced cognitive function. Neuroscience. Pergamon; 2006;140:823–33.
37. Vaynman S, Ying Z, Gomez-Pinilla F. Hippocampal BDNF mediates the efficacy of exercise on synaptic plasticity and cognition. Eur J Neurosci [Internet]. John Wiley & Sons, Ltd; 2004 [cited 2023 Oct 31];20:2580–90. Available from: https://onlinelibrary-wiley-com.mu.idm.oclc.org/doi/full/10.1111/j.1460-9568.2004.03720.x
38. Griseta C, Battista P, Castellana F, Colonna I, Sciarra S, Zupo R, et al. Serum levels of IL-6 are associated with cognitive impairment in the salus in apulia population-based study. Heliyon [Internet]. Heliyon; 2023 [cited 2023 Sep 8];9:e13972. Available from: https://pubmed.ncbi.nlm.nih.gov/36915478/
39. Elderkin-Thompson V, Irwin MR, Hellemann G, Kumar A. Interleukin-6 and memory functions of encoding and recall in healthy and depressed elderly adults. Am J Geriatr Psychiatry [Internet]. Am J Geriatr Psychiatry; 2012 [cited 2023 Sep 9];20:753–63. Available from: https://pubmed.ncbi.nlm.nih.gov/22892560/
40. McAfoose J, Baune BT. Evidence for a cytokine model of cognitive function. Neurosci Biobehav Rev. Pergamon; 2009;33:355–66.

41. Godbout JP, Johnson RW. Age and neuroinflammation: a lifetime of psychoneuroimmune consequences. Neurol Clin [Internet]. Neurol Clin; 2006 [cited 2023 Sep 8];24:521–38. Available from: https://pubmed.ncbi.nlm.nih.gov/16877122/
42. Pedersen BK, Steensberg A, Keller P, Keller C, Fischer C, Hiscock N, et al. Muscle-derived interleukin-6: lipolytic, anti-inflammatory and immune regulatory effects. Pflugers Arch-Eur J Physiol. 2003;446:9–16.
43. Bourgognon J-M, Cavanagh J. The role of cytokines in modulating learning and memory and brain plasticity. Brain Neurosci Adv [Internet]. SAGE Publications; 2020 [cited 2021 Feb 3];4:239821282097980. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7750764/
44. Landrigan J-F, Bell T, Crowe M, Clay OJ, Mirman D. Lifting cognition: a meta-analysis of effects of resistance exercise on cognition. Psychol Res 2019 845 [Internet]. Springer; 2019 [cited 2021 Sep 4];84:1167–83. Available from: https://link.springer.com/article/10.1007/s00426-019-01145-x
45. Cohn-Schwartz E. Pathways From Social Activities to Cognitive Functioning: The Role of Physical Activity and Mental Health. Innov Aging [Internet]. Oxford Academic; 2020 [cited 2024 Mar 6];4:1–10. Available from: https://dx.doi.org/10.1093/geroni/igaa015
46. Haslam C, Cruwys T, Haslam SA. "The we's have it": Evidence for the distinctive benefits of group engagement in enhancing cognitive health in aging. Soc Sci Med. Pergamon; 2014;120:57–66.
47. Bakker L, Ramakers IHGB, van Boxtel MPJ, Schram MT, Stehouwer CDA, van der Kallen CJH, et al. Associations between plasma kynurenines and cognitive function in individuals with normal glucose metabolism, prediabetes and type 2 diabetes: the Maastricht Study. Diabetologia [Internet]. Diabetologia; 2021 [cited 2023 Sep 8];64:2445–57. Available from: https://pubmed.ncbi.nlm.nih.gov/34409496/
48. Cysique LA, Jakabek D, Bracken SG, Allen-Davidian Y, Heng B, Chow S, et al. The kynurenine pathway relates to post-acute COVID-19 objective cognitive impairment and PASC. Ann Clin Transl Neurol [Internet]. John Wiley & Sons, Ltd; 2023 [cited 2023 Sep 8];10:1338–52. Available from: https://onlinelibrary.wiley.com/doi/full/10.1002/acn3.51825
49. Capuron L, Schroecksnadel S, Féart C, Aubert A, Higueret D, Barberger-Gateau P, et al. Chronic low-grade inflammation in elderly persons is associated with altered tryptophan and tyrosine metabolism: Role in neuropsychiatric symptoms. Biol Psychiatry. Elsevier; 2011;70:175–82.
50. Solvang SEH, Nordrehaug JE, Tell GS, Nygård O, McCann A, Ueland PM, et al. The kynurenine pathway and cognitive performance in community-dwelling older adults. The Hordaland Health Study. Brain Behav Immun. Academic Press Inc.; 2019;75:155–62.
51. Chouraki V, Preis SR, Yang Q, Beiser A, Li S, Larson MG, et al. Association of amine biomarkers with incident dementia and Alzheimer's disease in the Framingham Study. Alzheimer's Dement [Internet]. Alzheimers Dement; 2017 [cited 2023 Sep 8];13:1327–36. Available from: https://pubmed.ncbi.nlm.nih.gov/28602601/
52. Vécsei L, Szalárdy L, Fülöp F, Toldi J. Kynurenines in the CNS: Recent advances and new questions [Internet]. Nat. Rev. Drug Discov. Nat Rev Drug Discov; 2013 [cited 2021 Mar 29]. p. 64–82. Available from: https://pubmed.ncbi.nlm.nih.gov/23237916/
53. Potter MC, Elmer GI, Bergeron R, Albuquerque EX, Guidetti P, Wu HQ, et al. Reduction of endogenous kynurenic acid formation enhances extracellular glutamate, hippocampal plasticity, and cognitive behavior. Neuropsychopharmacology [Internet]. Nature Publishing Group; 2010 [cited 2021 Mar 29];35:1734–42. Available from: www.neuropsychopharmacology.org
54. Rózsa É, Robotka H, Vécsei L, Toldi J. The Janus-face kynurenic acid. J Neural Transm [Internet]. Springer; 2008 [cited 2021 Mar 29];115:1087–91. Available from: https://link.springer.com/article/10.1007/s00702-008-0052-5
55. Mavrikaki M, Lee JD, Solomon IH, Slack FJ. Severe COVID-19 is associated with molecular signatures of aging in the human brain. Nat Aging 2022 212 [Internet]. Nature Publishing Group; 2022 [cited 2023 Sep 8];2:1130–7. Available from: https://www.nature.com/articles/s43587-022-00321-w

CHAPTER 8

Hippocampal neurometabolic and structural changes from pre- to post-COVID-19: a case-series study

Authors: Wouter A.J. Vints, Kristina Valatkevičienė, Oron Levin, Akila Weerasekera, Simonas Jesmanas, Simona Kušleikienė, Vida J. Česnaitienė, Uwe Himmelreich, Jeanine A. Verbunt, Eva-Maria Ratai, Rymantė Gleiznienė, Nerijus Masiulis

Published in Magnetic Resonance Imaging, 2024, Vol. 109, p. 249-255
https://doi-org.mu.idm.oclc.org/10.1016/j.mri.2024.03.032

Abstract

Background: Neurological complications of the COVID-19 infection may be caused in part by local neurochemical and structural abnormalities that could not be detected during routine medical examinations. We examined within subject neurometabolic and structural brain alterations from pre-to post-COVID-19 in the hippocampal region of three elderly individuals (aged 63-68 years) who had a COVID-19 infection with mild symptoms. Patients were participating in an interventional study in which they were closely monitored at the time they were diagnosed with COVID-19. Patients 1 and 2 just completed 18-20 resistance training sessions prior to their diagnosis. Patient 3 was assigned to a non-training condition in the same study.

Methods: Whole brain magnetic resonance imaging (MRI) images and proton magnetic resonance spectroscopy (^{1}H-MRS) of the left hippocampus were collected before and after infection. Structural and spectroscopic imaging measures post-COVID-19 were contrasted to the pre-COVID-19 measures and were compared with values for Minimal Detectable Change at 95% (MDC_{95}) and 90% (MDC_{90}) confidence from a group of six elderly (aged 60-79 years) without COVID-19 that participated in the same study.

Results: After SARS-COV-2 infection, we observed a reduction of glutamate-glutamine (Glx) in Patients 1 and 2 (≥ 42.0 %) and elevation of myo-inositol (mIns) and N-acetyl-aspartate (NAA) in Patient 3 (≥ 36.4%); all > MDC_{90}. MRI findings showed increased (Patients 1 and 2) or unchanged (Patient 3) hippocampal volume.

Conclusions: Overall, findings from this exploratory study suggest that mild COVID-19 infection could be associated with development of local neuroinflammation and reduced glutamate levels in the hippocampus. Our ^{1}H-MRS findings may have clinical value for explaining chronic neurological and psychological complaints in COVID-19 long-haulers.

1. Introduction

Coronavirus 2019 disease (COVID-19) is characterized by multiple neurological symptoms including anosmia and ageusia, and in more severe cases, encephalitis, cerebral infarctions and syncope[1][2][3]. Recent neuroimaging studies and brain autopsies have shown structural and neurochemical brain abnormalities associated with COVID-19 that may lead to these complications[4][5][6][7][8][9]. The overall observations from these studies suggest that hemorrhagic infractions and moderate to severe microglial activation were the most frequent pathological findings in COVID-19 non-survivors[4][7]. Neuroimaging studies in living COVID-19 patients revealed, in addition to inflammatory and cerebrovascular brain lesions[5][6], also neurometabolic abnormalities in white matter[8] and prefrontal cortex[9][10].

Post-acute COVID-19 neuronal abnormalities such as hypoxic injury, hemorrhage, and non-specific inflammatory effects are likely to be more prevalent in patients with severe illness than in mild cases of COVID-19[8][11]. Long-term neurological and psychiatric conditions were also found in non-hospitalized COVID-19 patients which included besides mood and anxiety disorders[12], reduction in attention and working memory functions[11][12][13]. Cognitive and functional declines found in those COVID-19 long-haulers could be associated, at least in part, by local neurochemical abnormalities such as increased levels of myo-inositol[8] and decreased levels of glutamate and glutamate/glutamine ratio[9] that may not be detected during routine medical examinations. However, most neuroimaging studies on COVID-19 patients often overlook the effects of SARS-CoV-2 infection on brain metabolites even though a large body of evidence exists to support the links between neurometabolic abnormalities and cognitive dysfunctions as consequence of viral infections[14]. Here, we present preliminary observations from proton magnetic resonance spectroscopy (^{1}H-MRS) of the left hippocampus and MRI volumetric measurements of bilateral hippocampal regions in the same individuals before and after SARS-CoV-2 infection.

2. Methods

2.1 Patients and control subjects

Patients were three elderly adults (aged 63, 66 and 68 years; 2 females; Table 1) who, at the time of infection, were participating in a randomized controlled trial (RCT) that was specifically designed to examine the effects of resistance training on brain and blood biomarkers and cognitive/motor functions in elderly adults. All three patients were Lithuanian citizens from the region of Kaunas. All three patients were considered cognitively intact (all scored 28 or higher on the Montreal Cognitive Assessment - MoCA) and physically

abled-bodied with no severe morbidity conditions at the time of inclusion and had no medical history of COVID-19. The experimental protocol was approved by the local Medical Ethics Committee for Biomedical Research (No. BE-10-7), and a written informed consent was obtained from all participants prior to their inclusion in the original study. Before COVID-19 data collection was performed 60-79 days before participants were reported to be diagnosed with the SARS-CoV-2 virus infection. Exposure to high-risk contacts was 3-5 days before onset of symptoms in Patient 1 and 3 days in Patient 2 (no information exists for Patient 3). All three patients presented mild COVID-19 symptoms that did not require hospitalization. Reported illness durations were 12 to 19 days. The second (post COVID-19) ^{1}H-MRS and MRI scanning sessions, blood collection, and behavioral tests were performed 19-31 days after positive COVID-19 Polymerase Chain Reaction (PCR) test was taken, and 7-16 days after patients had been cleared by their general practitioners. Concerning the originally designed study, Patient 1 completed 20 resistance-training sessions and Patient 2 completed 18 resistance-training sessions prior to their diagnosis with COVID-19. Patient 3 was assigned as a passive control in the same study and did not undergo any training program prior to his infection. Clinical assessments for depression and anxiety diagnoses were performed by a qualified, mental health care specialist (co-author SK) using the Hospital anxiety and depression scale (HADS). None of the three patients presented neuro long-COVID symptoms after being declared healthy by their general practitioners. Lastly, we included six elderly adults (age range 60-79 years; 3 females; MoCA ≥ 26) that were randomly selected for the purpose of this study. They were all control subjects in the original RCT. These participants underwent the same data collection protocol as that of the experimental group but did not undergo any training. All six controls had no severe morbidity conditions at the time of their inclusion and, to our knowledge, were not diagnosed with or developed COVID-19 symptoms prior or during their participation in the RCT. The mean (SD) time between first and follow-up scanning sessions was 110 ± 13 days.

2.2 Neuroimage acquisition and processing

MR images and ^{1}H-MRS spectra were acquired on a Siemens 3T MAGNETOM Skyra scanner (Siemens Healthineers, Erlangen, Germany) with a 32-channel receiver head coil. The scanning protocol included: high resolution T1-weighted (T1W) whole brain scan (TR/TE = 2200/2.48 ms, 0.9 × 0.9 × 1.0 mm^3 voxels, field of view: 230 × 256 mm, 176 sagittal slices), T2-weighted (T2W) turbo-spin echo scan, fluid-attenuated inversion recovery (FLAIR), and susceptibility weighted imaging (SWI). Images were reviewed by an experienced radiologist with more than 10 years of experience (co-author KV). ^{1}H-MRS spectra were acquired using a Point Resolved Spectroscopy (PRESS) sequence with the following parameters: TR/TE = 2000/30 ms, 128 averages with and without water suppression, acquired from a 26 × 12 × 12 mm^3 voxel positioned in the left hippocampus. ^{1}H-MRS spectra from the three COVID-19 patients pre- and post-COVID-19 are illustrated in Figure 1.

Table 1: Clinical information and testing chronology of the three patients with COVID-19

Patient 1 (63 yr./F)	*Background:* The patient enrolled into the study in August 2020. She was in good health, has no known chronic illness, and was not taking any medication. First MRI session was performed on the 22nd of August 2020. Laboratory tests were performed on the 27th of August 2020. The patient completed 20 resistance-training sessions prior to her infection with COVID-19. Post COVID-19 MRI and laboratory tests were performed on the 5th and the 9th of December 2020, respectively. *COVID-19 diagnosis & symptoms:* Possible moment of a high risk contact was 3 to 5 days before symptoms. Polymerase chain reaction (PCR) test was performed, upon onset of symptoms, on the 9th of November 2020 and the patient was diagnosed with COVID-19 on the same day. Symptoms included anosmia, ageusia, arthralgia, myalgia, nausea and somnolence. No fever, no blood pressure changes. The patient recovered within 2 weeks and returned to work on the 23rd of November 2020. *Treatment & medication:* The patient was prescribed naproxen 550mg once per day for pain relief for 4 days.
Patient 2 (68 yr./F)	*Background:* The patient enrolled into the study in October 2020. She was diagnosed with glaucoma (medication: travaprost 40µg/mL, daily drops into eyes) but was otherwise in good health and was not taking any peroral medications. First MRI session was performed on the 17th of October, 2020. Laboratory tests were performed on the 23rd of October, 2020. The patient completed 18 resistance-training sessions prior to her infection with COVID-19. Post COVID-19 MRI and laboratory tests were performed on the 16th and the 21st of January 2021, respectively. *COVID-19 diagnosis & symptoms:* Possible moment of a high risk contact was 3 days before symptoms. PCR test was performed (upon onset of symptoms) on the 28th of December 2020 and the patient was diagnosed with COVID-19 on the same day. The symptoms included anosmia, ageusia, somnolence, subfebrile, and hypotension. The patient was declared healthy by her general practitioner (GP) on the 9th of January, 2021. *Treatment & medication:* paracetamol 500mg per day for two days and ibuprofen 400mg every 4 hours for two days. The patient took vitamin C, vitamin D and Zinc supplements.

Table 1: Continued

Patient 3 (66 yr./M)	*Background:* The patient enrolled into the study in October 2020. He has hypertension but was otherwise in good health and was taking ramipril 10mg daily (non-centrally active ACE inhibitor). First MRI session was performed on October 24th, 2020. Laboratory tests were performed on the 29th of October, 2020. The patient was enrolled as a control subject and did not undergo resistance training. Post COVID-19 MRI and laboratory tests were performed on the 23rd and the 27th of January 2021, respectively. *COVID-19 diagnosis & symptoms:* No reported high risk contacts. First symptoms (myalgia, arthralgia) appeared 2-3 days before PCR test. Patient reported subfebrile on the 22nd of December, 2020. PCR test was performed on the 23rd of December, 2020 and the patient was diagnosed with COVID-19 on the same day. The symptoms included anosmia, ageusia, somnolence, headache, subfebrile, and hypotension. The patient was declared healthy by his GP on the 11th of January, 2021. *Treatment & medication*: Single dose of paracetamol 500mg. Vitamin C, vitamin D and Zinc supplements.

Abbreviations: NAA = N-acetyl aspartate; Glx = glutamate-glutamine complex; Cho = total choline; mIns = myo-inositol; Cr = creatine + phosphocreatine.
$ICC_{2,1}$ was calculated using a two-way random model for absolute agreement. Negative ICC values are set to zero. n = number of samples. [1,2]Missing ^{1}H-MRS data from one (NAA, Cho, mIns, and Cr) or two (Glx) healthy controls; for details, see supplemental Table S1. [3]A blood-sample from one healthy control was not collected at follow-up.
Standard error of measurement (SEM) was calculated as follows: SEM = SD(before COVID-19) × $\sqrt{(1 - ICC_{2,1})}$.
Minimum detectable change (MDC) at 90% (MDC_{90}; not shown) and 95% (MDC_{95}) levels of confidence were calculated as follows: $MDC_{95} = 1.96 \times SEM \times \sqrt{2}$; $MDC_{90} = 1.645 \times SEM \times \sqrt{2}$.
Pre-to-post changes of > MDC_{95} were interpreted as true changes (**bold + underlined text**). Pre-to-post percentage difference of > 30% and/or > MDC_{90} were considered as trends (**bold text**).
†Values presented are the absolute (water-referenced) concentrations (in institutional units). Metabolic ratios to Cr are reported in Supplemental Table S2.
‡ Segmented subfield volumes are reported in Supplemental Table S3.
§ Hospital Anxiety and Depression Scale (HADS) scores considered normal if < 8.
# Interleukin 6 (IL-6) normal range: 5-15 pg/ml.

Spectra were processed using the LCModel software package, Version 6.3 (http://www.lcmodel.com/) and data were analyzed to quantify absolute (water-referenced) concentrations of N-acetyl-aspartate (NAA), choline (Cho), myo-inositol (mIns), glutamate-glutamine complex (Glx), and total creatine (Cr). Spectra with FWHM (full width at half maximum) > 0.1 ppm or SNR (signal-to-noise ratio) < 5 were excluded. All neurometabolites were quantified with a Cramér-Rao lower bound (CRLB) < 20%. We were interested specifically in examining changes in levels of NAA, mIns, and Glx from pre- to post-COVID-19 scanning sessions. NAA has been demonstrated to be a predictor of neuronal density and NAA levels decline can be taken as a marker for neuronal damage or neuronal loss[8][9]

[14]. Myo-inositol can be taken as an indicator for astrogliosis and inflammation[8][14]. Glx represents the sum of glutamate which is main neurotransmitter involved in cognitive/motor control[15][16] and glutamine. The aforementioned neurometabolites were found to be valuable biomarkers of cognitive and motor dysfunctions in normal aging[15][16][17] and neurodegenerative disorders[18][19]. One MRS scan from a healthy control was excluded due to low-quality (before COVID-19 scan: FWHM = 0.12 ppm, SNR = 3). Therefore, longitudinal ^{1}H-MRS data were available from only five healthy controls (see Table S1 in the Supplementary Appendix). Test-retest reliability for Glx were estimated from a sample of four subjects (one data point was considered as an outlier; see supplemental Table S1).

Hippocampal volume changes (both left and right) were analyzed using FreeSurfer v7.1.1 (Harvard, MA, USA, http://surfer.nmr.mgh.harvard.edu/). Isotropic 3D T1W images (0.9 mm slice thickness) from 18 scans of nine subjects (i.e., first and follow-up scans from the three COVID-19 patients and the six healthy controls) were first processed separately with the cross-sectional fully automated script. This step was then followed by joint analysis of each subjects' pairs of initial and follow-up scans in the automatic longitudinal analysis pipeline with an additional command to automatically segment the hippocampal subfields. Hippocampal volumes were divided by intracranial volume for each subject to adjust for differences in head size.

2.3 Interleukin-6 (IL-6)

Blood samples (10 ml) were collected via venipuncture at the antecubital vein, and were centrifuged immediately (10 min, at 3500 rpm, 4 °C). Serum was stored at -80°C until the analysis. IL-6 concentrations were measured using a commercially available ELISA kit (DIAsource ImmunoAssays S.A., Belgium, KAP1216). Lower limit of detection was 2 pg/ml. Absorbance was measured using spectrophotometer at 450 nm absorbance.

2.4 Statistical analysis

A single measure two-way random effects model for absolute agreement between first and follow-up measurements from the healthy controls was used to estimate the interclass correlation coefficient (ICC) for test/retest reliability of hippocampal metabolites and volumetric data. The standard error of measurement (SEM) and the minimal detectable change (MDC) values were calculated for each of the neurometabolites and left/right hippocampal volumes, serum IL-6 levels, and HADS scores (Table 2). The MDC values were calculated from SEM at 90% confidence (MDC_{90}) to indicate a trend and at 95% confidence (MDC_{95}) to indicate a real change for individual patients.

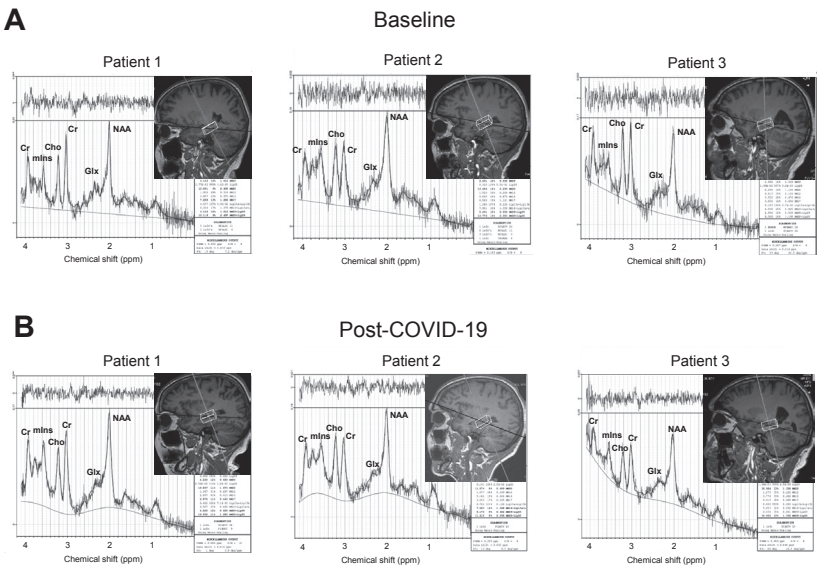


Figure 1. ^{1}H-MRS spectra from the left hippocampus of the three COVID-19 patients before SARS-COV-2 infection (A) and after recovery from COVID-19 (B). Spectral quantification was performed with LCModel (version 6.3). Pre MRS acquisitions were taken 79 days (Patient 1), 72 days (Patent 2), and 60 days (Patient 3) before infections. Post-COVID-19 MRS sessions were performed 12 days (Patient 1 and Patient 3) and 7 days (Patent 2) after the reported recovery (see Table 1 for details). Abbreviations: NAA = N-acetyl aspartate; Glx = glutamate-glutamine complex; Cho = total choline; mIns = myo-inositol; Cr = creatine + phosphocreatine. Corresponding spectrum (black) and LCModel fit (red) from each patient are illustrated.

3. Results

Pre- and post-COVID-19 (patients) or follow-up (controls) measures of the absolute water-referenced concentrations of NAA, Glx, Cr, Cho, and mIns are shown in Table 2A. Our findings indicated alterations in ^{1}H-MRS spectral profiles of the left hippocampus after recovery from COVID-19. Specifically, we observed reductions in Glx concentration in Patients 1 and 2 (≥ 42.0 % reduction from before COVID-19; both > MDC_{95}), a trend towards an increased mIns in Patient 1 (32.4 % elevation; < MDC_{90}) and a visible increase of mIns and NAA levels in Patient 3 (≥ 36.4 % elevation; both > MDC_{90}). No pre-to post-COVID-19 changes or trends were observed for Cho and Cr (all three patients), Glx (Patient 3), mIns (Patient 2), or NAA (Patients 1 and 2). Similar trends were observed when examining pre- and post-COVID measures of metabolite ratios to Cr (see supplemental Table S2) with the exception of reduction of Cho/Cr ratio in Patient 3 (16.6 % reduction; > MDC_{95}).

Both Patients 1 and 2 showed an overall increase in the volume of left hippocampus body (≥ 4.94 % increase from before COVID-19; both > MDC_{95}). A detailed inspection of the hippocampus subfields (see supplemental Table S3) revealed that these increases can be attributed primarily to enlargements of the left CA3-body, left CA4-body, left GC-ML-DG-body, and left parasubiculum regions (≥ 6.85%; all > MDC_{95}). An increase was also noted in the total volumes of left hippocampal head of Patient 1 (4.29 %) and right hippocampal body of Patient 2 (4.07 %); both > $MDC_{95.}$ There were no overall pre to post differences for the change in hippocampal volume of Patient 3. However, inspection of the hippocampus subfields revealed enlargements of right and left parasubiculum (≥ 9.16%) and reduction of the left subiculum body (7.46%); all > MDC_{95}.

Since an increased mIns concentration may be casually linked with elevated expressions of pro-inflammatory cytokines we further tested serum samples obtained from the three patients before and after recovery from COVID-19 to examine if there was an increase in serum levels of interleukin 6 (IL-6) relative to their pre-infection levels. The levels of IL-6 were observed to drop in all three patients (Table 2C). However, elevated levels of IL-6 were found in Patient 3 (76.7 pg/ml) before SARS-COV-2 infection. This suggests that Patient 3 may have developed an inflammatory condition prior to his inclusion in the RCT that was not worsened by the COVID-19 infection.

Finally, all three subjects showed higher scores on the HADS for depression and/or anxiety after recovery from COVID-19 as compared to their before COVID-19 levels (Table 2D). However, changes in HADS-anxiety and HADS-depression categories were not consistent across the three patients. Noticeably, the greatest increase in the combined anxiety and depression scores were observed in Patients 1 (total of + 7 point) and 2 (total of + 6 points) for whom Glx levels were found to decline following their recovery from COVID-19.

Table 2: Longitudinal comparison of left hippocampus metabolite concentrations

	Patient 1			Patient 2			Patient 3		
	Pre-COVID-19	Post-COVID-19		Pre-COVID-19	Post-COVID-19		Pre-COVID-19	Post-COVID-19	
(A) ^{1}H-MRS†									
			Δ (%)			Δ (%)			Δ (%)
NAA	5.33	5.94	+11.3	7.03	5.76	-18.1	4.81	6.57	**+36.4**
Glx	10.9	6.34	**-42.0**	9.18	4.64	**-49.5**	7.90	7.75	-1.89
Cho	1.34	1.58	+14.7	1.96	1.80	-8.36	1.55	1.39	-10.4
mIns	5.44	7.21	**+32.4**	7.60	6.35	-16.4	3.75	5.85	**+56.1**
Cr	6.06	6.50	+7.30	5.85	5.61	-4.17	5.27	5.66	+7.50
(B) MRI-based hippocampal volumetrics (mm^3)‡									
			Δ (%)			Δ (%)			Δ (%)
Left body	1402	1477	**+5.34**	1118	1173	**+4.94**	1245	1215	-2.42
Left head	2005	2092	**+4.29**	1549	1579	+1.97	1865	1866	+0.04
Left total	4119	4310	**+4.63**	3189	3273	+2.63	3641	3605	-0.98
Right body	1455	1470	+0.98	1144	1190	**+4.07**	1270	1279	+0.71
Right head	1816	1816	-0.01	1686	1745	+3.48	1976	2003	+1.41
Right total	4032	4062	+0.74	3441	3568	**+3.67**	3836	3892	+1.46
(C) Inflammatory biomarkers#									
			Δ			Δ			Δ
IL-6 (pg/ml)	10.8	8.80	-2.10	11.7	6.30	-5.4	76.7	39.6	**-37.1**
(D) HADS §									
			Δ			Δ			Δ
Depression	2	3	+1	1	5	**+4**	0	3	+3
Anxiety	2	8	**+6**	2	4	+2	4	4	0

(A), hippocampal volumes **(B)**, serum levels of interleukin 6 (IL-6) **(C)**, and Hospital Anxiety and Depression Scale (HADS) scoring **(D)** before and after infection with SARS-COV-2. Percentage changes relative to before (% Δ) or absolute changes (Δ) from before SARS-COV-2 infection are presented for the three COVID-19 patients (left-hand panel). Test-retest reliability assessments for first and follow-up data from six older controls with no history of COVID-19 (right-hand panel).

Table 2. Longitudinal comparison of left hippocampus metabolite concentrations (*continued*)

			Controls			
	Mean (SD) before	Mean (SD) follow-up	$ICC_{2,1}$ (n)	SEM	MDC_{95}	MDC_{95} (% before)
(A) ^{1}H-MRS†						
NAA	6.16 (0.66)	6.33 (0.50)	0.149 (n=5)[1]	0.61	1.69	27.5
Glx	9.55 (1.09)	9.88 (0.51)	0.527 (n=4)[2]	0.75	2.08	21.7
Cho	1.80 (0.22)	1.87 (0.26)	0 (n=5)	0.22	0.61	33.8
mIns	7.14 (0.90)	7.94 (1.54)	0 (n=5)	0.90	2.49	34.9
Cr	6.19 (0.92)	6.03 (0.64)	0 (n=5)	0.92	2.55	41.2
(B) MRI-based hippocampal volumetrics (mm^3)‡						
Left body	1094 (68)	1111 (69)	0.944 (n=6)	16.1	44.8	4.09
Left head	1656 (163)	1642 (194)	0.979 (n=6)	23.7	65.6	3.96
Left total	3279 (236)	3292 (272)	0.972 (n=6)	39.5	109.4	3.34
Right body	1152 (100)	1149 (113)	0.980 (n=6)	14.2	39.3	3.41
Right head	1701 (171)	1712 (198)	0.960 (n=6)	34.3	95.0	5.58
Right total	3456 (354)	3460 (390)	0.984 (n=6)	44.7	124	3.59
Inflammatory biomarkers#						
IL-6 (pg/ml)	17.2 (11.7)	17.6 (13.9)	0.350 (n=5)[3]	9.41	26.1	
(D) HADS §						
Depression	1.83 (3.06)	3.00 (3.41)	0.840 (n=6)	1.22	3.39	
Anxiety	4.00 (3.52)	4.83 (4.12)	0.601 (n=6)	2.22	6.17	

4. Discussion

We described findings from three recovered COVID-19 patients who underwent ^{1}H-MRS and anatomical MRI scanning of the brain to examine neurometabolic and structural changes that may be caused by the disease. The results of the post-COVID-19 scanning sessions were compared with pre-COVID-19 data, which were obtained from the same individuals prior to their infection by the SARS-CoV-2 virus. Two of the three patients showed a general reduction of Glx concentration in agreement with the findings of a case report by Yesilkaya et al.[9], who reported decreased prefrontal NAA, glutamate, and glutamate/glutamine ratio in a patient after acute COVID-19. In addition, Patient 1 and Patient 3 demonstrated elevated mIns levels, in line with observations from a ^{1}H-MRS study by Rapalino et al.[8] who reported elevated mIns levels in severe COVID-19 patients with leukoencephalopathy during the acute phase of the disease.

Increased levels of mIns is expected to be associated with an increased expression of pro-inflammatory cytokines in astrocytes and microglial cells similar to that seen in HIV infection[14], Alzheimer's disease[18] or neurodegenerative disorders such as amyotrophic lateral sclerosis[19]. Elevated IL-6 levels are commonly reported in COVID-19 patients as an expression of cytokine-triggered inflammatory reaction[20][21][22]. We examined whether increased levels of mIns in Patient 1 and Patient 3 would be accompanied by elevation of serum levels of IL-6. However, we found no association between elevation of mIns and increased serum levels of IL-6 in these two patients. The elevated levels of mIns (but not serum levels of IL-6) in two of three COVID-19 patients in our study could be interpreted as a marker of local neuro-inflammation without a (remaining) systemic inflammatory response; implying that blood samples may be insufficient to detect those neuroinflammatory responses in mild or asymptomatic COVID-19 cases. Therefore, we propose that ^{1}H-MRS should be considered as a diagnostic tool to detect inflammatory foci in the brain of COVID-19 patients with neurological symptoms. Specifically, mIns levels should be considered as potential biomarkers for neurodegenerative abnormalities[8] and persisting cognitive consequences of COVID-19.

Infection of the central nervous system (CNS) can be explained in part as a response to binding of the SARS-CoV-2 virus to angiotensin-converting enzyme 2 (ACE2) receptors in the CNS[3][23]. After binding, the virus is recognized by immune cells on the neural surface that then start an inflammatory response possibly leading to increased glial cell activity and glial inflammation[24][25][26]. Glial inflammation in the hippocampus could partly be associated with the observed elevation of mIns in Patient 3 and more generally explain the observed declines in memory and mental functions or hippocampal volume loss seen in COVID-19 patients during and/or after the acute phase[2][3][10][11][12][14][23][24][25][26][27]. In contrast to our expectation we found no hippocampal volume loss. Rather we observed a consistent increase of hippocampal volume (specifically but not exclusively in the left hippocampal body subfields) in two of our patients (Patients 1 and 2) who were included in a resistance-training program prior to their infection. The question remains open whether exercise can partly protect against pro-inflammatory processes and volume loss[28] that may be caused by COVID-19 infection.

The expression of ACE2 receptors in glutamatergic and GABAergic neurons[23] could suggest that inflammatory reactions triggered by the SARS-CoV-2 virus infection may also interrupt signaling pathways in the pre- and postsynaptic excitatory and inhibitory neurons which could possibly lead to glutamate excitotoxicity as seen in other viral infections[14][29]. Evidence for glutamate excitotoxicity as manifested by elevated levels of glutamate/glutamine in the brain tissue were previously reported in intubated COVID-19 patients[8]. However, we found no evidence for glutamate excitotoxicity in our patients as Glx levels measured in the post-COVID-19 scanning session decreased (Patients 1 and 2) or remained stable (Patient 3) relative to their pre-COVID-19 levels. The decrease in glutamatergic regulation is in line

with findings from TMS studies in COVID-19 long-haulers with neurological symptoms, where cortical hypoexcitability has been described [30][31]. However, to our knowledge, none of our participants developed neurological post-COVID-19 symptoms. Downregulation of glutamatergic and GABAergic neurotransmission (as well as lower levels of glutamate and GABA) has been implicated in a variety of age-related cognitive/motor declines[32], psychiatric disorders[33][34], and neuroplasticity[35] which may partly explain manifestation of persistent neuronal symptoms such as depression, attentional disorders and fatigue after the acute phase of the illness[5][27][36]. Based on the observations from this case study (specifically, increase of HADS-Anxiety and HADS-depression scores in Patients 1 and 2), we suggest that in addition to pro-oxidative and pro-inflammatory effects[37][38], cognitive changes observed in COVID-19 patients may be attributed partly to reduced hippocampal glutamate.

In addition to the possible role of inflammation, recent evidence from neuroimaging studies also suggested that alterations in brain structural and functional properties observed in COVID-19 patients could be caused by conditions with hypoperfusion (e.g., [39]; for a review see Cull et al., 2023 [40]). Thus, it is tempting to speculate that some of the metabolic changes observed in the current study could be explained at least in part by decreased cerebral blood flow (CBF) and hypoperfusion (e.g., [41]). For example, decreased NAA levels in Patient 2 or increased level of Cho in Patient 1. This presumption is supported by evidence from animal models [42][43] and observations in patients with circulatory impairments [44][45][46]). For example, findings from animal studies have shown that decreased cerebral blood flow (CBF) was accompanied by a significant reduction in hippocampal NAA concentration [42][43]. Interventional studies in patients with circulation impairments or at high risk of developing recurrent infraction reported that post-intervention recovery of blood reperfusion in the brain was associated with recovery of NAA signal [44][45] or reduction in choline Cho/Cr and increase of NAA/Cho ratios [46]. We need to emphasize, nevertheless, that the MRI protocol used in our study did not include monitoring of CBF. Therefore, our data cannot provide supporting evidence for a direct link between tissue metabolism and blood perfusion.

It should be noted that this study has several limitations: This study was not planned or preregistered before the results were obtained. Only three COVID-19 patients were included, all three of them suffered only mild symptoms, and we expect potential confounding effects of the exercise program on the results of two of the three patients, as was discussed. Finally, only ^{1}H-MRS changes in the left hippocampus were investigated. Therefore, our results should be regarded as an exploratory basis, which allows readers to draw hypotheses. However, readers should not draw final conclusions.

To summarize we have shown that SARS-CoV-2 infection may cause changes to the neurometabolic state of the hippocampus, indicating neuroinflammation and metabolic abnormalities that may persist beyond the acute phase of the disease. To the best of our knowledge, our study is the first one to offer observations from longitudinal

^{1}H-MRS measures that were acquired from the same subjects before their infection and immediately after the acute phase of COVID-19. The study has some limitations, specifically: small sample size, inclusion of only mild COVID-19 patients, and the possible confounding effects of exercise. Therefore, generalization of the present findings should be made with caution. However, they may serve others to formulate new hypotheses and guide future studies. In addition, we argue that more studies should examine the role of ^{1}H-MRS as a diagnostic, prognostic and predictive tool for assessment of long-lasting neurological and neuropsychiatric impacts of COVID-19.

Acknowledgments

We thank Prof. Kazimieras Pukėnas with his help in statistical analysis. We thank Dr. Mindaugas Kvedaras for the preparation and analysis of the blood samples.

Funding

This work was supported by the Research Council of Lithuania (Lietuvos mokslo taryba; Grant S-SEN-20-5). The sponsor was not involved in study design; collection, analysis and interpretation of the data; in writing of the report; nor in the decision to submit the article for publication.

Disclosure statement

The authors disclose no conflicts of interest

Author contributions

Preparation of first draft: W. Vints (WV) and O. Levin (OL). MRI scanning and processing and interpretation of MR images: K. Valatkevičienė (KV) and S. Jesmanas (SJ). Processing and interpretation of ^{1}H-MRS spectra: A. Weerasekera (AW). Recruitment and examination of patients: S. Kušleikienė (SK) and V. Česnaitienė (VC). Data management, and statistical analyses: SK, VC, and OL. Critical reading and commenting of the manuscript: E-M Ratai (EMR), U. Himmelreich (UH), J. Verbunt (JV), R. Gleiznienė (RG), and N. Masiulis (NM). Preparation of the final manuscript: WV, OL, JV, and NM. All authors were involved in the revision of the draft manuscript and have agreed to the final content.

References

[1] V. Montalvan, J. Lee, T. Bueso, J. De Toledo, K. Rivas, Neurological manifestations of COVID-19 and other coronavirus infections: A systematic review, Clin. Neurol. Neurosurg. 194 (2020) 105921. https://doi.org/10.1016/j.clineuro.2020.105921.

[2] R.E.A. Santos, M.G. da Silva, M.C.B. do Monte Silva, D.A.M. Barbosa, A.L. do V. Gomes, L.C.M. Galindo, R. da Silva Aragão, K.N. Ferraz-Pereira, Onset and duration of symptoms of loss of smell/taste in patients with COVID-19: A systematic review, Am. J. Otolaryngol. - Head Neck Med. Surg. 42 (2021). https://doi.org/10.1016/j.amjoto.2020.102889.

[3] A.S. Zubair, L.S. McAlpine, T. Gardin, S. Farhadian, D.E. Kuruvilla, S. Spudich, Neuropathogenesis and neurologic manifestations of the coronaviruses in the age of coronavirus disease 2019: A review, JAMA Neurol. 77 (2020) 1018–1027. https://doi.org/10.1001/jamaneurol.2020.2065.

[4] T. Coolen, V. Lolli, N. Sadeghi, A. Rovai, N. Trotta, F.S. Taccone, J. Creteur, S. Henrard, J.C. Goffard, O. Dewitte, G. Naeije, S. Goldman, X. De Tiège, Early postmortem brain MRI findings in COVID-19 non-survivors, Neurology. 95 (2020) e2016–e2027. https://doi.org/10.1212/WNL.0000000000010116.

[5] J. Helms, S. Kremer, H. Merdji, M. Schenck, F. Severac, R. Clere-Jehl, A. Studer, M. Radosavljevic, C. Kummerlen, A. Monnier, C. Boulay, S. Fafi-Kremer, V. Castelain, M. Ohana, M. Anheim, F. Schneider, F. Meziani, Delirium and encephalopathy in severe COVID-19: A cohort analysis of ICU patients, Crit. Care. 24 (2020) 1–11. https://doi.org/10.1186/s13054-020-03200-1.

[6] S. Katal, A. Gholamrezanezhad, Neuroimaging findings in COVID-19: A narrative review, Neurosci. Lett. 742 (2021). https://doi.org/10.1016/j.neulet.2020.135529.

[7] S.S. Mukerji, I.H. Solomon, What can we learn from brain autopsies in COVID-19?, Neurosci. Lett. 742 (2021) 135528. https://doi.org/10.1016/j.neulet.2020.135528.

[8] O. Rapalino, A. Weerasekera, S.J. Moum, K. Eikermann-Haerter, B.L. Edlow, D. Fischer, A. Torrado-Carvajal, M.L. Loggia, S.S. Mukerji, P.W. Schaefer, R.G. Gonzalez, M.H. Lev, E.M. Ratai, Brain MR spectroscopic findings in 3 consecutive patients with covid-19: Preliminary observations, Am. J. Neuroradiol. 42 (2021) 37–41. https://doi.org/10.3174/ajnr.A6877.

[9] U.H. Yesilkaya, M. Sen, Y.H. Balcioglu, COVID-19-related cognitive dysfunction may be associated with transient disruption in the DLPFC glutamatergic pathway, J. Clin. Neurosci. 87 (2021) 153–155. https://doi.org/10.1016/j.jocn.2021.03.007.

[10] G. Douaud, S. Lee, F. Alfaro-Almagro, C. Arthofer, C. Wang, F. Lange, J.L.R. Andersson, L. Griffanti, E. Duff, S. Jbabdi, B. Taschler, A. Winkler, T.E. Nichols, R. Collins, P.M. Matthews, N. Allen, K.L. Miller, S.M. Smith, Brain imaging before and after COVID-19 in UK Biobank, MedRxiv (Preprint Server). (2021). https://doi.org/10.1101/2021.06.11.21258690.

[11] A. Nalbandian, K. Sehgal, A. Gupta, M. V. Madhavan, C. McGroder, J.S. Stevens, J.R. Cook, A.S. Nordvig, D. Shalev, T.S. Sehrawat, N. Ahluwalia, B. Bikdeli, D. Dietz, C. Der-Nigoghossian, N. Liyanage-Don, G.F. Rosner, E.J. Bernstein, S. Mohan, A.A. Beckley, D.S. Seres, T.K. Choueiri, N. Uriel, J.C. Ausiello, D. Accili, D.E. Freedberg, M. Baldwin, A. Schwartz, D. Brodie, C.K. Garcia, M.S.V. Elkind, J.M. Connors, J.P. Bilezikian, D.W. Landry, E.Y. Wan, Post-acute COVID-19 syndrome, Nat. Med. 27 (2021) 601–615. https://doi.org/10.1038/s41591-021-01283-z.

[12] E.L. Graham, J.R. Clark, Z.S. Orban, P.H. Lim, A.L. Szymanski, C. Taylor, R.M. DiBiase, D.T. Jia, R. Balabanov, S.U. Ho, A. Batra, E.M. Liotta, I.J. Koralnik, Persistent neurologic symptoms and cognitive dysfunction in non-hospitalized Covid-19 "long haulers," Ann. Clin. Transl. Neurol. 8 (2021) 1073–1085. https://doi.org/10.1002/acn3.51350.

[13] E.A. Troyer, J.N. Kohn, S. Hong, Are we facing a crashing wave of neuropsychiatric sequelae of COVID-19? Neuropsychiatric symptoms and potential immunologic mechanisms, Brain. Behav. Immun. 87 (2020) 34–39. https://doi.org/10.1016/j.bbi.2020.04.027.

[14] L. Chelala, E.E. O'Connor, P.B. Barker, T.A. Zeffiro, Meta-analysis of brain metabolite differences in HIV infection, NeuroImage Clin. 28 (2020) 102436. https://doi.org/10.1016/j.nicl.2020.102436.
[15] A. Weerasekera, O. Levin, A. Clauwaert, K.-F. Heise, L. Hermans, R. Peeters, D. Mantini, K. Cuypers, I. Leunissen, U. Himmelreich, S.P. Swinnen, Neurometabolic Correlates of Reactive and Proactive Motor Inhibition in Young and Older Adults: Evidence from Multiple Regional 1H-MR Spectroscopy, Cereb. Cortex Commun. 1 (2020) 1–16. https://doi.org/10.1093/texcom/tgaa028.
[16] D.R. Roalf, V.J. Sydnor, M. Woods, D.A. Wolk, J.C. Scott, R. Reddy, P.J. Moberg, A quantitative meta-analysis of brain glutamate metabolites in aging, Neurobiol. Aging. 95 (2020) 240–249. https://doi.org/10.1016/j.neurobiolaging.2020.07.015.
[17] N.M. Zahr, D. Mayer, T. Rohlfing, S. Chanraud, M. Gu, E. V Sullivan, A. Pfefferbaum, In vivo glutamate measured with magnetic resonance spectroscopy: behavioral correlates in aging, Neurobiol. Aging. 34 (2013) 1265–1276. https://doi.org/10.1016/j.neurobiolaging.2012.09.014.
[18] D. Huang, D. Liu, J. Yin, T. Qian, S. Shrestha, H. Ni, Glutamate-glutamine and GABA in brain of normal aged and patients with cognitive impairment, Eur. Radiol. 27 (2017) 2698–2705. https://doi.org/10.1007/s00330-016-4669-8.
[19] A. Weerasekera, R. Peeters, D. Sima, T. Dresselaers, S. Sunaert, J. De Vocht, K. Claeys, S. Van Huffel, P. Van Damme, U. Himmelreich, Motor cortex metabolite alterations in amyotrophic lateral sclerosis assessed in vivo using edited and non-edited magnetic resonance spectroscopy, Brain Res. 1718 (2019) 22–31. https://doi.org/10.1016/j.brainres.2019.04.018.
[20] P. Berlit, J. Bösel, G. Gahn, S. Isenmann, S.G. Meuth, C.H. Nolte, M. Pawlitzki, F. Rosenow, B. Schoser, G. Thomalla, T. Hummel, "Neurological manifestations of COVID-19" - guideline of the German society of neurology, Neurol. Res. Pract. 2 (2020) 1–14. https://doi.org/10.1186/s42466-020-00097-7.
[21] E.A. Coomes, H. Haghbayan, Interleukin-6 in Covid-19: A systematic review and meta-analysis, Rev. Med. Virol. 30 (2020) 1–9. https://doi.org/10.1002/rmv.2141.
[22] H. Sun, P. Guo, L. Zhang, F. Wang, Serum interleukin-6 concentrations and the severity of COVID-19 pneumonia: A retrospective study at a single center in Bengbu City, Anhui Province, China, in January and February 2020, Med. Sci. Monit. 26 (2020). https://doi.org/10.12659/MSM.926941.
[23] R. Chen, K. Wang, J. Yu, D. Howard, L. French, Z. Chen, C. Wen, Z. Xu, The Spatial and Cell-Type Distribution of SARS-CoV-2 Receptor ACE2 in the Human and Mouse Brains, Front. Neurol. 11 (2021) 573095. https://doi.org/10.3389/fneur.2020.573095.
[24] L. Da Silva Chagas, P.C. Sandre, P.C. De Velasco, H. Marcondes, N.C.A. Ribeiro E Ribeiro, A.L. Barreto, L.B. Alves Mauro, J.H. Ferreira, C.A. Serfaty, Neuroinflammation and Brain Development: Possible Risk Factors in COVID-19-Infected Children, Neuroimmunomodulation. 28 (2021) 22–28. https://doi.org/10.1159/000512815.
[25] A.M. Mahalakshmi, B. Ray, S. Tuladhar, A. Bhat, S. Paneyala, D. Patteswari, M.K. Sakharkar, H. Hamdan, D.M. Ojcius, S.R. Bolla, M.M. Essa, S.B. Chidambaram, M.W. Qoronfleh, Does COVID-19 contribute to development of neurological disease?, Immunity, Inflamm. Dis. 9 (2021) 48–58. https://doi.org/10.1002/iid3.387.
[26] Y. Yachou, A. El Idrissi, V. Belapasov, S. Ait Benali, Neuroinvasion, neurotropic, and neuroinflammatory events of SARS-CoV-2: understanding the neurological manifestations in COVID-19 patients, Neurol. Sci. 41 (2020) 2657–2669. https://doi.org/10.1007/s10072-020-04575-3.
[27] E. Garrigues, P. Janvier, Y. Kherabi, A. Le Bot, A. Hamon, H. Gouze, L. Doucet, S. Berkani, E. Oliosi, E. Mallart, F. Corre, V. Zarrouk, J.D. Moyer, A. Galy, V. Honsel, B. Fantin, Y. Nguyen, Post-discharge persistent symptoms and health-related quality of life after hospitalization for COVID-19, J. Infect. 81 (2020) e4–e6. https://doi.org/10.1016/j.jinf.2020.08.029.
[28] C.W. Cotman, N.C. Berchtold, L.-A. Christie, Exercise builds brain health: key roles of growth factor cascades and inflammation, TRENDS Neurosci. 30 (2007) 464–472. https://doi.org/10.1016/j.tins.2007.06.011.

[29] A.M. Gorska, E.A. Eugenin, The Glutamate System as a Crucial Regulator of CNS Toxicity and Survival of HIV Reservoirs, Front. Cell. Infect. Microbiol. 10 (2020) 261. https://doi.org/10.3389/fcimb.2020.00261.

[30] P. Ortelli, F. Benso, D. Ferrazzoli, I. Scarano, L. Saltuari, L. Sebastianelli, V. Versace, R. Maestri, Global slowness and increased intra-individual variability are key features of attentional deficits and cognitive fluctuations in post COVID-19 patients, Sci. Reports 2022 121. 12 (2022) 1–9. https://doi.org/10.1038/s41598-022-17463-x.

[31] P. Manganotti, M. Michelutti, G. Furlanis, M. Deodato, A. Buoite Stella, Deficient GABABergic and glutamatergic excitability in the motor cortex of patients with long-COVID and cognitive impairment, Clin. Neurophysiol. 151 (2023) 83–91. https://doi.org/10.1016/J.CLINPH.2023.04.010.

[32] E.C. Porges, A.J. Woods, R.A.E. Edden, N.A.J. Puts, A.D. Harris, H. Chen, A.M. Garcia, T.R. Seider, D.G. Lamb, J.B. Williamson, R.A. Cohen, Frontal Gamma-Aminobutyric Acid Concentrations Are Associated With Cognitive Performance in Older Adults, Biol. Psychiatry Cogn. Neurosci. Neuroimaging. 2 (2017). https://doi.org/10.1016/j.bpsc.2016.06.004.

[33] J. Kaminski, L. Mascarell-Maricic, Y. Fukuda, T. Katthagen, A. Heinz, F. Schlagenhauf, Glutamate in the Dorsolateral Prefrontal Cortex in Patients With Schizophrenia: A Meta-analysis of 1H-Magnetic Resonance Spectroscopy Studies, Biol. Psychiatry. 89 (2021) 270–277. https://doi.org/10.1016/j.biopsych.2020.09.001.

[34] C. Wenneberg, M. Nordentoft, E. Rostrup, L.B. Glenthøj, K.B. Bojesen, B. Fagerlund, C. Hjorthøj, K. Krakauer, T.D. Kristensen, C. Schwartz, R.A.E. Edden, B.V. Broberg, B.Y. Glenthøj, Cerebral Glutamate and Gamma-Aminobutyric Acid Levels in Individuals at Ultra-high Risk for Psychosis and the Association With Clinical Symptoms and Cognition, Biol. Psychiatry Cogn. Neurosci. Neuroimaging. 5 (2020) 569–579. https://doi.org/10.1016/j.bpsc.2019.12.005.

[35] S.F. Cooke, T.V.P. Bliss, Plasticity in the human central nervous system, Brain. 129 (2006) 1659–1673. https://doi.org/10.1093/brain/awl082.

[36] A. Nath, B. Smith, Neurological issues during COVID-19: An overview, Neurosci. Lett. 742 (2021) 135533. https://doi.org/10.1016/j.neulet.2020.135533.

[37] A.P. Bouças, J. Rheinheimer, J. Lagopoulos, Why Severe COVID-19 Patients Are at Greater Risk of Developing Depression: A Molecular Perspective, Neuroscientist. (2020). https://doi.org/10.1177/1073858420967892.

[38] I. Crespo, A. Santos, B. Gómez-Ansón, O. López-Mourelo, P. Pires, Y. Vives-Gilabert, S.M. Webb, E. Resmini, Brain metabolite abnormalities in ventromedial prefrontal cortex are related to duration of hypercortisolism and anxiety in patients with Cushing's syndrome, Endocrine. 53 (2016) 848–856. https://doi.org/10.1007/s12020-016-0963-0.

[39] M. Ajčević, K. Iscra, G. Furlanis, M. Michelutti, A. Miladinović, A. Buoite Stella, M. Ukmar, M.A. Cova, A. Accardo, P. Manganotti, Cerebral hypoperfusion in post-COVID-19 cognitively impaired subjects revealed by arterial spin labeling MRI, Sci. Reports 2023 131. 13 (2023) 1–9. https://doi.org/10.1038/s41598-023-32275-3.

[40] O. Cull, L. Al Qadi, J. Stadler, M. Martin, A. El Helou, J. Wagner, D. Maillet, L. Chamard-Witkowski, Radiological markers of neurological manifestations of post-acute sequelae of SARS-CoV-2 infection: a mini-review, Front. Neurol. 14 (2023) 1233079. https://doi.org/10.3389/FNEUR.2023.1233079/BIBTEX.

[41] D. Lythgoe, A. Simmons, A. Pereira, M. Cullinane, S. Williams, H.S. Markus, Magnetic resonance markers of ischaemia: their correlation with vasodilatory reserve in patients with carotid artery stenosis and occlusion, J. Neurol. Neurosurg. Psychiatry. 71 (2001) 58–62. https://doi.org/10.1136/JNNP.71.1.58.

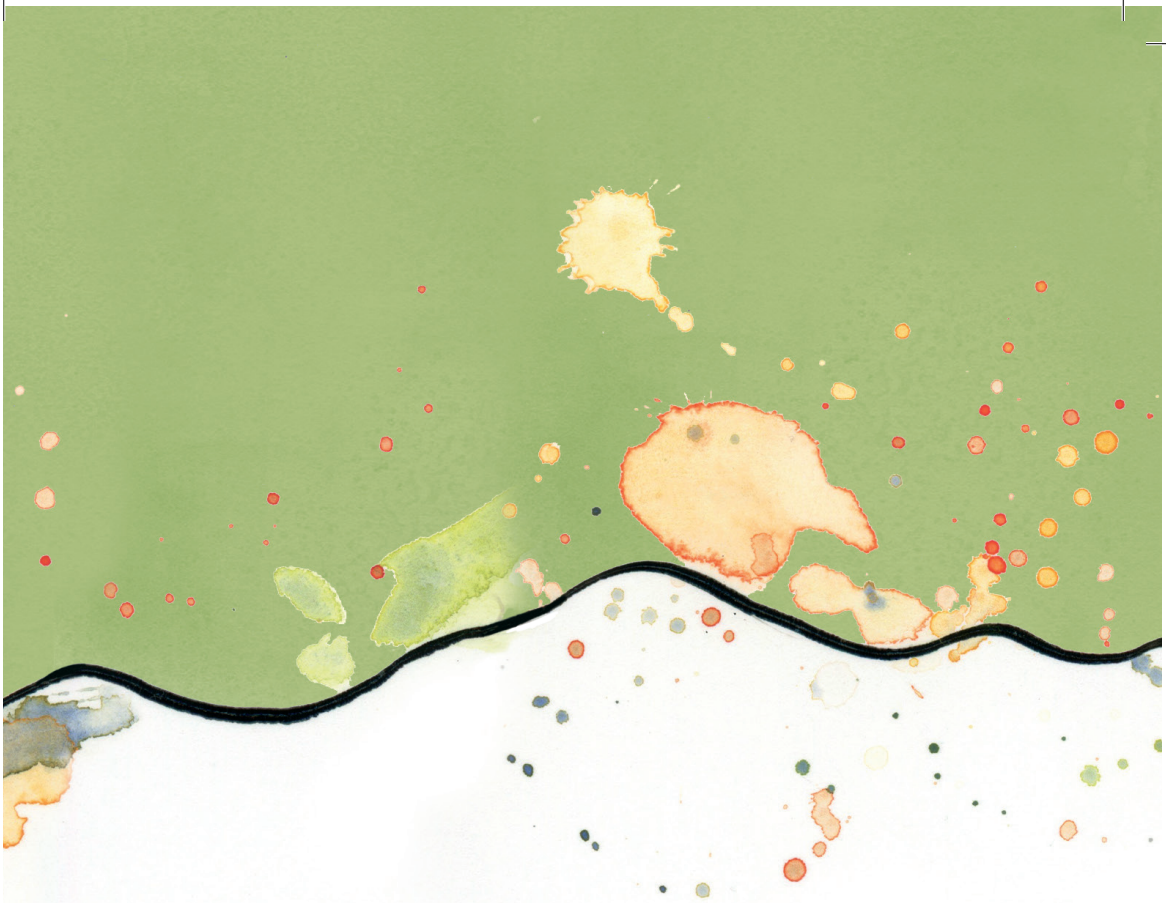
[42] S.O. Ajamu, R.C. Fenner, Y.N. Grigorova, D. Cezayirli, C.H. Morrell, E.G. Lakatta, M. Bouhrara, R.G. Spencer, O. V. Fedorova, K.W. Fishbein, Association of central arterial stiffness with hippocampal blood flow and N-Acetyl aspartate concentration in hypertensive adult Dahl salt sensitive rats, J. Hypertens. 39 (2021) 2113–2121. https://doi.org/10.1097/HJH.0000000000002899.

[43] H. Jian, W. Yi-Fang, L. Qi, H. Xiao-Song, Z. Gui-Yun, Cerebral blood flow and metabolic changes in hippocampal regions of a modified rat model with chronic cerebral hypoperfusion, Acta Neurol. Belg. 113 (2013) 313–317. https://doi.org/10.1007/S13760-012-0154-6/METRICS.

[44] Y.P. Cho, J.H. Lee, G.E. Kim, Metabolic Changes after Revascularization in a Patient with Innominate Artery Occlusion by Localized in vivo Proton Magnetic Resonance Spectroscopy, J. Korean Med. Sci. 18 (2003) 267–271. https://doi.org/10.3346/JKMS.2003.18.2.267.

[45] C. Laredo, A. Rodríguez, L. Oleaga, M. Hernández-Pérez, A. Renú, J. Puig, L.S. Román, A.M. Planas, X. Urra, Á. Chamorro, Adjunct Thrombolysis Enhances Brain Reperfusion following Successful Thrombectomy, Ann. Neurol. 92 (2022) 860–870. https://doi.org/10.1002/ANA.26474.

[46] G.E. Kim, H.J. Lee, Y.P. Cho, S.T. Kim, Metabolic changes in the ischemic penumbra after carotid endarterectomy in stroke patients by localized in vivo proton magnetic resonance spectroscopy (H-MRS), Cardiovasc. Surg. 9 (2001) 345–355. https://doi.org/10.1016/S0967-2109(01)00016-3.



PART IV

The exercise-cognition relationship applied to individuals with spinal cord injury

CHAPTER 9

Myokines may target accelerated cognitive aging in people with spinal cord injury: a systematic and topical review

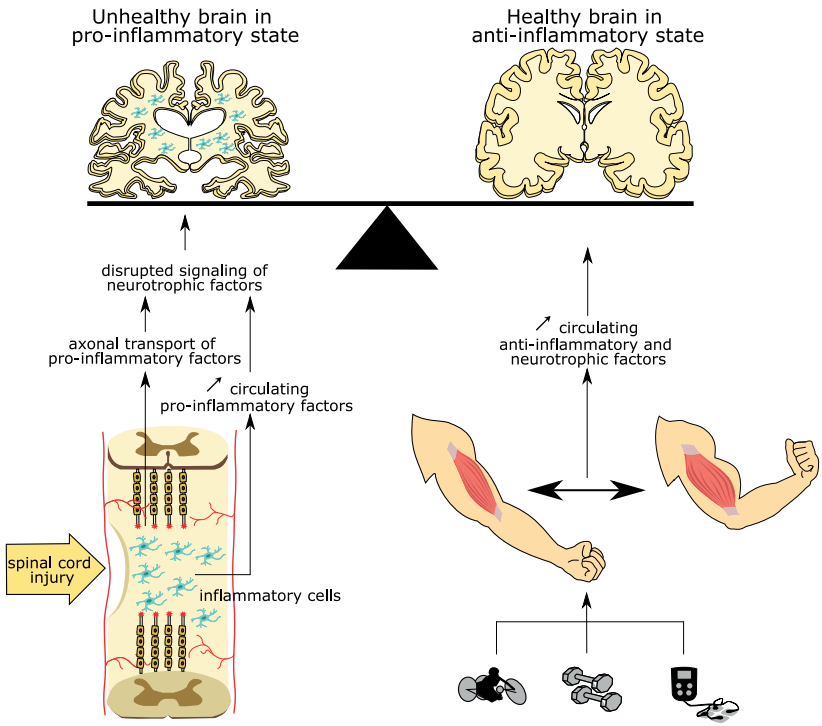
Authors: Wouter A.J. Vints, Oron Levin, Nerijus Masiulis, Jeanine Verbunt, Charlotte C.M. van Laake-Geelen

Published in Neuroscience & Biobehavioral reviews, 2023, Vol. 146, p.105065
https://doi-org.mu.idm.oclc.org/10.1016/j.neubiorev.2023.105065

Abstract

Persons with spinal cord injury (SCI) can suffer accelerated cognitive aging, even when correcting for mood and concomitant traumatic brain injury. Studies in healthy older adults have shown that myokines (i.e. factors released from muscle tissue during exercise) may improve brain health and cognitive function. Myokines may target chronic neuroinflammation, which is considered part of the mechanism of cognitive decline both in healthy older adults and SCI. An empty systematic review, registered in PROSPERO (CRD42022335873), was conducted as proof of the lack of current research on this topic in people with SCI. Pubmed, Embase, Cochrane and Web of Science were searched, resulting in 387 articles. None were considered eligible for full text screening. Hence, the effect of myokines on cognitive function following SCI warrants further investigation. An in-depth narrative review on the mechanism of SCI-related cognitive aging and the myokine-cognition link was added to substantiate our hypothetical framework. Readers are fully updated on the potential role of exercise as a treatment strategy against cognitive aging in persons with SCI.

Graphical abstract



Abbreviations

AIS, ASIA Impairment Scale; ASIA, American Spinal Injury Association; BDNF, brain-derived neurotrophic factor; CCL21, cysteine chemokine ligand 21; CT, computed tomography; FGF-21, fibroblast growth factor-21; FGF-2, fibroblast growth factor-2; HR, hazard ratio; fMRI, functional magnetic resonance imaging; IGF-1, insulin-like growth factor-1; IL-6, interleukin-6; LTP, long-term synaptic potentiation; MRI, magnetic resonance imaging; PHQ-9, Patient Health Questionnaire-9; SCI, spinal cord injury; SDMT, Symbol Digit Modalities Test; TBI, traumatic brain injury; TNF-α, tumor necrosis factor alpha.

1. Background

Spinal cord injury (SCI) is known to have an association with cognitive impairment (Sachdeva et al., 2018; Y. Li et al., 2020a). However, any cognitive impairment in persons with SCI has long been considered to be caused by coinciding traumatic brain injury (TBI) (Sachdeva et al., 2018; Y. Li et al., 2020a). In 2013, the World Health Organization reported that worldwide up to 90% of SCI cases are due to physical trauma, often resulting in concomitant TBI. Nevertheless, they noted that the proportion of non-traumatic cases is growing (World Health Organization (WHO), 2013). This might have led to recent findings that also patients with non-traumatic SCI suffer from cognitive impairment (Sachdeva et al., 2018; Y. Li et al., 2020a). While TBI remains the most important cause of cognitive impairment in persons with SCI, recent studies suggest a spreading secondary neuroinflammatory reaction arising from the SCI lesion site may be a major contributor to cognition decline, also in non-traumatic cases (Wu et al., 2016; F. Li et al., 2020). Indeed, the review of Sun et al. (2016) reports that persons with SCI suffer from chronic peripheral and central inflammation (Sun et al., 2016). Of interest, chronic systemic inflammation is known to impair neurotrophic signaling, synaptic plasticity and neurogenesis (Bourgognon and Cavanagh, 2020). As chronic low grade inflammation is also considered a major player in the process of age-related cognitive decline (i.e. cognitive aging) in healthy adults (Lind et al., 2020; Vints et al., 2022a), it was argued that ongoing systemic inflammation may cause an acceleration of cognitive aging in persons with SCI. This is supported by studies showing that brain structure and function in persons with SCI differ from healthy age-matched controls, but resembles that of much older subjects (Wrigley et al., 2009; Seif et al., 2018; Ziegler et al., 2018; Wylie et al., 2020).

In healthy adults, multiple studies have reported that exercise programs can improve cognitive function (Colcombe and Kramer, 2003). This beneficial effect may be caused by several anti-inflammatory and neurotrophic factors that seem to be released into the bloodstream during muscle contractions, also referred to as myokines. Myokines were suggested to potentiate neurotrophic signaling, synaptic plasticity, neurogenesis and cognitive function in an impressive array of research in healthy persons (Pedersen, 2019). Based on this evidence, we here propose a hypothetical model for muscle activity as a preventive or treatment strategy to boost myokine levels in the blood circulation, hereby possibly combatting SCI-associated accelerated cognitive aging (Figure 1).

In this systematic and topical review, our primary aim was to systematically search for evidence for the role of myokines as mediators of exercise-induced cognitive improvements in people with SCI. We searched the databases of Pubmed, Embase, Web of Science, and Cochrane. Our search yielded zero eligible studies (i.e. an empty systematic review), proving the lack of research on this topic. We subsequently decided to add a

topical review with the secondary aim to inform readers about the mechanisms behind accelerated cognitive aging in persons with SCI and the benefit that may result from physical exercise interventions. Finally, we argue that our in-depth work provides all evidence needed for readers to understand the potential of exercise-induced myokines as a non-pharmacological, low risk and low cost treatment against accelerated cognitive aging in people with SCI.

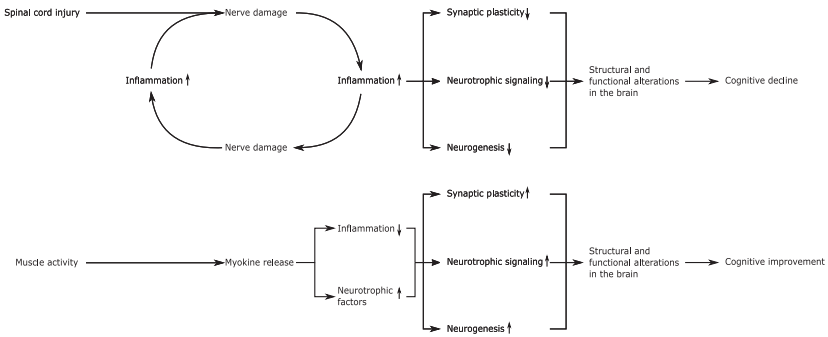


Figure 1. Hypothetical model for SCI-associated cognitive decline and muscle activity-induced cognitive improvement. Nerve damage in spinal cord injury leads to increased inflammation, which may result in a vicious circle that causes expanding nerve damage. Neuroinflammation impairs neurotrophic factor signaling, synaptic plasticity and neurogenesis, eventually resulting in structural and functional brain alterations and cognitive decline. Muscle activity counteracts this process via the release of myokines with anti-inflammatory and neurotrophic effects.

2. Empty systematic review

2.1 Methods

The methods of this systematic review were pre-registered with PROSPERO (CRD42022335873). Our research question was as follows: *Does the exercise-induced release of myokines induce cognitive improvements in spinal cord injury subjects?*

2.1.1 Eligibility criteria

We included human studies on acute (< 1 year postinjury) or chronic (> 1 year postinjury) spinal cord injury participants. Animal studies, or studies solely including patients with progressive neurological disorders such as multiple sclerosis, even if the neurological disorder may also possibly affect the spinal cord, were excluded. The study intervention needed to consist of a single bout (i.e. acute) or multiple bouts (i.e. chronic) of voluntary or electrically supported exercise. Included studies needed to be controlled, with a

control group undergoing rest, no exercise or another exercise intervention. Studies without pre-to-post exercise comparisons were excluded. The outcome of the included studies had to be a form of cognitive assessment or test score and the study needed to measure serum or plasma myokines levels.

2.1.2 Search details

We searched the databases of Pubmed, Embase, Web of Science and Cochrane. The last search was conducted on the 28th of May 2022. A full overview of the exact search terms we used is provided in Appendix 1. The search consisted of a merge of four key concepts, each combined using the AND-function, including exercise AND spinal cord injury AND myokines AND cognition. A filter was used to rule out animal studies where possible (i.e. in the Pubmed, Embase and Web of Science databases). A selection of myokines with probable effects on cognitive function were considered as search terms, based on current knowledge as suggested by previous literature (Pedersen et al., 2007; Woodbury and Ikezu, 2014; Sa-nguanmoo et al., 2016; Adhikary et al., 2019; Lin et al., 2019; Vints et al., 2022b). They included brain-derived neurotrophic factor (BDNF), insulin-like growth factor-1 (IGF-1), cytokines, adiponectin, cathepsin-B, irisin, apelin, fibroblast growth factor-21 (FGF-21), fibroblast growth factor-2 (FGF-2) and myostatin.

2.1.3 Study selection

Two of the authors reviewed the literature. One author (WV) screened the literature and the other author (CvL) checked the decisions. The second author was not blinded to the first author's decisions. In case of disagreement, a third author (OL) was requested to make the decision. The articles were first gathered in Endnote, where duplicates were removed. Next, title and abstract of all the remaining articles were transferred to Excel (presented in Appendix 2). Here, articles were marked included or excluded and the reason for in- or exclusion was recorded.

2.1.4 Data extraction

We planned to extract study design, participant characteristics (number, age, sex, time since spinal cord injury, American Spinal Injury Association (ASIA) Impairment Scale (AIS) grade), intervention characteristics (type of exercise, intensity, duration, frequency, type of control condition, exertion of musculature below or above level of injury, completely voluntary exertion or supported by e.g. electrical stimulation, etc.), and the main outcome findings. The main outcomes of interest were a pre-to-post exercise change in response time, throughput or accuracy on cognitive tests and/or a pre-to-post exercise change in serum or plasma myokine levels.

2.1.5 Quality assessment

The planned quality assessment as pre-registered with PROSPERO (CRD42022335873) was not performed as no studies were found eligible for inclusion.

2.2 Results

Our search resulted in 387 records. After removal of duplicates, 334 records remained to be screened. Based on title and abstract none of the articles remained eligible for full-text assessment (Figure 2). Reasons for exclusion were duplicate records (n=53), study population including animals or humans without spinal cord injury, n=233), study intervention not involving exercise (n=56), study outcome not including cognitive function (n=10) and the study design not involving pre-to-post exercise comparisons, also excluding review papers (n=35). As none of the articles were eligible for inclusion in our systematic review no quality assessment was performed.

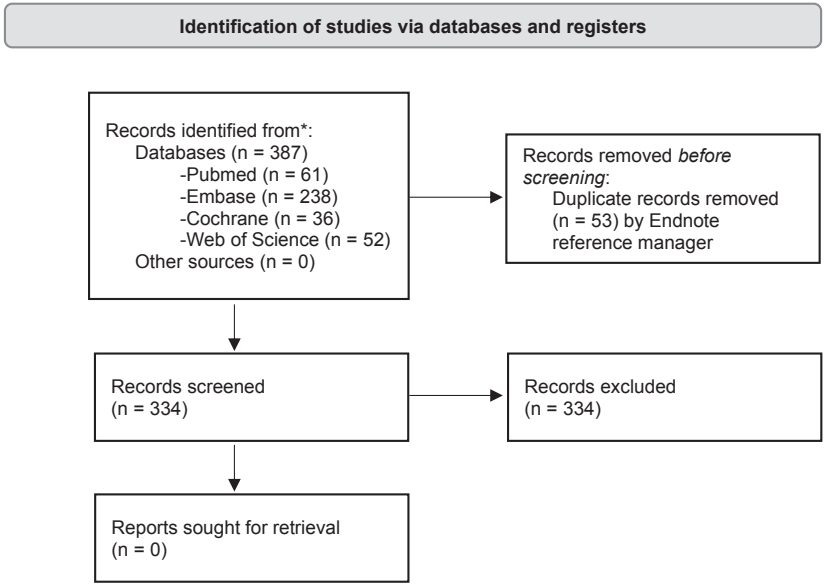


Figure 2. PRISMA 2020 flow diagram for new systematic reviews which includes searches of databases and registers

Table 1 Exercise-induced changes in myokine levels or cognition in people with SCI

Author	Participants (completeness, level of injury, total number, number in EG, male/female in EG, age, time since injury)	Exercise (type, intensity, duration, healthy/paretic muscles)
Exercise-myokine studies in SCI		
Alajam et al. 2020 (Alajam et al., 2020)	SCI (n = 11) , T1-L2, AIS A-D, m/f = 8/3, mean age 38.1, >1y post-injury mean 8.7y	Chronic, walking on treadmill with body-weight support and assistive gait training device, 8 weeks
An et al. 2020 (An et al., 2020)	SCI (n = 6) and amputation (n = 2) and polio (n = 1), total n = 9, m/f = 9/0, mean 34.5y old	Acute, wheelchair basketball competition and interval training
da Silva Alves et al. 2021 (Alves et al., 2021)	Physically active, SCI with complete paraplegia, wheelchair-bound below T8 (n = 9) and healthy (n = 8), m/f = 17/0, mean age 29.0 for SCI and 27.0 for healthy, > 1y post injury	Three treadmill exercise sessions (persons with SCI in wheelchair, able-bodied running) at ventilatory threshold 1, followed by (in random order) 15% below and 15% above VT1, for a mean of 30min
Donia et al., 2019 (Donia et al., 2019)	SCI (n = ?) and multiple sclerosis (n = ?), n = 13, m/f = 3/10, mean age 57, > 1y post-injury or diagnosis	Acute, recumbent hybrid arm-leg ergometer machine, 60% VO2peak, 30 min
Goldhardt et al. 2019 (Goldhardt et al., 2019)	SCI C4-L4 (n = 3 tetraplegia) AIS A or B, n = 10, m/f = 5/5, mean age 40.6, > 6 months (mean 3.5y) after injury	Acute, treadmill at 4.5km/h with body weight support, 60min, 1 week later, 60min gait training with a floor walker, at own pace but encouraged to go as fast as possible
Han et al., 2016 (Han et al., 2016)	SCI C4-L2 AIS A-D, n = 11 and able-bodied controls n = 11, m/f = 11/0, mean age 39.9, mean 14 years post-injury	Acute, arm ergometry cardiopulmonary exercise test with 10 W/min ramp protocol, 60-70 revolutions/min, until exhaustion
	SCI C4-L2 AIS A-D, n = 5, m/f = 5/0, mean age 39.6, mean 14 years post-injury	Chronic, arm ergometry exercise, around anaerobic threshold, 30 min/d, 3x/we, 12 weeks

	CG	Outcome measures	Outcome
	No control	HbA1c, HDL, LDL, CRP, IL-6 before and after the training period, at 24h, 2d, 3d, and 4d	↘ HbA1c, LDL, IL-6; ↗ HDL; ↔ CRP
	No rest group, controlled for supplement or placebo	IL-6, TNF-α, CK before and immediately after exercise	↘ IL-6 after exercise in the leucine-enriched essential amino acid-treated group compared to placebo
	No rest group, controlled for SCI versus healthy	IL-1ra, IL-1β, IL-2, IL-4, IL-6, IL-10, TNF-α before, immediately after and 30min after each exercise session	SCI vs healthy: ↗IL-2 and ↘IL-4 and IL-10 in SCI compared to healthy at before, immediately after and 30min after exercise, ↘ IL-1ra in SCI compared to healthy immediately after exercise in all three conditions; In healthy: ↗IL-6 immediately after exercise at VT1 compared to before exercise In SCI: ↔ IL-6
	No control	IL-6, TNF-α, IFN-γ, IL-1RA, TRP, KYN, KYN/TRP, before, immediately after and 1 hour post-exercise	↘ TNF-α 1h post-exercise compared with pre and immediately post exercise, ↔ IL-6, IFN-γ, IL-1RA, TRP, KYN, KYN/TRP
	No control	BDNF, histone acetylation status, oxidative stress markers, before and immediately after exercise	↔ BDNF and histone acetylation in all conditions, ↗ plasma advanced oxidation protein products (AOPPs), nitrite and thiobarbituric acid-reactive substances after treadmill training, and AOPP, nitrite concentrations, glutathione and catalase activity after floor walker training
	No rest group, controlled with able-bodied individuals	myostatin, IGF-1, and follistatin, pre vs post-exercise	↔ myostatin, IGF-1, follistatin post-exercise for SCI subjects; significant difference with control subjects for IGF-1 who showed significant ↗ in IGF-1
	No control	myostatin, IGF-1, and follistatin, pre vs post-exercise	↗ myostatin, ↔ IGF-1, follistatin post-exercise

Table 1 Continued

Author	Participants (completeness, level of injury, total number, number in EG, male/female in EG, age, time since injury)	Exercise (type, intensity, duration, healthy/paretic muscles)
Kouda et al. 2012 (Kouda et al., 2012)	Complete SCI C6-C7 AIS A, n = 8 and able-bodied controls n = 8, m/f = 8/0, mean age 37.1 for SCI and 32.4 for healthy, 70-76 months after injury	Acute, arm crank ergometer, at 60% VO2 max, 20min
Leech et al., 2017 (Leech and Hornby, 2017)	Incomplete SCI above T10, n = 11, m/f = 9/2, mean age 41, > 6m post-injury (mean 103m)	Acute, graded-intensity treadmill walking exercise, with measurements reported as at 33% of peak speed, at 66% of peak speed and at 100% of peak speed, duration ranged from 8-32min
Nishimura et al. 2022 (Nishimura et al., 2022)	Wheelchair athletes with SCI (n = 17), AIS A, m/f = 17/0, mean age 35 for cervical lesions and mean age 56 for the other patients, > 1y after injury mean 161 months for cervical lesions and 381 for the other patients	Acute, wheelchair half-marathon race
Ogawa et al. 2014 (Ogawa et al., 2014)	Wheelchair athletes with SCI (n = 14), 6 cervical lesions and 8 thoracic or lumbar, AIS A, m/f = 14/0, mean age 35.8 for cervical lesions and 42.7 for the other patients, >1y post-injury mean 162.3 months for cervical lesions and 307.8 for the other patients	Acute, wheelchair marathon race
Paulson et al. 2013 (Paulson et al., 2013)	Wheelchair athletes with SCI (n = 8 C6-C7, n = 10 T6-L1) and non-SCI controls (n = 8), AIS A, m/f = 26/0, mean age SCI 30, non-SCI 27, paraplegia mean 14y post-injury, tetraplegia mean 11 years post-injury	Acute, graded-intensity treadmill exercise in a wheelchair till exhaustion, around 5 min warm-up and 8 min graded-intensity exercise testing
Paulson et al. 2014 (Paulson et al., 2015)	Motor complete SCI (n = 5), T5-6, after completion of a 3month supervised FES-evoked training of 2-3d/week, m/f = 4/1, mean age 44, > 1y post-injury mean 8y	Acute, handcycling with and without functional electrical stimulation-evoked lower-limb cycling in random order with 7-14d in between sessions, 30min

	CG	Outcome measures	Outcome
	No rest group, controlled with able-bodied individuals	IL-6, adrenaline, prostaglandin E2, and cortisol immediately, 1h and 2h after exercise	SCI vs healthy: baseline ↗ IL-6, ↘ adrenaline and PGE2 in SCI; SCI: ↔ IL-6, adrenaline, cortisol, ↗ PGE2 immediately, 1h and 2h after exercise; healthy: ↗ IL-6 at 1h, ↗ adrenaline immediately, ↔ PGE2 and cortisol
	No control	BDNF, IGF-1 and blood lactate levels from 3 blood samples immediately before exercise, 1 during last 30s of each speed increment, 1 at 15min and 30min post-exercise	Highest % ↗ in BDNF during high-intensity (100% of peak speed), positively correlated with blood lactate levels, ↔ IGF-1
	No control	BDNF, adrenaline, noradrenaline, cortisol before, immediately after and 1h after exercise	↗ BDNF immediately after exercise, in patients with thoracic or lumbar lesion level also ↗ (nor)adrenaline and cortisol immediately and 1h after exercise
	No control	IL-6, TNF-α, adrenaline before, immediately after and 2h after	↗ IL-6 immediately after, in patients with thoracic and lumbar lesion level, also ↗ adrenaline immediately after exercise, ↘ TNF-α in patients with cervical lesion level 2h after exercise
	No rest group, comparisons between paraplegia, tetraplegia and non-SCI	IL-6, IL-10, IL-1ra, TNF-α, adrenaline, cortisol pre-, immediately post-, and 30min post-exercise	IL-6 ↗ post-, and 30min post-exercise in paraplegia and non-SCI; adrenaline ↗ immediately post-exercise in paraplegia and non-SCI; cortisol ↗ immediately post-, and 30min post-exercise in all groups; ↔ IL-10, IL-1ra, TNF-α
	No rest group, comparisons between exercise with or without functional electrical stimulation	IL-6, IL-10, IL-1ra, adrenaline, cortisol pre-, immediately post-, 1h post- and 2h post-exercise	↗ IL-6 1h after without and 1h and 2h after exercise in exercise with functional electrical stimulation, adrenaline and cortisol in both conditions immediately after exercise; ↔ IL-10, IL-1ra

Table 1 Continued

Author	Participants (completeness, level of injury, total number, number in EG, male/female in EG, age, time since injury)	Exercise (type, intensity, duration, healthy/paretic muscles)
Rojas Vega et al., 2008 (Rojas Vega et al., 2008)	Marathon athletes with SCI T4-T12 AIS A and B, n = 11, m/f = 11/0, mean age 41, chronic stage	Acute, handbiking, moderate intensity for 10min (54%max heart rate) followed by high intensity (89% max heart rate) for 42km
Rosety-Rodrigues et al. 2014 (Rosety-Rodriguez et al., 2014)	Complete SCI (n = 17), below T6, 9 exercise and 8 control, m/f = 17/0, mean age exercise group 29.6 control group 30.2, 4-5y post-injury	Chronic, arm-crank exercise, 3d/week, 10-15min warm-up 20-30min arm crank increasing 2min30s every 3 weeks, 50-65% HRR increasing 5% every 3 weeks 5-10min cool-dow, 12 weeks
Sasaki et al. 2014 (Sasaki et al., 2014)	Wheelchair athletes with SCI (n = 28), T7-L2, m/f = 28/0, mean age full marathon 40.9 half marathon 46.6	Acute, wheelchair marathon race (n = 16) or half marathon race (n = 12)
Umemoto et al. 2011 (Umemoto et al., 2011)	SCI T6-T10 (n = 6) and healthy (n = 7), m/f = 13/0, mean age SCI 30.7, healthy 29.4	Acute, arm crank ergometer, at 60% VO2 max, 120min
Zeller et al. 2015 (Zeller et al., 2015)	Wheelchair rugby athletes with SCI (n = 11), lesion level C5-7, AIS A-B, m/f = 11/0, mean age 31.7, active in wheelchair rugby for mean of 5.7y	Acute, warm-up consisting of continuous pushing 10min, submaximal sprints and agility drills, followed by the main training lasting 45min consisting of ball handling, passing drills, scrimmage activity and tactical practice, continuous pushing as cool-down, total duration 90min

	CG	Outcome measures	Outcome
	No control	BDNF, IGF-1, prolactin, cortisol Immediately following moderate intensity warm-up and immediately following marathon	Moderate intensity: ↗ BDNF, ↗ IGF-1, ↔ prolactin and cortisol High intensity for marathon distance: ↘ BDNF, ↗ IGF-1, prolactin and cortisol
	Rest group	leptin, adiponectin, plasminogen activator inhibitor-1, TNF-α, IL-6 before and after training	↘ leptin, TNF-α, IL-6 after the training program
	No control	IL-6, TNF-α, CRP before, immediately after, and 2h after exercise	↗ IL-6 immediately after in both groups and significantly higher in the full marathon group, ↔ TNF-α, CRP
	Rest vs exercise 1 week apart in random order, also controlled with able-bodied individuals	IL-6, TNF-α, CRP, adrenaline, noradrenaline, cortisol pre-, during exercise at 60min, immediately post- and 2h post-exercise	↗ IL-6 in SCI and healthy participants during, immediately post- and 2h post-exercise compared to pre-exercise and ↗ IL-6 in healthy at 2h post-exercise compared with rest, ↗ (nor)adrenaline during and immediately post-exercise compared with pre-exercise and with rest, ↔ CK, LDH, TNF-α, CRP, cortisol
	No control	BDNF before exercise and before cool-down	↔ BDNF

Table 1 Continued

Author	Participants (completeness, level of injury, total number, number in EG, male/female in EG, age, time since injury)	Exercise (type, intensity, duration, healthy/paretic muscles)
Exercise-cognition studies in SCI		
Ozturk et al. 2021 (Ozturk et al., 2021)	SCI n = 24, (≥ T4 n = 16, m/f = 15/1, mean age 28.2, AIS A-D with 3 patients motor incomplete, mean time since injury 18.6months < T4 n = 8, m/f = 8/0, mean age 30.3, AIS A or B, mean time since injury 26.5 months) and healthy n = 16, m/f = 10/6, mean age 28.8y	The persons with SCI underwent chronic, functional electrical stimulation-assisted full-body rowing exercise, 30-45min increments for 2-3d/week, for 6 months

Significant increases are marked with ↗, significant decreases with ↘, no significant change with ↔. Abbreviations: AIS, ASIA impairment scale; BDNF, brain-derived neurotrophic factor; C, cervical level; CG, control group; CK, creatine kinase; CRP, C-reactive protein; EG, exercise group; f, female; GDNF, glial cell-derived neurotrophic factor; HbA1c, hemoglobin A1c; HDL, high density lipoprotein;

Subsequently, we identified articles that investigated part of our research question, more specifically studies reporting either (1) the exercise-induced release of myokines or (2) the exercise-induced effects on cognitive function in persons with SCI. We found 18 articles in total, 17 investigated the exercise-myokine link in persons with SCI and one investigated the exercise-cognition link in persons with SCI. A description of these studies is presented in Table 1, and they are discussed in more detail in Appendix 3. In general, the heterogeneity in participant characteristics, exercise characteristics, timing of blood sample collection, and the low sample sizes, as well as the low quality of evidence of these studies makes it difficult to draw final conclusions.

2.3 Discussion

Our empty systematic review confirms that the exercise-induced effect on cognitive function mediated by myokines in people with SCI is an unexplored topic. We argue that the cause of this knowledge gap is twofold. First, we believe that cognitive dysfunctions in persons with SCI have long been underrecognized or attributed to concomitant TBI or mood disorders. Second, the link between gradual cognitive decline in persons with SCI and chronic inflammation has only recently been discovered (see section 3).

	CG	Outcome measures	Outcome
	Results for lesion level ≥T4 and <T4 are compared	Working memory using the n-back task before and after 3 and 6 months of exercise; healthy underwent only before measurements	Both persons with ≥T4 and <T4 SCI improved in reaction time after the exercise intervention

HRR, heart rate reserve; IFN-γ, interferon gamma; IGF-1, insulin-like growth factor-1; IL-1RA, interleukin 1 receptor antagonist; IL, interleukin; KYN, kynurenine; L, lumbar level; LDL, low density lipoprotein; m, male; NGF, nerve growth factor, NT, neurotrophin; SCI, spinal cord injury; T, thoracic level; TNF-α, tumor necrosis factor alpha; TRP, tryptophan; VT1, first ventilatory threshold.

3. Topical review

We seem to be the first to suggest that the anti-inflammatory effect of exercise, as well as the exercise-induced boost in neurotrophic factors can be used as a treatment strategy against SCI-associated cognitive aging. For this reason, we will provide an in depth discussion of the mechanisms underlying cognitive aging and the evidence for the beneficial effect of exercise on cognitive function, starting from basic principles known from healthy older adults and translating this evidence to people with SCI. Figure 3 represents an overview of the neuroinflammation versus exercise induced mechanisms.

3.1 Age-related alterations in the brain with impact on cognition

Cognitive function encompasses several domains, including memory, executive function, attention, processing speed, language comprehension and decoding (Barch et al., 2013). With aging, cognitive function typically declines on certain domains, including episodic and prospective memory, executive function, selective and divided attention, working memory and processing speed. In contrast, other domains, such as implicit, semantic memory, and sustained attention remain relatively stable into older age (Barch et al., 2013). Several studies have linked performance on different cognitive domains with brain volume, cortical thickness or white matter health (Salthouse, 2011). Although our brains shrink with age, and neurotransmitter function changes, cerebral networks have the potential to adapt in order to maintain cognitive function. For example, during demanding cognitive tasks, functional magnetic resonance imaging (fMRI) studies in

older adults have shown an increased activation of brain regions compared to younger individuals. This is thought to reflect compensatory neural recruitment, employed by the brain when cognitive resources are decreased, aiding to maintain a certain level of cognitive performance (Wylie et al., 2020). Furthermore, the brain can undergo structural reorganization, including neurogenesis, synaptogenesis and synaptic plasticity. This might also serve a compensatory function. Notably, these neuroplastic processes are essential in proper cognitive functioning and experience-dependent brain adaptation at any age. In this respect, the hippocampal region is most often examined (Jurkowski et al., 2020), but many other regions of the brain (Martin et al., 2000) and the spinal cord (Xin et al., 2006) may undergo neuroplastic changes. Neurotrophic factors, like brain-derived neurotrophic factor (BDNF) and insulin-like growth factor-1 (IGF-1), may boost synaptic plasticity via long-term synaptic potentiation (LTP) (Ding et al., 2006; Vints et al., 2022b), and increase neurogenesis and neural survival. Therefore, the well-known increase of these neurotrophic factors by exercise is expected to have a critical role for promoting neuroplasticity in older adults (Jurkowski et al., 2020). Indeed, it is apparent that more neurons are produced in the neurogenic zones of the hippocampus in response to physical exercise, while less are produced in conditions of stress, or with aging. Of interest, recent studies have found adult neurogenic zones outside the hippocampal region, including the hypothalamus, striatum, substantia nigra, cortex and amygdala. Further studies need to investigate the functional role of neurogenesis in these brain regions (Jurkowski et al., 2020). The age-related decrease in neurogenesis might be one of the reasons for volumetric brain loss (Denoth-Lippuner and Jessberger, 2021). It was reported that brain volume loss accounts for 25% to 100% of the differences in cognitive function between young and older adults (Fjell and Walhovd, 2010).

3.2 The underrecognized problem of cognitive impairment after spinal cord injury

Cognitive impairment is an important secondary problem after SCI. About 60% of persons with SCI suffer from cognitive deficits (Murray et al., 2007; Huang et al., 2017). The relative risk of cognitive impairment in persons with SCI is about 13 times higher than in the general population (Craig et al., 2017). A recent review paper further discussed the characteristics of cognitive impairment in persons with SCI. In summary, they described that persons with SCI show declines across multiple domains of cognitive functions during acute rehabilitation, including processing speed, new learning and memory, attention, concentration and executive function (F. Li et al., 2020). It was reported that these cognitive deficits are progressive over time, being already present in the subacute stage, but worsening in the chronic stage (Molina et al., 2018). Indeed, a network meta-analysis, including 12 studies investigating cognitive functions in persons with chronic SCI and only one study with persons with acute SCI, indicated that adults with (mostly chronic) SCI displayed impairments in attention and executive function when compared to

able-bodied individuals (Sandalic et al., 2022). Cohen et al. (2017) described that persons with chronic SCI scored especially poor on tasks dependent of processing speed and executive function (Cohen et al., 2017). In addition, it was found that the self-perceived cognitive ability of people with SCI was significantly lower in comparison with their pre-injury state (Murray et al., 2007). About half of the patients included in this study had recent injuries (mean 7 (SD 7) weeks post injury) and about half were at least one year after injury (mean 70 (SD 47) months post injury). It was demonstrated that there was no significant change in self-perceived cognitive ability between both groups (Murray et al., 2007). This highlights the importance of examining and addressing cognitive changes in patients with SCI during acute care, rehabilitation and at follow-up.

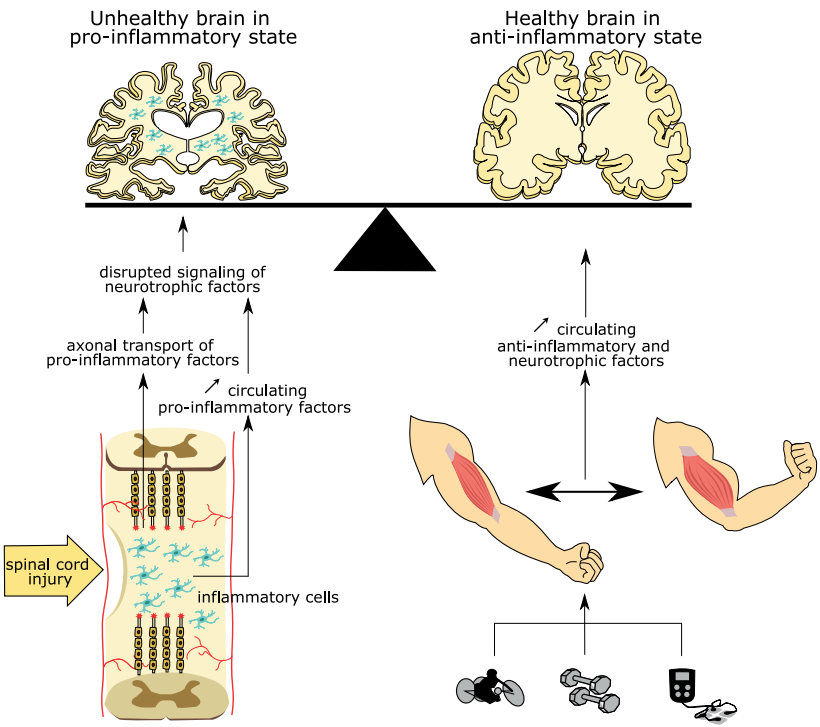


Figure 3. Balance between the detrimental effects of expanding chronic neuroinflammation following spinal cord injury and the beneficial effects of muscle activity on the brain

Following spinal cord injury, pro-inflammatory factors are transported via humoral and axonal routes, causing neuroinflammation to spread to the brain. Muscle activity or exercise in contrast causes the release of anti-inflammatory and neurotrophic factors from muscle cells (i.e. myokines) with the potential to restore brain health.

Notably, about 16-74% of persons with SCI also suffer from TBI which is also a major cause of post-injury cognitive dysfunction (Cohen et al., 2017). Consequently, TBI was long considered the reason for cognitive deficits in persons with SCI. Furthermore, it was reported that individuals with concomitant TBI and SCI responded less to cognitive rehabilitation than patients with TBI without associated SCI (Mollayeva et al., 2020). However, there is increasing evidence that patients with SCI without TBI also suffer from cognitive decline (Y. Li et al., 2020a). This is becoming more and more apparent, as the proportion of non-traumatic SCI cases is growing (World Health Organization (WHO), 2013). The main reason for this change in epidemiology is thought to be the increase in the proportion of older adults in our population (Molinares et al., 2022). The etiology of these non-traumatic SCI cases is heterogeneous, including degenerative changes to the vertebral column, tumors, multiple sclerosis, vascular, transverse myelitis, spina bifida, syringomyelia, and infections (New et al., 2002; Müller-Jensen et al., 2021). Especially in this growing group of non-traumatic SCI cases, assessments of cognitive function changes should receive more attention, as cognitive deficits in this often older population are commonly (falsely) explained by premorbid comorbidities, and are usually less apparent in the acute stage compared to cases with concomitant TBI. Therefore, SCI-associated cognitive decline is often neglected in rehabilitation centers, even though it may have a negative impact on the rehabilitation outcome.

Not only in the clinical setting, but also in research, cognitive impairment in persons with SCI without TBI have been underrecognized. Only a limited amount of studies investigating SCI-associated cognitive impairment excluded or adjusted for TBI when reporting patient's cognitive performances. Bombardier et al. (2016) were one of the first to suggest that other factors than TBI likely contribute to the cognitive impairment in persons with SCI. They found that the number of persons with SCI reporting cognitive deficits was 80% higher than the physician-rated presence of TBI (Bombardier et al., 2016). Furthermore, two studies reported that persons with SCI have an increased risk of Alzheimer's dementia (HR 1.92; 95% CI 1.16-3.15; n = 8508 with TBI excluded, during a 3 year follow-up and HR 1.94; 95% CI 1.41-2.67; n = 1038 adjusted for TBI, during a 7 year follow-up) (Huang et al., 2017; Yeh et al., 2018). In addition, Mahmoudi et al. (2021) found that the risk of Alzheimer disease and related dementia was approximately twice (HR 1.93; 95% CI 1.06-3.51) that of age-matched healthy controls in middle aged adults (45-65 years old) and 1.8 times (HR 1.77; 95% CI 1.55-2.02) that of age-matched older adults (65+ years old) during a follow-up period of 4 years. This study included 6083 age-matched subjects, only 3.12% of cases had associated TBI, and no significant increase in the hazard ratio for dementia was found when persons with SCI with or without TBI were compared (Mahmoudi et al., 2021). Another study that included persons with chronic (> 10 months post injury) traumatic SCI without diagnosis of TBI (i.e. normal Glasgow Coma Scale, no loss of consciousness, no post-traumatic amnesia, and no traumatic findings on brain

CT-scan) showed worse cognitive function and higher levels of depression in persons with SCI compared to healthy controls. When controlled for emotional factors (i.e. depressive symptoms measured with the Patient Health Questionnaire-9 (PHQ-9) instrument and psychological distress measured with the Brief Symptom Inventory), persons with SCI scored lower on tests of executive function, long-term memory, short-term memory and attention (Heled et al., 2020). Moreover, one study included persons with chronic (> 1 year post injury) SCI without history of TBI or coinciding TBI, diabetes mellitus, hypertension, stroke, epilepsy, or neurodegenerative or psychiatric disorders. They showed significant impairments on several verbal cognitive tests for information processing speed, new learning and memory, and verbal fluency in these persons with SCI. These results were similar to those in older adults (50-60 years old), but significantly worse compared with age-matched controls (30-40 years old). No differences were found on tests of attention or working memory (Chiaravalloti et al., 2020).

Importantly, recent MRI studies have also discovered structural and functional brain changes after SCI. These alterations were not only reported in the sensorimotor cortex, but also in brain regions that may not play a direct role in sensorimotor control (Wrigley et al., 2009; Seif et al., 2018; Ziegler et al., 2018; Wylie et al., 2020). Gray and/or white matter decreases were found in the cerebellum (Seif et al., 2018; Ziegler et al., 2018), limbic system (Seif et al., 2018), and anterior cingulate cortex (Ziegler et al., 2018) of persons with SCI without TBI. Wrigley et al. (2009) reported alterations in the anterior cingulate cortex and medial prefrontal cortex, but they unfortunately did not exclude patients with TBI (Wrigley et al., 2009). Interestingly, in chronic cervical spinal cord injury patients (mean age 35 ± 9, mean 8.9 years post-injury) Wylie et al. (2020) showed an overactivation of frontal, parietal and hippocampal brain areas during a processing speed task (Wylie et al., 2020). Patients with a history of TBI, stroke, epilepsy or neurodegenerative disorders were excluded from this study. Wylie et al. (2020) suggested that overactivation of brain regions might be a mechanism to compensate for SCI-associated cognitive impairment. Overactivation was not reported in age-matched controls, although a similar pattern of brain overactivation was also observed in older adults (mean age 60 ± 3) (Wylie et al., 2020). Another study also found evidence for accelerated brain atrophy by showing progressive enlargement of ventricles and cerebrospinal fluid volume after SCI similar to that observed in cognitive aging (Seif et al., 2018).

3.3 Potential causes of cognitive impairment in people with SCI

Sachdeva et al. (2018) reviewed potential causative factors for SCI-associated cognitive deficits. Next to the evident association with concurrent TBI, they reported an association between cognitive deficits and depression in three out of six included studies, pain or fatigue in three out of four included studies, hypotension in three out of five studies, and sleep apnea in both of the included studies. Since only three studies excluded

individuals with TBI, the contribution of TBI could not be dismissed from these results. However, similar effects were shown in the studies that included or excluded individuals with TBI (Sachdeva et al., 2018). The potential causes of cognitive impairment following SCI are described in more detail below, see also Table 2: (1) TBI is an important cause of immediate post-SCI cognitive impairment. Reported incidences of TBI within persons with SCI range between 16-74% (Cohen et al., 2017). (2) The link between depression and cognitive deficits is well known in the general population, and is even sometimes referred to as pseudodementia (Perini et al., 2019). In people with SCI, psychological alterations, such as depression and anxiety, are very common and were also suggested as potential causes of cognitive dysfunctions (Distel et al., 2020). In the context of SCI, this association may go in both directions, as it was reported that persons with SCI with lower cognitive capacity were more likely to experience depression, anxiety, fatigue and pain at 6 months after discharge from inpatient rehabilitation (Craig et al., 2015; Craig et al., 2017). (3) Chronic pain has been shown to cause functional and structural changes to the brain and is associated with impaired working memory and concentration in the general population (Mazza et al., 2018). Post-SCI pain was found to be a significant predictor of self-perceived cognitive functioning (Murray et al., 2007) and was associated with attention deficits measured with a traditional neuropsychological assessment (Carlozzi et al., 2021). Especially neuropathic pain is very common, being present in 40-50% of chronic SCI cases (Finnerup, 2013). (4) Autonomic dysreflexia, which may lead to wide fluctuations in blood pressure, was believed to be a potential cause of silent cerebral infarcts, that may over time cause cognitive deficits (Wecht and Bauman, 2013). In addition, hypotension was significantly associated with poor memory function and non-significantly with slow processing speed and with attention deficits in people with SCI (Jegede et al., 2010). A potential reason for the link between cognition and hypotension may be a lower and impaired regulation of cerebral blood flow, which was discovered in high thoracic and cervical SCI at rest compared to controls (Phillips et al., 2017; Sachdeva et al., 2019). This was also suggested in the study of Wecht et al.(2018), who found that an inadequate systemic and cerebral hemodynamic response to the Symbol Digit Modalities Test (SDMT) contributed significantly to the test score. (5) They suggested an association between level of SCI and cognitive function, as they found that that persons with tetraplegia, who often suffer impaired hemodynamics, scored lower on the SDMT compared to controls, but reported no difference between persons with paraplegia and controls (Wecht et al., 2018). Moreover, (6) Sleep apnea is known to be commonly associated with cognitive dysfunctions in the general population (Patil et al., 2007). It is very prevalent in people with SCI, especially in persons with tetraplegia (91% in complete and 56% in incomplete tetraplegia) (Berlowitz et al., 2012). Of interest, severe sleep apnea or nocturnal oxygen desaturations were associated with deficits in attention, information processing, working memory and cognitive flexibility in persons with SCI without TBI (Sajkov et al., 1998;

Schembri et al., 2017). Next to sleep apnea, respiratory failure with hypercapnia and hypoxemia are common in thoracic and cervical level injuries, due to reduced chest wall compliance, inefficient ventilation, reduced vital capacity, decreased peak cough flow and inspiratory pressure, and reduced forced expiratory volume (Brown et al., 2006). In pulmonary disorders, the severity of chronic respiratory failure is known to be associated with cognitive impairment, but in the SCI population the effect of respiratory function changes on cognition remains unknown (Areza-Fegyveres et al., 2010; Distel et al., 2020). (7) In addition to the potential causes of cognitive dysfunctions listed by Sachdeva et al. (2018), also substance abuse has been suggested to play a role in cognitive decline (Warren et al., 2008). Substance abuse is prevalent in the SCI population and may also be a contributor to the initial SCI (Tate et al., 2004). Furthermore, pharmacological agents may interfere with cognitive function, with polypharmacy being common in individuals with SCI (56% were prescribed 5 or more medications) and high-risks medications, such as benzodiazepines and other hypnotics, baclofen, opioids, antipsychotics, antidepressants, antiepileptics and anticholinergics are prescribed very often to patients with SCI (Kitzman et al., 2017; Distel et al., 2020). (8) A meta-analysis also pointed out that educational level was predictive of cognitive performance in people with SCI (Macciocchi et al., 2013). (9) Cardiovascular disease, often in the context of metabolic syndrome, obesity, dyslipidemia, hypertension or diabetes, is associated with brain atrophy, cerebral lesions and cognitive deficits in the general older population (Leritz et al., 2011). Nearly all cardiovascular risk factors tend to be more prevalent in people with SCI compared to able-bodied individuals. Notably, cardiovascular disease is now the primary cause of death in the SCI population, surpassing renal and pulmonary conditions that were the most important causes of mortality in the 20th century (Myers et al., 2007). The main reasons for the higher prevalence of cardiovascular diseases in people with SCI are autonomic dysfunction, associated with impaired hemodynamic regulation of blood pressure, heart rate variability and arrhythmias, as well as the high prevalence of sedentary behavior due to impaired motor function and the lack of accessibility or fewer opportunities to engage in physical activities (Myers et al., 2007; Soriano et al., 2022) (10) Intensive care admission can also be a reason for cognitive impairment. Irrespective of diagnosis, cognitive impairment is reported in 30-80% of patients after discharge from the intensive care. Possible players are anxiety, depression, post-traumatic stress disorder, hypotension, sedation, hypoxemia, prolonged mechanical ventilation, multiorgan failure, and systemic corticosteroids (Colbenson et al., 2019).

One final factor (11), recently suggested to contribute to SCI-associated cognitive decline and of high relevance for the next part of this paper, is chronic (neuro)inflammation (Sun et al., 2016; F. Li et al., 2020). In the acute phase following SCI, an inflammatory reaction is thought to arise from the injury site (see section 3.4) and spread throughout the body. Even though there is a difference in etiologic mechanism between traumatic and non-

traumatic SCI, neuroinflammation at the injury site is found to be present in both cases (Molinares et al., 2022). For example, studies from Wu and colleagues have linked SCI-associated neuroinflammation to neurodegeneration and cognitive decline in mice (Wu et al., 2016). Persistent neuroinflammation is reported to play a role in secondary neuronal loss and neurological deficits following TBI (Kumar et al., 2017). However, measuring neuroinflammation in humans requires special techniques like magnetic resonance spectroscopy (Vints et al., 2022a), which is still only sporadically used due to the specific expertise it requires. Therefore, most studies measure peripheral inflammation, which was found to be a good surrogate for neuroinflammation as inflammatory markers readily cross the blood-brain barrier (Barrientos et al., 2015). Peripheral inflammation in the context of SCI has been linked to many secondary problems following SCI such as depression, chronic pain, autonomic dysreflexia, respiratory failure and cardiovascular disease (Sun et al., 2016). Chronic peripheral inflammation is also frequently described to be linked to sleep apnea (Unnikrishnan et al., 2015). In general, it remains unclear if chronic inflammation is the cause or a consequence of secondary problems following SCI. In any case, these relationships with chronic inflammation may suggest that neuroinflammation could be a mechanism linking many of the secondary problems in SCI to cognitive impairment, in addition to the mechanisms described in the previous paragraph. We should also note that individuals that have incurred their SCI at older age often present with premorbid comorbidities that may also be associated with chronic inflammation (Molinares et al., 2022). Moreover, the suggested role of chronic inflammation as a cause of progressive (Molina et al., 2018) cognitive decline following SCI is highlighted by its resemblance with the process of progressive cognitive decline in normal aging (Chiaravalloti et al., 2020). In the context of normal aging, chronic (neuro)inflammation is generally accepted as a mediator of cognitive decline (Sartori et al., 2012). The only difference between the general population and people with SCI is that chronic (neuro)inflammation may start at a younger age (at the time of injury). Chronic (neuro)inflammation is also consistently described in neurodegenerative diseases, with a higher risk of developing these diseases being reported for people with SCI (Huang et al., 2017; Yeh et al., 2018; Mahmoudi et al., 2021). Indeed, multiple studies have reported associations between chronic peripheral inflammation and poor performance on cognitive tests or Alzheimer's dementia (Sartori et al., 2012; Allison et al., 2017; F. Li et al., 2020). Considering that relatively young individuals with SCI show cognitive dysfunctions, brain atrophy and alterations in functional brain activation similar to healthy older adults (Seif et al., 2018; Chiaravalloti et al., 2020; Wylie et al., 2020), the common finding of chronic peripheral and neuroinflammation in both populations might be of interest for further exploration. This raises the hypothesis that chronic neuroinflammation might underlie an acceleration of cognitive aging after SCI, associated with progressive cognitive decline, as reported by Molina et al. (2018).

Taken together, the available evidence derived from these studies suggests that other contributors than the neuronal deficits caused by TBI need to be considered as possible causes of cognitive dysfunction in SCI and that neuroinflammation may be an important mechanism of progressive cognitive decline in people with SCI. We would advise other researchers to rule out or at least document all these associated conditions and take into account the effect of comorbidities or the secondary consequences of SCI when reporting results on cognitive function in people with SCI.

Table 2 Potential causes or predictors of cognitive impairment for people with SCI

1.	Concomitant traumatic brain injury
2.	Psychological alterations • Depression • Anxiety
3.	Chronic pain
4.	Hemodynamic dysregulation • Autonomic dysreflexia • Hypotension
5.	Level of injury (tetraplegia vs paraplegia)
6.	Respiratory failure • Sleep apnea • Hypercapnia and hypoxemia
7.	Substance (ab)use • Alcohol and drugs • Polypharmacy and medication acting on the central nervous system
8.	Educational level
9.	Cardiovascular disease • Metabolic syndrome • Obesity • Dyslipidemia • Hypertension • Diabetes
10.	Intensive care admission
11.	Neuroinflammation

3.4 Chronic neuroinflammation mediates cognitive decline following spinal cord injury

In the brain, the main cellular components of the innate immune system are microglia. Microglia have two main phenotypes, the neurotoxic proinflammatory M1 and anti-inflammatory M2 phenotype, respectively secreting pro- or anti-inflammatory cytokines. The latter also secretes neurotrophic factors and contributes to wound healing and tissue remodeling (Wu et al., 2014). With normal aging, the number of microglia was found to increase in the frontal and temporal cortex (Terry et al., 1987). Moreover, microglia were reported to change to the M1 phenotype with aging. These changes were found to be associated with age-related cognitive decline (Cohen and Torres, 2019).

Neuroinflammation in SCI was found to be mediated in part by chemokines like cysteine-cysteine chemokine ligand 21 (CCL21) which is expressed at the injury site (Figure 4). These chemokines attract and activate microglia (Chen et al., 2020). Multiple studies have reported that CCL21 can be transported by axons to cause distant activation of microglia (Y. Li et al., 2020a). Subsequently, microglia release pro-inflammatory cytokines that may damage surrounding neurons. In turn, these damaged neurons may also start to release CCL21. Eventually, this may cause an ascending chronic neuroinflammatory reaction (Wu et al., 2016). Another cause for the spread of neuroinflammation that is seen after SCI might be mediated by the microglia themselves. In the context of TBI, microglia were found to release microparticles that were suggested to play a role in expanding neuroinflammation (Kumar et al., 2017). Future studies should further investigate the mediators of the progressive neuroinflammation following SCI.

Pro-inflammatory cytokines and M1-type microglia were reported to suppress hippocampal neurogenesis (Sung et al., 2020). A recent review has summarized the multiple pathways mediated by inflammatory cytokines that might modulate cognition, synaptic plasticity and neurogenesis (Bourgognon and Cavanagh, 2020). Of interest, Bourgognon and Cavanagh (2020) reported that a chronic neuroinflammatory state may impair synaptic plasticity, synaptogenesis and neurogenesis by the disturbing effect of proinflammatory cytokines on neurotrophic growth factor signaling (Figure 1) (Cotman et al., 2007; Bourgognon and Cavanagh, 2020). Interestingly, Bourgognon and Cavanagh (2020) describe that the effect is dependent of the intensity and duration of the inflammatory activity. Most detrimental are states of long-term elevated pro-inflammatory cytokine levels such as seen following SCI, whereas short-term increases may have beneficial effect (Bourgognon and Cavanagh, 2020).

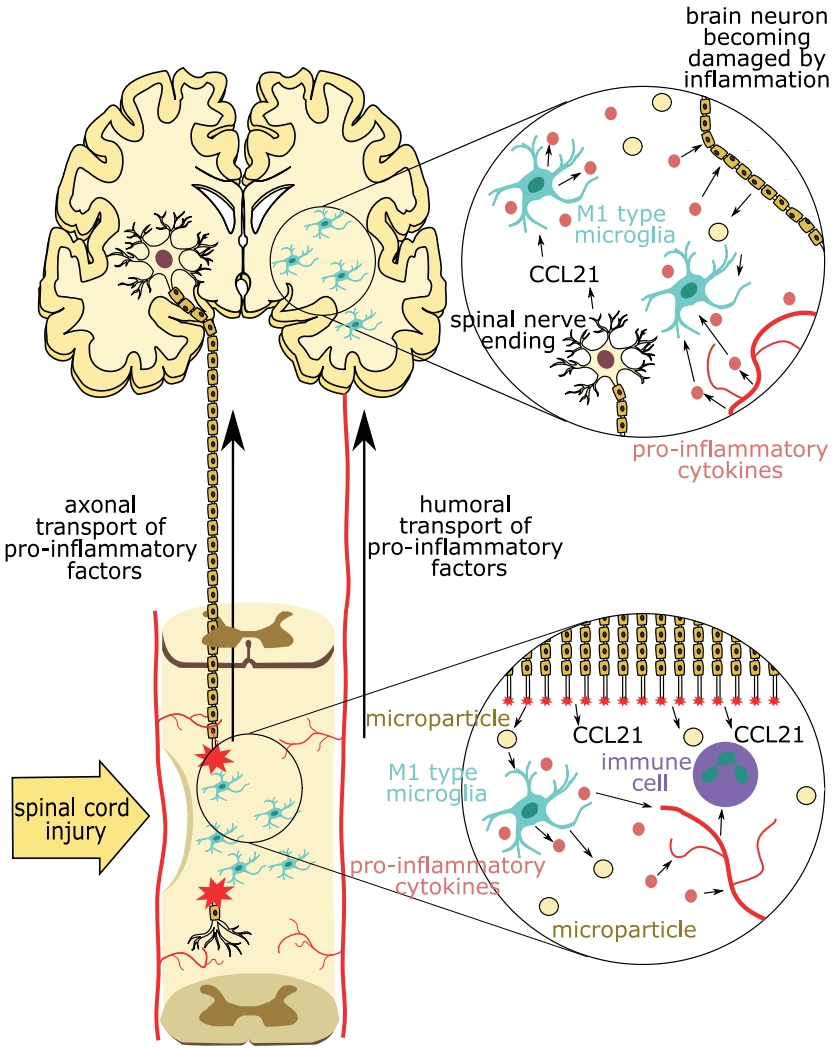


Figure 4. Routes of expanding chronic neuroinflammation following spinal cord injury

Damaged neurons following spinal cord injury release microparticles and CCL21 who attract immune cells and activate microglia. These cells aggravate the inflammatory reaction. The reaction spreads to the brain via humoral and axonal routes, causing also inflammatory damage to brain neurons.

So far, we have focused on the role of neuroinflammation in relation to cognition and brain plasticity. However, both in normal aging and after SCI, this chronic inflammatory state is reported throughout the whole body (Sartori et al., 2012; Sun et al., 2016). Of interest, systemic elevations of pro-inflammatory cytokines were found to upregulate indoleamine 2,3 dioxygenase activity in the liver. This enzyme converts tryptophan into kynurenine. Tryptophan is also a precursor for serotonin. Therefore, the upregulation of kynurenine levels may go at the expense of sufficient serotonin levels. Some authors consider this may be one of the etiologic explanations for depression (Allison et al., 2017). In addition, kynurenine may cross the blood brain barrier. There, it was found to be detrimental for the LTP process (Vécsei et al., 2013; Vints et al., 2022b) and we have found an association between serum kynurenine levels and neurodegeneration and neuroinflammation (Vints et al., 2022a).

In the context of spinal cord injury, Wu and colleagues reported a chronic increase in the number of activated microglia in the cerebral cortex and hippocampus of mice. This was associated with local CCL21 elevations. Furthermore, they found that moderate and severely injured mice were cognitively impaired, and showed a reduction in hippocampal neurogenesis and increased neuronal endoplasmic reticulum dysfunction. The latter results in intracellular protein misfolding, which eventually leads to neuronal cell death (Wu et al., 2016). Allison et al. (2017) examined the effect of a 3-month intervention with an anti-inflammatory diet on pro-inflammatory cytokine and kynurenine levels in persons with SCI. They found a reduction in inflammation, but no change in memory and verbal learning function. They suggest the reduction in inflammation might not have been large enough to induce cognitive changes (Allison et al., 2017). Furthermore, a recent animal study showed that pharmacological inhibition of microglial activity reduces SCI-associated neuroinflammation in mice. This resulted in improved cognition and motor function, and decreased depression-like animal behavior (Y. Li et al., 2020b).

3.5 Physical exercise inhibits inflammation and cognitive decline through the release of myokines

In contrast to dietary or pharmacological interventions, exercise has been proposed as a low-cost and low-risk intervention to inhibit peripheral and neural inflammation in people without SCI (Mee-inta et al., 2019). Furthermore, exercise exerts beneficial effects on brain structure, brain function and cognition (Mandolesi et al., 2018), while a sedentary life-style is a well-known risk factor for cognitive decline (Kirk-Sanchez and McGough, 2013). In people with SCI, exercising has been reported to have neuroprotective and neuro-regenerative effects (Sandrow-Feinberg and Houlé, 2015).

The mechanism underlying the beneficial effect of exercise on brain health and cognition is of increasing interest. However, we found only one study who assessed the effect of exercise on cognition in persons with SCI (Ozturk et al., 2021). Recent studies with able-bodied individuals have advocated that myokines probably mediate at least

part of the effect of exercise on brain health and cognition (Pedersen, 2019; Vints et al., 2022b). The most extensively studied examples are BDNF and IGF-1. Both were consistently reported to improve cognition (Berg and Bang, 2004; Máderová et al., 2019) and induce synaptic plasticity, neurogenesis and neural survival (Ding et al., 2006; Jurkowski et al., 2020). These neurotrophic factors can be released from muscle tissue, but are also released from the brain itself (Berg and Bang, 2004; Máderová et al., 2019; Pedersen, 2019). Of interest, other myokines were suggested to stimulate the exercise-induced release of BDNF in the brain and possibly indirectly induce LTP (Vints et al., 2022b). For example, irisin (Wrann, 2016), cathepsin-B (Moon et al., 2016), apelin (Kwak et al., 2019) and adiponectin (Dai et al., 2013) were reported to induce BDNF transcription in the hippocampus. Adiponectin is mainly released from adipose tissue during exercise, but it was also found to be expressed in skeletal muscle (Dai et al., 2013). In contrast, other myokines, like myostatin are generally decreased in skeletal muscle during and following exercise (Baczek et al., 2020). Our systematic review included some preliminary evidence that also in people with SCI, neurotrophic factors are induced by acute or chronic exercise interventions (Rojas Vega et al., 2008; Zeller et al., 2015; Han et al., 2016; Leech and Hornby, 2017; Goldhardt et al., 2019; Nishimura et al., 2022). In addition, exercise induced several pathways that modulate inflammation, as reviewed by others (Cotman et al., 2007). The most extensively studied pro-inflammatory marker is IL-6, which is released from muscle tissue during exercise (Pedersen, 2019). During a single bout of exercise, it was found that both pro- and anti-inflammatory cytokines increased, keeping inflammation in balance (Flynn et al., 2007). As we reported earlier, acute elevations of IL-6 were suggested to be beneficial for hippocampal neurogenesis. In contrast, chronic elevations of IL-6 and other pro-inflammatory factors are found to be detrimental for cognition and brain plasticity (Bourgognon and Cavanagh, 2020). Fortunately, a vast array of research has provided evidence that regularly exercising induces reductions in the baseline circulating levels of pro-inflammatory cytokines (Flynn et al., 2007). Also in people with SCI quite some studies provide preliminary evidence for similar changes in IL-6 levels, as was described in our systematic review (Umemoto et al., 2011; Kouda et al., 2012; Paulson et al., 2013; Ogawa et al., 2014; Paulson et al., 2014; Sasaki et al., 2014; Rosety-Rodriguez et al., 2014; Alajam et al., 2020; An et al., 2020; Alves et al., 2021).

4. Conclusion

Based on recent literature, it has been suggested that an expanding chronic neuroinflammatory process following SCI may cause an acceleration of cognitive aging (Wu et al., 2016; Molina et al., 2018; Wylie et al., 2020). Although evidence exists that physical exercise could be implemented as a therapeutic approach to decrease systemic

inflammation and increase the levels of neurotrophic factors in persons with SCI (Rojas Vega et al., 2008; Umemoto et al., 2011; Kouda et al., 2012; Paulson et al., 2013; Ogawa et al., 2014; Paulson et al., 2014; Rosety-Rodriguez et al., 2014; Sasaki et al., 2014; Zeller et al., 2015; Han et al., 2016; Leech and Hornby, 2017; Donia et al., 2019; Alves et al., 2021; Goldhardt et al., 2019; Alajam et al., 2020; An et al., 2020; Nishimura et al., 2022). To our knowledge, only one study also reported improvements in cognitive function with exercise in people with SCI (Ozturk et al., 2021). However, none of the studies combined both the evaluation of myokines and cognitive function as was initially the aim to find in our systematic review. This proves that there is currently a knowledge gap in this topic. We argue that the current paper describes enough evidence to suggest that physical activity will probably be beneficial for the (cognitive) health of persons with SCI. Furthermore, we hope this paper may raise awareness about the underrecognized problem of cognitive decline in individuals with SCI and the potential benefit of physical exercise, especially because sedentarism levels are high in people with SCI (Soriano et al., 2022).

5. Implications for future research

We believe that our work will serve as an incentive for other researchers to further explore the promising effects of exercise as a treatment modality against cognitive aging and improve the existing rehabilitation strategies for patients with SCI (and other patients with neurological or neurodegenerative disorders). At present, evidence quality of studies assessing the effect of exercise on cognition or of exercise on myokine levels is very low in the context of SCI. More insight in the mechanism of expanding neuroinflammation would aid in the understanding of cognitive aging following SCI, but likely also following TBI and probably even in the context of normal aging. Given that we are currently facing an aging society, a process that is prospected to continue in the next decades, the problem of cognitive aging is becoming increasingly relevant (Dall et al., 2013; Molinares et al., 2022). While the mechanism of cognitive aging in people with SCI is starting to become clear, more research on cognitive dysfunctions in people with SCI is warranted, especially in cases without TBI. More attention needs to be directed to the role of comorbidities and secondary consequences of SCI as risk factors for the development of cognitive impairment (listed in Table 2). It is important that researchers will take these factors into account or at least document them as possible influencers of cognitive performance in people with SCI. Eventually, we hope that clinicians will also become more aware of the importance of assessing cognitive performance both in traumatic and non-traumatic SCI cases in the acute phase, in the rehabilitation phase, and especially during follow-up.

Declarations of interest

None

Funding

This work was supported by the Research Council of Lithuania under Grant number S-MIP-21-37.

The sponsor was not involved in study design; collection, analysis and interpretation of the data; in writing of the report; nor in the decision to submit the article for publication.

Acknowledgements

Not applicable

References

Adhikary, S., Choudhary, D., Tripathi, A.K., Karvande, A., Ahmad, N., Kothari, P., Trivedi, R., 2019. FGF-2 targets sclerostin in bone and myostatin in skeletal muscle to mitigate the deleterious effects of glucocorticoid on musculoskeletal degradation. Life Sci. 229, 261–276. https://doi.org/10.1016/J.LFS.2019.05.022

Alajam, R.A., Alqahtani, A.S., Moon, S., Sarmento, C.V.M., Frederick, J., Smirnova, I. V., Liu, W., 2020. Effects of walking training on risk markers of cardiovascular disease in individuals with chronic spinal cord injury. J. Spinal Cord Med. 0, 1–9. https://doi.org/10.1080/10790268.2020.1853332

Allison, D.J., Josse, A.R., Gabriel, D.A., Klentrou, P., Ditor, D.S., 2017. Targeting inflammation to influence cognitive function following spinal cord injury: A randomized clinical trial. Spinal Cord 55, 26–32. https://doi.org/10.1038/sc.2016.96

Alves, E. da S., dos Santos, R.V.T., de Lira, F.S., Almeida, A.A., Edwards, K., Benvenutti, M., Tufik, S., De Mello, M.T., 2021. Effects of intensity-matched exercise at different intensities on inflammatory responses in able-bodied and spinal cord injured individuals. J. Spinal Cord Med. 44, 920–930. https://doi.org/10.1080/10790268.2020.1752976

An, Y.H., Kim, J., Kim, H.-J., Lim, K., 2020. Effects of leucine-enriched essential amino acid supplementation on muscular fatigue and inflammatory cytokines in wheelchair basketball players. Phys. Act. Nutr. 24, 38–46. https://doi.org/10.20463/pan.2020.0013

Areza-Fegyveres, R., Kairalla, R.A., Carvalho, C.R.R., Nitrini, R., 2010. Cognition and chronic hypoxia in pulmonary diseases. Dement. Neuropsychol. 4, 22. https://doi.org/10.1590/S1980-57642010DN40100003

Baczek, J., Silkiewicz, M., Wojszel, Z.B., 2020. Myostatin as a biomarker of muscle wasting and other pathologies-state of the art and knowledge gaps. Nutrients. https://doi.org/10.3390/nu12082401

Barch, D.M., Burgess, G.C., Harms, M.P., Petersen, S.E., Schlaggar, B.L., Corbetta, M., Glasser, M.F., Curtiss, S., Dixit, S., Feldt, C., Nolan, D., Bryant, E., Hartley, T., Footer, O., Bjork, J.M., Poldrack, R., Smith, S., Johansen-Berg, H., Snyder, A.Z., Van Essen, D.C., 2013. Function in the human connectome: Task-fMRI and individual differences in behavior. Neuroimage 80, 169–189. https://doi.org/10.1016/j.neuroimage.2013.05.033

Barrientos, R.M., Kitt, M.M., Watkins, L.R., Maier, S.F., 2015. Neuroinflammation in the normal aging hippocampus. Neuroscience. https://doi.org/10.1016/j.neuroscience.2015.03.007

Berg, U., Bang, P., 2004. Exercise and Circulating Insulin-Like Growth Factor I. Horm. Res. Paediatr. 62, 50–58. https://doi.org/10.1159/000080759

Berlowitz, D.J., Spong, J., Gordon, I., Howard, M.E., Brown, D.J., 2012. Relationships Between Objective Sleep Indices and Symptoms in a Community Sample of People With Tetraplegia. Arch. Phys. Med. Rehabil. 93, 1246–1252. https://doi.org/10.1016/J.APMR.2012.02.016

Bombardier, C.H., Lee, D.C., Tan, D.L., Barber, J.K., Hoffman, J.M., 2016. Comorbid Traumatic Brain Injury and Spinal Cord Injury: Screening Validity and Effect on Outcomes. Arch. Phys. Med. Rehabil. 97, 1628–1634. https://doi.org/10.1016/j.apmr.2016.03.008

Bourgognon, J.-M., Cavanagh, J., 2020. The role of cytokines in modulating learning and memory and brain plasticity. Brain Neurosci. Adv. 4, 239821282097980. https://doi.org/10.1177/2398212820979802

Brown, R., DiMarco, A.F., Hoit, J.D., Garshick, E., 2006. Respiratory dysfunction and management in spinal cord injury. Respir. Care 51, 853–870.

Carlozzi, N.E., Graves, C.M., Troost, J.P., Ehde, D.M., Miner, J.A., Kratz, A.L., 2021. Association of Physical and Mental Symptoms With Cognition in People With Spinal Cord Injury. Rehabil. Psychol. 66, 532–540. https://doi.org/10.1037/REP0000416

Chen, Y., Liang, L., Cao, S., Hou, G., Zhang, Q., Ma, H., Shi, B., 2020. Serum CCL21 as a Potential Biomarker for Cognitive Impairment in Spinal Cord Injury. Biomed Res. Int. 2020, 21. https://doi.org/10.1155/2020/6692802

Chiaravalloti, N.D., Weber, E., Wylie, G., Dyson-Hudson, T., Wecht, J.M., 2020. Patterns of cognitive deficits in persons with spinal cord injury as compared with both age-matched and older individuals without spinal cord injury. J. Spinal Cord Med. 43, 88–97. https://doi.org/10.1080/10790268.2018.1543103

Cohen, J., Torres, C., 2019. Astrocyte senescence: Evidence and significance. Aging Cell. https://doi.org/10.1111/acel.12937

Cohen, M.L., Tulsky, D.S., Holdnack, J.A., Carlozzi, N.E., Wong, A., Magasi, S., Heaton, R.K., Heinemann, A.W., 2017. Cognition among community-dwelling individuals with spinal cord injury. Rehabil. Psychol. 62, 425–434. https://doi.org/10.1037/rep0000140

Colbenson, G.A., Johnson, A., Wilson, M.E., 2019. Post-intensive care syndrome: impact, prevention, and management. Breathe 15, 98–101. https://doi.org/10.1183/20734735.0013-2019

Colcombe, S., Kramer, A.F., 2003. Fitness Effects on the Cognitive Function of Older Adults: A Meta-Analytic Study. Psychol. Sci. 14, 125–130. https://doi.org/10.1111/1467-9280.t01-1-01430

Cotman, C.W., Berchtold, N.C., Christie, L.-A., 2007. Exercise builds brain health: key roles of growth factor cascades and inflammation. TRENDS Neurosci. 30, 464–472. https://doi.org/10.1016/j.tins.2007.06.011

Craig, A., Guest, R., Tran, Y., Middleton, J., 2017. Cognitive Impairment and Mood States after Spinal Cord Injury. J. Neurotrauma 34, 1156–1163. https://doi.org/10.1089/neu.2016.4632

Craig, A., Nicholson Perry, K., Guest, R., Tran, Y., Dezarnaulds, A., Hales, A., Ephraums, C., Middleton, J., 2015. Prospective Study of the Occurrence of Psychological Disorders and Comorbidities after Spinal Cord Injury. Arch. Phys. Med. Rehabil. 96, 1426–1434. https://doi.org/10.1016/j.apmr.2015.02.027

Dai, Y., Pang, J., Gong, H., Fan, W., Zhang, T.M., 2013. Roles and tissue source of adiponectin involved in lifestyle modifications. Journals Gerontol. - Ser. A Biol. Sci. Med. Sci. 68, 117–128. https://doi.org/10.1093/gerona/gls131

Dall, T.M., Gallo, P.D., Chakrabarti, R., West, T., Semilla, A.P., Storm, M. V., 2013. An Aging Population And Growing Disease Burden Will Require A Large And Specialized Health Care Workforce By 2025. Health Aff. 32, 2013–2020. https://doi.org/10.1377/hlthaff.2013.0714

Denoth-Lippuner, A., Jessberger, S., 2021. Formation and integration of new neurons in the adult hippocampus. Nat. Rev. Neurosci. 1–14. https://doi.org/10.1038/s41583-021-00433-z

Ding, Q., Vaynman, S., Akhavan, M., Ying, Z., Gomez-Pinilla, F., 2006. Insulin-like growth factor I interfaces with brain-derived neurotrophic factor-mediated synaptic plasticity to modulate aspects of exercise-induced cognitive function. Neuroscience 140, 823–833. https://doi.org/10.1016/j.neuroscience.2006.02.084

Distel, D.F., Amodeo, M., Joshi, S., Abramoff, B.A., 2020. Cognitive Dysfunction in Persons with Chronic Spinal Cord Injuries. Phys. Med. Rehabil. Clin. N. Am. 31, 345–368. https://doi.org/10.1016/J.PMR.2020.04.001

Donia, S.A., Allison, D.J., Gammage, K.L., Ditor, D.S., 2019. The effects of acute aerobic exercise on mood and inflammation in individuals with multiple sclerosis and incomplete spinal cord injury. NeuroRehabilitation 45, 117–124. https://doi.org/10.3233/NRE-192773

Finnerup, N.B., 2013. Pain in patients with spinal cord injury. Pain 154, S71–S76. https://doi.org/10.1016/J.PAIN.2012.12.007

Fjell, A.M., Walhovd, K.B., 2010. Structural brain changes in aging: Courses, causes and cognitive consequences. Rev. Neurosci. https://doi.org/10.1515/REVNEURO.2010.21.3.187

Flynn, M.G., McFarlin, B.K., Markofski, M.M., 2007. State of the Art Reviews: The Anti-Inflammatory Actions of Exercise Training. Am. J. Lifestyle Med. 1, 220–235. https://doi.org/10.1177/1559827607300283

Goldhardt, M., Bard, A., Dorneles, G., da Silva, I., Pochmann, D., Peres, A., Elsner, V., 2019. Does a single bout of exercise impacts BDNF, oxidative stress and epigenetic markers in spinal cord injury patients? Funct. Neurol. 34, 158–166.

Han, D.S., Hsiao, M.Y., Wang, T.G., Chen, S.Y., Yang, W.S., 2016. Association of serum myokines and aerobic exercise training in patients with spinal cord injury: An observational study. BMC Neurol. 16. https://doi.org/10.1186/s12883-016-0661-9

Heled, E., Tal, K., Zeilig, G., 2020. Does lack of brain injury mean lack of cognitive impairment in traumatic spinal cord injury? J. Spinal Cord Med. https://doi.org/10.1080/10790268.2020.1847564

Huang, S.W., Wang, W. Te, Chou, L.C., Liou, T.H., Lin, H.W., 2017. Risk of dementia in patients with spinal cord injury: A nationwide population-based cohort study. J. Neurotrauma 34, 615–622. https://doi.org/10.1089/neu.2016.4525

Jegede, A.B., Rosado-Rivera, D., Bauman, W.A., Cardozo, C.P., Sano, M., Moyer, J.M., Brooks, M., Wecht, J.M., 2010. Cognitive performance in hypotensive persons with spinal cord injury. Clin. Auton. Res. 20, 3–9. https://doi.org/10.1007/s10286-009-0036-z

Jurkowski, M.P., Bettio, L., K. Woo, E., Patten, A., Yau, S.Y., Gil-Mohapel, J., 2020. Beyond the Hippocampus and the SVZ: Adult Neurogenesis Throughout the Brain. Front. Cell. Neurosci. https://doi.org/10.3389/fncel.2020.576444

Kirk-Sanchez, N.J., McGough, E.L., 2013. Physical exercise and cognitive performance in the elderly: Current perspectives. Clin. Interv. Aging 9, 51–62. https://doi.org/10.2147/CIA.S39506

Kitzman, P., Cecil, D., Kolpek, J.H., 2017. The risks of polypharmacy following spinal cord injury. J. Spinal Cord Med. 40, 147–153. https://doi.org/10.1179/2045772314Y.0000000235

Kouda, K., Furusawa, K., Sugiyama, H., Sumiya, T., Ito, T., Tajima, F., Shimizu, K., 2012. Does 20-min arm crank ergometer exercise increase plasma interleukin-6 in individuals with cervical spinal cord injury? Eur. J. Appl. Physiol. 112, 597–604. https://doi.org/10.1007/s00421-011-2004-2

Kumar, Alok, Stoica, B.A., Loane, D.J., Yang, M., Abulwerdi, G., Khan, N., Kumar, Asit, Thom, S.R., Faden, A.I., 2017. Microglial-derived microparticles mediate neuroinflammation after traumatic brain injury. J. Neuroinflammation 14, 47. https://doi.org/10.1186/s12974-017-0819-4

Kwak, S.E., Cho, S.C., Bae, J.H., Lee, J., Shin, H.E., Di Zhang, D., Lee, Y. Il, Song, W., 2019. Effects of exercise-induced apelin on muscle function and cognitive function in aged mice. Exp. Gerontol. 127, 110710. https://doi.org/10.1016/j.exger.2019.110710

Leech, K.A., Hornby, T.G., 2017. High-Intensity Locomotor Exercise Increases Brain-Derived Neurotrophic Factor in Individuals with Incomplete Spinal Cord Injury. J. Neurotrauma 34, 1240–1248. https://doi.org/10.1089/neu.2016.4532

Leritz, E.C., McGlinchey, R.E., Kellison, I., Rudolph, J.L., Milberg, W.P., 2011. Cardiovascular Disease Risk Factors and Cognition in the Elderly. Curr. Cardiovasc. Risk Rep. 5, 412. https://doi.org/10.1007/S12170-011-0189-X

Li, F., Huo, S., Song, W., 2020. Multidimensional review of cognitive impairment after spinal cord injury. Acta Neurol. Belg. https://doi.org/10.1007/s13760-020-01507-y

Li, Y., Cao, T., Ritzel, R.M., He, J., Faden, A.I., Wu, J., 2020a. Dementia, Depression, and Associated Brain Inflammatory Mechanisms after Spinal Cord Injury. Cells. https://doi.org/10.3390/cells9061420

Li, Y., Ritzel, R.M., Khan, N., Cao, T., He, J., Lei, Z., Matyas, J.J., Sabirzhanov, B., Liu, S., Li, H., Stoica, B.A., Loane, D.J., Faden, A.I., Wu, J., 2020b. Delayed microglial depletion after spinal cord injury reduces chronic inflammation and neurodegeneration in the brain and improves neurological recovery in male mice. Theranostics 10, 11376–11403. https://doi.org/10.7150/thno.49199

Lin, Y.S., Lin, F.Y., Hsiao, Y.H., 2019. Myostatin Is Associated With Cognitive Decline in an Animal Model of Alzheimer's Disease. Mol. Neurobiol. 56, 1984–1991. https://doi.org/10.1007/s12035-018-1201-y

Lind, A., Boraxbekk, C.-J., Petersen, E.T., Paulson, O.B., Siebner, H.R., Marsman, A., 2020. Regional Myo-Inositol, Creatine, and Choline Levels Are Higher at Older Age and Scale Negatively with Visuospatial Working Memory: A Cross-Sectional Proton MR Spectroscopy Study at 7 Tesla on Normal Cognitive Ageing. J. Neurosci. 40, 8149–8159. https://doi.org/10.1523/JNEUROSCI.2883-19.2020

Macciocchi, S.N., Seel, R.T., Thompson, N., 2013. The Impact of Mild Traumatic Brain Injury on Cognitive Functioning Following Co-occurring Spinal Cord Injury. Arch. Clin. Neuropsychol. 28, 684–691. https://doi.org/10.1093/ARCLIN/ACT049

Máderová, D., Krumpolec, P., Slobodová, L., Schön, M., Tirpáková, V., Kovaničová, Z., Klepochová, R., Vajda, M., Šutovský, S., Cvečka, J., Valkovič, L., Turčáni, P., Kršňák, M., Sedliak, M., Tsai, C.L., Ukropcová, B., Ukropec, J., 2019. Acute and regular exercise distinctly modulate serum, plasma and skeletal muscle BDNF in the elderly. Neuropeptides 78. https://doi.org/10.1016/j.npep.2019.101961

Mahmoudi, E., Lin, P., Peterson, M.D., Meade, M.A., Tate, D.G., Kamdar, N., 2021. Traumatic Spinal Cord Injury and Risk of Early and Late Onset Alzheimer's Disease and Related Dementia: Large Longitudinal Study. Arch. Phys. Med. Rehabil. 102, 1147–1154. https://doi.org/10.1016/J.APMR.2020.12.019

Mandolesi, L., Polverino, A., Montuori, S., Foti, F., Ferraioli, G., Sorrentino, P., Sorrentino, G., 2018. Effects of physical exercise on cognitive functioning and wellbeing: Biological and psychological benefits. Front. Psychol. https://doi.org/10.3389/fpsyg.2018.00509

Martin, S.J., Grimwood, P.D., Morris, R.G.M., 2000. Synaptic Plasticity and Memory: An Evaluation of the Hypothesis. Annu. Rev. Neurosci. 23, 649–711.

Mazza, S., Frot, M., Rey, A.E., 2018. A comprehensive literature review of chronic pain and memory. Prog. Neuro-Psychopharmacology Biol. Psychiatry 87, 183–192. https://doi.org/10.1016/J.PNPBP.2017.08.006

Mee-inta, Zhao, Kuo, 2019. Physical Exercise Inhibits Inflammation and Microglial Activation. Cells 8, 691. https://doi.org/10.3390/cells8070691

Molina, B., Segura, A., Serrano, J.P., Alonso, F.J., Molina, L., Pérez-Borrego, Y.A., Ugarte, M.I., Oliviero, A., 2018. Cognitive performance of people with traumatic spinal cord injury: a cross-sectional study comparing people with subacute and chronic injuries. Spinal Cord 56, 796–805. https://doi.org/10.1038/s41393-018-0076-0

Molinares, D.M., Gater, D.R., Daniel, S., Pontee, N.L., 2022. Nontraumatic Spinal Cord Injury: Epidemiology, Etiology and Management. J. Pers. Med. 12, 1872. https://doi.org/10.3390/JPM12111872

Mollayeva, T., Sutton, M., Escobar, M., Hurst, M., Colantonio, A., 2020. The Impact of a Comorbid Spinal Cord Injury on Cognitive Outcomes of Male and Female Patients with Traumatic Brain Injury. PM R. https://doi.org/10.1002/pmrj.12456

Moon, H.Y., Becke, A., Berron, D., Becker, B., Sah, N., Benoni, G., Janke, E., Lubejko, S.T., Greig, N.H., Mattison, J.A., Duzel, E., van Praag, H., 2016. Running-Induced Systemic Cathepsin B Secretion Is Associated with Memory Function. Cell Metab. 24, 332–340. https://doi.org/10.1016/j.cmet.2016.05.025

Müller-Jensen, L., Ploner, C.J., Kroneberg, D., Schmidt, W.U., 2021. Clinical Presentation and Causes of Non-traumatic Spinal Cord Injury: An Observational Study in Emergency Patients. Front. Neurol. 12, 701927. https://doi.org/10.3389/FNEUR.2021.701927

Murray, R.F., Asghari, A., Egorov, D.D., Rutkowski, S.B., Siddall, P.J., Soden, R.J., Ruff, R., 2007. Impact of spinal cord injury on self-perceived pre- and postmorbid cognitive, emotional and physical functioning. Spinal Cord 45, 429–436. https://doi.org/10.1038/sj.sc.3102022

Myers, J., Lee, M., Kiratli, J., 2007. Cardiovascular disease in spinal cord injury: An overview of prevalence, risk, evaluation, and management. Am. J. Phys. Med. Rehabil. 86, 142–152. https://doi.org/10.1097/PHM.0B013E31802F0247

New, P.W., Rawicki, H.B., Bailey, M.J., 2002. Nontraumatic spinal cord injury: Demographic characteristics and complications. Arch. Phys. Med. Rehabil. 83, 996–1001. https://doi.org/10.1053/apmr.2002.33100

Nishimura, Y., Nakamura, T., Kamijo, Y. ichiro, Arakawa, H., Umemoto, Y., Kinoshita, T., Sakurai, Y., Tajima, F., 2022. Increased serum levels of brain-derived neurotrophic factor following wheelchair half marathon race in individuals with spinal cord injury. J. Spinal Cord Med. 45, 455–460. https://doi.org/10.1080/10790268.2020.1816402

Ogawa, T., Nakamura, T., Banno, M., Sasaki, Y., Umemoto, Y., Kouda, K., Kawasaki, T., Tajima, F., 2014. Elevation of interleukin-6 and attenuation of tumor necrosis factor-α during wheelchair half marathon in athletes with cervical spinal cord injuries. Spinal Cord 52, 601–605. https://doi.org/10.1038/sc.2014.88

Ozturk, E.D., Lapointe, M.S., Kim, D. Il, Hamner, J.W., Tan, C.O., 2021. Effect of 6-Month Exercise Training on Neurovascular Function in Spinal Cord Injury. Med. Sci. Sports Exerc. 53, 38–46. https://doi.org/10.1249/MSS.0000000000002452

Patil, S.P., Schneider, H., Schwartz, A.R., Smith, P.L., 2007. Adult Obstructive Sleep Apnea: Pathophysiology and Diagnosis. Chest 132, 325–337. https://doi.org/10.1378/CHEST.07-0040

Paulson, T.A.W., Bishop, N.C., Smith, B.M., Goosey-Tolfrey, V.L., 2014. Inflammation-mediating cytokine response to acute handcycling exercise with/without functional electrical stimulation-evoked lower-limb cycling. J. Rehabil. Res. Dev. 51, 645–654. https://doi.org/10.1682/JRRD.2013.08.0184

Paulson, T.A.W., Goosey-Tolfrey, V.L., Leicht, C.A., Bishop, N.C., 2015. Plasma cytokine and exertional responses in relation to exercise intensity and volume of exercising muscle mass during arm-crank ergometry. Appl. Physiol. Nutr. Metab. 40, 782–787. https://doi.org/10.1139/apnm-2014-0537

Paulson, T.A.W., Goosey-Tolfrey, V.L., Lenton, J.P., Leicht, C.A., Bishop, N.C., 2013. Spinal cord injury level and the circulating cytokine response to strenuous exercise. Med. Sci. Sports Exerc. 45, 1649–1655. https://doi.org/10.1249/MSS.0b013e31828f9bbb

Pedersen, B.K., 2019. Physical activity and muscle–brain crosstalk. Nat. Rev. Endocrinol. https://doi.org/10.1038/s41574-019-0174-x

Pedersen, B.K., Åkerström, T.C.A., Nielsen, A.R., Fischer, C.P., 2007. Role of myokines in exercise and metabolism. J. Appl. Physiol. 103, 1093–1098. https://doi.org/10.1152/JAPPLPHYSIOL.00080.2007

Perini, G., Ramusino, M.C., Sinforiani, E., Bernini, S., Petrachi, R., Costa, A., 2019. Cognitive impairment in depression: recent advances and novel treatments. Neuropsychiatr. Dis. Treat. 15, 1258. https://doi.org/10.2147/NDT.S199746

Phillips, A.A., Squair, J.R., Currie, K.D., Tzeng, Y.C., Ainslie, P.N., Krassioukov, A. V., 2017. 2015 ParaPan American Games: Autonomic Function, But Not Physical Activity, Is Associated with Vascular-Cognitive Impairment in Spinal Cord Injury. J. Neurotrauma 34, 1283–1288. https://doi.org/10.1089/NEU.2016.4751

Rojas Vega, S., Abel, T., Lindschulten, R., Hollmann, W., Bloch, W., Strüder, H.K., 2008. Impact of exercise on neuroplasticity-related proteins in spinal cord injured humans. Neuroscience 153, 1064–1070. https://doi.org/10.1016/j.neuroscience.2008.03.037

Rosety-Rodriguez, M., Camacho, A., Rosety, I., Fornieles, G., Rosety, M.A., Diaz, A.J., Bernardi, M., Rosety, M., Ordonez, F.J., 2014. Low-grade systemic inflammation and leptin levels were improved by arm cranking exercise in adults with chronic spinal cord injury. Arch. Phys. Med. Rehabil. 95, 297–302. https://doi.org/10.1016/j.apmr.2013.08.246

Sa-nguanmoo, P., Chattipakorn, N., Chattipakorn, S.C., 2016. Potential roles of fibroblast growth factor 21 in the brain. Metab. Brain Dis. https://doi.org/10.1007/s11011-015-9789-3

Sachdeva, R., Gao, F., Chan, C.C.H., Krassioukov, A. V., 2018. Cognitive function after spinal cord injury: A systematic review. Neurology. https://doi.org/10.1212/WNL.0000000000006244

Sachdeva, R., Nightingale, T.E., Krassioukov, A. V., 2019. The Blood Pressure Pendulum following Spinal Cord Injury: Implications for Vascular Cognitive Impairment. Int. J. Mol. Sci. https://doi.org/10.3390/ijms20102464

Sajkov, D., Marshall, R., Walker, P., Mykytyn, I., McEvoy, R.D., Wale, J., Flavell, H., Thornton, A.T., Antic, R., 1998. Sleep apnoea related hypoxia is associated with cognitive disturbances in patients with tetraplegia. Spinal Cord 36, 231–239. https://doi.org/10.1038/sj.sc.3100563

Salthouse, T.A., 2011. Neuroanatomical substrates of age-related cognitive decline. Psychol. Bull. 137, 753–784. https://doi.org/10.1037/a0023262

Sandalic, D., Craig, A., Tran, Y., Arora, M., Pozzato, I., McBain, C., Tonkin, H., Simpson, G., Gopinath, B., Kaur, J., Shetty, S., Weber, G., Middleton, J., 2022. Cognitive Impairment in Individuals With Spinal Cord Injury. Neurology 99, e1779–e1790. https://doi.org/10.1212/WNL.0000000000200957

Sandrow-Feinberg, H.R., Houlé, J.D., 2015. Exercise after spinal cord injury as an agent for neuroprotection, regeneration and rehabilitation. Brain Res. https://doi.org/10.1016/j.brainres.2015.03.052

Sartori, A.C., Vance, D.E., Slater, L.Z., Crowe, M., 2012. The impact of inflammation on cognitive function in older adults: Implications for healthcare practice and research. J. Neurosci. Nurs. https://doi.org/10.1097/JNN.0b013e3182527690

Sasaki, Y., Furusawa, K., Tajima, F., Nakamura, T., Kouda, K., Kanno, N., Kawasaki, T., Umemoto, Y., Shimizu, K., 2014. Wheelchair marathon creates a systemic anti-inflammatory environment in persons with spinal cord injury. Clin. J. Sport Med. 24, 295–301. https://doi.org/10.1097/JSM.0000000000000015

Schembri, R., Spong, J., Graco, M., Berlowitz, D.J., 2017. Neuropsychological function in patients with acute tetraplegia and sleep disordered breathing. Sleep 40, 1–6. https://doi.org/10.1093/sleep/zsw037

Seif, M., Ziegler, G., Freund, P., 2018. Progressive Ventricles Enlargement and Cerebrospinal Fluid Volume Increases as a Marker of Neurodegeneration in Patients with Spinal Cord Injury: A Longitudinal Magnetic Resonance Imaging Study. J. Neurotrauma 35, 2941–2946. https://doi.org/10.1089/neu.2017.5522

Soriano, J.E., Squair, J.W., Cragg, J.J., Thompson, J., Sanguinetti, R., Vaseghi, B., Emery, C.A., Grant, C., Charbonneau, R., Larkin-Kaiser, K.A., Phillips, A.A., Dujic, Z., 2022. A national survey of physical activity after spinal cord injury. Sci. Reports 2022 121 12, 1–10. https://doi.org/10.1038/s41598-022-07927-5

Sun, X., Jones, Z.B., Chen, X. ming, Zhou, L., So, K.F., Ren, Y., 2016. Multiple organ dysfunction and systemic inflammation after spinal cord injury: A complex relationship. J. Neuroinflammation. https://doi.org/10.1186/s12974-016-0736-y

Sung, P.S., Lin, P.Y., Liu, C.H., Su, H.C., Tsai, K.J., 2020. Neuroinflammation and neurogenesis in alzheimer's disease and potential therapeutic approaches. Int. J. Mol. Sci. https://doi.org/10.3390/ijms21030701

Tate, D.G., Forchheimer, M.B., Krause, J.S., Meade, M.A., Bombardier, C.H., 2004. Patterns of alcohol and substance use and abuse in persons with spinal cord injury: Risk factors and correlates. Arch. Phys. Med. Rehabil. 85, 1837–1847. https://doi.org/10.1016/J.APMR.2004.02.022

Terry, R.D., DeTeresa, R., Hansen, L.A., 1987. Neocortical cell counts in normal human adult aging. Ann. Neurol. 21, 530–539. https://doi.org/10.1002/ana.410210603

Umemoto, Y., Furusawa, K., Kouda, K., Sasaki, Y., Kanno, N., Kojima, D., Tajima, F., 2011. Plasma IL-6 levels during arm exercise in persons with spinal cord injury. Spinal Cord 49, 1182–1187. https://doi.org/10.1038/sc.2011.74

Unnikrishnan, D., Jun, J., Polotsky, V., 2015. Inflammation in Sleep Apnea: An Update. Rev. Endocr. Metab. Disord. 16, 34. https://doi.org/10.1007/S11154-014-9304-X

Vécsei, L., Szalárdy, L., Fülöp, F., Toldi, J., 2013. Kynurenines in the CNS: Recent advances and new questions. Nat. Rev. Drug Discov. https://doi.org/10.1038/nrd3793

Vints, W.A.J., Kušleikienė, S., Sheoran, S., Šarkinaitė, M., Valatkevičienė, K., Gleiznienė, R., Kvedaras, M., Pukėnas, K., Himmelreich, U., Česnaitienė, V.J., Levin, O., Verbunt, J., Masiulis, N., 2022a. Inflammatory blood biomarker kynurenine is linked with elevated neuroinflammation and neurodegeneration in older adults: evidence from two 1H-MRS post-processing analysis methods. Front. Psychiatry. https://doi.org/10.3389/FPSYT.2022.859772

Vints, W.A.J., Levin, O., Fujiyama, H., Verbunt, J., Masiulis, N., 2022b. Exerkines and long-term synaptic potentiation: Mechanisms of exercise-induced neuroplasticity. Front. Neuroendocrinol. 66, 100993. https://doi.org/10.1016/J.YFRNE.2022.100993

Warren, A.M., Pullins, J., Elliott, T.R., 2008. Concomitant Cognitive Impairment in Persons with Spinal Cord Injuries in Rehabilitation Settings, in: Gontovsky, S.T., Golden, C.J. (Eds.), Neuropsychology within the Inpatient Rehabilitation Environment. Nova Science Publishers, Inc., New York, pp. 79–98.

Wecht, J.M., Bauman, W.A., 2013. Decentralized cardiovascular autonomic control and cognitive deficits in persons with spinal cord injury. J. Spinal Cord Med. https://doi.org/10.1179/2045772312Y.0000000056

Wecht, J.M., Weir, J.P., Katzelnick, C.G., Wylie, G., Eraifej, M., Nguyen, N., Dyson-Hudson, T., Bauman, W.A., Chiaravalloti, N., 2018. Systemic and Cerebral Hemodynamic Contribution to Cognitive Performance in Spinal Cord Injury. J. Neurotrauma 35, 2957–2964. https://doi.org/10.1089/NEU.2018.5760

Woodbury, M.E., Ikezu, T., 2014. Fibroblast Growth Factor-2 Signaling in Neurogenesis and Neurodegeneration. J. Neuroimmune Pharmacol. 9, 92–101. https://doi.org/10.1007/s11481-013-9501-5

World Health Organization (WHO), 2013. Spinal cord injury fact sheet [WWW Document]. URL https://www.who.int/news-room/fact-sheets/detail/spinal-cord-injury (accessed 4.4.21).

Wrann, C.D., 2016. FNDC5/Irisin – Their Role in the Nervous System and as a Mediator for Beneficial Effects of Exercise on the Brain. Brain Plast. 1, 55–61. https://doi.org/10.3233/bpl-150019

Wrigley, P.J., Gustin, S.M., Macey, P.M., Nash, P.G., Gandevia, S.C., Macefield, V.G., Siddall, P.J., Henderson, L.A., 2009. Anatomical changes in human motor cortex and motor pathways following complete thoracic spinal cord injury. Cereb. Cortex 19, 224–232. https://doi.org/10.1093/cercor/bhn072

Wu, J., Zhao, Z., Kumar, A., Lipinski, M.M., Loane, D.J., Stoica, B.A., Faden, A.I., 2016. Endoplasmic reticulum stress and disrupted neurogenesis in the brain are associated with cognitive impairment and depressive-like behavior after spinal cord injury. J. Neurotrauma 33, 1919–1935. https://doi.org/10.1089/neu.2015.4348

Wu, J., Zhao, Z., Sabirzhanov, B., Stoica, B.A., Kumar, A., Luo, T., Skovira, J., Faden, A.I., 2014. Spinal cord injury causes brain inflammation associated with cognitive and affective changes: Role of cell cycle pathways. J. Neurosci. 34, 10989–11006. https://doi.org/10.1523/JNEUROSCI.5110-13.2014

Wylie, G.R., Chiaravalloti, N.D., Weber, E., Genova, H.M., Dyson-Hudson, T.A., Wecht, J.M., 2020. The Neural Mechanisms Underlying Processing Speed Deficits in Individuals Who Have Sustained a Spinal Cord Injury: A Pilot Study. Brain Topogr. 33, 776–784. https://doi.org/10.1007/s10548-020-00798-x

Xin, W.-J., Gong, Q.-J., Xu, J.-T., Yang, H.-W., Zang, Y., Zhang, T., Li, Y.-Y., Liu, X.-G., 2006. Role of phosphorylation of ERK in induction and maintenance of LTP of the C-fiber evoked field potentials in spinal dorsal horn. J. Neurosci. Res. 84, 934–943. https://doi.org/10.1002/jnr.21013

Yeh, T.-S., Ho, Y.-C., Hsu, C.-L., Pan, S.-L., 2018. Spinal cord injury and Alzheimer's disease risk: a population-based, retrospective cohort study. Spinal Cord 56, 151–157. https://doi.org/10.1038/s41393-017-0009-3

Zeller, S., Abel, T., Rojas-Vega, S., Foitschik, T., Strueder, H.K., 2015. Brain-derived neurotrophic factor concentrations in tetraplegic athletes. Spinal Cord 53, 791–794. https://doi.org/10.1038/sc.2015.94

Ziegler, G., Grabher, P., Thompson, A., Altmann, D., Hupp, M., Ashburner, J., Friston, K., Weiskopf, N., Curt, A., Freund, P., 2018. Progressive neurodegeneration following spinal cord injury: Implications for clinical trials. Neurology 90, e1257–e1266. https://doi.org/10.1212/WNL.0000000000005258

CHAPTER 10

Acute effects of neuromuscular electrical stimulation on cognitive performance and IGF-1 levels in individuals with chronic spinal cord injury: a randomized cross-over study

Author: Wouter A.J. Vints, Ugnė Mazuronytė, Orgesa Qipo, Oron Levin, Jeanine Verbunt, Charlotte C.M. van Laake-Geelen, Vaida Pokvytytė, Nerijus Masiulis

Submitted for publication

Abstract

Introduction: Individuals with spinal cord injuries (SCI) exhibit a more pronounced and accelerated age-related cognitive decline compared to healthy individuals, even after adjusting for factors such as mood and concomitant traumatic brain injury. We hypothesized that neuromuscular electrical stimulation (NMES) on hamstring and gluteal muscles may induce a dose-dependent increase in insulin-like growth factor-1 (IGF-1) and temporarily enhance cognition.

Methods: Twenty-two individuals with chronic SCI participated in a randomized cross-over study, receiving NMES on one of both visits. Participants randomly underwent a single session of 30 or 60-minute NMES. Lactate, insulin-like growth factor-1 (IGF-1) levels and processing speed were tested before, immediately after and 30 minutes after intervention or 60 minutes rest.

Results: Lactate levels increased significantly under NMES conditions compared to control. Lactate increases were greater in the 30-minute NMES group compared to the 60-minute NMES group, consistent with the higher current amplitude applied in the former. IGF-1 did not significantly increase, and the effect was further attenuated by a longer time since injury in the 60-minute NMES group. SDMT performance improved over time at all measurement points, except immediately after the 30-minute NMES session. Additionally, a higher level of injury was associated with smaller SDMT improvements following the 60-minute NMES session.

Conclusion: Acute NMES did not induce changes in IGF-1 levels or cognitive performance in individuals with SCI. Further research is required to explore various NMES protocols and their impact on cognitive domains in individuals with SCI.

Abbreviations

AIS, ASIA Impairment Scale; ASIA, American Spinal Injury Association; BDNF, brain-derived neurotrophic factor; BMI, body mass index; DABEST, Data Analysis with Bootstrap-coupled ESTimation; ELISA, enzyme-linked immunosorbent assay; FES, functional electrical stimulation; GABA, γ-aminobutyric acid; IGF-1, insulin-like growth factor 1; NA, information not available; NMDA, N-methyl-D-aspartate; NMES, neuromuscular electrical stimulation; SDMT, Symbol Digit Modalities Test; TBI, traumatic brain injury; VEGF, vascular endothelial growth factor.

1. Introduction

Cognitive function impairments are present in about 60% of all individuals with a spinal cord injury (SCI) [1,2]. Their relative risk for cognitive impairment is 13 times higher than that of the general population [3] and their hazard ratio for Alzheimer's dementia is twice as high [2,4]. Even when excluding individuals with concomitant traumatic brain injury (TBI) and accounting for mood influences, individuals with SCI demonstrate reduced performance in information processing speed, executive function, verbal fluency, short-term memory, long-term memory and attention tasks compared to age-matched healthy adults [5,6]. Moreover, these impairments have been reported to worsen over time, indicating an accelerated cognitive aging process [7]. Individuals with SCI aged 30-40 years generally exhibit cognitive test results and brain activation patterns during cognitive tasks that closely resemble those of older adult controls aged 50-60 years, while differing significantly from their age-matched counterparts [6,8].

Physical exercise may attenuate the cognitive aging process [9,10,11]. Even a single session of (i.e. acute) physical exercise may temporarily enhance cognitive performance [12,13,14]. This effect may partly arise from the release of myokines, factors released into the blood stream from contracting muscle tissue [11,12,15]. More than 1,000 putative myokines have been described, some of which may cross the blood-brain barrier and exert neurotrophic effects [11,16]. Examples of widely studied myokines are insulin-like growth factor-1 (IGF-1) and brain-derived neurotrophic factor (BDNF), which have repeatedly been reported to influence neuroplastic processes and cognitive performance [11,17,18]. During and following an exercise training session, myokines can activate signaling pathways in the brain, facilitating long-term potentiation-like processes that are critical in several cognitive function domains [11,19,20].

However, individuals with SCI may face challenges in engaging in leisure-time physical exercise, depending on the level and completeness of their injury [9]. Therefore, neuromuscular electrical stimulation (NMES) is proposed as a promising alternative or adjunct to physical exercise for inducing muscle contractions in this population. Moreover,

muscle activation with NMES has been shown to induce the release of myokines in healthy individuals [21,22,23], but no studies have been conducted in individuals with SCI. Additionally, it has not been previously investigated whether NMES can induce cognitive changes in any population.

Our objective was to investigate if a single session of NMES on gluteal and hamstring muscles of individuals with SCI can induce (a) changes in cognitive performance on the Symbol Digit Modalities Test (SDMT) and (b) changes in IGF-1. Secondarily, as an indicator of dose-response, participants were randomly allocated to either 30-minute or 60-minute NMES. We hypothesized that 60 minutes would cause the largest increase in IGF-1, associated with a larger benefit on cognitive performance.

2. Methods

2.1 Participant characteristics

A total of 22 participants with SCI were recruited from local SCI associations and activity clubs in Lithuania (see Figure 1). Participants were aged between 25 and 54 years, with a chronic traumatic SCI (between 7 and 37 years since injury), injury level L1 or higher and an American Spinal Cord Injury Association (ASIA) Impairment Scale (AIS) classification A to C [24]. Reasons for exclusion were the inability to lie in prone position for the duration of the NMES session, flaccid paresis (areflexia) which might indicate lower motor neuron disease, a history of severe autonomic dysreflexia, insufficient mastery of the Lithuanian language, severe cognitive or communicative disorders, intolerance or contra-indication for electrical stimulation (including current cancer, pregnancy, or metal implants in the stimulation area), recent or current participation in an electrical stimulation program (up to 6 months prior to inclusion), recent or current pressure ulcer category 2 or higher, and infections within 3 weeks prior to blood sample collection. Participants were allowed to withdraw from the study at any time. Written informed consent was obtained from all participants or their legal representatives before inclusion in the study.

2.2 Study protocol

Participants were enrolled in a randomized AB/BA cross-over study. Randomization was done by means of random number calculator in Excel. Participants were randomly allocated to 30 minutes (n = 10) or 60 minutes (n = 12) NMES and randomly determined to either undergo the control condition first and the experimental condition second, or vice-versa (see Figure 1). The arrival order of the participants (time of the day measurements took place) was determined randomly by one researcher unaware of the testing day schedule or allocation of the participant. They were invited to visit the Lithuanian Sports University in Kaunas, Lithuania on two separate days with exactly 48h between both visits. Before the

first visit, participants were informed about the study content by the recruiting researcher (author V.P.), and a participant information form, including an informed consent form, was provided electronically. On the first visit, they were first asked to complete the informed consent form before the start of the experiments. Subsequently, participants underwent lactate assessments, blood collection and cognitive assessments in this order. This was followed by 30-minute NMES, 60-minute NMES, or 60 minutes of rest in a waiting room, according to the participant allocation. Immediately after intervention or rest and 30min later, assessments of lactate, blood collection and cognitive tests were repeated in the same order. On the first visit, participants were asked to complete the questionnaires and undergo the clinical tests. On the second visit, participants did not undergo any additional tests apart from the intervention and outcome measures (see Figure 2). During testing and interventions, participants and assessors were aware of the participants' group allocation.

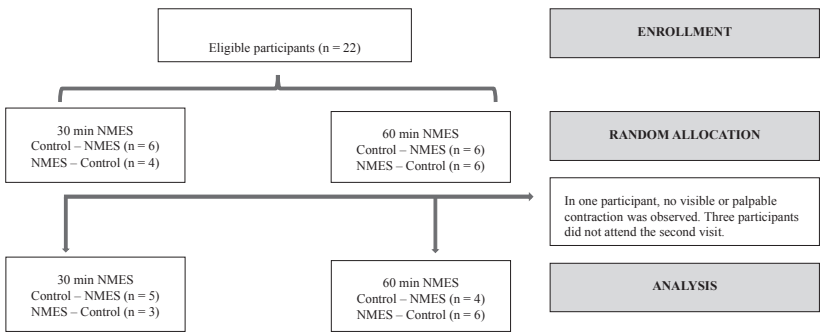


Figure 1. Participant flow diagram

Abbreviations: NMES, neuromuscular electrical stimulation.

10

2.3 Demographic and clinical characteristics

Participants were asked to provide a medical history file from their general practitioner including a description of chronic illnesses and the level and completeness of spinal cord injury based on an AIS classification [24] measured at least two years after the date of injury. If this information was not available, the AIS-classification was re-evaluated by a trained rehabilitation physician (author W.V.). Furthermore, participants were specifically asked to report a history of traumatic brain injury (concomitant or non-concomitant to the spinal cord injury), diabetes mellitus, oncologic disease, systemic inflammatory disease, infections in the last 3 weeks, wounds in the last 3 weeks, or autonomic dysreflexia. We asked participants to report their age, gender, drug or alcohol abuse, smoking status, time since spinal cord injury, highest educational degree (i.e. primary education, secondary

education, high school, university bachelor, or university master), current job or indication of unemployment or retirement, and participation in physical exercise (type, sessions per week, duration per session). Finally, we calculated body mass index (BMI) for each participant based on the measured body weight and self-reported body length.

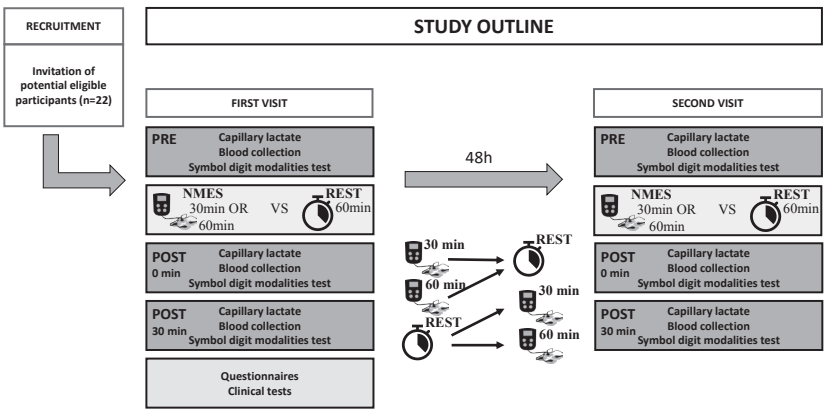


Figure 2. AB/BA cross-over study outline

2.4 Health-related questionnaires

Traumatic brain injury (TBI)-4 questionnaire: The probability of concomitant traumatic brain injury at the moment of the SCI was assessed with the TBI-4 questionnaire screening tool [25,26]. Participants were asked if they lost consciousness or had a temporary loss of memory at the time of injury. If they indicated that this was not the case, concomitant TBI probability was ranked 'improbable'. If they did not lose consciousness but reported other mental status changes immediately following SCI, TBI probability was ranked 'possible'. If they lost consciousness, they were asked to specify the duration of unconsciousness, with a duration below 30 minutes, longer than 30 minutes, or more than 24 hours considered indicative of mild, moderate or severe TBI respectively. It should be noted that the TBI-4 questionnaire has good sensitivity, but poor specificity. Therefore, the results are merely indicative of a possible TBI risk [25,26].

2.5 Lactate assessment

Capillary lactate levels were measured using the Lactate Pro 2 (Arkray Co., Ltd.) from a drop of blood collected on a test strip after a pin-prick on the fingertip. The first drop of blood was discarded and wiped away with a sterile tissue. Lactate measurements were performed before, immediately after, and 30 minutes after the intervention or control condition.

2.6 Blood biomarker assessment

Insulin-like growth factor-1 (IGF-1) was measured from venous blood samples drawn at the antecubital vein before, immediately after and 30min after the intervention or control condition. Only IGF-1 levels at baseline and IGF-1 levels immediately after intervention or control were measured. Additionally, we only assessed levels from the 17 participants from whom we had a complete set of blood samples (pre and post on both the first and second visits). The reasons for missing samples included not showing up for the second visit (n=3), insufficient blood collected for processing (n=1) and refusal of follow-up blood collection (n=1). Participants were measured throughout the whole day. On the second visit, arrival time was kept the same and participants were asked to take the same meal in order to account for diurnal or nutritional influences on blood biomarker levels. Blood samples were collected in serum separator tubes. After blood collection, the tubes were gently inverted 8-10 times and allowed to clot for exactly 30 minutes at room temperature. Then, they were centrifuged for 15 minutes at 4000 x g. After centrifugation, serum was aliquoted into 1.5 mL polypropylene tubes, and subsequently stored at -80 °C until further analysis. IGF-1 levels were assessed using an enzyme-linked immunosorbent assay (ELISA) from a commercially available ELISA kit (IBL International, GMBH, Germany, MD58011, with a lower limit of detection 0.09 ng/mL). The ELISA measurement was performed by a trained researcher with a background in pharmacology (author O.Q.).

2.7 Cognitive assessment

The symbol digit modalities test (SDMT) [27]: This test measures processing speed, complex visual tracking and working memory. The oral version of this test has been proven to be reliable in spinal cord injury patients [28]. It has been validated for the Lithuanian population, including among multiple sclerosis patients [29]. In this test procedure, the subject is given a sheet of paper at the top of which is printed the key (9 abstract symbols and 9 corresponding numbers). The key is available to the participants throughout the test. A sequence of 120 symbols, each printed in a square, is presented below the key. Empty squares are located below the symbol containing squares. In the oral version of this test, the examiner, on a copy of the test sheet, records in the empty squares the numbers the participant associates, orally, with the symbols. In the first test phase, the participant can complete 10 trial symbol-number associations without a time limit; the examiner corrects the participant's errors. After this familiarization phase, the test begins. Then, participant get 90 seconds to complete as many associations as possible. The outcome measure is the number of correct associations made in the given time frame. We had three versions of this test available, each with an unique legend containing slightly different symbols. The SDMT test was administered before, immediately after, and 30 minutes after the intervention or control condition. The order of the versions used was 1-2-3 for the first visit and 2-1-3 for the second visit.

2.8 NMES intervention

NMES was applied using a battery-powered portable stimulator with surface electrodes (CEFAR REHAB X2, CEFAR Medical AB, Sweden). For each leg, one electrode was placed on the proximal side of the gluteal muscles, and a second electrode was positioned over the mid-part of the hamstrings. NMES induced a tetanic contraction of the gluteal and hamstring muscles. Participants underwent one of the two possible interventions (i.e. 30-minute or 60-minute NMES). For both NMES interventions, electrical stimulation involved a biphasic 6-second to 18-second activation-rest cycle, 50 Hz frequency, and a 400 µs pulse duration. The activation within the activation-rest cycle consisted of a 1.5-second ramp-up, a 3-second full activation and 1.5-second ramp-down followed by 18 seconds of rest. The current amplitude was gradually increased until at least a visible or palpable muscle contraction was achieved. Then, the amplitude was further increased as long as it did not cause discomfort in the participant. However, as some participants with SCI may never start to feel any discomfort upon increasing intensity, it was predetermined that the current amplitude would never be elevated above 40 mA in the 60-minute intervention group, and above 100 mA in the 30-minute intervention group. The reason for this decision was that we expected a risk of muscle fatigue when longer durations would be used at higher intensities.

Participants in the control condition stayed in the waiting room for 60 minutes between assessments. They arrived on the same time of the day and measurement protocols and assessors were the same between visits. Furthermore, they were instructed to keep activities and nutrition as equal as possible between visits.

2.9 Statistical analysis and sample size calculation

IBM SPSS Statistics 27 (IBM Inc., Chicago, IL, USA) was used to perform all analyses. The graphical presentation of the data and the estimation of effect sizes with 95% confidence intervals was performed with Data Analysis with Bootstrap-coupled ESTimation (DABEST, version 2023.02.14) [30]. First, the data was inspected for outliers and normality. Extreme outliers, defined as values lying more than 3× the interquartile range away from the median were excluded. For this reason, we excluded one outlier in lactate results (on the third measurement in control condition), two outliers in IGF-1 results (one on the second measurement in the control condition and one on the second measurement in the 60-minute condition) and one outlier in the SDMT results (on the third measurement in control condition). Normality was defined as a value of kurtosis and skewness between -2 and +2. Additionally, normality was visually checked using P-P plots and histograms. Data not meeting one of the normality assumptions were log transformed. Homoscedasticity was tested with Levene's test.

Differences between 30-minute and 60-minute NMES groups at baseline were analyzed using independent t-tests and Chi² tests (or Fisher Exact tests, if the expected count in any of the cells was below 5) for continuous and categorical variable respectively. A three-way repeated measures ANOVA was conducted for the evaluation of changes in cognitive, lactate and IGF-1 levels caused by the intervention, with one between-group factor (30-minute NMES versus 60-minute NMES) and two within-group factors (pretest versus first posttest versus second posttest; and NMES versus control). Finally, an explorative analysis of the moderating role of certain participant characteristics on the change in the outcome measures was performed using Pearson's correlations for continuous parameters and independent samples t-tests for categorical parameters.

The sample size needed to have sufficient power for performing a three-way repeated measures ANOVA and find meaningful differences in our primary outcome, changes in SDMT test performance, was estimated with a Shiny app, using a simulation-based method to estimate power for ANOVA tests [31]. As we could not find previous studies testing acute NMES effects on the SDMT test, we based our simulation on the study of Parthimos et al. including 60 male semi-professional basketball players who underwent basketball exercise or one hour inactive resting, with measurements of SDMT test before and after the intervention [32]. Based on the results of this study we were only able to assess the sample size needed for a two-way ANOVA test with two within factors. Therefore, we estimated the sample size needed for running this test twice, once for the 30-minute and once for the 60-minute NMES group separately. For the simulation, we entered a high expected correlation among within-subjects factors estimated to be 0.9, a standard deviation of 4.47 and mean values in the exercise group pre 64.93 and post 68.76 and in the control group pre 64.77 and post 65.42 based on the abovementioned article [32]. The results of the simulation showed that with a total of 18 participants, 9 participants in the 30 minutes and 9 participants in the 60 minutes group, we would have a power of 86.96 to find differences between exercise and control group, a power of 97.56 to find differences between pre- and post-tests and a power of 80.14 to find significant interaction effects between the two within factors.

3. Results

3.1 Participant characteristics

Table 1. Demographic characteristics

	60 min NMES (n = 12)	30 min NMES (n = 10)	Total (n = 22)	Significance
Age (years)	43.1 (5.8)	35.8 (7.6)	39.7 (7.5)	0.019*
Gender				NA
• Male	8 (66.7%)	10 (100%)	18 (81.8%)	
• Female	4 (33.3%)	0 (0%)	4 (18.2%)	
BMI (kg/m²)	24.5 (5.1)	21.7 (5.5)	23.2 (5.3)	0.240
Smoking				0.783
• Never	5 (41.7%)	5 (50.0%)	10 (45.5%)	
• Past	4 (33.3%)	2 (20.0%)	6 (27.3%)	
• Currently	3 (25.0%)	3 (30.0%)	6 (27.3%)	
Alcohol				0.699
• Never	2 (16.7%)	3 (30.0%)	5 (22.7%)	
• Sporadically	8 (66.7%)	5 (50.0%)	13 (59.1%)	
• Regularly	2 (16.7%)	3 (30.0%)	4 (18.2%)	
Education				0.305
• Primary education	1 (8.3%)	0 (0%)	1 (4.5%)	
• Secondary education	4 (33.3%)	5 (50.0%)	9 (40.9%)	
• High school	0 (0%)	1 (10.0%)	1 (4.5%)	
• University bachelor	2 (16.7%)	3 (30.0%)	5 (22.7%)	
• University master	5 (41.7%)	1 (10.0%)	6 (27.3%)	
Employment				0.840
• Not currently	2 (16.7%)	2 (20.0%)	4 (18.2%)	
• Currently	10 (83.3%)	8 (80.0%)	18 (81.8%)	
Physical activity				NA
• Sedentary	7 (58.3%)	10 (100%)	17 (77.3%)	
• Regularly exercising	5 (41.7%)	0 (0%)	5 (22.7%)	
Time since injury (years)	21 (8.6)	14.4 (7.2)	17.9 (8.5)	0.075
Completeness of injury				
• AIS A	9 (75%)	8 (80%)	17 (77.3%)	0.594
• AIS C	3 (25%)	2 (20%)	5 (22.7%)	
Diabetes mellitus	0 (0%)	0 (0%)	0 (0%)	NA
Reported history of TBI	0 (0%)	3 (30.0%)	3 (13.6%)	NA

Table 1. Continued

	60 min NMES (n = 12)	30 min NMES (n = 10)	Total (n = 22)	Significance
TBI-4 questionnaire on concomitant TBI:				0.292
• Improbable TBI	5 (41.7%)	6 (60.0%)	11 (50.0%)	
• Possible TBI	2 (16.7%)	0 (0%)	2 (9.1%)	
• Mild TBI	3 (25.0%)	3 (30.0%)	6 (27.3%)	
• Moderate TBI	0 (0%)	1 (10.0%)	1 (4.5%)	
• Severe TBI	2 (16.7%)	0 (0%)	2 (9.1%)	

Continuous variables are presented as mean (standard deviation) and unpaired t-tests are used to check for differences between high and low intensity groups (per protocol). Categorical variables are presented as n (%) and Chi² or Fisher's Exact tests are used to check for differences between high and low intensity groups. If one of the values was 0, a p-value could not be calculated, and "NA" was reported instead. Abbreviations: BMI, body mass index; NA, not applicable; NMES, neuromuscular electrical stimulation; TBI, traumatic brain injury

Of the 22 participants included in the study, 19 (86.4%) attended both testing sessions at the Lithuanian Sport University laboratory. The three participants who did not attend the second visit are listed as participants 20-22 in Table 2. Their characteristics or outcome measures did not significantly differ from other participants. Demographic characteristics of the participants are presented in Table 1 and SCI-related characteristics in Table 2. Baseline and follow-up values of the individual outcome measures are depicted in Figure 3. The absolute values of the outcome measures are presented in Table A.1 (supplemental materials). The participants' ages ranged from 25 to 54 years, and their BMI varied from 15.1 to 33.5 kg/m². Participants in the 30-minute NMES group were significantly younger than participants allocated to 60-minute NMES group (p =0.019).

3.2 NMES effects

Visible or palpable contractions were observed in all participants except one female (participant 7 in Table 2). This participant was allocated to the 60-minute intervention group. She was not included in further analysis. All participants in the 60-minute intervention group received the predetermined maximum intensity of 40 mA. Similarly, all participants in the 30-minute intervention group tolerated the predefined maximum intensity of 100 mA. The ANOVA test results are presented in Table 3. For lactate, the two within-group factors and their interaction were significant (Time, p = 0.032; control vs NMES, p = 0.036; Time*control vs NMES, p = 0.004). There were no significant findings for IGF-1 changes. Performance on the SDMT only significantly improved over time (p < 0.001). No significant effects or interactions were observed for duration of NMES (30-minute vs 60-minute) for any of the three outcome measures (all p ≥ 0.259). Notably, performance on the SDMT test tended to decrease immediately after 30-minute NMES.

Table 2. Individual SCI-related characteristics of included participants

Number	Level and completeness of injury	Years since injury	Cause	Group
1.	Th10 AIS A	18	Traumatic	Control – 30 min NMES
2.	Th11 AIS A	25	Traumatic	60 min NMES – Control
3.	Th5 AIS A	13	Traumatic	Control – 30 min NMES
4.	Th4 AIS A	13	Traumatic	Control – 60 min NMES
5.	C6 AIS C	22	Traumatic	60 min NMES – Control
6.	C5 AIS A	10	Traumatic	30 min NMES – Control
7.	Th8 AIS A	21	Traumatic	Control – 60 min NMES
8.	Th5 AIS A	7	Traumatic	Control – 60 min NMES
9.	Th8 AIS A	21	Traumatic	60 min NMES – Control
10.	Th12 AIS C	32	Traumatic	60 min NMES – Control
11.	Th4 AIS A	7	Traumatic	30 min NMES – Control
12.	Th10 AIS A	11	Traumatic	Control – 60 min NMES
13.	Th6 AIS C	10	Traumatic	Control – 30 min NMES
14.	C4 ASIA C	23	Traumatic	Control – 60 min NMES
15.	Th12 AIS A	21	Traumatic	Control – 60 min NMES
16.	C5 AIS A	19	Traumatic	Control – 30 min NMES
17.	L1 AIS A	37	Traumatic	60 min NMES – Control
18.	Th6 AIS A	21	Traumatic	Control – 30 min NMES
19.	Th12 AIS A	7	Traumatic	30 min NMES – Control
20.	Th11 AIS A	22	Traumatic	30 min NMES – Control
21.	C5 AIS C	27	Traumatic	Control – 30 min NMES
22.	L1 AIS A	23	Traumatic	60 min NMES – Control

The level of completeness is presented as the AIS score. The time since injury is presented in years. Abbreviations: AIS, ASIA Impairment Scale; ASIA, American Spinal Injury Association.

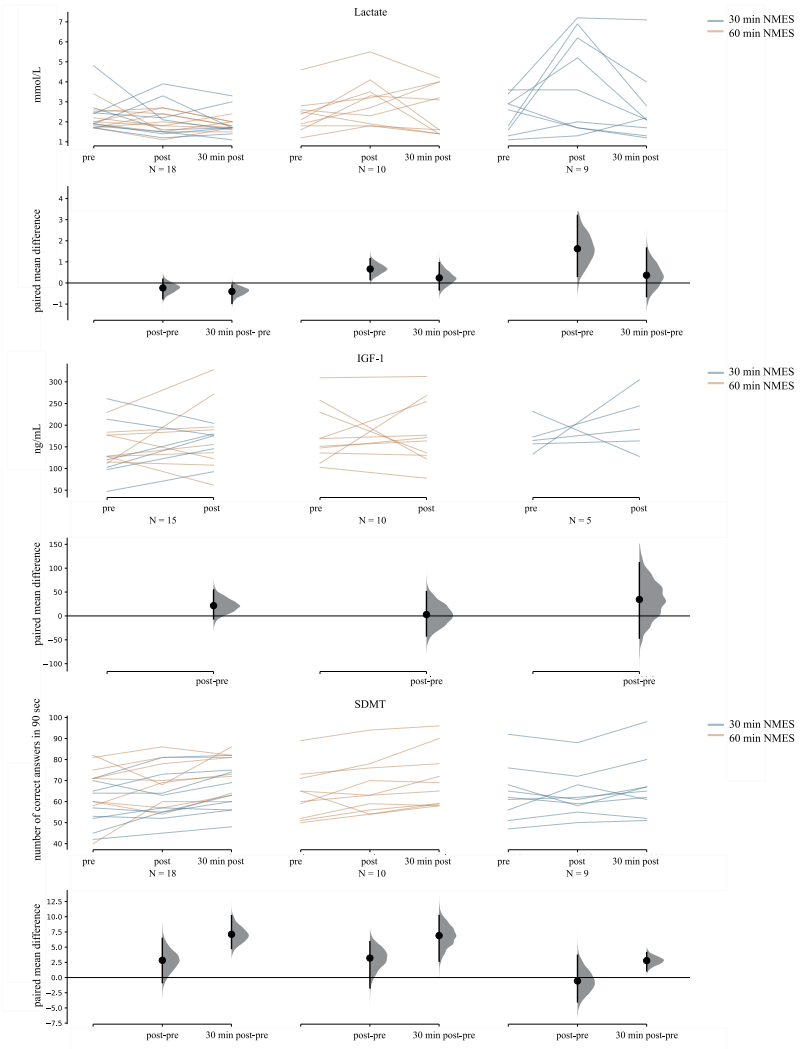


Figure 3. Individual outcome values and effect sizes
The individual values per participant are presented for A) lactate, B) IGF-1, C) SDMT performance over time at baseline (pre), immediately after intervention or control (post) and 30min after intervention or control (post2), for participants with complete datasets of the respective outcome measure, taking into account missing values and exclusion of outliers. The number (N) of participants with complete data for each condition is shown. Blue lines reflect participants that underwent a 30-minute and orange lines reflect participants that underwent a 60-minute NMES session. Control and experimental conditions are presented separately. The effect sizes of the change per condition and per duration group (paired mean differences) with their 95% confidence interval are presented in comparison with baseline values of the same testing day. Abbreviations: IGF-1, insulin-like growth factor 1; NMES, neuromuscular electrical stimulation; SDMT, Symbol Digit Modalities Test.

Table 3. Three-way repeated measures ANOVA results

	Time		Condition (control vs NMES)		Duration (30 vs 60-minute NMES)	
	p	$_p\eta^2$	p	$_p\eta^2$	p	$_p\eta^2$
Lactate	0.032	0.184	0.036	0.233	0.403	0.041
IGF-1	0.231	0.117	0.348	0.074	0.668	0.016
SDMT	<0.001	0.451	0.458	0.035	0.337	0.058

Dependent variables are presented in the first column. P-values and effect sizes (partial eta squared) are given for the Time effect (pre versus post for IGF-1; pre versus immediately post versus 30min post for lactate and SDMT), condition effect (NMES versus control)

The baseline SDMT results on the second testing day were significantly higher than those on the first testing day, irrespective of whether the NMES intervention took place during the first ($p < 0.001$) or second ($p = 0.005$) visit. IGF-1 levels were significantly higher at the baseline of the second testing day compared to the first if the first testing day was a control condition ($p=0.029$).

3.3 Exploratory analysis of the role of participant characteristics

Pearson's correlations were used to examine the relationships between age, time since injury, injury level, or BMI related and the changes in outcome measures (lactate, IGF-1, and SDMT results). Independent samples t-tests were conducted to evaluate how gender, smoking status, alcohol consumption, educational level, participation in physical activity, completeness of injury (AIS A versus C) and loss of consciousness during spinal injury influenced changes in outcome measures from baseline to immediately after NMES (see Table A.2 in the supplemental materials). The analyses revealed that participants who regularly consumed alcohol exhibited significantly higher lactate increases ($p =0.017$) compared to participants who consumed alcohol sporadically or not at all. For participants in the 60-minute NMES group, IGF-1 levels increased significantly less in older individuals ($R^2 =0.684$, $p =0.011$) and individuals with longer time since injury ($R^2 =0.524$, $p =0.042$), and SMDT performance increased more in participants with lower injury level ($R^2 =0.651$, $p =0.003$).

Time * Condition		Time * Duration		Condition * Duration		Time * Condition * Duration	
p	$_p\eta^2$	p	$_p\eta^2$	p	$_p\eta^2$	p	$_p\eta^2$
<u>0.004</u>	<u>0.275</u>	0.371	0.057	0.685	0.010	0.262	0.076
0.808	0.005	0.641	0.019	0.432	0.052	0.259	0.105
0.347	0.061	0.454	0.048	0.464	0.034	0.892	0.004

and duration effect (30 minutes versus 60 minutes) and their interactions are presented. Significant values are underlined. Abbreviations: IGF-1, insulin-like growth factor 1; p, p-value; $_p\eta^2$, partial eta squared value; SDMT, Symbol Digit Modalities Test.

4. Discussion

This is the first study to investigate the effects of a single session of NMES on cognitive performance in individuals with SCI. Based on data from 18 participants, our findings do not provide evidence of an effect of NMES on SDMT performance, a measure of processing speed. The intervention was administered at two different durations (30 or 60 minutes of NMES), but the current amplitude was higher in the 30-minute NMES group, which limits direct comparability. However, no significant differences were observed in lactate level changes between the two groups. Therefore, since both intervention durations were ineffective and no significant differences were identified between groups for any of the outcomes, they are not discussed separately in the subsequent text.

Given the limited number of studies on this topic, we can only speculate as to why our hypothesis was not confirmed. We found one other study that evaluated effects of muscle training on cognitive function in persons with SCI [33]. This study reported improvements in a working memory task after 6 months of functional electrical stimulation (FES)-assisted rowing exercise. It is unclear whether this effect was partly caused by the FES-assisted exercise intervention or only the result of a practice effect, as there was no control group included in the study [33].

A secondary aim of this study was to evaluate the role of IGF-1 in enhancing cognitive performance in relation to NMES. Physical exercise has been shown to enhance cognitive function, with IGF-1 proposed as one of the potential players underlying this effect [34]. Previous studies have shown that intramuscular IGF-1 expression was upregulated after acute NMES in rats [35] and chronic NMES in humans [36]. Electrical stimulation in rats even induced a larger intramuscular release of IGF-1 compared to aerobic exercise [35]. However, circulating levels of IGF-1 did not significantly increase in the NMES condition compared to control in our study.

Consistent with previous studies [22], our results demonstrated a significant increase in lactate levels following NMES. Interestingly, it has been suggested previously that lactate can also have positive effects on cognition [37]. Lactate can serve as a precursor to glutamate and γ-aminobutyric acid (GABA), the main excitatory and inhibitory neurotransmitters in the human brain [38], and potentiate N-methyl-D-aspartate (NMDA) receptors [39]. Via these pathways, it was argued that lactate may enhance neuroplastic processes such as long-term synaptic potentiation, potentially boosting learning and memory formation [11,38,39]. Furthermore, lactate was described to stimulate the release of BDNF in the blood circulation [22,40] and vascular endothelial growth factor (VEGF) in the brain [41], myokines that respectively play a role in neural survival and angiogenesis. Interestingly, lactate levels were reported to increase more with NMES than with voluntary muscle contractions, since NMES non-selectively activates both lactate-producing fast-twitch and slow-twitch muscle fibers, while with voluntary exercise first the slow twitch fibers are activated according to the Henneman's size principle [23]. One study in young healthy adults argued that higher lactate levels were the reason they found higher elevations in BDNF levels following NMES compared to voluntary exercise with the same integrated force of muscle contraction [22]. Despite these mechanisms suggest that NMES might enhance cognition via the release of lactate, our results are not in line with this hypothesis, given that in the 30-minute NMES group, who had higher lactate increases, the normal practice effect seemed to be diminished compared to both the 60min NMES and control conditions.

It is important to note that the role of myokines and lactate in the temporary enhancement of cognitive performance observed after a single bout of physical exercise is still under debate, in contrast to their potential to induce more significant changes following chronic physical exercise interventions [15]. An alternative hypothesis is that acute (high-intensity) physical exercise temporary enhances cognitive performance due to increased arousal caused by elevated levels of catecholamines [42]. Also after NMES, increases in adrenaline have been described in healthy adults [43]. Yet again, in our study SDMT performance tended to decrease immediately after the 30-minute NMES session, where participants received stimulation with a higher current amplitude. Maybe this contradiction is in line with physical exercise studies, where it was reported that high-intense physical exercise may either enhance or impair cognitive performance depending on the type of exercise, the type of cognitive task, and the recovery time after exercise and before cognitive testing [13]. It is possible that the higher current amplitude in this condition caused discomfort or fatigue, which may have counteracted the typical practice effect observed in cognitive testing. Importantly, none of the participants had autonomic complaints or skin reactions due to NMES. Yet, in some participants the NMES evoked spasticity. Based on these reflections, we advise to use NMES of an current amplitude that is sufficiently high, but still easily tolerated by the participant, similar to what is tolerated by able-bodied individuals.

Second, it is possible that stimulation of paretic muscle tissue may have different effects on the release of myokines and catecholamines than stimulation of healthy muscle tissue. Interestingly, older age and longer time since injury were related to a significantly lower increase in IGF-1 levels following 60-minute stimulation (R = -0.827 and R = -0.724, respectively; see Table A.2 in the supplemental materials). Older age was highly correlated to time since injury (R^2 = 0.664, p = 0.001). Participants in the 60-minute NMES group were significantly older than those in the 30-minute NMES group. The correlation test findings suggest an attenuation of the release of myokines by NMES in case of long-term paresis, possibly associated with larger muscle atrophy.

Third, it may be argued that the cognitive test we used for investigating cognitive changes may not have been the most responsive to changes caused by NMES. However, based on knowledge from exercise studies [44,45], we expected a good likelihood to find NMES effects on SDMT performance. Firstly, the SDMT is an information processing task with a working memory component, which is known to be one of the domains that is often impaired in people with SCI [5,6]. Secondly, meta-analyses investigating cognitive changes induced by a single bout of physical exercise have repeatedly confirmed an enhancement of processing speed and executive functions, which includes working memory [14,44,45,46]. Furthermore, the SDMT test is considered to be of appropriate difficulty, as it has been shown to be crucial to select a cognitive task that is neither too easy nor too challenging, thereby increasing the likelihood of detecting significant effects [47]. Finally, we discovered that individuals with higher level of injury improved less on the SDMT test compared to individuals with lower level of injury in the 60-minute group. In line with this finding, Wecht et al. (2018) found that tetraplegic participants scored lower on a SDMT test compared to paraplegic participants and controls. They attributed this difference to impaired hemodynamic responses in the tetraplegic group, as an inadequate systemic and cerebral hemodynamic response to the SDMT test related to the test score [48]. It is possible that hemodynamics also explain our finding that SDMT test changes in participants with higher level SCI are less responsive to an intervention with NMES.

This study has several limitations. First, the study design had different criteria for selecting current amplitude between the conditions with different NMES duration. Second, we did also not measure the level of electrically evoked force, which may vary greatly between individuals due to variability in individuals' intrinsic tissue properties [49]. As a result, the force produced by a participant receiving 40 mA could have been higher than that produced by someone receiving 100 mA. The effectiveness of NMES depends on the level of electrically evoked force, which is strongly related to lactate concentrations [50]. Lactate levels significantly differed between the control and NMES conditions, but no significant differences were observed between the different NMES duration conditions. Third, it could be argued that measuring additional outcomes might have provided more comprehensive insights from this study, such as including blood assessments of myokines like BDNF, catecholamine levels, and evaluating changes across a broader range of cognitive domains using a larger neurocognitive test battery.

5. Conclusion

We conclude that a single session of NMES on the gluteal and hamstrings muscles below the level of injury in individuals with SCI induced elevations in lactate levels. On average, this increase was larger in participants receiving higher current amplitudes. We did not find significant changes in IGF-1 levels or SDMT performance induced by a single session of NMES. After a 60-minute NMES session, longer time since injury attenuated the release of IGF-1 levels and higher injury level was associated with smaller SDMT performance improvements. Further research is needed to determine whether the absence of a positive effect can be attributed to participant characteristics, the nature of the intervention, or aspects of the outcome assessments. Future research should explore potential moderators of NMES effects on cognitive function and investigate the impact of long-term NMES interventions over multiple weeks.

Data availability

The data collected in this study has been made available in Open Science Framework (OSF) via the following link https://osf.io/xc9ap/?view_only=147a2d573cca4fad8712cc816d6061b5.

Declarations

The study methods were approved by the Lithuanian Sports University local bioethics committee (No. 2021 07 07 NR. BNL- KIN(B)-2021-402)

Acknowledgements

We thank Mindaugas Kvedaras for his help in preparing the blood samples after blood collection. His work involved centrifugating the blood samples followed by aliquoting serum and storing the serum samples in the Lithuanian Sports University refrigerator compartments until further analysis.

Funding

This work was supported by the Research Council of Lithuania (grant number S-MIP-22-12). The sponsor was not involved in study design, collection, analysis or interpretation of the data, in writing the report, nor in the decision to submit the article for publication.

References

1. Murray RF, Asghari A, Egorov DD, et al. Impact of spinal cord injury on self-perceived pre- and postmorbid cognitive, emotional and physical functioning. Spinal Cord. 2007;45:429–436. Available at: https://pubmed.ncbi.nlm.nih.gov/17228355/. Accessed March 13, 2021.
2. Huang SW, Wang W Te, Chou LC, Liou TH, Lin HW. Risk of dementia in patients with spinal cord injury: A nationwide population-based cohort study. J. Neurotrauma. 2017;34:615–622. Available at: https://pubmed.ncbi.nlm.nih.gov/27539630/. Accessed March 13, 2021.
3. Craig A, Guest R, Tran Y, Middleton J. Cognitive Impairment and Mood States after Spinal Cord Injury. J. Neurotrauma. 2017;34:1156–1163. Available at: https://pubmed.ncbi.nlm.nih.gov/27717295/. Accessed March 14, 2021.
4. Yeh T-S, Ho Y-C, Hsu C-L, Pan S-L. Spinal cord injury and Alzheimer's disease risk: a population-based, retrospective cohort study. Spinal Cord. 2018;56:151–157. Available at: https://doi.org/10.1038/s41393-017-0009-3. Accessed March 24, 2021.
5. Heled E, Tal K, Zeilig G. Does lack of brain injury mean lack of cognitive impairment in traumatic spinal cord injury? J. Spinal Cord Med. 2020. Available at: https://www.tandfonline.com/doi/abs/10.1080/10790268.2020.1847564. Accessed March 24, 2021.
6. Chiaravalloti ND, Weber E, Wylie G, Dyson-Hudson T, Wecht JM. Patterns of cognitive deficits in persons with spinal cord injury as compared with both age-matched and older individuals without spinal cord injury. J. Spinal Cord Med. 2020;43:88–97. Available at: /pmc/articles/PMC7006756/. Accessed March 26, 2021.
7. Molina B, Segura A, Serrano JP, et al. Cognitive performance of people with traumatic spinal cord injury: a cross-sectional study comparing people with subacute and chronic injuries. Spinal Cord. 2018;56:796–805. Available at: https://pubmed.ncbi.nlm.nih.gov/29472608/. Accessed March 28, 2021.
8. Wylie GR, Chiaravalloti ND, Weber E, Genova HM, Dyson-Hudson TA, Wecht JM. The Neural Mechanisms Underlying Processing Speed Deficits in Individuals Who Have Sustained a Spinal Cord Injury: A Pilot Study. Brain Topogr. 2020;33:776–784. Available at: https://doi.org/10.1007/s10548-020-00798-x. Accessed March 14, 2021.
9. Vints WAJ, Levin O, Masiulis N, Verbunt J, van Laake-Geelen CCM. Myokines may target accelerated cognitive aging in people with spinal cord injury: A systematic and topical review. Neurosci. Biobehav. Rev. 2023;146:105065. Available at: https://linkinghub.elsevier.com/retrieve/pii/S0149763423000349. Accessed January 31, 2023.
10. Mee-inta, Zhao, Kuo. Physical Exercise Inhibits Inflammation and Microglial Activation. Cells. 2019;8:691. Available at: /pmc/articles/PMC6678635/. Accessed March 14, 2021.
11. Vints WAJ, Levin O, Fujiyama H, Verbunt J, Masiulis N. Exerkines and long-term synaptic potentiation: Mechanisms of exercise-induced neuroplasticity. Front. Neuroendocrinol. 2022;66:100993. Available at: https://linkinghub.elsevier.com/retrieve/pii/S0091302222000164. Accessed March 15, 2022.
12. Tsai C-L, Ukropec J, Ukropcová B, Pai M-C. An acute bout of aerobic or strength exercise specifically modifies circulating exerkine levels and neurocognitive functions in elderly individuals with mild cognitive impairment. NeuroImage Clin. 2018;17:272–284. Available at: http://creativecommons.org/licenses/BY-NC-ND/4.0/. Accessed February 20, 2019.
13. Lambourne K, Tomporowski P. The effect of exercise-induced arousal on cognitive task performance: A meta-regression analysis. Brain Res. 2010;1341:12–24. Available at: https://www.sciencedirect.com/science/article/pii/S0006899310007547. Accessed July 8, 2019.

14. Wilke J, Giesche F, Klier K, Vogt L, Herrmann E, Banzer W. Acute Effects of Resistance Exercise on Cognitive Function in Healthy Adults: A Systematic Review with Multilevel Meta-Analysis. Sport. Med. 2019 496. 2019;49:905–916. Available at: https://link.springer.com/article/10.1007/s40279-019-01085-x. Accessed September 4, 2021.
15. El-Sayes J, Harasym D, Turco C V., Locke MB, Nelson AJ. Exercise-Induced Neuroplasticity: A Mechanistic Model and Prospects for Promoting Plasticity. Neuroscientist. 2019;25:65–85. Available at: http://journals.sagepub.com/doi/10.1177/1073858418771538. Accessed March 21, 2019.
16. Vints WAJ, Gökçe E, Langeard A, et al. Myokines as mediators of exercise-induced cognitive changes in older adults: protocol for a comprehensive living systematic review and meta-analysis. Front. Aging Neurosci. 2023;15:1213057.
17. Berg U, Bang P. Exercise and Circulating Insulin-Like Growth Factor I. Horm. Res. Paediatr. 2004;62:50–58. Available at: https://www.karger.com/Article/FullText/80759. Accessed November 28, 2020.
18. Ding Q, Vaynman S, Akhavan M, Ying Z, Gomez-Pinilla F. Insulin-like growth factor I interfaces with brain-derived neurotrophic factor-mediated synaptic plasticity to modulate aspects of exercise-induced cognitive function. Neuroscience. 2006;140:823–833.
19. Martin SJ, Grimwood PD, Morris RGM. Synaptic Plasticity and Memory: An Evaluation of the Hypothesis. Annu. Rev. Neurosci. 2000;23:649–711. Available at: https://web-a-ebscohost-com.ezproxy.ub.unimaas.nl/ehost/pdfviewer/pdfviewer?vid=1&sid=93ada247-f922-4b91-a057-a1632a738fcd%40sdc-v-sessmgr02. Accessed December 18, 2020.
20. Müller P, Duderstadt Y, Lessmann V, Müller NG. Lactate and BDNF: Key Mediators of Exercise Induced Neuroplasticity? J. Clin. Med. 2020;9:1136. Available at: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7230639/. Accessed January 9, 2021.
21. Sanchis-Gomar F, Lopez-Lopez S, Romero-Morales C, Maffulli N, Lippi G, Pareja-Galeano H. Neuromuscular Electrical Stimulation: A New Therapeutic Option for Chronic Diseases Based on Contraction-Induced Myokine Secretion. Front. Physiol. 2019;10. Available at: https://pubmed.ncbi.nlm.nih.gov/31849710/. Accessed March 6, 2021.
22. Kimura T, Kaneko F, Iwamoto E, Saitoh S, Yamada T. Neuromuscular electrical stimulation increases serum brain-derived neurotrophic factor in humans. Exp. Brain Res. 2019;237:47–56. Available at: https://pubmed.ncbi.nlm.nih.gov/30306243/. Accessed March 6, 2021.
23. Miyamoto T, Kou K, Yanamoto H, et al. Effect of Neuromuscular Electrical Stimulation on Brain-derived Neurotrophic Factor. Int. J. Sports Med. 2018;39:5–11. Available at: http://www.thieme-connect.de/DOI/DOI?10.1055/s-0043-120343. Accessed March 21, 2019.
24. Betz R, Biering-Sørensen F, Burns SP, et al. The 2019 revision of the International Standards for Neurological Classification of Spinal Cord Injury (ISNCSCI)-What's new? Spinal Cord. 2019;57:815–817. Available at: https://doi.org/10.1038/s41393-019-0350-9. Accessed November 6, 2023.
25. Bombardier CH, Lee DC, Tan DL, Barber JK, Hoffman JM. Comorbid Traumatic Brain Injury and Spinal Cord Injury: Screening Validity and Effect on Outcomes. Arch. Phys. Med. Rehabil. 2016;97:1628–1634. Available at: https://pubmed.ncbi.nlm.nih.gov/27084266/. Accessed March 28, 2021.
26. Brenner LA, Homaifar BY, Olson-Madden JH, et al. Prevalence and screening of traumatic brain injury among veterans seeking mental health services. J. Head Trauma Rehabil. 2013;28:21–30. Available at: https://pubmed.ncbi.nlm.nih.gov/23288308/. Accessed May 1, 2022.
27. Smith A. Symbol Digit Modalities Test (SDMT). Manual (Revised). 1982. Available at: https://www.scirp.org/(S(vtj3fa45qm1ean45vvffcz55))/reference/ReferencesPapers.aspx?ReferenceID=975903. Accessed June 15, 2021.
28. Nightingale TE, Lim CAR, Sachdeva R, Zheng MMZ, Phillips AA, Krassioukov A. Reliability of Cognitive Measures in Individuals With a Chronic Spinal Cord Injury. PM R. 2019;11:1278–1286. Available at: https://pubmed.ncbi.nlm.nih.gov/30900829/. Accessed March 26, 2021.

29. Giedraitienė N, Kizlaitienė R, Kaubrys G. The BICAMS battery for assessment of Lithuanian-speaking multiple sclerosis patients: Relationship with age, education, disease disability, and duration. Med. Sci. Monit. 2015;21:3853–3859. Available at: https://pubmed.ncbi.nlm.nih.gov/26655632/. Accessed June 15, 2021.
30. Ho J, Tumkaya T, Aryal S, Choi H, Claridge-Chang A. Moving beyond P values: data analysis with estimation graphics. Nat. Methods. 2019;16:565–566. Available at: https://www.nature.com/articles/s41592-019-0470-3. Accessed October 27, 2023.
31. Lakens D, Caldwell A. Simulation-Based Power Analysis for Factorial Analysis of Variance Designs. Adv. Methods Pract. Psychol. Sci. 2021;4.
32. Parthimos TP, Schulpis KH, Karousi AD, Loukas YL, Dotsikas Y. The relationship between neurotransmission-related amino acid blood concentrations and neuropsychological performance following acute exercise. Appl. Neuropsychol. Adult. 2022. Available at: https://www.tandfonline.com/doi/abs/10.1080/23279095.2022.2043327. Accessed October 9, 2023.
33. Ozturk ED, Lapointe MS, Kim D Il, Hamner JW, Tan CO. Effect of 6-Month Exercise Training on Neurovascular Function in Spinal Cord Injury. Med. Sci. Sports Exerc. 2021;53:38–46.
34. Cotman CW, Berchtold NC, Christie L-A. Exercise builds brain health: key roles of growth factor cascades and inflammation. TRENDS Neurosci. 2007;30:464–472. Available at: www.sciencedirect.com. Accessed October 20, 2020.
35. Kido K, Ato S, Yokokawa T, Makanae Y, Sato K, Fujita S. Acute resistance exercise-induced IGF1 expression and subsequent GLUT4 translocation. Physiol. Rep. 2016;4:e12907. Available at: https://pubmed.ncbi.nlm.nih.gov/27550988/. Accessed January 6, 2025.
36. Vivodtzev I, Debigaré R, Gagnon P, et al. Functional and muscular effects of neuromuscular electrical stimulation in patients with severe COPD: A randomized clinical trial. Chest. 2012;141:716–725. Available at: http://journal.chestnet.org/article/S0012369212601627/fulltext. Accessed January 6, 2025.
37. Sugimoto T, Suga T, Tomoo K, et al. Blood Flow Restriction Improves Executive Function after Walking. Med. Sci. Sports Exerc. 2021;53:131–138. Available at: https://pubmed.ncbi.nlm.nih.gov/32694372/. Accessed November 24, 2021.
38. Kleppner SR, Tobin AJ. GABA. In: Ramachadran VS, ed. Encyclopedia of the human brain. 2nd ed. California: Academic Press; 2002:353–367.
39. Yang J, Ruchti E, Petit JM, et al. Lactate promotes plasticity gene expression by potentiating NMDA signaling in neurons. Proc. Natl. Acad. Sci. U. S. A. 2014;111:12228–12233. Available at: /pmc/articles/PMC4143009/?report=abstract. Accessed January 9, 2021.
40. Proia P, di Liegro CM, Schiera G, Fricano A, Di Liegro I. Lactate as a Metabolite and a Regulator in the Central Nervous System. Int. J. Mol. Sci. 2016, Vol. 17, Page 1450. 2016;17:1450. Available at: https://www.mdpi.com/1422-0067/17/9/1450/htm. Accessed November 1, 2023.
41. Morland C, Andersson KA, Haugen ØP, et al. Exercise induces cerebral VEGF and angiogenesis via the lactate receptor HCAR1. Nat. Commun. 2017;8:1–9.
42. McMorris T, Collard K, Corbett J, Dicks M, Swain JP. A test of the catecholamines hypothesis for an acute exercise–cognition interaction. Pharmacol. Biochem. Behav. 2008;89:106–115. Available at: https://linkinghub.elsevier.com/retrieve/pii/S0091305707003498. Accessed March 21, 2019.
43. Moreau D, Dubots P, Boggio V, Guilland JC, Cometti G. Effects of electromyostimulation and strength training on muscle soreness, muscle damage and sympathetic activation. J. Sports Sci. 1995;13:95–100. Available at: https://pubmed.ncbi.nlm.nih.gov/7595984/. Accessed November 1, 2023.
44. McMorris T, Hale BJ. Differential effects of differing intensities of acute exercise on speed and accuracy of cognition: A meta-analytical investigation. Brain Cogn. 2012;80:338–351. Available at: https://linkinghub.elsevier.com/retrieve/pii/S0278262612001224. Accessed May 18, 2019.

45. Chang YK, Labban JD, Gapin JI, Etnier JL. The effects of acute exercise on cognitive performance: A meta-analysis. Brain Res. 2012;1453:87–101. Available at: https://www.sciencedirect.com/science/article/pii/S0006899312004003. Accessed July 8, 2019.
46. Ludyga S, Gerber M, Brand S, Holsboer-Trachsler E, Pühse U. Acute effects of moderate aerobic exercise on specific aspects of executive function in different age and fitness groups: A meta-analysis. Psychophysiology. 2016;53:1611–1626. Available at: http://www.ncbi.nlm.nih.gov/pubmed/27556572. Accessed February 20, 2019.
47. Green CS, Bavelier D. Exercising Your Brain: A Review of Human Brain Plasticity and Training-Induced Learning. Psychol. Aging. 2008;23:692–701. Available at: /pmc/articles/PMC2896818/. Accessed August 18, 2023.
48. Wecht JM, Weir JP, Katzelnick CG, et al. Systemic and Cerebral Hemodynamic Contribution to Cognitive Performance in Spinal Cord Injury. J. Neurotrauma. 2018;35:2957–2964. Available at: https://pubmed.ncbi.nlm.nih.gov/30113243/. Accessed December 5, 2022.
49. Lieber RL, Kelly MJ. Factors influencing quadriceps femoris muscle torque using transcutaneous neuromuscular electrical stimulation. Phys. Ther. 1991;71:715–723. Available at: https://pubmed.ncbi.nlm.nih.gov/1946610/. Accessed January 6, 2025.
50. Maffiuletti NA. Physiological and methodological considerations for the use of neuromuscular electrical stimulation. Eur. J. Appl. Physiol. 2010;110:223–234. Available at: https://pubmed.ncbi.nlm.nih.gov/20473619/. Accessed March 6, 2021.

CHAPTER 11

Neuromuscular electrical stimulation to combat cognitive aging in people with spinal cord injury: protocol for a single case experimental design study

Authors: Wouter A.J. Vints, Oron Levin, Martijn van Griensven, Johan W. S. Vlaeyen, Nerijus Masiulis, Jeanine Verbunt, Charlotte C.M. van Laake-Geelen

Published in BMC Neurology, 2024, Vol. 24, p. 197
https://doi-org.mu.idm.oclc.org/10.1186/s12883-024-03699-9

Abstract

Introduction: Individuals with spinal cord injury (SCI) can experience accelerated cognitive aging. Myokines (factors released from muscle cells during contractions), such as brain-derived neurotrophic factor (BDNF), are thought to have beneficial effects on cognition. Neuromuscular electrical stimulation (NMES) was shown to elicit a large release of myokines. However, the effects of NMES on cognitive function have not been studied.

Objective: To present the study protocol for a clinical trial evaluating the effects of NMES aimed at improving cognition and BDNF.

Methods: A replicated randomized three-phases single-case experimental design (SCED) with sequential multiple baseline time series and a single-armed prospective trial will be conducted with 15 adults with chronic SCI (>12 months after injury) above L1 neurological level undergoing 30-minute quadriceps NMES, 3 days per week for 12 weeks.

Main study endpoints: Primary endpoint is cognitive performance (assessed by a smartphone test) conducted three times per week during the baseline phase with random duration of 3 to 8 weeks, the intervention phase of 12 weeks, and the follow-up phase of 3 weeks after a no measurement rest period of 12 weeks. Secondary endpoints are changes in BDNF levels and cognitive performance measured before the baseline period, before and after intervention and after a 12 weeks follow-up.

Conclusion: This will be the first study investigating the effects of 12 weeks NMES on both cognition and BDNF levels in individuals with SCI. The SCED results provide information on individual treatment effect courses which may direct future research.

Trial registration: ClinicalTrials.gov (NCT05822297, 12/01/2023)

Abbreviations

AIS, ASIA Impairment Scale; ASIA, American Spinal Cord Injury Association; BDNF, brain-derived neurotrophic factor; BMI, body mass index; COWAT, controlled word association test; ELISA, enzyme-linked immunosorbent assay; FES, functional electrical stimulation; FVC, forced vital capacity; GDPR, General Data Protection Regulation; HADS, Hospital Anxiety Depression Scale; ICC, interclass correlation coefficient; NMES, neuromuscular electrical stimulation; SCED, single case experimental design; NAP, nonoverlap of pairs; PEF, peak expiratory flow; SCI, spinal cord injury; PRPM, perceived resistance to passive movement; RAVLT, Rey Auditory Verbal learning Test; SDMT, symbol digit modalities test; SEM, standard error of measurement; SPIRIT, Standard Protocol Items: Recommendations for Interventional Trials; TBI, traumatic brain injury; TMT, trail making test; USER-P, Utrecht Scale for Evaluation of Rehabilitation-Participation; VAS, visual analogue scale.

1. Introduction

Cognitive problems in persons with spinal cord injury (SCI) have long been underrecognized and were usually explained by concomitant traumatic brain injury (TBI). However, since less than five years, it has been noted that individuals with SCI suffer from accelerated cognitive aging, even when correcting for mood factors and in the absence of TBI [1][2][3][4]. Individuals with SCI face a 13-fold increase in the risk of cognitive impairment compared to the general population [5] and they are twice as likely to develop Alzheimer's dementia [6][7][8]. The cognitive domains most affected following SCI are executive functions, long-term memory, short-term memory, attention [2], processing speed and verbal fluency [1] when controlling for mood factors. This cognitive impairment begins in the subacute phase and seems to worsen over time [9]. It was found that individuals with SCI have cognitive impairments and brain activation patterns that are similar to healthy adults that are on average 20 years older [1][10]. A proposed mechanism for this cognitive decline is neuroinflammation [4][11]. Neuroinflammation plays a role in normal age-related cognitive decline [12][13][14] and following spinal injury, chronic elevation of neuroinflammation is found in the whole central nervous system [15][16][17].

Physical exercise interventions have been shown to have anti-inflammatory effects, induce an elevation of neurotrophic factors in the bloodstream, potentiate neuroplastic processes in the brain, increase brain volume and benefit cognitive function [18][19][20][21]. The anti-inflammatory effect and increase in neurotrophic factors during exercise results in part from the release of products from contracting muscle cells (i.e. myokines) [20]. This release of myokines has been reported following all sorts of muscle training, including electrical stimulation [22][20]. It is often difficult for persons with SCI to sufficiently

engage in physical exercise, with only 50% of SCI patients engaging in any leisure-time physical activity at all [23]. This difficulty may depend on the level of injury and the severity of motor function loss, as well as the availability of sport opportunities for wheelchair users. Electrical stimulation has been proposed as an interesting addition or alternative for increasing circulating myokine levels in humans with difficulty to participate in regular exercise programs [22]. Electrical stimulation can be used alone, i.e. neuromuscular electrical stimulation (NMES), or to assist voluntary exercise, i.e. functional electrical stimulation (FES). NMES induces contraction of myocytes similarly to exercise, which results in the release of myokines in the circulation, as recently reviewed by Sanchis-Gomar et al. (2019) [22]. A widely studied myokine is brain-derived-neurotrophic factor (BDNF), which is released from neurons [24], astrocytes [25], platelets [26], and skeletal muscle [27] and can trigger neuroprotective and neurotrophic effects [28]. Some studies suggest that most of the circulating BDNF is released from the brain [29]. Some other myokines, like insulin-like growth factor-1 and irisin were suggested to induce the release of brain-derived BDNF [20]. Remarkably, one study in young healthy adults reported that circulating levels of the myokine named BDNF increased more following a single bout of NMES than voluntary exercise with the same integrated force of muscle contraction [30]. Similarly, in a rat study, assessing able-bodied rats, the increase in BDNF levels was two times higher after four weeks of NMES compared to four weeks of running [31]. Also in human studies, long-term interventions with NMES were reported to increase circulating levels of BDNF, for example in older adults with type 2 diabetes [32]. BDNF can be considered one of the most important exercise-induced factors as it has direct beneficial effects on synaptic plasticity and neurogenesis, and it was linked with brain atrophy and cognitive function in a wide array of research [33]. So far, however, we were unable to find any studies reporting a beneficial effect of NMES on cognitive function. To the best of our knowledge, only one other study has evaluated the effects of FES [34] and only one study evaluated the effects of NMES [35] on cognitive function. The FES study is also the only study investigating exercise effects on cognition in SCI patients. In this study, improvements on a working memory task were reported after 6 months of functional electrical stimulation (FES)-assisted rowing exercise in SCI patients. This study did not measure myokine levels [34]. The authors of the NMES study evaluated the effect of a short intervention of 15 days of NMES in coronary bypass patients and discovered that patients in the experimental group showed increases in functional connectivity in the brain frontoparietal, salience and sensorimotor networks [35]. Overall, there is a lack of knowledge on the effects of electrical stimulation to improve cognitive function and the dose-response needed to attain such effect.

We will use a randomized replicated ABC single case experimental design (SCED) [36]. Primarily, we will examine to what extent and after how many weeks a 12 week intervention with NMES may change performance of people with SCI on the momentary digital symbol substitution task, an information processing task that will repeatedly be

administered via a smartphone application. NMES will be applied to the quadriceps muscles of individuals with SCI. An intervention of 12 weeks is considered to be sufficient to induce both a myokine response and muscular changes. Secondarily, we will assess changes in the myokine BDNF before and after the 12 week intervention with NMES and after a 12 week follow-up without NMES. We hypothesize that NMES will have a beneficial effect on cognitive performance starting from the first weeks and that 12 weeks of NMES will induce an elevation of BDNF levels. This study tests a new treatment strategy, accessible even to individuals with low physical exercise possibilities, with the potential to slow down or prevent further cognitive decline in persons with SCI. The explorative findings may guide future research. Furthermore, it may help to raise awareness of the process of accelerated cognitive decline in persons with SCI.

2. Methods

The Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) guidelines (see Appendix) and RoBiNT scale for risk of bias in SCED studies were used in designing and describing this clinical trial [37][38].

The trial was registered with ClinicalTrials.gov (NCT05822297) on the 12th of January 2023, sponsored by Adelante Zorggroep and approved by the Medical Ethical Testing Committee (reference number W23.071) of Maxima Medical Center at Veldhoven, the Netherlands on the 9th of October 2023. In case of important protocol modifications, they will be notified to the Medical Ethical Testing Committee and updated in the trial registry.

2.1 Participants

Participants and recruitment: Participants will be recruited from the outpatient clinic of the rehabilitation center Adelante Centre of Expertise in Rehabilitation and Audiology, locations Hoensbroek and Maastricht University Medical Center (MUMC+)., the Netherlands. The rehabilitation physician will inform potential participants about the study and flyers with information will be handed over. The flyers will include the contact details of one of the researchers. Alternatively, participants can give permission to the physicians that their contact information can be sent to the researchers.

Inclusion criteria: Participants are eligible if they are 18 years or older, have SCI since at least one year (chronic phase), the injury level is L2 or higher (meaning that the quadriceps muscle is likely affected to some extent, since this muscle is innervated by radicular nerves L3-L4), completeness of injury has been scored according to the American Spinal Injury Association (ASIA) Impairment Scale (AIS) [39] as either A, B or C, if they are able to use apps on their smartphones, if they are able to use NMES device safely at home, and if they speak Dutch at a native level. We will exclude participants if we cannot

induce visible or palpable contractions of the quadriceps muscles with NMES or if they cannot tolerate the NMES intervention. We will exclude participants with a diagnosis of cancer, neurodegenerative or psychiatric disorders, current pressure ulcers, a history of severe autonomic dysreflexia (systolic blood pressure elevations above 200mmHg), have metal implants in the stimulation site, an intrathecal baclofen pump, or are currently pregnant. Finally, we exclude participants who have taken part in a program with electrical stimulation in the last 6 months prior to the study.

2.2 Study design and procedures

2.2.1 Study design

This study will apply a randomized replicated sequential ABC single-case experimental design (SCED), with a baseline (A), intervention (B) and follow-up (C) phase [36][40]. SCED study designs demonstrate strong internal validity to determine the likelihood of a causal relationship between the intervention and outcomes. One entity is observed repeatedly over a certain time period under different levels of at least one independent variable. The power in SCEDs is related to the number of data points for each participant and not the number of participants. Each participant serves as his/her own comparison, thus controlling for confounding variables that can impact outcome and allowing heterogeneous clinical presentations. The internal validity of the SCED results can be improved by randomization techniques such as randomization of the starting time of the intervention. The downside is that the generalizability of the study results to the total population still depends on the number of participants included (i.e. replication of the findings) [41][40]. The SCED lend itself perfectly to reflect on the reasons for the (likely) variability in the onset of NMES-induced effects on cognitive performance, looking at individual participant's intervention courses. Thus, allowing to reflect on the existence of different potentially moderating characteristics. Several potential moderators have been suggested previously [4] and will be measured within this study. These include concomitant traumatic brain injury, psychological alterations, chronic pain, hemodynamic dysregulation, level of injury, respiratory failure, substance (ab)use, educational level, cardiovascular diseases, and intensive care admission [4]. In addition, a SCED design allows us to interpret after how many weeks the NMES leads to changes in performance on the momentary digital symbol substitution task. The aforementioned advantages of SCED studies indicate how this design offers additional information that is well fitted for an explorative study in a new topic.

In the context of SCED studies, randomization does not refer to individuals being randomly allocated to treatment groups, but to the random onset of the intervention phase which is usually set within a fixed window of time in order to make this randomization more feasible [38]. In this study, a window of 9 to 24 measurement points (i.e. 3 to 8 weeks at the set frequency of three test assessments per week) will be employed in which the change of phase from baseline to intervention phase will be randomly determined.

This determination will be done with a random number generator in Excel set to give a random number between 9 and 24. There will be a baseline phase ranging from 3 to 8 weeks, followed by an intervention phase of 12 weeks and a final follow-up phase of 3 weeks after a 12 week rest period with no measurements and no interventions. As we will exclude the first 4 measurements in the baseline phase from analysis, see section 2.7, this leaves us at least 5 measurement points in this phase, 36 measurement points in the intervention phase and 9 measurement points in the follow-up phase. Neither participants nor researchers will be blinded to the phase of the intervention. Replication of the experiment will be done in 15 participants.

Finally, additional measurements will be done in a repeated manner according to a single-armed prospective design before baseline phase, at the end of the baseline phase, at the end of the intervention phase and at the start of the follow-up phase.

2.2.2 Study procedures

The experimental design is illustrated in Figure 1. Upon the first appointment (T1), participants will sign an informed consent and undergo baseline examinations consisting of questionnaires and clinical tests. A smartphone application for repeated monitoring of cognitive function will be installed on their smartphones and they will be familiarized with the cognitive tests and neuromuscular electrical stimulation intervention. On a separate day (T2), within seven days from the first appointment, they will undergo a series of cognitive tests and venous blood samples will be collected. Furthermore, they will fill in a second list of questionnaires and undergo a clinical evaluation of strength and spasticity . This will be followed by a no-treatment baseline period with a random length of 3-8 weeks. Upon the third (T3), fourth (T4) and fifth (T5) visit, the oral cognitive tests, venous blood sample collection and questionnaires from T2 will be repeated. During the third visit (T3) the participants will receive their own NMES device for home-based training and they will be asked to return it 12 weeks later on the fourth visit (T4). All participants will undergo the same intervention. During the fourth visit (T4) the clinical evaluation of strength and spasticity that was done at T2 will be repeated. Between T2 and T4 and between T5 and T6 participants will undergo smartphone cognitive tests 3 times per week. At the end of the study, no additional tests will be done. Participants will be requested to write in a diary the specifications of the NMES session (duration, intensity) after each session and indicate when they performed a cognitive test. They can also write down any problems they encountered using the NMES device or smartphone application. Participants receive a telephone call once a week by one of the researchers to assess how they feel, be reminded of the diary and to assure there are no technical issues with the NMES device or smartphone application. Participants are allowed to withdraw from the study at any time. Available data from these participants will be included in the data analyses whenever possible.

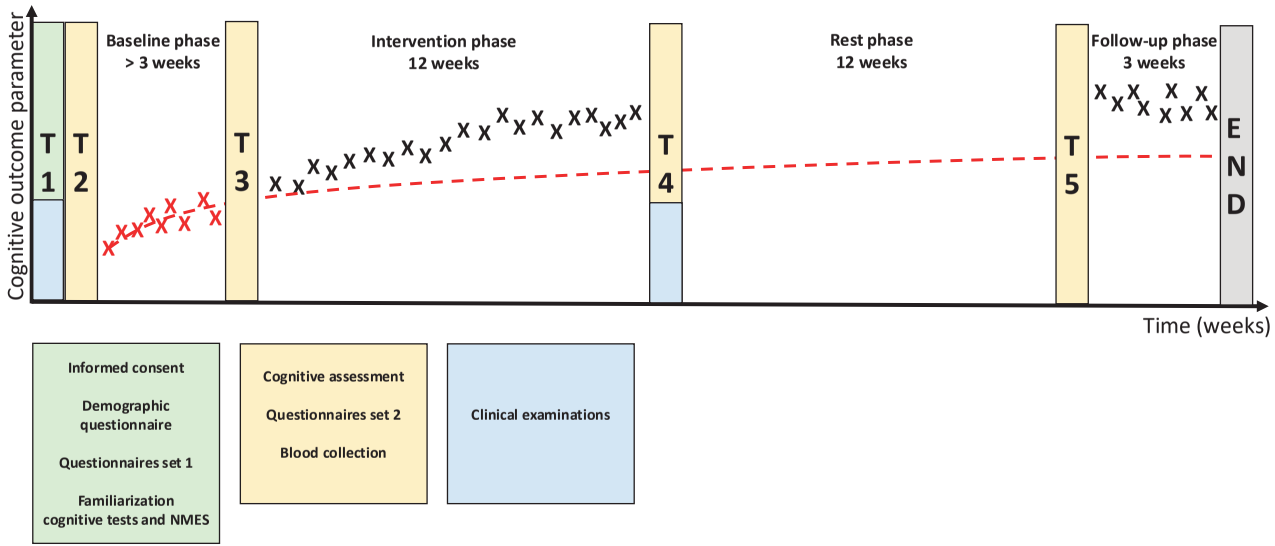


Figure 1. Experimental design

T1-5 represent measurement time points. During T2-5 the same measurements are repeated, expect the addition of clinical examination in time point T4. The red line is the expected change in cognitive test performance on the smartphone-based cognitive test without intervention, which we expect to show a fast increase in the first ±3 sessions followed by a slight increase over time due to a learning effect. The X's mark how cognitive test results are hypothesized to change due to the intervention.

2.3 Intervention

The intervention consists of 12 weeks NMES which will be done using the Pierensymphony M, serie A, article number 104800 (10003566) (manufactured by Pierenkemper GmbH and distributed by schwa-medico Nederland B.V.), a two-channel NMES device designed, and CE marked for muscular electrical stimulation (CE 0482). After a familiarization session, the intervention will be home-based, three times a week, with at least 1 day between stimulation sessions and 2 days between a stimulation session and testing day. Electrical stimulation will be done on the quadriceps muscles of both legs simultaneously. For each leg, one electrode is placed on the proximal lateral side and one on the distal medial side of the quadriceps muscle (see Figure 2). Electrical stimulation will take 30min, at a stimulation frequency of 50Hz, and a pulse width of 400μs. We will choose the highest intensity that is easily supported by the participant without inducing discomfort with a maximum intensity of 100mA. We should at least see a visible or palpable contraction or the participant will be excluded. The activation within the activation-rest cycle consists of a 1s ramp-up, 7s full activation and 1s ramp-down, followed by 18s rest. Every 4 weeks the rest period will be diminished with 3s until the rest phase is equal to the activation phase (9s). Participants may continue their normal treatments, which may consist of physiotherapy. However, they will be asked not to change their physical activity habits during the experiment.

2.4 Cognitive assessments

2.4.1 Smartphone-based cognitive assessment

To study the primary aim of our study, participants will undergo a cognitive test for 3 times a week during the baseline, intervention and follow-up phase of the experiment. This repetitive cognitive test will be administered using a secured smartphone application, which was designed for use in clinical settings and research (m-Path, https://m-path.io/landing/). It will be programmed to emit an auditory signal three times a week signaling the availability of a new cognitive test between 7.30 AM and 10.30 PM on the days that participants do not undergo NMES sessions. The test is the momentary digital symbol substitution task, which is a measure of processing speed and short-term working memory function. For this test, participants have 30 seconds time to complete as many trials as possible where they need to correctly select the figure representing the number given as depicted in the legend provided on the top of the screen. The outcome is presented as response time derived from the number of trials/30s (speed, in ms) and the percentage of correct trials (accuracy). Before the test starts, participants will be informed that they should be ready to respond as fast and accurate as possible. Daniëls et al. (2020) have validated this test for repeated use in healthy adults. They reported a significant learning effect for number of correct answers in 30 seconds between the first and 48th trial ($B = 0.32$, $SE = 0.04$, $p < 0.001$; with number of correct answers as the dependent variable and the logarithmic transformation of the session number (between 1 and 48) as the independent variable) [42].

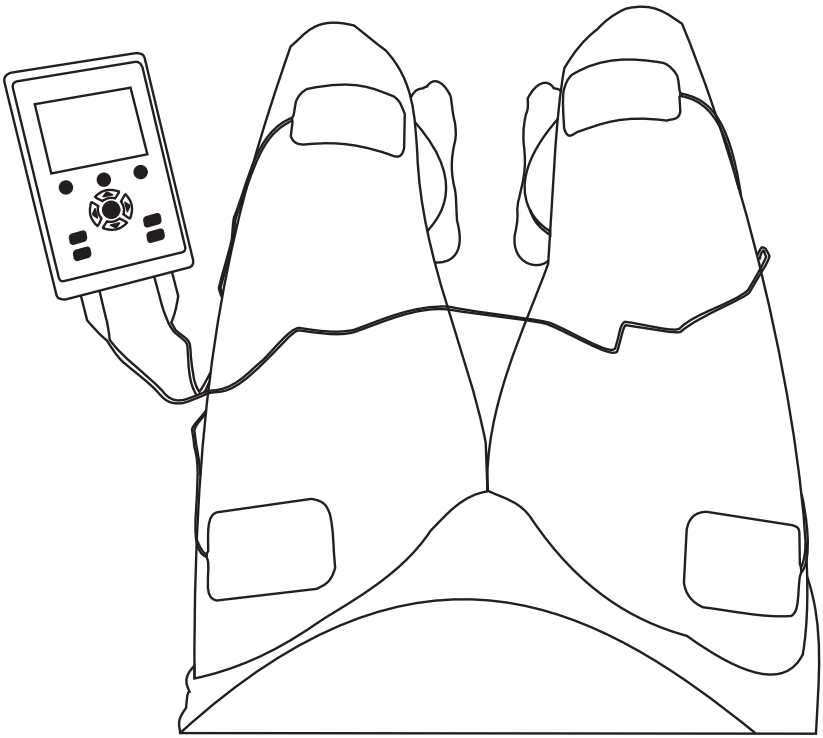


Figure 2. Placement of the electrodes on the quadriceps muscles

2.4.2 Oral cognitive test battery

At four time points, participants will undergo an oral cognitive test battery consisting of seven tests that were validated for repeated use in people with SCI, even with impaired hand function [43]. The Dutch version of these tests will be used, whenever the test depends on language. The tests have a good-to-excellent test-retest reliability, except the RAVTL recognition score which has been shown to have a poor test-retest reliability [43]. This test was still used as the interference recall and delayed recall scores of this test have a good test-retest reliability in people with SCI [43]. To further decrease learning effects of repeated administration of the cognitive tests, different versions will be used on different visits if available. The test versions will be numbered and on their first visit participants will be asked to draw a random number from an envelope including a number for every existing version of the cognitive test, until the envelope is empty. This is repeated for every cognitive test. The sequence will be reported for the next visits. The cognitive tests are presented in Table 1.

Table 1. Oral cognitive assessments

Cognitive test	Description
Information processing speed	
SDMT	This test is similar to the m-Path smartphone test. However, now, the participant is asked to verbally match the number representing the symbol given as depicted in the legend provided on the top of the page. The researcher writes down the answers on a separate page. The first 10 trials are used for familiarization, then the participant is given 90 seconds to verbally match as many numbers to symbols as possible. The final score is obtained by subtracting the number of errors from the number of items completed within the given timeframe [34]. To minimize learning effects, three different versions of this test will be used.
Verbal fluency	
COWAT	For this test participants are asked three times to name as many words starting with a specific letter in one minute time. Words cannot be named more than once and non-existing words are not counted. In addition, names of persons and numbers are also not allowed. There are three versions of this test (DAT, KOM, PGR) in the Dutch language. The total score is the sum of the number of words named for each of the three letters [35][34].
Attention/concentration	
Digit Span Forward	Participants will be given a series of numbers, starting with 2 numbers and progressing to 9 numbers, read by an assessor. Participants will be asked to repeat the numbers in the same order. They get two trials before progressing to a larger series of numbers. When both series are answered incorrectly, the test is ended. The total score is the number of correctly answered series [34]. There are two versions of this test available at our institution.
Executive functions	
Digit Span Backward	This version of the digit span adds a working memory component to the test when compared to the forward digit span. Participants will be given a series of numbers, starting with 2 numbers and progressing to 9 numbers, read by an assessor. Participants will be asked to repeat the numbers backwards. They get two trials before progressing to a larger series of numbers. When both series are answered incorrectly, the test is ended. The total score is the number of correctly answered series [34]. There are two versions of this test available at our institution.
TMT A & B	This test assesses switching ability. This test encompasses two parts. In trial A, the participant verbally count from 1 to 25 as quickly as possible. In trial B, the participant will be instructed to verbally alternate between numbers and letters of the alphabet until 13. The time difference between trial A and B will be recorded. This test lasts approximately 3 minutes [36][34].

Table 1. Continued

Cognitive test	Description
Stroop test	This test assesses cognitive inhibition, but also attention and processing speed. This test consists of three lists that the participant must read out loud; the first list has the names of colors printed in black ink, the second list with the colors printed in colored ink, and the final list having the name of colors printed in incongruent colored ink (e.g., the word red is printed in blue ink). The time to complete second list, third list, as well as the time difference between these latter two lists will be recorded. The test takes approximately 5 minutes. There is one version of this test available [37][34].
Memory	
15 word test (RAVLT)	The 15 word test is the RAVLT. This test consists of a list of 15 words that is read to the participant by an assessor. After reading, the participant must try to recall as many words as they can. This read and recall process is repeated an additional four times. A distractor list (second list) is presented with a recall attempt, followed by a timed recall of the first list. The timed recall measures the participant's ability to recall information despite the intervening list. After 10 minutes, the participant performs another timed recall of the first list of words. Afterwards the participant is given a recognition task where they identify the 15 words from the initial list from a paragraph containing 30 underlined words. This final trial distinguishes memory storage from inefficient recall [34]. There are two versions of this test available at our institution.

Abbreviations: COWAT, controlled word association test; RAVLT, Rey Auditory Verbal Learning Test; SDMT, Symbol Digit Modalities Test; TMT, Trail Making Test.

2.5 Blood analysis

Venous blood samples will be taken at the antecubital vein. Blood samples will be collected in two 5mL serum separator tubes. After blood collection, the tubes will be gently inverted 6-8 times and within 90 min be centrifuged. Quantitative determination of serum BDNF will be measured using enzyme-linked immunosorbent assays (ELISA). Blood will be drawn four times (T2-5) per participant during the whole study project. To decrease errors due to ELISA kit differences, we will store the blood samples in a refrigerator compartment at the Maastricht University laboratory site at -80°C until analysis, using the same kit for all blood measurements of the same participant at the end of the project.

2.6 Demographic and clinical assessments

At baseline, we will collect information on participant's age, gender, smoking status, years of education, cause of the SCI, date of the SCI, drug/alcohol use, body mass index (BMI), medical history such as diabetes mellitus or hemodynamic regulation problems (hypertension, hypotension, autonomic dysreflexia) or respiratory problems (nocturnal apnea or use of a device for sleep apnea, signs of restricted or obstructive lung disease on

spirometry test) or history of intensive care admission, medication use, history of TBI, and infections or wounds in the past 3 weeks. Some clinical tests will also be conducted upon baseline, while others are also measured at follow-up since we expect they may change due to our intervention. The latter are therefore seen as secondary outcomes and may be evaluated in a separate study.

The following clinical tests will be conducted:
At measurement timepoint 1

- SCI classification: Functional impairment in terms of sensation and strength of key muscles will be assessed using the AIS classification [39]. It is a structured clinical assessment of the level and completeness of the spinal cord injury. Intra- and interobserver correlation coefficients are generally around 0.9 [44].
- Lung spirometry: A portable spirometer (Vitalograph In2itive) will be used to assess forced expiratory volume in one second (FEV1), forced vital capacity (FVC) and peak cough flow (PCF) (L/s).
- Heart rate and blood pressure measurement using an automated device will be measured at the left or right arm in seated position.

At measurement timepoint 1 and 4

- Spasticity level: The Perceived Resistance to Passive Movement (PRPM) test is recommended by the Dutch guidelines for measuring spinal spasticity [45]. The scale ranges from (0) 'no increased resistance' to (4) 'movement of the limb impossible'. Resistance of elbow flexors, elbow extensors, wrist flexors, wrist extensors, hip adductors, knee flexors, knee extensors, ankle plantar flexors (both with extended and with a 90° flexed knee) will be assessed in supine position. A sum of the scores will be used as the outcome measure, ranging from 0 to 32.
- Electrically-evoked muscle strength: The MicroFET2 (Hoggan Scientific, LLC) muscle strength testing system is an handheld dynamometer, which will be used to measure electrical stimulation-evoked quadriceps force. The electrical stimulation protocol will be equal to that used during the 12 week intervention. The dynamometer will be affixed to a band, which in turn will be secured around the wheelchair and the distal part of the participant's lower leg. The MicroFET2 dynamometer demonstrates a high intra-rater reliability for knee extension, with an interclass correlation coefficient (ICC) of 0.93, a standard error of measurement (SEM) of 17.2N and a minimal detectable change of 47.5N [46].

The following questionnaires will be used:
At measurement timepoint 1

- Brief Physical Activity Assessment Tool: This tool is a two question physical assessment. It has a validity similar to that of more detailed self-report measures of physical activity. It can be used efficiently in routine primary healthcare services to identify insufficiently active patients who may need physical activity advice [47].
- TBI-4 questionnaire: This questionnaire is specifically designed as a self-report tool to determine the likelihood of TBI in traumatic SCI patients utilizing just two questions. The outcome is presented as improbable TBI, possible TBI, mild TBI, moderate TBI or severe TBI. At the cut-off "possible TBI" this tool had a sensitivity of 83% and a specificity of 51% to detect mild TBI based on medical reports, indicating this questionnaire can be used as a guide in the unavailability of medical reports, but should be interpreted with care [48].

At measurement timepoints 2 till 5

- Cognitive Failures Index: This test can reliably evaluate how participants experience their own cognitive function. It consists of 25 questions, each scored on a 5-point scale ranging from never (0) to very often (5) [49].
- McGill Pain Questionnaire: Participants will be given a figure of a person, where they can color the zone of pain. They will be asked to describe the pain and score it on a 10 point visual analogue scale (VAS) and will receive some questions related to the impact of pain on their daily life [50].
- Hospital Anxiety and Depression Scale (HADS): this scale is a widely used and reliable screening instrument to assess the severity of anxiety and depression [51]. A subtest score of more than 8 on 21 denotes considerable symptoms of anxiety or depression.
- Fatigue Severity Scale: This questionnaire encompasses nine questions and has previously been used to recognize and diagnose fatigue in patients with neurological disorders [52].
- Pittsburgh Sleep Quality Index: This index consists of 19 questions concerning seven domains related to sleep quality in the preceding month. Every domain receives a score of 0-3, with a total score of 0-21. A score of more than 5 indicates a poor sleep quality [53].
- Utrecht Scale for Evaluation of Rehabilitation – Participation (USER-P): This test is widely used to assess participation in daily life activities in rehabilitation contexts. It contains 32 questions related to the frequency of participation, participation restrictions and satisfaction with participation, with a total score of 0-100 for each of these three domains. A higher score indicates a better participation. [54].

The baseline measurements of potential moderators of the effect of our intervention on cognitive and blood outcomes and risk factors for cognitive impairments according to a previous review paper will be entered in the statistical analysis [4]. The risk factors for cognitive impairments will be entered as a combined risk score with a maximum score of 10, indicating the highest risk. Participants receive a score between zero and one for each of the following: 1 point if self-reported history of TBI or mild to severe concomitant TBI according to TBI4 questionnaire [48], 0.5 points for a score of >8/21 for anxiety and 0.5 points for a score of >8/21 for depression on the HADS scale [51], 1 point for chronic pain indicated on the McGill pain questionnaire to exist longer than 3 months and is scored at its minimum a VAS score of 3 [50], 0.5 points for a history of autonomic dysreflexia and 0.5 points for a blood pressure below 90/60 upon measurement, 1 point for tetraplegic participants compared to 0 points for paraplegic participants, 0.33 points for self-reported sleep apnea, 0.33 points for a FVC value below 0.85% of the predicted value, and 0.33 points for a PCF below 4.5L/s, 0.25 points for regular alcohol use (more than once per week), 0.25 points for drug abuse, 0.25 points for polypharmacy (more than 7 prescribed drugs), 0.25 points for any medication acting on the central nervous system, 1 point for participants who finished only basic education, 0.5 points for participants who finished only secondary education, 0.5 points for diabetes mellitus, 0.5 points for a BMI equal to or above 25kg/m^2 [55], 1 point for previous intensive care admission.

2.7 Statistical analysis

2.7.1 Single-case data

For our primary aim, the smartphone cognitive assessment, statistical analysis will be performed using the Shiny app for Single-Case Data Analysis (Shiny SCDA) [56]. A total of 54-69 measurements per participant will be attained.

2.7.1.1 Visual analysis

First, measurement points will be plotted and visually inspected per participant. Six features will be visually examined according to the guidelines of Lane and Gast (2014): cognitive performance in the different phases, variability in cognitive performance both within and between phases, trends in the data, immediacy of effect, overlap of data points between phases, and consistency of data patterns across participants [57]. Instead of inspecting only immediate effects, also the existence of a potential delay of the effect and consistency of this delay between participants will be explored [58].

2.7.1.2 Effect size measures

Finally, effect size measures will be calculated for each participant. This will be done by calculating the percentage of non-overlapping pairs between phases [59]. The NAP (Nonoverlap of All Pairs) value equals the number of comparison pairs showing no overlap, divided by the total number of comparisons, and can be considered an area under the curve percentage from a receiver operating characteristic analysis [60].

2.7.1.3 Randomization test

Subsequently, randomization tests will be performed to test the null hypothesis that NMES does not have an effect on participant's cognitive function. The observations of the baseline phase will be compared to those of the intervention and follow-up phases respectively. The test statistic "means of phase A minus means of phase B" will be chosen as the primary outcome of this study. In case visual analysis suggests delayed effects, the randomization test will be repeated with lagged data until the lowest p-value has been reached (one effect lag equals one day). The first 4 measurements in the baseline phase will be removed before calculating the means, since we expect a learning effect on the first trials, leaving between 5 and 20 measurement points depending on the randomly decided length of the baseline period. Studies that tested the statistical properties of a randomization test used in this type of design showed that the Type I error probability of the randomization test was maintained at an acceptable level [61].

2.7.1.4 Missing data

Missing values from the repeated smartphone-based cognitive test will be treated with the randomized marker method [62]. In this method, the missing value is removed from calculation of the mean in the randomization test. In addition, the position of the missing value will be randomly reshuffled within possible randomization schemes of the study protocol. In this study, this means that a missing value in the baseline or beginning of the intervention phase may be reshuffled to be part of the other phase if this falls within the possibilities (i.e. if it falls within the first 4 to 8 weeks of the study). This method was found to be more effective at controlling type I error and resulted in higher power than multiple imputation and single imputation using a time series model in a SCED simulation study [63].

2.7.2 Secondary data

For the secondary study parameters (i.e. BDNF levels and results of the oral cognitive test battery) which are measured within a single-armed prospective design, statistical analysis will be done using IBM SPSS Statistics 27.

2.7.2.1 Secondary statistics

Before analysis, the data will be checked for outliers, defined as values lying further than three times the interquartile range away from the median value. The normality assumption will be checked based on visual representations of the data using histograms and measurements of skewness and kurtosis (normality assumed if the data values lie between -2 and +2) [64]. Homoskedasticity will be tested using the Levene's test. Descriptive statistics will be used. Furthermore, the Friedman test will be

used for analysis of repeated measurements. The False Discovery Rate (FDR) method will be applied to correct for the multiple testing problem. In the FDR method, every p-value is compared against a sequentially weighted threshold on all p-values [65].

2.7.2.2 Missing data

Missing pre-post values will not be replaced by specific values, but be excluded from statistical analysis.

2.8 Sample size calculation

2.8.1 Single-case data

The sample size needed for the abovementioned statistical analyses is different for the SCED and single-armed prospective study design. For the SCED design, the minimum sample size is n = 1. Instead, the power of the analysis depends on the number of observations [41][40]. For randomization tests the lowest attainable p-value is calculated by dividing 1 by the number of possible permutations. In this study, the baseline phase, after removing the first four values because of a learning effect, can contain 5 to 24 measurement points, allowing 20 possible permutations. This corresponds with a lowest attainable p-value of 0.05 [56]. Of note, successful replication of the single-case experiment in additional participants with similar symptoms will improve the generalizability of the results [41][40].

2.8.2 Secondary data

For the single-armed prospective study design we have estimated the sample size needed in order to have sufficient power (Power = 0.80) for evaluating a Time effect (over 4 time points) of cognitive test performance changes with repeated measures ANOVA. We found no previous studies examining the effect of any muscle activity intervention in spinal cord injury subjects. Therefore, the required effect size was estimated to be similar to that from a meta-analysis examining the effect of resistance exercise interventions on general cognitive function in healthy adults [66]. The overall effect size (Cohen's d) was 0.71 (0.30-1.12) for resistance exercise. The correlation among repeated measures for this cognitive test battery that was found to be ≥ 0.77 in a previous SCI study [43]. G*Power 3.1.9.7 estimated that the minimum total sample size should be 7. Taking into account potential drop-outs and the uncertainty of the effect, we decided to aim for a total sample of 15 included participants.

2.9 Data management

All the participant data will be coded and pseudomized to protect their privacy. The repeated cognitive test will be administered using a secured smartphone application (see www.m-path.io/landing), which was designed for use in clinical settings and research

and is General Data Protection Regulation (GDPR) compliant. Participants will register to m-Path with their study code instead of their personal name. With a password, one of the researchers can access a dashboard where data from all included participants will be visible. Other test results including participant identification details will be stored at Adelante Hoensbroek in a locked cabinet with restricted access. The key will be kept in another locked cabinet. The test results with participant identification will only be accessed again after coding and de-identifying the data in order to hand them over to participants requesting their test results. The data will be stored for 15 years. Blood samples will be destroyed after analysis, which is expected to take place within one year after blood collection. We intend to make pseudomized data from our study available according to the FAIR principle, such that it is Findable, Accessible, Interoperable and Reusable. Both negative and positive results will be made public. Our results will be presented on the Dutch national rehabilitation medicine congress and international rehabilitation/neuroscience congresses so that rehabilitation professionals are more aware of the benefit of muscle training or maintaining a healthy body overall on the brain and cognitive function, and so that neuroscientists will understand the relevance of neuromuscular electrical stimulation as one of the muscle training strategies to investigate the exercise-cognition link. The results will be conveyed locally to spinal cord injury subjects in our rehabilitation center in Hoensbroek and in the Maastricht University Medical Center (MUMC+). We will inform other national spinal cord injury clinics of the results and we will discuss the findings in the national work group of the Dutch rehabilitation society for movement and sport. Finally, the results will be submitted to a peer-reviewed journal.

2.10 Risks and harms

The use of electrical stimulation is generally safe. It has even been used unsupervised in spinal cord injury subjects during sleep [67]. However some adverse events or inconveniences that have been reported previously in literature are a red, raised or itchy skin; muscle pain; increased neuropathic pain; uncomfortable feeling; orthostatic hypotension (dizziness, light-headedness, blurred vision, palpitation or shortness of breath); in some cases pain from a spasm may occur. The adverse events reported are temporary added risks that disappear once the stimulus/stimulation has stopped [68]. In case the subject feels uncomfortable during electrical stimulation, they will be informed that the stimulation can be interrupted. Participants with a lesion level above T6 may experience autonomic dysreflexia in response to the electrical stimulation. Whenever this occurs, stopping the electrical stimulation should solve the problem. Participants with lesion levels above T6 will be explained how they can recognize signs of autonomic dysreflexia. Whenever autonomic dysreflexia has occurred during the NMES training period participants are asked to contact the medical professional who's telephone number is

provided to them at the beginning of the project and in the participant information. The sponsor has a liability insurance which covers for damage to research participants. All adverse events reported spontaneously by a participant or observed by the investiga¬tor will be recorded.

Acknowledgements

None

Funding

This work was supported by University Fund Limburg/SWOL (reference CoBes 22.055) awarded to Wouter Vints. Johan W.S. Vlaeyen is supported by the 'Asthenes' long-term structural funding Methusalem grant by the Flemish Government, Belgium (METH/15/011) and an NOW Open Competition grant (nr. 406.20.GO.032). The sponsors were not involved in study design, collection, analysis or interpretation of the data, in writing the report, nor in the decision to submit the article for publication.

Data availability

Not applicable

Declarations

- **Declarations of Interest**
  - None
- **Ethics approval**
  - The study was approved by the Medical Ethical Testing Committee (reference number W23.071) of Maxima Medical Center at Veldhoven, the Netherlands on the 9th of October 2023.
- **Consent to participate**
  - Not applicable
- **Consent for publication**
  - All authors have approved publication of this manuscript.
- **Authors' contributions**
  - WV, NM, OL, JVe and CvL were involved in the conception and design of the manuscript. WV, JVl and MG contributed to the description of the methods. WV wrote the main manuscript and prepared the figure. All authors reviewed and approved the manuscript text.

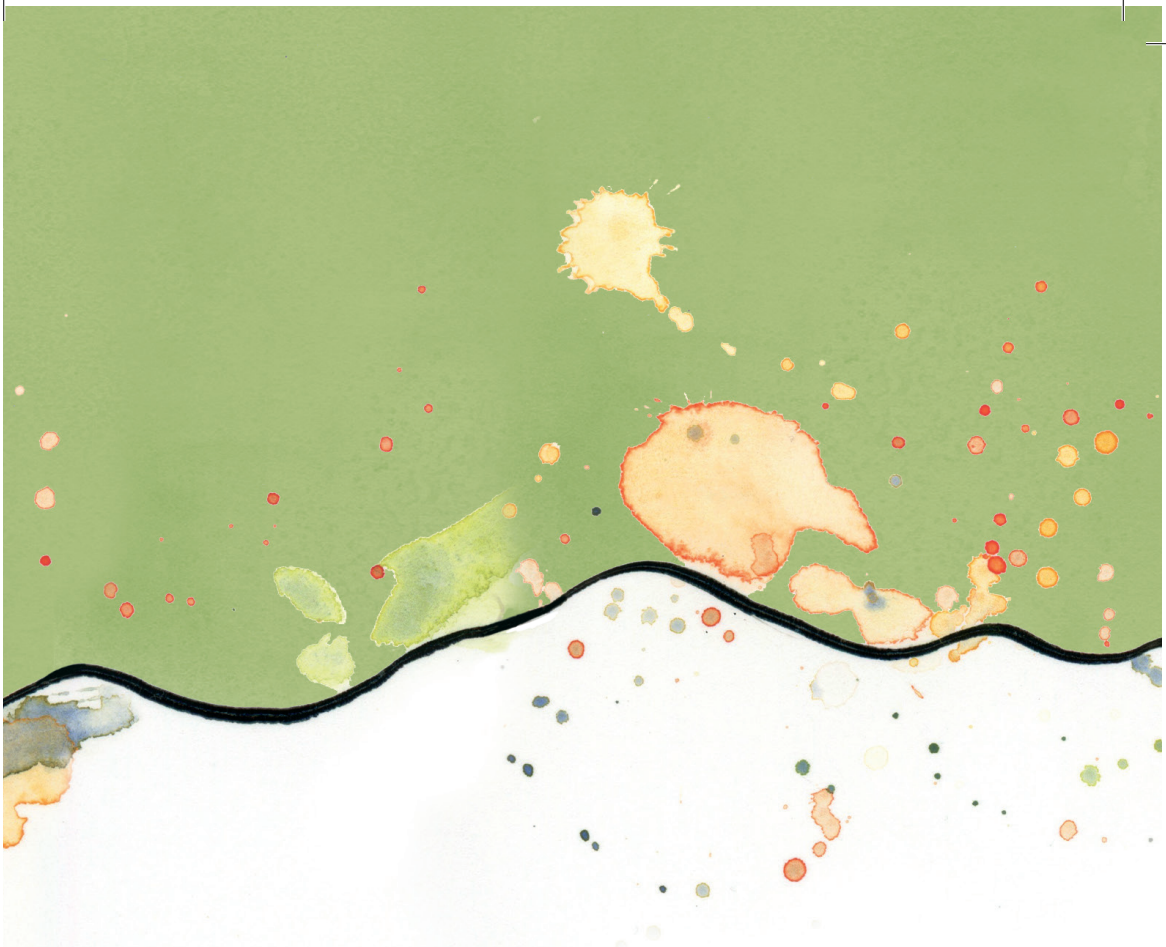
References

1. Chiaravalloti ND, Weber E, Wylie G, Dyson-Hudson T, Wecht JM. Patterns of cognitive deficits in persons with spinal cord injury as compared with both age-matched and older individuals without spinal cord injury. J Spinal Cord Med. 2020;43:88–97.
2. Heled E, Tal K, Zeilig G. Does lack of brain injury mean lack of cognitive impairment in traumatic spinal cord injury? J Spinal Cord Med. 2020. https://doi.org/10.1080/10790268.2020.1847564.
3. Li Y, Cao T, Ritzel RM, He J, Faden AI, Wu J. Dementia, Depression, and Associated Brain Inflammatory Mechanisms after Spinal Cord Injury. Cells. 2020;9.
4. Vints WAJ, Levin O, Masiulis N, Verbunt J, van Laake-Geelen CCM. Myokines may target accelerated cognitive aging in people with spinal cord injury: A systematic and topical review. Neurosci Biobehav Rev. 2023;146:105065.
5. Craig A, Guest R, Tran Y, Middleton J. Cognitive Impairment and Mood States after Spinal Cord Injury. J Neurotrauma. 2017;34:1156–63.
6. Huang SW, Wang W Te, Chou LC, Liou TH, Lin HW. Risk of dementia in patients with spinal cord injury: A nationwide population-based cohort study. J Neurotrauma. 2017;34:615–22.
7. Yeh T-S, Ho Y-C, Hsu C-L, Pan S-L. Spinal cord injury and Alzheimer's disease risk: a population-based, retrospective cohort study. Spinal Cord. 2018;56:151–7.
8. Mahmoudi E, Lin P, Peterson MD, Meade MA, Tate DG, Kamdar N. Traumatic Spinal Cord Injury and Risk of Early and Late Onset Alzheimer's Disease and Related Dementia: Large Longitudinal Study. Arch Phys Med Rehabil. 2021;102:1147–54.
9. Molina B, Segura A, Serrano JP, Alonso FJ, Molina L, Pérez-Borrego YA, et al. Cognitive performance of people with traumatic spinal cord injury: a cross-sectional study comparing people with subacute and chronic injuries. Spinal Cord. 2018;56:796–805.
10. Wylie GR, Chiaravalloti ND, Weber E, Genova HM, Dyson-Hudson TA, Wecht JM. The Neural Mechanisms Underlying Processing Speed Deficits in Individuals Who Have Sustained a Spinal Cord Injury: A Pilot Study. Brain Topogr. 2020;33:776–84.
11. Alcántar-Garibay O V., Incontri-Abraham D, Ibarra A. Spinal cord injury-induced cognitive impairment: A narrative review. Neural Regen Res. 2022;17:2649–54.
12. Sartori AC, Vance DE, Slater LZ, Crowe M. The impact of inflammation on cognitive function in older adults: Implications for healthcare practice and research. Journal of Neuroscience Nursing. 2012;44:206–17.
13. Cleeland C, Pipingas A, Scholey A, White D. Neurochemical changes in the aging brain: A systematic review. Neuroscience and Biobehavioral Reviews. 2019;98:306–19.
14. Vints WAJ, Kušleikienė S, Sheoran S, Šarkinaitė M, Valatkevičienė K, Gleiznienė R, et al. Inflammatory blood biomarker kynurenine is linked with elevated neuroinflammation and neurodegeneration in older adults: evidence from two 1H-MRS post-processing analysis methods. Front Psychiatry. 2022;13:859772.
15. Li F, Huo S, Song W. Multidimensional review of cognitive impairment after spinal cord injury. Acta Neurologica Belgica. 2020;121.
16. Sun X, Jones ZB, Chen X ming, Zhou L, So KF, Ren Y. Multiple organ dysfunction and systemic inflammation after spinal cord injury: A complex relationship. Journal of Neuroinflammation. 2016;13.
17. Wu J, Zhao Z, Kumar A, Lipinski MM, Loane DJ, Stoica BA, et al. Endoplasmic reticulum stress and disrupted neurogenesis in the brain are associated with cognitive impairment and depressive-like behavior after spinal cord injury. J Neurotrauma. 2016;33:1919–35.

18. Erickson KI, Hillman C, Stillman CM, Ballard RM, Bloodgood B, Conroy DE, et al. Physical Activity, Cognition, and Brain Outcomes: A Review of the 2018 Physical Activity Guidelines. Medicine and Science in Sports and Exercise. 2019;51:1242–51.
19. Mee-inta, Zhao, Kuo. Physical Exercise Inhibits Inflammation and Microglial Activation. Cells. 2019;8:691.
20. Vints WAJ, Levin O, Fujiyama H, Verbunt J, Masiulis N. Exerkines and long-term synaptic potentiation: Mechanisms of exercise-induced neuroplasticity. Front Neuroendocrinol. 2022;66:100993.
21. Sheoran S, Vints WAJ, Valatkevičienė K, Kušleikienė S, Gleiznienė R, Česnaitienė VJ, et al. Strength gains after 12 weeks of resistance training correlate with neurochemical markers of brain health in older adults: a randomized control 1H-MRS study. GeroScience. 2023;:1–19.
22. Sanchis-Gomar F, Lopez-Lopez S, Romero-Morales C, Maffulli N, Lippi G, Pareja-Galeano H. Neuromuscular Electrical Stimulation: A New Therapeutic Option for Chronic Diseases Based on Contraction-Induced Myokine Secretion. Frontiers in Physiology. 2019;10.
23. Martin Ginis KA, Latimer AE, Arbour-Nicitopoulos KP, Buchholz AC, Bray SR, Craven BC, et al. Leisure Time Physical Activity in a Population-Based Sample of People With Spinal Cord Injury Part I: Demographic and Injury-Related Correlates. Arch Phys Med Rehabil. 2010;91:722–8.
24. Lessmann V, Gottmann K, Malcangio M. Neurotrophin secretion: current facts and future prospects. Prog Neurobiol. 2003;69:341–74.
25. Numakawa T, Suzuki S, Kumamaru E, Adachi N, Richards M, Kunugi H. BDNF function and intracellular signaling in neurons. Histol Histopathol. 2010;25:237–58.
26. Le Blanc J, Fleury S, Boukhatem I, Bélanger J-C, Welman M, Lordkipanidzé M. Platelets Selectively Regulate the Release of BDNF, But Not That of Its Precursor Protein, proBDNF. Front Immunol. 2020;0:2994.
27. Máderová D, Krumpolec P, Slobodová L, Schön M, Tirpáková V, Kovaničová Z, et al. Acute and regular exercise distinctly modulate serum, plasma and skeletal muscle BDNF in the elderly. Neuropeptides. 2019;78.
28. Knaepen K, Goekint M, Heyman EM, Meeusen R. Neuroplasticity - Exercise-Induced Response of Peripheral Brain-Derived Neurotrophic Factor: A Systematic Review of Experimental Studies in Human Subjects. Sport Med. 2010;40:765–801.
29. Serra-Millàs M. Are the changes in the peripheral brain-derived neurotrophic factor levels due to platelet activation? World J Psychiatry. 2016;6:84.
30. Kimura T, Kaneko F, Iwamoto E, Saitoh S, Yamada T. Neuromuscular electrical stimulation increases serum brain-derived neurotrophic factor in humans. Exp Brain Res. 2019;237:47–56.
31. Dalise S, Cavalli L, Ghuman H, Wahlberg B, Gerwig M, Chisari C, et al. Biological effects of dosing aerobic exercise and neuromuscular electrical stimulation in rats. Sci Rep. 2017;7.
32. Miyamoto T, Iwakura T, Matsuoka N, Iwamoto M, Takenaka M, Akamatsu Y, et al. Impact of prolonged neuromuscular electrical stimulation on metabolic profile and cognition-related blood parameters in type 2 diabetes: A randomized controlled cross-over trial. Diabetes Res Clin Pract. 2018;142:37–45.
33. Müller P, Duderstadt Y, Lessmann V, Müller NG. Lactate and BDNF: Key Mediators of Exercise Induced Neuroplasticity? J Clin Med. 2020;9:1136.
34. Ozturk ED, Lapointe MS, Kim D Il, Hamner JW, Tan CO. Effect of 6-Month Exercise Training on Neurovascular Function in Spinal Cord Injury. Med Sci Sports Exerc. 2021;53:38–46.
35. Lo Re V, Russelli G, Lo Gerfo E, Alduino R, Bulati M, Iannolo G, et al. Cognitive outcomes in patients treated with neuromuscular electrical stimulation after coronary artery bypass grafting. Front Neurol. 2023;14:1209905.
36. Krasny-Pacini A, Evans J. Single-case experimental designs to assess intervention effectiveness in rehabilitation: A practical guide. Ann Phys Rehabil Med. 2018;61:164–79.

37. Tetzlaff JM, Moher D, Chan AW. Developing a guideline for clinical trial protocol content: Delphi consensus survey. Trials. 2012;13:176.
38. Tate RL, Perdices M, Rosenkoetter U, Wakim D, Godbee K, Togher L, et al. Revision of a method quality rating scale for single-case experimental designs and n-of-1 trials: The 15-item Risk of Bias in N-of-1 Trials (RoBiNT) Scale. Neuropsychol Rehabil. 2013;23:619–38.
39. Betz R, Biering-Sørensen F, Burns SP, Donovan W, Graves DE, Guest J, et al. The 2019 revision of the International Standards for Neurological Classification of Spinal Cord Injury (ISNCSCI)-What's new? Spinal Cord. 2019;57:815–7.
40. Vlaeyen JWS, Onghena P, Vannest KJ, Kratochwill TR. Single-Case Experimental Designs: Clinical Research and Practice. Compr Clin Psychol (Second Ed. 2022;3:1–28.
41. Kazdin AE. Single-case experimental designs. Evaluating interventions in research and clinical practice. Behav Res Ther. 2019;117:3–17.
42. Daniëls NEM, Bartels SL, Verhagen SJW, Van Knippenberg RJM, De Vugt ME, Delespaul PAEG. Digital assessment of working memory and processing speed in everyday life: Feasibility, validation, and lessons-learned. Internet Interv. 2020;19:100300.
43. Nightingale TE, Lim CAR, Sachdeva R, Zheng MMZ, Phillips AA, Krassioukov A. Reliability of Cognitive Measures in Individuals With a Chronic Spinal Cord Injury. PM R. 2019;11:1278–86.
44. Roberts TT, Leonard GR, Cepela DJ. Classifications In Brief: American Spinal Injury Association (ASIA) Impairment Scale. Clin Orthop Relat Res. 2017;475:1504.
45. Nederlandse Vereniging van Revalidatieartsen. Evaluatie van spasticiteit - Cerebrale en/of spinale spasticiteit. 2016. https://richtlijnendatabase.nl/richtlijn/cerebrale_en_of_spinale_spasticiteit/cerebrale_en_of_spinale_spasticiteit_-_startpagina.html. Accessed 26 Feb 2023.
46. Jackson SM, Cheng MS, Smith AR, Kolber MJ. Intrarater reliability of hand held dynamometry in measuring lower extremity isometric strength using a portable stabilization device. Musculoskelet Sci Pract. 2017;27:137–41.
47. Marshall AL, Smith BJ, Bauman AE, Kaur S. Reliability and validity of a brief physical activity assessment for use by family doctors. Br J Sports Med. 2005;39:294–7.
48. Brenner LA, Homaifar BY, Olson-Madden JH, Nagamoto HT, Huggins J, Schneider AL, et al. Prevalence and screening of traumatic brain injury among veterans seeking mental health services. J Head Trauma Rehabil. 2013;28:21–30.
49. Wassenaar A, De Reus J, Donders ART, Schoonhoven L, Cremer OL, De Lange DW, et al. Development and Validation of an Abbreviated Questionnaire to Easily Measure Cognitive Failure in ICU Survivors: A Multicenter Study. Crit Care Med. 2018;46:79–84.
50. Melzack R. The McGill Pain Questionnaire: major properties and scoring methods. Pain. 1975;1:277–99.
51. Spinhoven P, Ormel J, Sloekers PPA, Kempen GIJM, Speckens AEM, Van Hemert AM. A validation study of the Hospital Anxiety and Depression Scale (HADS) in different groups of Dutch subjects. Psychol Med. 1997;27:363–70.
52. Rietberg MB, Van Wegen EEH, Kwakkel G. Measuring fatigue in patients with multiple sclerosis: reproducibility, responsiveness and concurrent validity of three Dutch self-report questionnaires. Disabil Rehabil. 2010;32:1870–6.
53. Buysse DJ, Reynolds CF, Monk TH, Berman SR, Kupfer DJ. The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research. Psychiatry Res. 1989;28:193–213.
54. Van Der Zee CH, Post MW, Brinkhof MW, Wagenaar RC. Comparison of the Utrecht Scale for Evaluation of Rehabilitation-Participation with the ICF Measure of Participation and Activities Screener and the WHO Disability Assessment Schedule II in persons with spinal cord injury. Arch Phys Med Rehabil. 2014;95:87–93.
55. Laughton GE, Buchholz AC, Martin Ginis KA, Goy RE. Lowering body mass index cutoffs better identifies obese persons with spinal cord injury. Spinal Cord 2009 4710. 2009;47:757–62.
56. De TK, Michiels B, Vlaeyen JWS, Onghena P. Shiny SCDA (Computer software). 2020.

57. Lane JD, Gast DL. Visual analysis in single case experimental design studies: Brief review and guidelines. Neuropsychol Rehabil. 2014;24:445–63.
58. Ledford JR, Lane JD, Severini KE. Systematic Use of Visual Analysis for Assessing Outcomes in Single Case Design Studies. Brain Impair. 2018;19:4–17.
59. Parker RI, Vannest KJ, Davis JL. Effect Size in Single-Case Research: A Review of Nine Nonoverlap Techniques. Behav Modif. 2011;35:303–22.
60. Parker RI, Vannest K. An Improved Effect Size for Single-Case Research: Nonoverlap of All Pairs. Behav Ther. 2009;40:357–67.
61. Levin JR, Ferron JM, Kratochwill TR. Nonparametric statistical tests for single-case systematic and randomized ABAB...AB and alternating treatment intervention designs: new developments, new directions. J Sch Psychol. 2012;50:599–624.
62. De TK, Onghena P. The randomized marker method for single-case randomization tests: Handling data missing at random and data missing not at random. Behav Res Methods. 2022;54:2905–38.
63. De TK, Michiels B, Tanious R, Onghena P. Handling missing data in randomization tests for single-case experiments: A simulation study. Behav Res Methods. 2020;52:1355–70.
64. George D, Mallery P. SPSS for Windows Step by Step: A Simple Guide and Reference, 17.0 update. 10th edition. Boston: Allyn & Bacon; 2010.
65. Benjamini Y, Hochberg Y. Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. J R Stat Soc Ser B. 1995;57:289–300.
66. Landrigan J-F, Bell T, Crowe M, Clay OJ, Mirman D. Lifting cognition: a meta-analysis of effects of resistance exercise on cognition. Psychol Res 2019 845. 2019;84:1167–83.
67. Smit CAJ, Berenpas F, de Groot S, Stolwijk-Swuste JM, Janssen TWJ. Feasibility of overnight electrical stimulation-induced muscle activation in people with a spinal cord injury. A Pilot study. Spinal cord Ser cases. 2020;6:5.
68. Wijker BJ, de Groot S, van Dongen JM, van Nassau F, Adriaansen JJE, Achterberg-Warmer WJ, et al. Electrical stimulation to prevent recurring pressure ulcers in individuals with a spinal cord injury compared to usual care: the Spinal Cord Injury PREssure VOLTage (SCI PREVOLT) study protocol. Trials. 2022;23:156.



PART V

Discussion and summary

CHAPTER 12

GENERAL DISCUSSION

1. Summary of the main findings

The primary aim of this dissertation was to further our understanding on the mechanisms underlying the beneficial effects of resistance exercise on brain health and cognitive function in older adults, by exploring the role of exerkines (growth factors, myokines, cytokines, metabolites, hormones and neuropeptides released from bodily organs during physical exercise) on (exercise-induced changes in) neuroplasticity. Secondarily, the beneficial effects of neuromuscular electrical stimulation on cognitive function in individuals with spinal cord injury were evaluated.

In **part I** of this dissertation, the exerkines suggested to mediate neuroplastic and cognitive changes of acute and chronic exercise were discussed. *Chapter 1* presents a narrative review highlighting the influence of 16 exerkines on the molecular pathways involved in long-term synaptic potentiation (LTP) [1]. LTP is a complex neurophysiological process resulting in the increase in synaptic transmission from one neuron to the next. The evidence presented in this review paper can be considered indicative of the neurobiological actions of these exerkines from the perspective of molecular neuroscience. Given the existing neurobiological background of these 16 exerkines, they were considered promising candidates for further evaluation in the following studies. The 16 exerkines included: the growth factors BDNF (brain-derived neurotrophic factor), IGF-1 (insulin-like growth factor-1), and GH (growth hormone); pro-inflammatory cytokines, such as interleukin (IL)-1β, IL6 and TNF-α (tumor necrosis factor-alpha), anti-inflammatory cytokines, such as IL-4 and IL-10, and the inflammation marker kynurenine; the myokines irisin, cathepsin-B, apelin and adiponectin, the metabolites lactate and β-hydroxybutyrate, and other exerkines including osteocalcin, orexin-A, ghrelin and VIP (vasoactive intestinal peptide). In general, the circulating levels of these exerkines increased following acute and chronic exercise, except of pro-inflammatory cytokines and kynurenine, who increased after acute, but decreased after chronic exercise interventions. Commonly reported moderators of the effect of physical exercise on exerkine levels in previous studies were the type of exercise (resistance exercise, cardiovascular exercise, multimodal exercise, mind-body exercises, balance exercises, etc.), the intensity of exercise, the exercise duration or the volume of the exercise program, the amount of weight loss associated with the intervention and the age, sex, or comorbidities of the included study participants. There was a large heterogeneity between moderators across studies and more often than not, these moderating factors were not well described in the studies we reviewed.

Chapter 2 described a protocol for a comprehensive living systematic review and meta-analysis in which we will evaluate the mediating role of myokines (i.e. exerkines derived from muscle tissue) on cognitive function in older adults [2]. A list of 1126 putative myokines was retrieved from several secretome and transcriptome studies on human skeletal muscle. It is likely that not all of the identified secretory products enter the bloodstream and only few of them can (in)directly influence neuroplasticity within the brain.

In **part II** of this dissertation, the relationships between inflammatory (interleukin-6, IL-6; kynurenine) and neurotrophic (insulin-like growth factor-1, IGF-1) biomarkers in blood serum and neurometabolites associated with neuroinflammation and neurodegeneration in older adults were examined. In *chapter 3*, serum kynurenine was reported to correlate with neuroinflammation and neurodegeneration in multiple brain regions [3]. Interestingly, kynurenine is a circulating inflammatory marker converted from tryptophan by the enzyme indolamine 2,3-dioxygenase, in response to elevated levels of C-reactive protein (CRP), IL-1β, IL-6, TNF-α and interferon-γ and it decreases in response to anti-inflammatory cytokines IL-4 and IL-10 [4][5][6][7]. This suggest that kynurenine could serve as a general marker of heightened inflammatory states. Next, *chapter 4* further explored the role of participant characteristics, such as obesity and sarcopenia on blood serum and brain health markers. A hyperbolic relationship between body fat percentage and total gray matter volume was found, indicating that normal body fat percentage and overweight were associated with larger gray matter volumes than underweight and obesity. Furthermore, handgrip strength was negatively associated with kynurenine levels and total gray matter volume, and positively associated with neurometabolic markers of neural health [8]. This indicates a potential role of handgrip strength and kynurenine as surrogates of brain health, while underweight and obesity may be unhealthy conditions for the brain [3][8].

In **part III** of this dissertation, older adults' blood, brain and cognitive outcomes were evaluated before and after resistance exercise or a waiting list control condition. *Chapter 5* describes the effect of a single bout of Smith machine squat strength training with high relative loads (n=19) compared to a control group (n=18) on cognitive function (tested with a set of computerized cognitive tests and a postural dual task) [9]. We found improved performance on the mathematical processing task, a working memory test, in the experimental group but not in the control group. Performance on the postural dual task, which consisted of standing one feet behind the other (tandem Romberg stance) on a force plate while performing a similar mathematical processing task, however, did not improve. We had to reject our hypothesis that improvements in cognitive function would coincide with improvements in balance control. *Chapter 6 and 7* report the effect of a 12-week intervention with progressive, moderate to high-intensity, lower limb resistance exercises in seventy older adults with intact cognitive function and older adults with high risk of mild cognitive impairment (MCI) [10][11]. The older adults with high risk of MCI had higher kynurenine levels and lower subiculum volumes compared to cognitively healthy adults. Resistance exercise tended to increase IL-6 levels [10][11] and hippocampal total N-acetylaspartate levels and tended to prevent further age-related change in gray matter volume of the dentate gyrus [10]. Finally, our results showed significant improvements on the Go/No-go test, a cognitive inhibition test, following resistance exercise compared to the control group, dependent on the cognitive status of the older adults, being most

apparent in older adults with high risk of MCI. There was a relationship between cognitive improvements and exerkine level changes over time. Specifically, IGF-1 level increases in the exercise group were associated with improved performance on the mathematical processing task and IL-6 level increases were associated with improvements in response time on the memory search test in the total group of participants (both working memory tests) [11]. *Chapter 8* discusses the influence of the COVID-19 pandemic on our neurological findings [12]. In this study including three cases with COVID-19, neurometabolic changes in the hippocampus, related to neuroinflammation and cortical hypoexcitability, were discovered immediately after symptomatic recovery from the infection compared to before infection. Interestingly, the two resistance exercise participants showed increases in hippocampus volume despite COVID-19.

In **part IV** of this dissertation, our focus shifted to individuals with spinal cord injury. *Chapter 9* summarizes the existing evidence for an accelerated age-related cognitive decline, probably at least partly caused by a chronic neuroinflammatory response arising from the lesion site. An empty systematic review underscored the lack of intervention studies evaluating the exercise-cognition relationship in this population [13]. Therefore, we conducted a first exercise intervention study with low- or high-intense neuromuscular electrical stimulation and discovered that lactate increased significantly after both interventions (*chapter 10*). However, there was no significant increase in IGF-1 and no significant improvement in cognitive performance on the information processing speed test we used [14]. Interestingly, a longer time since spinal cord injury was associated with smaller changes in IGF-1 in the low-intensity group. Higher injury level was associated with smaller improvements in information processing speed, suggesting that individuals with cervical lesions would respond less to this intervention than individuals with thoracic lesions. Notably, large differences in tolerance to the low- or high-intensity stimulation were observed among the participants, indicating that 'same protocol' may not signify 'same intensity' for these participants due to differences in the remaining muscle mass. Therefore, future project should probably use other methods to keep intensity equal among participants in this population, for example by using a Borg scale. Overall, this study enhanced our understanding of administering this type of intervention to individuals with spinal cord injury, as well as the key factors to consider in data analysis. Using this information, we finally designed a new intervention study with a chronic (12 week) intervention using neuromuscular electrical stimulation, as presented in *chapter 11* [15].

2. Methodological considerations

One of the key methodological strengths of the papers included in this dissertation is the comprehensive, multidisciplinary approach used to investigate the mechanisms underlying the role of exerkines in the exercise-brain/cognition relationship. In the theoretical framework we tested (Figure 1), exerkines cause functional changes in the brain, subsequently resulting in structural brain changes and eventually explaining how physical exercise can diminish or prevent age-related deteriorations in brain health and cognitive function. So far, studies have mainly shown effects of exercise on one of these steps, but only few studies examined interrelationships.

A limitation associated with this holistic approach is the problem of p-hacking or multiple testing. The large amount of statistical tests increases the probability of false positive findings [16]. Therefore, we had to apply a correction using the false discovery rate (FDR) or Benjamini-Hochberg adjustment [17]. Another limitation of the original studies included in this dissertation was that they took place during the COVID-19 pandemic. It has previously been shown that (even mild) COVID-19 may alter brain neurometabolites and structure and cause neurological symptoms, which may have influenced our findings [12][18][19]. Even though only three participants were diagnosed with COVID-19 while in the study, fear for infection may have had impact on the results of some participants, and has caused additional dropouts.

Of the multiple methods that were used in this dissertation, the most recurring were the use of enzyme-linked immunosorbent assays (ELISA) for the evaluation of blood exerkine levels, magnetic resonance imaging (MRI) for the evaluation of (regional and total) gray matter volumes, proton magnetic resonance spectroscopy (^{1}H-MRS) for the evaluation of neurometabolites and the Montreal Cognitive Assessment (MoCA) and Automated Neuropsychological Assessment Metrics, version 4 (ANAM4) computerized cognitive test battery. For further methodological considerations related to ELISA, I refer to section 12.3.5. Briefly, this method uses antibodies that react to specific antigens and this reaction can be quantified. It is a time-consuming and precise work, but is considered the golden standard method for the estimation of biomarker levels. It is important that the antibodies are specific to the biomarkers tested and do not show cross-reactivity with other biomarkers. In the studies included in this dissertation, commercially available ELISA kits were used with good characteristics to detect the biomarkers needed. Concerning the estimation of gray matter volumes from MRI data, we used the FreeSurfer longitudinal pipeline. FreeSurfer is an automated, open-source segmentation software, and is therefore ideal for processing large datasets. It is the most popular surface-base morphometry package, providing information about cortex volume, cortical thickness, cortical surface area and gyrification. In contrast, voxel-based morphometry (VBM) analysis is an alternative method to calculate gray matter volume. It is argued that

the information provided by surface or voxel-based morphometry is complementary for the detection of morphological changes to the cortex, for a review see Goto et al. 2022 [20]. The main reasons for choosing the FreeSurfer package were the large amount of good quality data it provides and the availability of the package and its longitudinal pipeline to make pre-post comparisons at our institution. Furthermore, the radiologists who performed the data analysis were familiar with this package. The subregional gray matter volumes we analyzed, corresponded to the ^{1}H-MRS voxels we placed for evaluation of neurometabolites. This way, their interrelationship could be assessed more appropriately. The regions of interest were the left hippocampus, left middle temporal cortex, right dorsolateral prefrontal cortex, dorsal posterior cingulate cortex and the left primary sensorimotor cortex. The temporal cortex, including the hippocampus was selected since it is considered the most plastic region, and therefore it could be the first region to respond to physical exercise. The prefrontal cortex was measured since it is known to be the first to atrophy with aging, together with the hippocampus [21]. The dorsal posterior cingulate cortex was found to play a role in attentional focus and abnormalities in this region were associated with cognitive aging and Alzheimer's disease [22]. The primary sensorimotor cortex was selected because we used an intervention with physical exercise, although we expected this region to remain relatively stable. The MRI image acquisitions were done with a 3 Tesla (T) Siemens scanner. This type of scanner is now commonly used in hospitals, while more performant scanners of 7T are also available in some research centers. These 7T scanners (besides increasing image spatial resolution, contrast and signal-to-noise ratios) can measure the neurometabolites with more details. For example allowing differentiation between glutamate and glutamine instead of only the combined Glx signal. ^{1}H-MRS results are presented as spectra, which are then converted to values using a post-processing software for spectral fitting. Again, several software options exist, including Tarquin, LCModel, jMRUI-AMARES, jMRUI-QUEST and Gannet. Gannet is specifically designed for processing of edited MR spectra and jMRUI requires more manual adjustments which makes it suboptimal for use in large datasets. Therefore, Tarquin and LCModel were considered the most relevant options for our study design. In our first participant study, we compared Tarquin to LCModel and found a moderate-to-strong agreement between the results for 4 out of the 5 measured neurometabolites (i.e. total creatine, total choline, total N-acetylaspartate and myo-inositol). Only glutamate-glutamine complex (Glx) levels showed very low agreement between the two post-processing tools. Therefore, we advised researchers to consider the post-processing tool used when comparing ^{1}H-MRS results across studies, especially for Glx [3]. Finally, I would like to discuss the cognitive tests used in our studies. The MoCA test is used as a global cognitive screen and applied in our study to stratify participants by low or high MoCA score. A cutoff score of 26 was used, as this was previously suggested to differentiate older adults with mild cognitive impairment from older adults with

maintained cognitive function [23]. Furthermore, the ANAM4 cognitive test battery was used in our studies. This is a commonly used computerized neurocognitive assessment in students and the military, but it is less frequently used in older adults [24]. However, due to its computerized format, the response times measured are very precise and the computer presents the tests equally for all participants. Therefore, it is considered sensitive to discover small changes. The tests we selected from a library of 28 test options who mainly measure attention, processing speed and executive functions. The tests we chose included the 2-choice reaction time test, the Go/No-go test, the memory search test and a mathematical processing test, respectively measuring processing speed, response inhibition (a subdomain of executive functioning), short-term/working memory (a subdomain of executive functioning) and working memory with computational skills. The selection of these tests was based on the knowledge that the effect size of improvement on executive functions was previously reported to be the largest of all cognitive domains following cardiovascular type of exercise [25]. A further discussion of future directions regarding the methods used in this dissertation is presented in section 12.3.6.

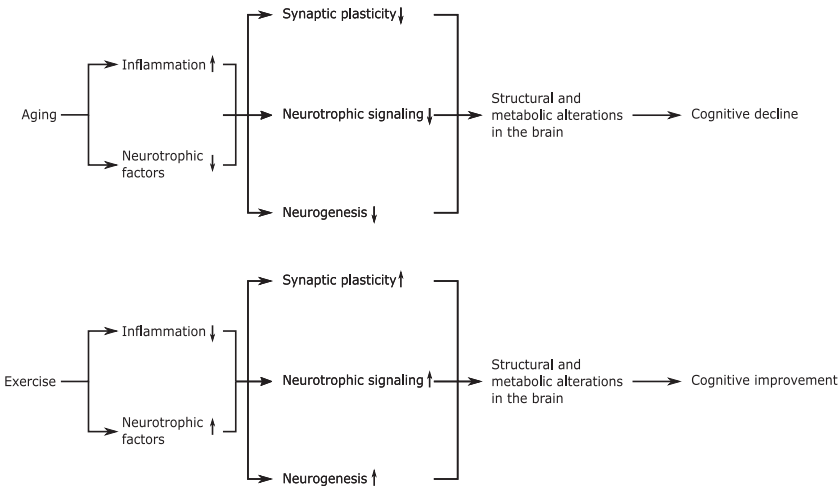


Figure 1. Theoretical frameworks of the aging and exercise-related changes resulting in cognitive decline and cognitive improvement, respectively, illustrating their similarities.

3. Further thematical discussion and future directions

Overall, this dissertation illustrates the complexity of the processes involved in age-related cognitive decline and of the mechanisms underlying exercise-induced changes in cognitive function. The reason for this complexity is caused by the lack of knowledge regarding related neurophysiological processes, as well as the heterogeneity of participant and exercise characteristics and of methodological choices made in studies in this research field. Thus, the publications included in this dissertation are only a few of the pieces of a very large puzzle. However, during the process of conducting these studies, presenting our findings at international conferences and interacting with prominent researchers of this field, I have developed a deeper understanding of the topic, its relevance, existing concerns and disagreements, as well as remaining knowledge gaps. I have used this section as a way to discuss these themes and tell the principal story beyond the content of the papers published by our research group. I will answer six questions who are being taught to journalism students as the questions you should be able to answer in order to fully convey a topic: who, what, when, where, why and how [26].

This thematical discussion will include in this order:

1) WHAT: The magnitude of the exercise effect on cognitive function. Significant results do not always mean strong effects. What are the sizes of these effects and what is the clinical relevance of these findings?
2) WHY: Proposed mechanisms of exercise-induced cognitive changes. This why question was an important part of this dissertation. The role of exerkines may explain partly why we see a change in cognitive performance following physical exercise. However, other mechanisms may also play a role. Here, I provided an overview of the most commonly reported potential mechanisms for the exercise-cognition relationship.
3) WHO: Individualization of exercise prescriptions for combatting age-related cognitive decline. Individuals with risk factors for age-related cognitive decline may have increased benefit of physical exercise programs designed to prevent or delay this decline. However, it remains unclear if there exists a one size fits all program or the optimal exercise prescription depends on personal characteristics. Here, I differentiate between cardiovascular and resistance exercise effects from the exerkine perspective and suggest specific targets.
4) WHEN: The exercise-cognition relationship throughout life. This dissertation included mainly studies in older adults. However, physical exercise may exert a greater protective effect if already started earlier in life. In order to give a more holistic picture of the role of physical exercise throughout life, I briefly discuss the existing evidence from studies in children, young adults and middle aged adults and describe the need for more studies evaluating methods to enhance intrinsic capacity and resilience in middle-aged and older adults.

5) HOW: Blood sampling and methodological considerations when examining exerkine levels. How to correctly measure exerkine levels does not seem as easy as it sounds. It feels required to address some issues related to measuring exerkines in this dissertation. Here, I will describe some influencing factors that may bias the results of exerkine studies.
6) WHERE: Future possibilities for research on the role of exerkines in the exercise-cognition relationship. This paragraph will discuss where the field is heading towards and the more futuristic applications of exerkine research.

3.1 WHAT? The magnitude of the exercise effect on cognitive function

In the last decade, the number of publications showing evidence for a beneficial effect of acute (i.e. a single bout) or chronic (i.e. multiple bouts over time) exercise on cognition has exploded, but it remains a topic of debate. In 2018, a study reported that only 76.1% of scientists who published manuscripts related to the effects of physical activity on cognitive performance endorsed the statement that physical activity has positive effects on cognition in humans [27]. By now, several meta-analyses have confirmed both acute and chronic exercise effects in humans [28][29][30][31], so that the World Health Organization now recommends regular physical exercise as a means to maintain a healthy cognitive state across the lifespan [32]. Specifically, physical exercise has been reported to cause improvements in executive functions [33], cognitive inhibition [34], cognitive flexibility [35], information processing speed [36], memory [37], and visuospatial ability [38]. Recently, an umbrella review summarized the findings of existing meta-analyses on the effect of chronic exercise on cognition in healthy adults across the life span. They also performed a meta-analysis on the primary randomized-controlled trials included in the revised meta-analyses and found small effect sizes (Cohen's d = 0.22 to 0.36). The largest effect sizes were found for changes in executive functions, but also in other cognitive domains such as memory and attention. However, the authors of this umbrella review state that many of the previous meta-analyses did not sufficiently account for key moderators and publication bias, and the exercise-related benefits on cognition become negligible and the evidence at best inconclusive after correcting for these factors [28]. The methodological choices that were made in this umbrella review have been criticized. For instance, because they included only studies with healthy populations and excluded studies with mind-body exercise programs and for the lack of consideration of key moderators [39]. Interestingly, another umbrella review specific to older adults aged 55 and above, on both acute and chronic exercise studies, found an overall significant small positive effects of chronic exercise on all cognitive domains compiled (Cohen's d = 0.24; SE = 0.04; mind-body exercise such as tai chi, yoga, pilates or qi gong Cohen's d = 0.48, resistance exercise Cohen's d = 0.24, cardiovascular exercise Cohen's d = 0.17) but no significant effect of acute exercise interventions. The effect of all exercise interventions

compiled was the largest for changes in global cognition (Cohen's d = 0.43; SE = 0.11), followed by executive functions (Cohen's d = 0.26), processing speed (Cohen's d = 0.21), memory (Cohen's d = 0.20) and attention (Cohen's d = 0.20) [40]. Finally, an umbrella review specifically evaluating the effect of physical exercise on adults with mild cognitive impairment found the largest effects for resistance exercise training, but with low certainty of evidence based on four randomized-controlled trials (Cohen's d = 0.80; 95% CI 0.29-1.31) [41]. Of note, no umbrella reviews exist related to the effects of physical exercise on exerkines or brain outcomes.

A common conclusion of meta-analyses is that the effect of exercise on cognition (but also on other outcomes) depends on several moderators, including characteristics of the population, exercise protocol, and cognitive tests or subdomains, so that research should further examine if specific exercise interventions may be valuable to specific populations with specific aims (see also the section about the WHO question). However, studies comparing different age cohorts, cognitive outcomes, exercise modes (aerobic, resistance, balance, exergames, coordination, etc.) or exercise doses (intensities, durations or number of sessions per week) have often not been able to find significant differences (e.g. Borde et al., 2015; Levin et al., 2017; Netz, 2019; Gallardo-Gómez et al., 2022; Ciria et al., 2023). Notably, the extent of the involvement of specific moderators to the exercise-cognition effect may be affected by other moderators, causing it almost impossible to predict the individual effect of a specific intervention. Advanced or new techniques, such as artificial intelligence based on big databases may be needed to further increase our understanding [46]. Moreover, time-dependent variation in cognitive test results could be controlled for by using highly repeated measurements of the main outcome, such as used in single-case experimental designs [15]. This can also increase our knowledge on (moderators that influence) the duration of an intervention needed before an effect can be expected. Additionally, this type of design can learn us more about moderators in specific populations.

Furthermore, I would like to come back to the finding from umbrella reviews that the effect of exercise interventions in improving cognitive function is in general considered to be modest [28][40] and ask to reflect if small effects are not in line with what we expected? It is common knowledge that physical exercise has mental and physical health benefits and aids to prevent age-related co-morbidities of various kinds. It is great to find positive effects of short-term exercise interventions on cognitive performance, and at least exciting to find transient effects already after a single exercise bout. However, while exercise intervention studies offer insight in the underlying mechanisms, maybe it is only logical that the real change in age-related (cognitive) decline can only be expected after years, or a lifetime of, healthy life choices/behavior. The findings from our research group also suggests that resistance exercise has small beneficial effects and prevents or diminishes age-related neurodegeneration and cognitive decline rather than restoring these functions [11][10]. This

is in line with a previous meta-analysis which indicated that exercise interventions with a duration of 6 months or more stopped or diminished hippocampal volume loss compared to the control group [47]. Moreover, literature has shown that multimodal strategies, combining exercise, diet, cognitive training and social engagement have larger effects than individual behavioral interventions [48]. See Zhang et al. 2022 for an umbrella review on modifiable risk factors for incident dementia [49]. Hence, there has been critique that exercise-cognition intervention studies are merely explorative in nature and lack a consensus on a theoretical framework leading to heterogeneity and lack of progress towards the development of (individualized) treatment programs [28]. I will elaborate on the theoretical frameworks in the section about the WHY question. Here, I would like to add that several meta-analyses have indeed confirmed and encouraged physical activity as a 'lifelong approach' to reduce the incidence of mild cognitive impairment and dementia [50][51][52][53]. Especially the continuation of regular physical activity throughout the life span into mid and late adulthood decreased the odds of cognitive decline at older age and both maintaining regular physical activity into late adulthood and starting new habits of physical activity during mid or late adulthood strongly decreased these odds. Hence, physical exercise at any age has protective effects, and for non-exercisers, it is never too late to start [54]. A second critique, related to the size of physical exercise benefits on the effect on cognitive tests, is that (changes in performance on) these tests not always reflect a clinical meaningfulness for the individual. Next to the abovementioned finding that physical activity diminishes the odds to develop mild cognitive impairment and dementia, which is definitely clinically meaningful, another outcome marker that could be more clinically meaningful and be applied in intervention studies is a measurement of subjective cognitive complaints. Examples of such complaints are forgetting why you went from one part of the house to the other, failing to remember people's names, to notice signposts on the road or forgetting if you turned off a light or locked the door [55]. It was shown that neither self-reported nor informant-reported subjective cognitive complaint scores related to neuropsychological test results in cognitively unimpaired and mild cognitive impaired participants and only informants' subjective cognitive complaints scores correlated with neuropsychological test results in for individuals with dementia [56]. Interestingly, a study showed that low physical activity levels are associated with more severe subjective cognitive complaints in adults aged above 18 years old [57], but an intervention study did not report significant effects on subjective cognitive complaints measured with the cognitive failures questionnaire [58]. We advise researchers to include subjective cognitive complaints questionnaires in future studies [15].

3.2 WHY? Proposed mechanisms of exercise-induced cognitive changes

Several mechanisms have been hypothesized as possible explanations for the exercise-cognition link, including the exerkine hypothesis who was the theoretical framework that was explored in this dissertation. A point of critique is the lack of consensus on a

theoretical framework for these effects [28]. In agreement with this critique, it remains unclear if all of these different mechanisms contribute to the effect of exercise at the same time or to what extent each of the suggested mechanisms plays a role. However, it is likely that several of these mechanisms occur synergistically. A non-exhaustive list including the most commonly cited hypotheses is described below:

a) The effort hypothesis: According to this hypothesis, there is a bidirectional relationship between effortful control and the regular practice of effortful exercise [59]. On the one hand, the ability to plan and remember your next physical training, to maintain and update in-program or in-session training goals and to inhibit the temptation to stop when the feeling of discomfort or fatigue is rising, two tasks requiring strong executive functioning, benefit the adherence to effortful exercise. On the other hand, chronic physical exercise enhances concentration and willpower, improving executive functions and effortful control. During effortful physical exercise, the central executive brain network, also described as the frontoparietal network, is activated similarly as during cognitive tasks requiring executive functions [59][60]. The salience network, also known as the cingulo-opercular network, is involved in switching between the default mode network (activated in a task-negative activity profile) and the central executive network when effortful exercise is initiated and each time an individual is distracted or disengages his or her attention from the task. Activating the central executive and salience network during effortful exercise is thought to benefit the connectivity between and within these networks and enhance the efficiency of executive functions [59].
b) The cerebrovascular or cardiovascular hypothesis : This hypothesis suggests that the increase in cerebral perfusion and regional cerebral blood flow seen following physical exercise enhances the transportation of oxygen and nutrients to the brain. This is considered to cause improved efficiency of neural networks underlying cognitive functions [61]. Between the age of 30 and 70, cerebral blood flow reduces 28-50%, which was found to predict cognitive decline [62][63]. Acute physical exercise is associated with blood vessel dilatation by the release of nitric oxide (NO) [64]. Chronic exercise can improve the bioavailability of NO which is linked to decreased blood vessel stiffness and improved arterial compliance [65][66]. Chronic exercise also enhances cerebrovascular status by stimulating the formation of new blood vessels. This process is called angiogenesis, mediated in part by the release of vascular endothelial growth factor (VEGF) [67][68]. Moreover, endothelial cell apoptosis is diminished by physical exercise's beneficial effect on endothelial cell telomere-stabilizing proteins [69]. Finally, chronic exercise improves cerebrovascular health by its preventative effect on the formation of atheromatous plaques and by reducing high blood pressure [64].

c) The catecholamine hypothesis: During and already immediately before exercise, the brainstem and hypothalamus activate the sympathoadrenal system, resulting in the release of the catecholamine dopamine, which can be converted into noradrenaline, or lead to the release of adrenaline from the adrenal gland [70]. Furthermore, catecholamine levels are augmented by the effect of exercise-induced, but not stress-induced, cortisol [71]. The catecholamine hypothesis states that following acute exercise, these catecholamines would enhance arousal and thereby transiently increase mental focus during cognitive tasks [72]. However, during vigorous acute exercise, the beneficial effect on cognition may be dampened by a feeling of fatigue, depending on a person's motivation, perception of effort and perceived availability of resources. This is further explained in the interoception model presented by McMorris 2021 [73]. It should be noted that there is at present insufficient evidence to support a mechanistic role of catecholamines in the beneficial effects on cognition induced by chronic exercise [70].

d) The stress-adaptation hypothesis: Chronic stress contributes to the development of cognitive decline and Alzheimer's dementia, and glucocorticoid stress hormones were shown to induce neuronal injury, affecting the structure and function of specific brain regions and networks, especially of the hippocampus [74]. While physical exercise is an acute stressor, regular participation in exercise was found to decrease diurnal levels of the glucocorticoid stress hormone cortisol [75] and improve stress reactivity, leading to a better homeostatic stability through stressful events. Thereby inducing a preventative effect against glucocorticoid stress hormone induced neuronal injury and cognitive decline [76].

e) The cognitive engagement hypothesis or "use it or lose it" hypothesis: Many types of physical exercise require cognitive effort, such as controlling behavior, paying attention, planning, problem solving or learning to master certain skills, which is considered a form of cognitive training [77]. Participation in physical exercise (with cognitive components) often activates similar networks as needed for cognitive demanding tasks (see also the effort hypothesis for the case of executive functions). The regular use of these neurons and neuronal networks is thought to improve their efficiency [78]. Interestingly, it is reported that simultaneous physical and cognitive training have a synergistic effect. Indeed, a network meta-analysis reported that the effect of physical exercise in combination with cognitive training was more effective in enhancing cognitive function in older adults than physical exercise or cognitive training alone [79].

f) The social enrichment hypothesis or intellectual engagement hypothesis: Animal research has shown that an enriched environment, which provides a combination of social, cognitive and physical stimulation, improves brain health and cognition [80]. Also in human studies, a more complex environment, with diverse social and physical stimuli was related to enhanced cognitive development across the lifespan [81]. According to this hypothesis, engagement in a larger variety of activities that

are novel or multimodal (e.g., combined mental and physical stimulation, see also the cognitive engagement hypothesis) would have the largest impact on preventing age-related cognitive decline. A specific addition of this hypothesis is that also social interaction (e.g. group training) may play a role in the beneficial effect of physical exercise intervention on cognitive functioning [81][82].

g) The exerkine hypothesis (It includes the myokine hypothesis and is a combination of the neurotrophic hypothesis and the inflammatory hypothesis): according to this hypothesis, a beneficial effect on cognition and brain health is induced by the neuroplastic effect of neurotrophic and anti-inflammatory endocrine factors released from muscle tissue (a.k.a. myokines) during muscle contractions [83], or from other bodily tissues such as the liver (i.e. hepatokines), fat tissue (i.e. adipokines), bone (i.e. osteokines) or the nervous system (i.e. neurokines), generally referred to as 'exerkines' [1]. The exerkines are thought to create a more optimal neural environment for neuroplastic processes to take place [1].

In my opinion, all of these hypotheses have a plausible theoretical background, but there are some differences between them. While the catecholamine hypothesis is limited to acute exercise effects, most other hypotheses are related to effects that can only be expected after chronic exercise interventions. The cardiovascular and exerkine hypotheses may be the exceptions that can both induce transient acute and longer lasting chronic effects [61][1]. Interestingly, there is some evidence that the accumulation of acute effects could result in the chronic effects on cognitive performance seen following exercise [84]. Concerning the role of exerkines, we reviewed that acute effects can arise from a temporary enhanced responsiveness to neuroplastic processes due to functional changes, while chronic effects reflect structural changes within the central nervous system [1]. The impact of these different frameworks could be explored further by designing protocols where one group cannot experience an effect of a single mechanism while another group can. Additionally, researchers tend to continue adding more evidence to their own hypotheses, while an effort should be made to gain a more holistic view on the field. This may be achieved by designing studies that assess multiple frameworks at the same time and evaluate their interrelationship from a biopsychosocial perspective. To the best of my knowledge, no such analyses exist.

3.3 WHO? Individualization of exercise prescriptions for combatting age-related cognitive decline

The exercise-cognition research field is advancing towards an individualization of the exercise prescription in order to improve the effectiveness of the intervention. A next step in this kind of precision might be attained by linking conditions associated with cognitive decline and neurodegeneration with a preferrable exercise mode i.e. cardiovascular or

resistance training. Based on newly gained insights from our research studies including the review papers presented in this dissertation, a model for individualized exercise training from the viewpoint of the exerkine hypothesis can be proposed (Figure 2). In this model, specific chronic conditions who are associated with disturbances in baseline exerkine levels, and a preferred exercise type (i.e. either cardiovascular or resistance exercise) to restore the disturbances in exerkine levels are presented. The discussion is limited to exerkines included in our 2022 narrative review paper [1]. The indication of a preferred exercise type does not mean that the other exercise mode is insufficient. Notably, combined exercise approaches, biopsychosocial influences and combinations of influential moderators were not considered in the current model. All of the cognitive impairment-associated conditions in the model generally occur during normal aging. Hence, to target aging in general, a combined exercise approach, including social enrichment, might be advised, as described in the previous section and suggested by other studies [43]. First, cognitive decline was linked with an age-related decrease in growth factors, with brain-derived neurotrophic factor (BDNF) as the cornerstone [85] [86]. At this point, some authors suggest that myokines, which include growth factors and BDNF-stimulating factors could be better stimulated by resistance exercise than by cardiovascular exercise [87][88][89][90][91][92]. The only other factor depicted in Figure 2 that is not a myokine but also involved in BDNF signaling pathways, is osteocalcin, which is secreted by osteoblasts. However, osteocalcin was also found to increase more after chronic resistance exercise than following cardiovascular exercise [93]. Second, an aging-associated evolution toward a more pro-inflammatory state was found to damage neurons and impair neurotrophic factor signaling [94][95][96][13]. So far, there are only some studies showing a decrease of inflammatory markers following resistance exercise [97]. In our own intervention study, IL-6 levels even tended to increase following resistance exercise [10]. In contrast, the beneficial effect of cardiovascular exercise against chronic inflammation was found in a growing array of research [98]. A meta-analysis showed that all modes of exercise training had small to moderate positive effects on markers of inflammation in older adults with or without chronic diseases. Subgroup analysis showed that TNF-α levels were significantly diminished by cardiovascular exercise training, while CRP decreased significantly both by cardiovascular and resistance training [99]. One condition associated with systemic inflammation is obesity. Adipose tissue is considered largely contributing to systemic inflammation [100][101] and obesity is linked to cognitive decline [102][103]. Adding to the existing evidence, we also discovered that obesity was associated with decreased levels of N-acetylaspartate/Creatine, a biochemical marker of neural health, in the sensorimotor and dorsolateral prefrontal cortex in older adults [8]. A meta-analysis showed a benefit of cardiovascular exercise compared to resistance exercise training to reduce inflammation levels in overweight and obese populations [104]. While it seems from current meta-analytic evidence that cardiovascular exercise is

also better at inducing fat loss than resistance exercise [105][106], the number of studies on the effect of resistance exercise on fat loss are increasing and some studies suggest a benefit in resistance exercise given it does not have the same problem of fat regain as with cardiovascular exercise when exercise training is stopped [107]. Another population where chronic inflammation is hypothesized to cause cognitive aging is in individuals with central nervous system injury, such as spinal cord injury, traumatic brain injury and stroke [13][108][109]. I could not find any RCTs or review papers evaluating the effect of resistance exercise training compared to control or cardiovascular exercise on inflammation in any of these populations. Next, many authors have linked cognitive deficit with sarcopenia [110][111]. The exact mechanism for sarcopenia-associated cognitive impairment is not well elucidated. A recent review suggested that the decreased release of myokines in sarcopenia might be the missing link [111]. We discovered that handgrip strength in older adults was positively related to N-acetylaspartate/Creatine levels in the dorsal posterior cingulate and dorsolateral prefrontal cortex [8]. To combat sarcopenia, resistance exercise is generally advised. Moreover, evidence has shown that acute resistance exercise might induce greater increases of GH [112] and IGF-1 [88][89] and chronic resistance exercise causes higher levels of IGF-1 [90] and osteocalcin [93]. Finally, other risk factors of cardiovascular disease, such as hypercholesterolemia, hypertension, diabetes and smoking were associated with cognitive decline [113][114][115] and structural brain alterations [116]. Here, exercise might also benefit the brain by improving cardiovascular health, metabolic regulation, aerobic capacity, mood and sleep, which were not reviewed in this dissertation, but well-explained by others [117]. In addition, in individuals with cardiovascular disease, cognitive deficit was suggested to result from impaired cerebral blood flow, which could be improved by regular cardiovascular exercise [118]. However, muscle mass and regular resistance training are also related to cardiovascular health, dyslipidemia, and glycemic control [97][119]. Part of this effect is mediated through the resistance exercise-associated release of myokines that affect metabolic regulation and systemic inflammation, as reviewed by Fiuza-Luces et al. (2018) [119]. Some additional examples of cardiovascular health-associated blood products that are altered by exercise are levels of cholesterol, triglycerides, antioxidants and reactive oxygen species [120][117]. To researchers in the field, we put forward the idea of considering this model when designing the optimal exercise protocol for investigating certain exerkines. An assessment of age, baseline levels of BDNF, inflammatory markers, fat percentage or handgrip strength might be used to create subgroups that could benefit from different exercise modes. This could provide new insight in the individualization of exercise training with the ultimate goal of improving cognitive function.

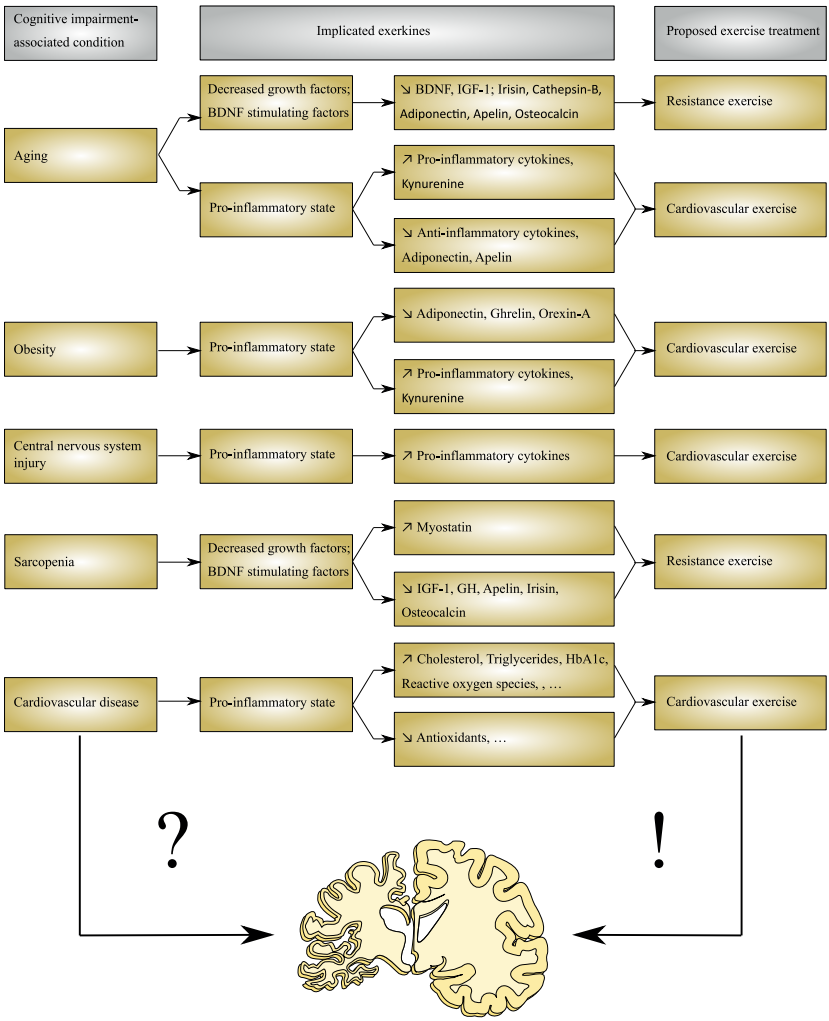


Figure 2. Preliminary model for individualized exercise training

3.4 WHEN? The exercise-cognition relationship throughout life.

Exercise interventions have shown beneficial effects on cognitive function across the lifespan. Most evidence exists for children aged between 6 and 13 years of age and older adults aged over 50 years old [121]. However, meta-analyses have also shown that acute and chronic exercise interventions also enhance academic performance in adolescents

and young adults [122] and chronic exercise was reported to benefit cognitive performance in young and middle-aged adults [123]. Additionally, some studies reported also acute exercise effects on cognition in middle-aged adults [124][125]. Hence, there seems to be a consistent exercise-cognition relationship across the lifespan and across populations, which illustrates the robustness of the effect [121].

Given the continuously increasing amount of older adults with declined self-reliance, and the decreasing health care resources to help these patients and their families, with cognitive decline being the most rapidly increasing cause of disability, showing also the second largest increment for causes of death [126], the World Health Organization (WHO) has developed an Integrated Care for Older People (ICOPE) program [127]. Stating that "health care that addresses the multidimensional demands of older age in an integrated way is more effective than services that merely react to specific diseases", they aim to move the clinical field towards prevention. Cognitive function, together with psychological function, locomotion, sensory function and vitality form the five most commonly cited pillars of older adults' intrinsic capacity [128], defined as the "composite of all of the physical and mental capacities that an individual can draw on" [127]. From this perspective, it is relevant to study enhancing cognitive ability before the development of deficits. An interesting approach is to study a person's resilience, defined as "a characteristic at a whole-person level which determines an individual's ability to resist functional decline or recover physical health following a stressor. It not only requires having sufficient physiological reserves, but also draws on resources from the person's social environment, their mindset, and ability to manifest adaptive behavior, as well as support resources provided through care or interventions."

Therefore, not only the evaluation of exercise-induced cognitive performance increases in middle-aged adults, but also the effect of this intervention on the enhancement of resilience and the prevention of cognitive decline in late adulthood is of major importance. Interestingly, it has been proposed that the implementation of physical exercise in midlife could exert clinically meaningful protective effects against age-related cognitive decline [129]. It is hypothesized that middle-aged individuals can build cognitive and brain reserve, increasing their resilience against age-related neurodegenerative processes in late adulthood [130]. As discussed above (see section related to the WHAT question), supportive findings exist in literature, indicating that both continued and new habits of physical activity in mid and late adulthood diminish the odds of cognitive deficits at older age [54]. There is a need for more studies directed towards the evaluation of physical exercise effects on intrinsic capacity domains and resilience in order to guide public health initiatives in developing healthy aging programs.

3.5 HOW? Blood sampling and methodological considerations when examining exerkine levels

Over the last years, there has been growing evidence that numerous methodological decisions can influence exerkine levels. Knowledge about these influencing factors is of critical importance when designing a research protocol. We advise that potential influencers should be kept as equal as possible between pre- and post-intervention measurements and between groups. It is generally a good research practice to strive for "cateris paribus", meaning that except for the intervention, all other things should be equal between groups. During the course of this PhD we have searched for a way to reduce variability in exerkine level results. Especially for BDNF, we measured very large standard deviations at baseline in our study with older adults (results not published). Subsequently, we piloted measuring in plasma instead of serum, we spoke others at conferences who indicated similar problems and searched the literature for a solution. This is what we learned:

A first indicator of quality of the measurement is the lower detection limit of the ELISA kit that is being used to assess the levels of the exerkine. If this detection limit is not appropriate, it may happen that a large amount of sample values are unmeasurable. If only a few values are missing, it is advised to impute the value that corresponds to half the detection limit. However, when more than 10% of the observations are below the detection limit, more advanced techniques, such as multiple imputation, maximum likelihood estimations or regression models are needed, especially if one decides to use parametric analysis methods [131]. If more than 20% of the observations are below the detection limit, one should seriously consider if the data can be used for analysis. To prevent these situations from happening, researchers should carefully review the literature to estimate the expected detection limit needed for the exerkine they intend to measure.

However, some exerkines are largely influenced by endogenous or exogenous factors as well as methodological choices, so that their levels may vary largely between studies. For example, a study showed that plasma BDNF levels are influenced by the menstrual cycle in women, with plasma BDNF levels increasing from early follicular phase to a pre-ovulatory peak (day 14) and showing a second rise during mid-luteal phase to peak at around day 24. BDNF levels correlated positively with estradiol and progesterone levels and postmenopausal women had significantly lower BDNF levels than their fertile counterparts. Interestingly, hormone replacement therapy restored BDNF levels of postmenopausal women to the early follicular phase levels of fertile women [132]. Fertile women taking oral contraceptive therapy also had plasma BDNF levels similar to follicular levels. Plasma BDNF levels showed a peak around 8am followed by a constant decrease throughout the day in the follicular phase of fertile women and in women undergoing oral contraceptive therapy [133]. Two other studies described a similar diurnal

variation in plasma BDNF levels in men. There were no diurnal variations in serum BDNF levels and in women, in either the follicular or luteal phases of the menstrual cycle [134] [135]. Serum BDNF levels were also reported to show seasonal variation, with higher concentrations in the spring-summer period. The analysis was done in individuals with depression and showed a significant correlation between BDNF serum concentrations and the number of sunshine hours in the week of blood collection and the 10 weeks prior to the blood collection [136]. Furthermore, several genetic determinants may influence the individual variation in circulating BDNF levels [137]. Most commonly reported is the Val66Met polymorphism, where a substitution of one or two Val alleles with a Met allele was shown to decrease exercise-dependent BDNF expression in the hippocampus in mice [138] and decrease exercise-dependent improvements in cognition in older adults [139].

BDNF levels were also reported to be influenced by how the samples were handled before analysis as shown by Gejl et al. 2019 [140]. Significant differences were observed between serum, plasma or platelet poor plasma levels of BDNF, with serum levels being larger than plasma levels and plasma levels being larger than platelet poor plasma levels. This caused 34% of the observations from platelet poor plasma falling below the lower limit of detection of the ELISA kit in this study. The serum levels did not correlate to plasma or platelet poor plasma levels. Furthermore, large differences were found between serum BDNF levels measured after 30 minutes or 60 minutes clotting time. The longer delay between sample collection and centrifugation corresponded to about a 33% increase in measured BDNF levels. Further extending clotting time did not change BDNF level measurements compared to the 60 minutes interval and the same differences were not reported for plasma or platelet poor plasma [140]. Another study compared similar conditions for IL-6 [141]. Gong et al. 2019 reported a good correlation between plasma and serum IL-6 levels, with higher values in plasma than in serum. When samples were stored in 4°C both plasma and serum levels remained constant. However, serum levels increased significantly with storage time when samples were stored at room temperature or at 37°C, both in samples stored in these conditions before centrifugation (4.53 and 20.45 times increase after 24h respectively) and after centrifugation (3.28 and 9.55 times increase after 24h respectively) [141].

Another very popular myokine in literature that requires to be discussed here is irisin, described in 2012 as a cleaved and secreted part of the transmembrane protein fibronectin type III domain containing 5 (FNDC5) [142]. In PubMed, over 2000 articles have been published mentioning irisin in their title or abstract. This myokine has been suggested to regulate several metabolic and cognitive health benefits of physical exercise [143][144]. However, in 2014 the existence of irisin was questioned due to heterogeneity and discrepancy of results from commercially available ELISA kits [145]. Albrecht et al. 2015 revealed prominent cross-reactivity of the polyclonal antibodies used in ELISA kits with non-specific proteins in human and animal serum samples, questioning if it is really

irisin researchers had been measuring and if irisin really exists [146]. Furthermore, almost a complete lack of agreement was found between newer irisin ELISA kits claiming to be validated against western-blot analysis and mass spectrometry and older kits [147]. Today, to our knowledge, there is still advise against the use of ELISA kits for the detection of irisin in humans, with Albrecht et al. stating in 2020 that available antibodies still bind to unspecific serum proteins. They argue that irisin can be found with mass spectroscopy, but as the multi-step sample preparation required for mass spectroscopy introduces uncontrollable variations, irisin can still not reliably be quantified with presently available methods [148]. This discussion around irisin arised from its popularity, but imposes the question if the same issues do not also affect other, less commonly used or newer exerkines.

3.6 WHERE? Future possibilities for research on the role of exerkines in the exercise-cognition relationship.

As mentioned above in the methodological considerations section, one of the unique features of the research included in this dissertation is the amount of outcomes we evaluated, and the exploration of relationships between these outcomes. This approach has the advantage of offering a more holistic view on existing mechanisms and theoretical frameworks. Especially ^{1}H-MRS has only infrequently been used in previous research. Before us, only Lind et al. 2021 had published evidence for an association between exercise-induced changes in inflammatory markers and neurometabolites [149]. More similar research is needed to confirm this concept. Additionally, technical advancements and artificial intelligence will increase the possibilities to gain a holistic understanding of the mechanisms underlying the exercise-cognition relationship. For example, while we were able to measure subregional volumes in the whole brain using MRI, ^{1}H-MRS is a voxel-based technique requiring long scanning times and limiting the number of brain regions that can be explored at one time. It still has a lower resolution than MRI data. Recently, whole brain ^{1}H-MRS, resolution upscaling algorithms and automatic spectral quality checks are being used to improve the feasibility of this method for clinical purposes, such as for the detection of 2-hydroxyglutarate, a metabolite that is abundantly produced in brain tumors (gliomas) with a IDH1 mutation [150]. These techniques need further validation for detection of other metabolites and in other populations. Furthermore, we measured single blood biomarkers using ELISA, while there may exist thousands of exerkines [2]. With multiplex technology, dozens of blood biomarkers can be measured simultaneously from small volume of samples. It has advantages in terms of efficient use of resources, costs, and time, but the development of specific and sensitive multiplex immunoassays with improved reproducibility, and limited cross-reactivity and analytical variability can be challenging and relatively few multiplex immunoassays have been validated. Therefore, for now, singleplex ELISA remains the golden standard [151][152].

In sum, the abovementioned technical possibilities will certainly aid to enhance our understanding of the complex interplay of existing mechanisms behind the exercise-cognition relationship. Yet, these explorative findings should always be corrected for multiple testing and confirmation of the discovered relationships will need further confirmation in hypothesis-driven research in large study samples.

Another future direction that exerkine research is heading towards is the development of the exercise pill. Given the well-known benefit of exercise in prevention and treatment of up to 26 diseases such as cognitive impairment, but also for example obesity, type 2 diabetes mellitus and even cancer, in 2021, Gubert and Hannan proposed to develop 'exercise pills', pharmacological drugs containing so-called 'Exercise Mimetics' to mimic the health benefits of physical exercise [153][154]. Exercise Mimetics research is growing gradually in recent years. For example, Findeisen et al. 2019 described how the beneficial characteristics of interleukin-6 (IL-6) and ciliary neurotrophic factor (CNTF) on metabolic homeostasis were merged to design the gp130 ligand IC7Fc for treatment of type 2 diabetes. In mice, IC7Fc improved glucose tolerance and hyperglycemia, and prevented weight gain, loss of skeletal muscle mass and liver steatosis [155]. Furthermore, based on the knowledge of exercise-dependent increases in IL15Rα^{+} CD8 T cells, and the tumor-protective effects of physical exercise mediated by mobilization of immune cells, the IL-15 super-agonist, NIZ985, was trialed as a potential treatment against pancreatic tumors in mice. It was discovered that NIZ985 attenuated tumor growth and prolonged survival. Moreover, immune checkpoint blockade (ICB) therapy, showing responses only rarely in pancreatic tumor patients, led to an effective reduction in tumor growth when combined with exercise, NIZ985 treatment or both, while there was no effect on tumor size of ICB therapy alone [156]. Despite very promising advances in the use of exercise mimetics in pharmacological drugs, none of the exercise pills have yet attained market approval [154]. Furthermore, it is important to recognize that exercise pills are limited to replicating specific biological or physiological processes, whereas physical exercise offers broader health benefits, likely stemming not only from biological factors but also from psychological and social aspects associated with exercise. Anyway, to advance this emerging field, it is crucial to further investigate the unique roles of exerkines. For a review on this topic, I refer to Zhu et al. 2024 [154].

4. General conclusion and recommendations

The primary aim of this dissertation was to gain understanding concerning the mechanisms underlying the beneficial effects of resistance exercise on brain health and cognitive function in older adults, with specific focus on the role of exerkines as mediators of these effects. Secondarily, we examined the effects of neuromuscular electrical stimulation on

cognitive function in individuals with spinal cord injury, focusing again on the role of exerkines. While it is important to acknowledge the vastness of this research field and the complexity of the neurophysiological processes involved, I can affirm that this effort has undoubtedly expanded our knowledge in several key areas.

Specifically, we described the molecular pathways activated by 16 principal exerkines which were found to regulate neuroplasticity, providing a neurobiological basis for the mechanisms [1]. Additionally, we retrieved 1126 putative myokines, most with unknown effects, underscoring the extent of the remaining knowledge gap [2]. A notable exerkine in our further analysis was kynurenine. This exerkine marks a state of high inflammation and was found to correlate with neuroinflammation and neurodegeneration in multiple brain regions [3]. In the second and third part of this dissertation, kynurenine levels were found to be higher in older adults with high MCI risk compared to older adults with low MCI risk after adjusting for age [11]. Moreover, kynurenine levels were elevated in older adults with lower handgrip strength after adjusting for age, sex and body fat percentage [8]. Interestingly, lower handgrip strength was also associated with total brain volume loss and diminished levels of a marker of neural density (N-acetylaspartate) in the dorsal posterior cingulate cortex and right dorsolateral prefrontal cortex [8]. Finally, underweight and obesity were also associated with total brain volume loss [8]. This suggests that kynurenine, handgrip strength and body fat percentage are markers of brain health and potential targets for (monitoring the effect of) intervention studies. Indeed, in the third part of this dissertation, I showed that an intervention with high-intensity, lower limb resistance exercises can boost up executive functioning. This can be seen by an increased performance on a mathematical processing task following acute exercise in older adults [9], and performance improvements on a Go/No-go test after 12 weeks in older adults with high MCI risk [11]. Additionally, IGF-1 level increases were associated with improvements in performance on the mathematical processing test in the exercise group. Furthermore, 12 weeks of resistance exercise tended to prevent age-related declines in hippocampal volume and neural density [10]. Finally, in the last part of this dissertation, I focused on individuals with spinal cord injury, who have been described to experience an accelerated cognitive aging process, potentially partly due to a chronic neuroinflammatory response following the spinal cord lesion [13]. Further, findings from a pilot study on a group of individuals with spinal cord injury revealed that acute neuromuscular electrical stimulation elevated lactate, one of the 16 exerkines we previously reported, but did not affect processing speed. Adding to the abovementioned finding, our results indicated that myokine responses may be smaller in individuals with longer time since spinal cord injury and cognitive effects may be smaller in individuals with higher injury level [14].

Based on these findings, a few general recommendations can be made. First, I recommend individuals to stay physically active throughout life in order to prevent or delay cognitive decline at older age. Based on current knowledge, a multimodal

group-based exercise program of at least moderate intensity is most likely to produce beneficial effects, as it may activate a combination of several hypothesized pathways and biopsychosocial influences. Second, I recommend that researchers and clinicians may consider kynurenine or handgrip strength as markers of brain health and future research may direct its focus on developing exercise programs for preventing cognitive decline in older adults with obesity or sarcopenia. Finally, I recommend that researchers implement longer lasting intervention periods to more comprehensively assess the benefits of physical exercise on preventing age-related neurodegeneration and cognitive decline and establish international collaborations to increase sample sizes.

References

1. Vints WAJ, Levin O, Fujiyama H, Verbunt J, Masiulis N. Exerkines and long-term synaptic potentiation: Mechanisms of exercise-induced neuroplasticity. Front Neuroendocrinol [Internet]. Academic Press; 2022 [cited 2022 Mar 15];66:100993. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0091302222000164
2. Vints WAJ, Gökçe E, Langeard A, Pavlova I, Çevik ÖS, Ziaaldini MM, et al. Myokines as mediators of exercise-induced cognitive changes in older adults: protocol for a comprehensive living systematic review and meta-analysis. Front Aging Neurosci. Frontiers Media SA; 2023;15:1213057.
3. Vints WAJ, Kušleikienė S, Sheoran S, Šarkinaitė M, Valatkevičienė K, Gleiznienė R, et al. Inflammatory blood biomarker kynurenine is linked with elevated neuroinflammation and neurodegeneration in older adults: evidence from two 1H-MRS post-processing analysis methods. Front Psychiatry [Internet]. Frontiers; 2022 [cited 2022 Mar 17];13:859772. Available from: https://www.frontiersin.org/articles/10.3389/fpsyt.2022.859772/abstract
4. Chiarugi A, Calvani M, Meli E, Traggiai E, Moroni F. Synthesis and release of neurotoxic kynurenine metabolites by human monocyte-derived macrophages. J Neuroimmunol [Internet]. J Neuroimmunol; 2001 [cited 2021 Dec 16];120:190–8. Available from: https://pubmed.ncbi.nlm.nih.gov/11694334/
5. Lustgarten MS, Fielding RA. Metabolites Associated With Circulating Interleukin-6 in Older Adults. Journals Gerontol Ser A [Internet]. Oxford Academic; 2017 [cited 2021 Dec 16];72:1277–83. Available from: https://academic.oup.com/biomedgerontology/article/72/9/1277/2629966
6. Kindler J, Lim CK, Weickert CS, Boerrigter D, Galletly C, Liu D, et al. Dysregulation of kynurenine metabolism is related to proinflammatory cytokines, attention, and prefrontal cortex volume in schizophrenia. Mol Psychiatry 2019 2511 [Internet]. Nature Publishing Group; 2019 [cited 2021 Dec 16];25:2860–72. Available from: https://www.nature.com/articles/s41380-019-0401-9
7. Pedraz-Petrozzi B, Elyamany O, Rummel C, Mulert C. Effects of inflammation on the kynurenine pathway in schizophrenia - A systematic review. J Neuroinflammation [Internet]. BioMed Central Ltd.; 2020 [cited 2021 Dec 16];17:1–17. Available from: https://jneuroinflammation.biomedcentral.com/articles/10.1186/s12974-020-1721-z
8. Vints WAJ, Kušleikienė S, Sheoran S, Valatkevičienė K, Gleiznienė R, Himmelreich U, et al. Body fat and components of sarcopenia relate to inflammation, brain volume, and neurometabolism in older adults. Neurobiol Aging [Internet]. 2023;127:1–11. Available from: https://doi.org/10.1016/j.neurobiolaging.2023.02.011
9. Vints WAJ, Ghafelzadeh Ahwaz F, Westhof-Jacobs C, Drozdova-Statkevičienė M, Česnaitienė VJ, Ziv G, et al. The effects of a single bout of high-intense strength exercise on cognitive function and postural dual-task control in older adults. Int J Sport Exerc Psychol [Internet]. Routledge; 2023 [cited 2023 Dec 5]; Available from: https://www.tandfonline.com/action/journalInformation?journalCode=rijs20
10. Vints WAJ, Šeikinaitė J, Gökçe E, Kušleikienė S, Šarkinaite M, Valatkeviciene K, et al. Resistance exercise effects on hippocampus subfield volumes and biomarkers of neuroplasticity and neuroinflammation in older adults with low and high risk of mild cognitive impairment: a randomized controlled trial. GeroScience [Internet]. Geroscience; 2024 [cited 2024 Jun 26]; Available from: https://pubmed.ncbi.nlm.nih.gov/38478179/
11. Vints WAJ, Gökçe E, Šeikinaitė J, Kušleikienė S, Česnaitienė VJ, Verbunt J, et al. Resistance training's impact on blood biomarkers and cognitive function in older adults with low and high risk of mild cognitive impairment: a randomized controlled trial. Eur Rev Aging Phys Act [Internet]. BMC; 2024 [cited 2024 Jul 4];21:9. Available from: /pmc/articles/PMC11005144/

12. Vints WAJ, Valatkevičienė K, Levin O, Weerasekera A, Jesmanas S, Kušleikienė S, et al. Hippocampal neurometabolic and structural changes from pre-to post-COVID-19: A case-series study. Magn Reson Imaging. Elsevier; 2024;109:249–55.
13. Vints WAJ, Levin O, Masiulis N, Verbunt J, van Laake-Geelen CCM. Myokines may target accelerated cognitive aging in people with spinal cord injury: A systematic and topical review. Neurosci Biobehav Rev [Internet]. Pergamon; 2023 [cited 2023 Jan 31];146:105065. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0149763423000349
14. Vints WAJ, Mazuronytė U, Qipo O, Levin O, Verbunt J, van Laake-Geelen CCM, et al. Acute neuromuscular electrical stimulation effects on lactate, insulin-like growth factor-1 and processing speed in spinal cord injured individuals: a randomized cross-over study. unpublished.
15. Vints WAJ, Levin O, van Griensven M, Vlaeyen JWS, Masiulis N, Verbunt J, et al. Neuromuscular electrical stimulation to combat cognitive aging in people with spinal cord injury: protocol for a single case experimental design study. BMC Neurol. 2024;24:197.
16. Jafari M, Ansari-Pour N. Why, When and How to Adjust Your P Values? Cell J [Internet]. Royan Institute; 2019 [cited 2024 Sep 17];20:604–7. Available from: /pmc/articles/PMC6099145/
17. Benjamini Y, Hochberg Y. Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. J R Stat Soc Ser B [Internet]. John Wiley & Sons, Ltd; 1995 [cited 2022 Feb 12];57:289–300. Available from: https://onlinelibrary.wiley.com/doi/full/10.1111/j.2517-6161.1995.tb02031.x
18. Douaud G, Lee S, Alfaro-Almagro F, Arthofer C, Wang C, Lange F, et al. Brain imaging before and after COVID-19 in UK Biobank. medRxiv (preprint server) [Internet]. Cold Spring Harbor Laboratory Press; 2021 [cited 2021 Aug 13]; Available from: https://www.medrxiv.org/content/10.1101/2021.06.11.21258690v1
19. Rapalino O, Weerasekera A, Moum SJ, Eikermann-Haerter K, Edlow BL, Fischer D, et al. Brain MR spectroscopic findings in 3 consecutive patients with covid-19: Preliminary observations. Am J Neuroradiol [Internet]. American Society of Neuroradiology; 2021 [cited 2021 Jun 4];42:37–41. Available from: https://pubmed.ncbi.nlm.nih.gov/33122208/
20. Goto M, Abe O, Hagiwara A, Fujita S, Kamagata K, Hori M, et al. Advantages of Using Both Voxel- and Surface-based Morphometry in Cortical Morphology Analysis: A Review of Various Applications. Magn Reson Med Sci [Internet]. Japanese Society for Magnetic Resonance in Medicine; 2022 [cited 2024 Sep 17];21:41–57. Available from: /pmc/articles/PMC9199978/
21. Fjell AM, Walhovd KB. Structural brain changes in aging: Courses, causes and cognitive consequences [Internet]. Rev. Neurosci. Freund Publishing House Ltd; 2010 [cited 2021 Apr 17]. p. 187–221. Available from: https://pubmed.ncbi.nlm.nih.gov/20879692/
22. Leech R, Sharp DJ. The role of the posterior cingulate cortex in cognition and disease. Brain [Internet]. Oxford University Press; 2014 [cited 2024 Sep 17];137:12–32. Available from: /pmc/articles/PMC3891440/
23. Nasreddine ZS, Phillips NA, Bédirian V, Charbonneau S, Whitehead V, Collin I, et al. The Montreal Cognitive Assessment, MoCA: A brief screening tool for mild cognitive impairment. J Am Geriatr Soc [Internet]. Blackwell Publishing Inc.; 2005 [cited 2020 Nov 4];53:695–9. Available from: www.mocatest.
24. Center for the Study of Human Operator Performance. C-SHOP ANAM4: User Manual. University of Oklahoma: Center for the Study of Human Operator Performance (C-SHOP); 2007.
25. Colcombe S, Kramer AF. Fitness Effects on the Cognitive Function of Older Adults: A Meta-Analytic Study. Psychol Sci [Internet]. 2003 [cited 2019 May 18];14:125–30. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12661673
26. Bleyer WG. Structure and Style in News Stories. Newsp Writ Ed. Cambridge, Massachusetts: The Riverside Press Cambridge; 1913. p. 66.

27. Nazlieva N, Mavilidi M-F, Baars M, Paas F. Establishing a Scientific Consensus on the Cognitive Benefits of Physical Activity. Int J Environ Res Public Heal 2020, Vol 17, Page 29 [Internet]. Multidisciplinary Digital Publishing Institute; 2019 [cited 2021 Aug 26];17:29. Available from: https://www.mdpi.com/1660-4601/17/1/29/htm
28. Ciria LF, Román-Caballero R, Vadillo MA, Holgado D, Luque-Casado A, Perakakis P, et al. An umbrella review of randomized control trials on the effects of physical exercise on cognition. Nat Hum Behav 2023 76 [Internet]. Nature Publishing Group; 2023 [cited 2023 Aug 25];7:928–41. Available from: https://www.nature.com/articles/s41562-023-01554-4
29. Chang YK, Labban JD, Gapin JI, Etnier JL. The effects of acute exercise on cognitive performance: A meta-analysis. Brain Res [Internet]. Elsevier; 2012 [cited 2019 Jul 8];1453:87–101. Available from: https://www.sciencedirect.com/science/article/pii/S0006899312004003
30. Levin O, Netz Y, Ziv G. Behavioral and Neurophysiological Aspects of Inhibition—The Effects of Acute Cardiovascular Exercise. J Clin Med [Internet]. Multidisciplinary Digital Publishing Institute (MDPI); 2021 [cited 2021 Sep 12];10:282. Available from: /pmc/articles/PMC7828827/
31. Griebler N, Schröder N, Artifon M, Frigotto M, Pietta-Dias C. The effects of acute exercise on memory of cognitively healthy seniors: A systematic review. Arch Gerontol Geriatr [Internet]. Arch Gerontol Geriatr; 2022 [cited 2023 Jan 22];99:104583. Available from: https://pubmed.ncbi.nlm.nih.gov/34861591/
32. Bull FC, Al-Ansari SS, Biddle S, Borodulin K, Buman MP, Cardon G, et al. World Health Organization 2020 guidelines on physical activity and sedentary behaviour. Br J Sports Med [Internet]. BMJ Publishing Group; 2020 [cited 2024 Aug 7];54:1451–62. Available from: /pmc/articles/PMC7719906/
33. Chen FT, Etnier JL, Chan KH, Chiu PK, Hung TM, Chang YK. Effects of Exercise Training Interventions on Executive Function in Older Adults: A Systematic Review and Meta-Analysis. Sport Med [Internet]. Springer; 2020 [cited 2023 Jan 22];50:1451–67. Available from: https://link.springer.com/article/10.1007/s40279-020-01292-x
34. Boucard GK, Albinet CT, Bouquet CA, Clarys D, Audiffren M, Bugaiska A. Impact of Physical Activity on Executive Functions in Aging: A Selective Effect on Inhibition Among Old Adults. J Sport Exerc Psychol [Internet]. 2012 [cited 2023 Jan 22];34:808–27. Available from: www.JSEP-Journal.com
35. Lerche S, Gutfreund A, Brockmann K, Hobert MA, Wurster I, Sünkel U, et al. Effect of physical activity on cognitive flexibility, depression and RBD in healthy elderly. Clin Neurol Neurosurg [Internet]. Clin Neurol Neurosurg; 2018 [cited 2023 Jan 22];165:88–93. Available from: https://pubmed.ncbi.nlm.nih.gov/29331872/
36. Lin TY, Hsieh SS, Chueh TY, Huang CJ, Hung TM. The effects of barbell resistance exercise on information processing speed and conflict-related ERP in older adults: a crossover randomized controlled trial. Sci Reports 2021 111 [Internet]. Nature Publishing Group; 2021 [cited 2023 Jan 22];11:1–14. Available from: https://www.nature.com/articles/s41598-021-88634-5
37. Wu J, Wang X, Ye M, Wang L, Zheng G. Effect of regular resistance training on memory in older adults: A systematic review. Exp Gerontol. Pergamon; 2021;150:111396.
38. Nemoto M, Sasai H, Yabushita N, Tsuchiya K, Hotta K, Fujita Y, et al. A Novel Exercise for Enhancing Visuospatial Ability in Older Adults with Frailty: Development, Feasibility, and Effectiveness. Geriatr (Basel, Switzerland) [Internet]. Geriatrics (Basel); 2020 [cited 2023 Jan 22];5:29. Available from: https://pubmed.ncbi.nlm.nih.gov/32375239/
39. Dupuy O, Ludyga S, Ortega FB, Hillman CH, Erickson KI, Herold F, et al. Do not underestimate the cognitive benefits of exercise. Nat Hum Behav [Internet]. Nature Publishing Group; 2024 [cited 2024 Sep 18];8:1460–3. Available from: https://www.nature.com/articles/s41562-024-01949-x

40. Blomstrand P, Tesan D, Nylander EM, Ramstrand N. Mind body exercise improves cognitive function more than aerobic- and resistance exercise in healthy adults aged 55 years and older – an umbrella review. Eur Rev Aging Phys Act [Internet]. BioMed Central Ltd; 2023 [cited 2023 Aug 25];20:1–22. Available from: https://eurapa.biomedcentral.com/articles/10.1186/s11556-023-00325-4
41. Demurtas J, Schoene D, Torbahn G, Marengoni A, Grande G, Zou L, et al. Physical Activity and Exercise in Mild Cognitive Impairment and Dementia: An Umbrella Review of Intervention and Observational Studies. J Am Med Dir Assoc. Elsevier; 2020;21:1415-1422.e6.
42. Borde R, Hortobágyi T, Granacher U. Dose-Response Relationships of Resistance Training in Healthy Old Adults: A Systematic Review and Meta-Analysis. Sports Med [Internet]. Sports Med; 2015 [cited 2023 Jan 22];45:1693–720. Available from: https://pubmed.ncbi.nlm.nih.gov/26420238/
43. Levin O, Netz Y, Ziv G. The beneficial effects of different types of exercise interventions on motor and cognitive functions in older age: a systematic review. Eur Rev aging Phys Act [Internet]. BioMed Central; 2017 [cited 2019 Jun 25];14:20. Available from: http://www.ncbi.nlm.nih.gov/pubmed/29276545
44. Netz Y. Is There a Preferred Mode of Exercise for Cognition Enhancement in Older Age?-A Narrative Review. Front Med [Internet]. Frontiers Media SA; 2019 [cited 2019 Jun 25];6:57. Available from: http://www.ncbi.nlm.nih.gov/pubmed/30984760
45. Gallardo-Gómez D, del Pozo-Cruz J, Noetel M, Álvarez-Barbosa F, Alfonso-Rosa RM, del Pozo Cruz B. Optimal dose and type of exercise to improve cognitive function in older adults: A systematic review and bayesian model-based network meta-analysis of RCTs. Ageing Res Rev. Elsevier; 2022;76:101591.
46. Herold F, Törpel A, Hamacher D, Budde H, Zou L, Strobach T, et al. Causes and Consequences of Interindividual Response Variability: A Call to Apply a More Rigorous Research Design in Acute Exercise-Cognition Studies. Front Physiol [Internet]. Frontiers Media S.A.; 2021 [cited 2024 Aug 1];12:682891. Available from: www.frontiersin.org
47. Wilckens KA, Stillman CM, Waiwood AM, Kang C, Leckie RL, Peven JC, et al. Exercise interventions preserve hippocampal volume: A meta-analysis. Hippocampus. 2021;31:335–47.
48. Lee KJ, Bae HJ. What have clinical trials taught us about brain health? Cereb Circ - Cogn Behav [Internet]. Elsevier; 2024 [cited 2024 Aug 7];6:100199. Available from: /pmc/articles/PMC10792690/
49. Zhang YR, Xu W, Zhang W, Wang HF, Ou YN, Qu Y, et al. Modifiable risk factors for incident dementia and cognitive impairment: An umbrella review of evidence. J Affect Disord [Internet]. J Affect Disord; 2022 [cited 2024 Aug 7];314:160–7. Available from: https://pubmed-ncbi-nlm-nih-gov.mu.idm.oclc.org/35863541/
50. Sofi F, Valecchi D, Bacci D, Abbate R, Gensini GF, Casini A, et al. Physical activity and risk of cognitive decline: A meta-analysis of prospective studies. J Intern Med [Internet]. J Intern Med; 2011 [cited 2021 Mar 14];269:107–17. Available from: https://pubmed.ncbi.nlm.nih.gov/20831630/
51. Iso-Markku P, Kujala UM, Knittle K, Polet J, Vuoksimaa E, Waller K. Physical activity as a protective factor for dementia and Alzheimer's disease: systematic review, meta-analysis and quality assessment of cohort and case-control studies. Br J Sports Med [Internet]. Br J Sports Med; 2022 [cited 2023 Jan 22];56:701–9. Available from: https://pubmed.ncbi.nlm.nih.gov/35301183/
52. Yoneda T, Lewis NA, Knight JE, Rush J, Vendittelli R, Kleineidam L, et al. The Importance of Engaging in Physical Activity in Older Adulthood for Transitions Between Cognitive Status Categories and Death: A Coordinated Analysis of 14 Longitudinal Studies. J Gerontol A Biol Sci Med Sci [Internet]. J Gerontol A Biol Sci Med Sci; 2021 [cited 2024 Aug 1];76:1668–77. Available from: https://pubmed.ncbi.nlm.nih.gov/33099603/

53. López-Ortiz S, Lista S, Valenzuela PL, Pinto-Fraga J, Carmona R, Caraci F, et al. Effects of physical activity and exercise interventions on Alzheimer's disease: an umbrella review of existing meta-analyses. J Neurol [Internet]. Springer Science and Business Media Deutschland GmbH; 2023 [cited 2024 Aug 7];270:711–25. Available from: https://link-springer-com.mu.idm.oclc.org/article/10.1007/s00415-022-11454-8
54. Makino K, Raina P, Griffith LE, Lee S, Harada K, Katayama O, et al. Lifetime Physical Activity and Late-Life Mild Cognitive Impairment in Community-Dwelling Older Adults. J Am Med Dir Assoc [Internet]. Elsevier Inc.; 2024 [cited 2024 Aug 1];25:488–93. Available from: http://www.jamda.com/article/S152586102301037X/fulltext
55. Wassenaar A, De Reus J, Donders ART, Schoonhoven L, Cremer OL, De Lange DW, et al. Development and Validation of an Abbreviated Questionnaire to Easily Measure Cognitive Failure in ICU Survivors: A Multicenter Study. Crit Care Med [Internet]. Crit Care Med; 2018 [cited 2023 Feb 27];46:79–84. Available from: https://pubmed.ncbi.nlm.nih.gov/29068855/
56. Peng SW, Wang CY, Lin SY, Lee YL, Lin YC, Lin YJ, et al. Subjective Cognitive Complaints: Comparing the Relation between Self-Reported Versus Informant-Reported Subjective Cognitive Complaints and Cognitive Performances in Cognitively Unimpaired, Mild Cognitive Impairment and Populations with Dementia. J Prev Alzheimer's Dis [Internet]. J Prev Alzheimers Dis; 2023 [cited 2024 Aug 1];10:562–70. Available from: https://pubmed-ncbi-nlm-nih-gov.mu.idm.oclc.org/37357298/
57. Felez-Nobrega M, Haro JM, Erickson KI, Koyanagi A. Physical Activity is Associated With Fewer Subjective Cognitive Complaints in 47 Low- and Middle-Income Countries. J Am Med Dir Assoc. Elsevier; 2020;21:1423-1429.e2.
58. Chou CC, Chien LY, Lin MF, Wang CJ, Liu PY. Effects of Aerobic Walking on Memory, Subjective Cognitive Complaints, and Brain-Derived Neurotrophic Factor Among Older Hypertensive Women. https://doi.org/101177/10998004221098974 [Internet]. SAGE PublicationsSage CA: Los Angeles, CA; 2022 [cited 2024 Aug 1];24:484–92. Available from: https://journals.sagepub.com/doi/abs/10.1177/10998004221098974
59. Audiffren M, André N. The exercise–cognition relationship: A virtuous circle. J Sport Heal Sci [Internet]. Shanghai University of Sport; 2019 [cited 2024 Jun 19];8:339–47. Available from: /pmc/articles/PMC6620209/
60. Seeley WW, Menon V, Schatzberg AF, Keller J, Glover GH, Kenna H, et al. Dissociable Intrinsic Connectivity Networks for Salience Processing and Executive Control. J Neurosci [Internet]. Society for Neuroscience; 2007 [cited 2024 Sep 18];27:2349–56. Available from: https://www.jneurosci.org/content/27/9/2349
61. Tarumi T, Zhang R. The role of exercise-induced cardiovascular adaptation in brain health. Exerc Sport Sci Rev [Internet]. Lippincott Williams and Wilkins; 2015 [cited 2024 Jun 19];43:181–9. Available from: https://journals-lww-com.mu.idm.oclc.org/acsm-essr/fulltext/2015/10000/the_role_of_exercise_induced_cardiovascular.2.aspx
62. Chao LL, Buckley ST, Kornak J, Schuff N, Madison C, Yaffe K, et al. ASL Perfusion MRI Predicts Cognitive Decline and Conversion From MCI to Dementia. Alzheimer Dis Assoc Disord [Internet]. NIH Public Access; 2010 [cited 2024 Jun 19];24:19–27. Available from: /pmc/articles/PMC2865220/
63. Portugal E, Vasconcelos P, Souza R, Lattari E, Monteiro-Junior R, Machado S, et al. Aging process, cognitive decline and Alzheimer`s disease: can strength training modulate these responses? CNS Neurol Disord - Drug Targets. Bentham Science Publishers Ltd.; 2015;14:1209–13.
64. Agbangla NF, Fraser SA, Albinet CT. An Overview of the Cardiorespiratory Hypothesis and Its Potential Contribution to the Care of Neurodegenerative Disease in Africa. Medicina (B Aires) [Internet]. Multidisciplinary Digital Publishing Institute (MDPI); 2019 [cited 2024 Jun 19];55:601. Available from: /pmc/articles/PMC6780802/

65. Steppan J, Sikka G, Jandu S, Barodka V, Halushka MK, Flavahan NA, et al. Exercise, Vascular Stiffness, and Tissue Transglutaminase. J Am Hear Assoc Cardiovasc Cerebrovasc Dis [Internet]. Wiley; 2014 [cited 2024 Jun 19];3:e000599. Available from: /pmc/articles/PMC4187484/
66. Montero D, Vinet A, Roberts CK. Effect of combined aerobic and resistance training versus aerobic training on arterial stiffness. Int J Cardiol [Internet]. Int J Cardiol; 2015 [cited 2024 Jun 19];178:69–76. Available from: https://pubmed-ncbi-nlm-nih-gov.mu.idm.oclc.org/25464222/
67. Bullitt E, Rahman FN, Smith JK, Kim E, Zeng D, Katz LM, et al. The Effect of Exercise on the Cerebral Vasculature of Healthy Aged Subjects as Visualized by MR Angiography. AJNR Am J Neuroradiol [Internet]. American Society of Neuroradiology; 2009 [cited 2024 Jun 19];30:1857. Available from: /pmc/articles/PMC7051270/
68. Chapman SB, Aslan S, Spence JS, DeFina LF, Keebler MW, Didehbani N, et al. Shorter term aerobic exercise improves brain, cognition, and cardiovascular fitness in aging. Front Aging Neurosci [Internet]. Frontiers Media SA; 2013 [cited 2024 Jun 19];5. Available from: /pmc/articles/PMC3825180/
69. Werner C, Fürster T, Widmann T, Pöss J, Roggia C, Hanhoun M, et al. Physical exercise prevents cellular senescence in circulating leukocytes and in the vessel wall. Circulation [Internet]. Circulation; 2009 [cited 2024 Jun 19];120:2438–47. Available from: https://pubmed-ncbi-nlm-nih-gov.mu.idm.oclc.org/19948976/
70. McMorris T. Exercise and cognitive function: a neuroendocrinological explanation. In: McMorris T, Tomporowski P, Audriffren M, editors. Exerc Cogn Funct. Chichester: Wiley-Blackwell; 2009. p. 40–68.
71. Chen C, Nakagawa S, An Y, Ito K, Kitaichi Y, Kusumi I. The exercise-glucocorticoid paradox: How exercise is beneficial to cognition, mood, and the brain while increasing glucocorticoid levels. Front Neuroendocrinol. Academic Press; 2017;44:83–102.
72. Cooper CJ. Anatomical and Physiological Mechanisms of Arousal, with Special Reference to the Effects of Exercise. Ergonomics [Internet]. Taylor & Francis Group; 1973 [cited 2021 Aug 25];16:601–9. Available from: https://www.tandfonline.com/doi/abs/10.1080/00140137308924551
73. McMorris T. The acute exercise-cognition interaction: From the catecholamines hypothesis to an interoception model. Int J Psychophysiol. Elsevier; 2021;170:75–88.
74. Csernansky JG, Dong H, Fagan AM, Wang L, Xiong C, Holtzman DM, et al. Plasma cortisol and progression of dementia in subjects with Alzheimer-type dementia. Am J Psychiatry. American Psychiatric Association; 2006;163:2164–9.
75. Ho RTH, Fong TCT, Chan WC, Kwan JSK, Chiu PKC, Yau JCY, et al. Psychophysiological Effects of Dance Movement Therapy and Physical Exercise on Older Adults With Mild Dementia: A Randomized Controlled Trial. Journals Gerontol Ser B [Internet]. Oxford Academic; 2020 [cited 2024 Jun 20];75:560–70. Available from: https://dx.doi.org/10.1093/geronb/gby145
76. Molina-Hidalgo C, Stillman CM, Collins AM, Velazquez-Diaz D, Ripperger HS, Drake JA, et al. Changes in stress pathways as a possible mechanism of aerobic exercise training on brain health: a scoping review of existing studies. Front Physiol [Internet]. Frontiers Media SA; 2023 [cited 2024 Jun 20];14:1273981. Available from: /pmc/articles/PMC10598664/
77. Schooler C. The effects of the cognitive complexity of occupational conditions and leisure-time activities on the intellectual functioning of older adults. In: Kramer AF, Chodzko-Zajko WJ, Poon LW, editors. Enhancing Cogn Funct Brain Plast [Internet]. Champaign, IL: Human Kinetics; 2009 [cited 2021 Aug 25]. p. 15–34. Available from: https://experts.illinois.edu/en/publications/enhancing-cognitive-functioning-and-brain-plasticity
78. Marmeleira J. An examination of the mechanisms underlying the effects of physical activity on brain and cognition. Eur Rev Aging Phys Act 2012 102 [Internet]. BioMed Central; 2012 [cited 2021 Jul 22];10:83–94. Available from: https://eurapa.biomedcentral.com/articles/10.1007/s11556-012-0105-5

79. Bruderer-Hofstetter M, Rausch-Osthoff AK, Meichtry A, Münzer T, Niedermann K. Effective multicomponent interventions in comparison to active control and no interventions on physical capacity, cognitive function and instrumental activities of daily living in elderly people with and without mild impaired cognition – A systematic review and network meta-analysis. Ageing Res Rev. Elsevier; 2018;45:1–14.
80. van Praag H, Kempermann G, Gage FH. Neural consequences of environmental enrichment. Nat Rev Neurosci [Internet]. Nat Rev Neurosci; 2000 [cited 2024 Jun 19];1:191–8. Available from: https://pubmed-ncbi-nlm-nih-gov.mu.idm.oclc.org/11257907/
81. Voss MW, Vivar C, Kramer AF, van Praag H. Bridging animal and human models of exercise-induced brain plasticity. Trends Cogn Sci [Internet]. NIH Public Access; 2013 [cited 2024 Jun 19];17:525–44. Available from: /pmc/articles/PMC4565723/
82. Karp A, Paillard-Borg S, Wang HX, Silverstein M, Winblad B, Fratiglioni L. Mental, physical and social components in leisure activities equally contribute to decrease dementia risk. Dement Geriatr Cogn Disord [Internet]. Dement Geriatr Cogn Disord; 2006 [cited 2024 Jun 19];21:65–73. Available from: https://pubmed-ncbi-nlm-nih-gov.mu.idm.oclc.org/16319455/
83. Pedersen BK. Physical activity and muscle–brain crosstalk [Internet]. Nat. Rev. Endocrinol. Nature Publishing Group; 2019 [cited 2020 Sep 12]. p. 383–92. Available from: https://pubmed.ncbi.nlm.nih.gov/30837717/
84. Voss MW, Weng TB, Narayana-Kumanan K, Cole RC, Wharff C, Reist L, et al. Acute exercise effects predict training change in cognition and connectivity. Med Sci Sports Exerc [Internet]. NIH Public Access; 2020 [cited 2023 Sep 1];52:131–40. Available from: /pmc/articles/PMC7753185/
85. El-Sayes J, Harasym D, Turco C V., Locke MB, Nelson AJ. Exercise-Induced Neuroplasticity: A Mechanistic Model and Prospects for Promoting Plasticity. Neuroscientist [Internet]. A.J. Nelson, Department of Kinesiology, McMaster University, Hamilton, ON, Canada; 2019 [cited 2019 Mar 21];25:65–85. Available from: http://journals.sagepub.com/doi/10.1177/1073858418771538
86. Erickson KI, Prakash RS, Voss MW, Chaddock L, Heo S, McLaren M, et al. Brain-derived neurotrophic factor is associated with age-related decline in hippocampal volume. J Neurosci [Internet]. Society for Neuroscience; 2010 [cited 2021 Feb 19];30:5368–75. Available from: https://www.jneurosci.org/content/30/15/5368
87. Kim S, Choi JY, Moon S, Park DH, Kwak HB, Kang JH. Roles of myokines in exercise-induced improvement of neuropsychiatric function [Internet]. Pflugers Arch. Eur. J. Physiol. Springer Verlag; 2019 [cited 2021 Jan 13]. p. 491–505. Available from: https://link.springer.com/article/10.1007/s00424-019-02253-8
88. de Alcantara Borba D, da Silva Alves E, Rosa JPP, Facundo LA, Costa CMA, Silva AC, et al. Can IGF-1 serum levels really be changed by acute physical exercise? A systematic review and meta-analysis. J Phys Act Heal [Internet]. Human Kinetics Publishers Inc.; 2020 [cited 2021 Jan 25];17:575–84. Available from: https://journals.humankinetics.com/view/journals/jpah/17/5/article-p575.xml
89. Gulick CN, Peddie M, Rehrer NJ. Does Exercise Impact Insulin-like Growth Factor 1? : Systematic Review & Meta-Analysis. Open J Heal Sci Med [Internet]. 2020 [cited 2021 Jan 31];1:104. Available from: https://www.researchgate.net/publication/345990546
90. Jiang Q, Lou K, Hou L, Lu Y, Sun L, Tan SC, et al. The effect of resistance training on serum insulin-like growth factor 1(IGF-1): A systematic review and meta-analysis. Complement. Ther. Med. Churchill Livingstone; 2020. p. 102360.
91. Marinus N, Hansen D, Feys P, Meesen R, Timmermans A, Spildooren J. The Impact of Different Types of Exercise Training on Peripheral Blood Brain-Derived Neurotrophic Factor Concentrations in Older Adults: A Meta-Analysis [Internet]. Sport. Med. Springer International Publishing; 2019 [cited 2021 Jan 24]. p. 1529–46. Available from: https://pubmed.ncbi.nlm.nih.gov/31270754/

92. Tsuchiya Y, Ando D, Takamatsu K, Goto K. Resistance exercise induces a greater irisin response than endurance exercise. Metabolism. W.B. Saunders; 2015;64:1042–50.
93. Lester ME, Urso ML, Evans RK, Pierce JR, Spiering BA, Maresh CM, et al. Influence of exercise mode and osteogenic index on bone biomarker responses during short-term physical training. Bone [Internet]. Bone; 2009 [cited 2021 Feb 15];45:768–76. Available from: https://pubmed-ncbi-nlm-nih-gov.ezproxy.ub.unimaas.nl/19520194/
94. Cotman CW, Berchtold NC, Christie L-A. Exercise builds brain health: key roles of growth factor cascades and inflammation. TRENDS Neurosci [Internet]. 2007 [cited 2020 Oct 20];30:464–72. Available from: www.sciencedirect.com
95. Bourgognon J-M, Cavanagh J. The role of cytokines in modulating learning and memory and brain plasticity. Brain Neurosci Adv [Internet]. SAGE Publications; 2020 [cited 2021 Feb 3];4:239821282097980. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7750764/
96. Scheiblich H, Trombly M, Ramirez A, Heneka MT. Neuroimmune Connections in Aging and Neurodegenerative Diseases [Internet]. Trends Immunol. Elsevier Ltd; 2020 [cited 2021 Mar 31]. p. 300–12. Available from: https://doi.org/10.1016/j.it.2020.02.002
97. Mejías-Peña Y, Estébanez B, Rodriguez-Miguelez P, Fernandez-Gonzalo R, Almar M, de Paz JA, et al. Impact of resistance training on the autophagy-inflammation-apoptosis crosstalk in elderly subjects. Aging (Albany NY) [Internet]. Impact Journals LLC; 2017 [cited 2021 Feb 26];9:408–18. Available from: www.aging-us.com
98. Flynn MG, McFarlin BK, Markofski MM. State of the Art Reviews: The Anti-Inflammatory Actions of Exercise Training. Am J Lifestyle Med [Internet]. SAGE PublicationsSage CA: Los Angeles, CA; 2007 [cited 2020 Oct 20];1:220–35. Available from: http://journals.sagepub.com/doi/10.1177/1559827607300283
99. Khalafi M, Akbari A, Symonds ME, Pourvaghar MJ, Rosenkranz SK, Tabari E. Influence of different modes of exercise training on inflammatory markers in older adults with and without chronic diseases: A systematic review and meta-analysis. Cytokine [Internet]. Cytokine; 2023 [cited 2023 Sep 8];169:156303. Available from: https://pubmed.ncbi.nlm.nih.gov/37467710/
100. Woods JA, Wilund KR, Martin SA, Kistler BM. Exercise, inflammation and aging [Internet]. Aging Dis. International Society on Aging and Disease; 2012 [cited 2020 Oct 20]. p. 130–40. Available from: /pmc/articles/PMC3320801/?report=abstract
101. Yudkin JS. Inflammation, obesity, and the metabolic syndrome. Horm Metab Res [Internet]. © Georg Thieme Verlag KG Stuttgart · New York; 2007 [cited 2020 Nov 11];39:707–9. Available from: http://www.thieme-connect.de/DOI/DOI?10.1055/s-2007-985898
102. Dye L, Boyle NB, Champ C, Lawton C. The relationship between obesity and cognitive health and decline. Proc Nutr Soc [Internet]. Cambridge University Press; 2017 [cited 2021 Feb 19]. p. 443–54. Available from: https://doi.org/10.1017/S0029665117002014
103. Alford S, Patel D, Perakakis N, Mantzoros CS. Obesity as a risk factor for Alzheimer's disease: weighing the evidence. Obes Rev [Internet]. Blackwell Publishing Ltd; 2018 [cited 2021 Feb 26];19:269–80. Available from: http://doi.wiley.com/10.1111/obr.12629
104. Guo Y, Qian H, Xin X, Liu Q. Effects of different exercise modalities on inflammatory markers in the obese and overweight populations: unraveling the mystery of exercise and inflammation. Front Physiol [Internet]. Front Physiol; 2024 [cited 2024 Aug 12];15:1405094. Available from: https://pubmed.ncbi.nlm.nih.gov/38933362/
105. Yarizadeh H, Eftekhar R, Anjom-Shoae J, Speakman JR, Djafarian K. The Effect of Aerobic and Resistance Training and Combined Exercise Modalities on Subcutaneous Abdominal Fat: A Systematic Review and Meta-analysis of Randomized Clinical Trials. Adv Nutr. Elsevier; 2021;12:179–96.
106. Ismail I, Keating SE, Baker MK, Johnson NA. A systematic review and meta-analysis of the effect of aerobic vs. resistance exercise training on visceral fat. Obes Rev. 2012;13:68–91.

107. Del Vecchio FB, Coswig VS, Cabistany LD, Orcy RB, Gentil P. Effects of exercise cessation on adipose tissue physiological markers related to fat regain: A systematic review. SAGE Open Med [Internet]. SAGE Publications; 2020 [cited 2021 Nov 15];8:2050312120936956. Available from: /pmc/articles/PMC7331762/
108. Kumar A, Stoica BA, Loane DJ, Yang M, Abulwerdi G, Khan N, et al. Microglial-derived microparticles mediate neuroinflammation after traumatic brain injury. J Neuroinflammation [Internet]. BioMed Central Ltd.; 2017 [cited 2021 Mar 15];14:47. Available from: https://jneuroinflammation.biomedcentral.com/articles/10.1186/s12974-017-0819-4
109. Schaechter JD, Hightower BG, Kim M, Loggia ML. A pilot [11C]PBR28 PET/MRI study of neuroinflammation and neurodegeneration in chronic stroke patients. Brain, Behav Immun - Heal [Internet]. Elsevier; 2021 [cited 2024 Aug 1];17:100336. Available from: /pmc/articles/PMC8474408/
110. Chang KV, Hsu TH, Wu WT, Huang KC, Han DS. Association Between Sarcopenia and Cognitive Impairment: A Systematic Review and Meta-Analysis. J Am Med Dir Assoc [Internet]. Elsevier Inc.; 2016 [cited 2021 Feb 26];17:1164.e7-1164.e15. Available from: http://www.jamda.com/article/S1525861016304285/fulltext
111. Scisciola L, Fontanella RA, Surina, Cataldo V, Paolisso G, Barbieri M. Sarcopenia and cognitive function: Role of myokines in muscle brain cross-talk. Life. MDPI AG; 2021. p. 1–12.
112. Consitt LA, Bloomer RJ, Wideman L. The effect of exercise type on immunofunctional and traditional growth hormone. Eur J Appl Physiol [Internet]. Eur J Appl Physiol; 2007 [cited 2021 Feb 26];100:321–30. Available from: https://pubmed-ncbi-nlm-nih-gov.ezproxy.ub.unimaas.nl/17357792/
113. Jefferson AL, Hohman TJ, Liu D, Haj-Hassan S, Gifford KA, Benson EM, et al. Adverse vascular risk is related to cognitive decline in older adults. J Alzheimer's Dis. IOS Press; 2015;44:1361–73.
114. Viticchi G, Falsetti L, Buratti L, Boria C, Luzzi S, Bartolini M, et al. Framingham risk score can predict cognitive decline progression in Alzheimer's disease. Neurobiol Aging. Elsevier Inc.; 2015;36:2940–5.
115. Chatterjee S, Peters SAE, Woodward M, Arango SM, Batty GD, Beckett N, et al. Type 2diabetes as a risk factor for dementia in women compared with men: A pooled analysis of 2.3 million people comprising more than 100,000 cases of dementia. Diabetes Care [Internet]. American Diabetes Association Inc.; 2016 [cited 2021 Feb 26];39:300–7. Available from: http://care.diabetesjournals.org/lookup/
116. Cox SR, Lyall DM, Ritchie SJ, Bastin ME, Harris MA, Buchanan CR, et al. Associations between vascular risk factors and brain MRI indices in UK Biobank. Eur Heart J [Internet]. Oxford University Press; 2019 [cited 2021 Feb 24];40:2290–300. Available from: https://academic.oup.com/eurheartj/article/40/28/2290/5371095
117. Tyndall A V., Clark CM, Anderson TJ, Hogan DB, Hill MD, Longman RS, et al. Protective Effects of Exercise on Cognition and Brain Health in Older Adults. Exerc Sport Sci Rev [Internet]. 2018 [cited 2018 Nov 24];46:215–23. Available from: http://insights.ovid.com/crossref?an=00003677-201810000-00004
118. Thomas BP, Tarumi T, Sheng M, Tseng B, Womack KB, Munro Cullum C, et al. Brain Perfusion Change in Patients with Mild Cognitive Impairment after 12 Months of Aerobic Exercise Training. J Alzheimer's Dis. IOS Press; 2020;75:617–31.
119. Fiuza-Luces C, Santos-Lozano A, Joyner M, Carrera-Bastos P, Picazo O, Zugaza JL, et al. Exercise benefits in cardiovascular disease: beyond attenuation of traditional risk factors [Internet]. Nat. Rev. Cardiol. Nature Publishing Group; 2018 [cited 2021 Feb 24]. p. 731–43. Available from: www.nature.com/nrcardio
120. Tran ZV, Weltman A. Differential Effects of Exercise on Serum Lipid and Lipoprotein Levels Seen With Changes in Body Weight: A Meta-analysis. JAMA J Am Med Assoc [Internet]. American Medical Association; 1985 [cited 2021 Feb 20];254:919–24. Available from: https://jamanetwork.com/journals/jama/fullarticle/400078

121. Erickson KI, Hillman C, Stillman CM, Ballard RM, Bloodgood B, Conroy DE, et al. Physical Activity, Cognition, and Brain Outcomes: A Review of the 2018 Physical Activity Guidelines. Med Sci Sports Exerc [Internet]. NIH Public Access; 2019 [cited 2024 Aug 7];51:1242–51. Available from: /pmc/articles/PMC6527141/
122. Haverkamp BF, Wiersma R, Vertessen K, van Ewijk H, Oosterlaan J, Hartman E. Effects of physical activity interventions on cognitive outcomes and academic performance in adolescents and young adults: A meta-analysis. J Sports Sci [Internet]. Routledge; 2020 [cited 2024 Aug 7];38:2637–60. Available from: https://www.tandfonline.com/doi/abs/10.1080/02640414.2020.1794763
123. Zhang J, Tam WWS, Hounsri K, Kusuyama J, Wu VX. Effectiveness of Combined Aerobic and Resistance Exercise on Cognition, Metabolic Health, Physical Function, and Health-related Quality of Life in Middle-aged and Older Adults With Type 2 Diabetes Mellitus: A Systematic Review and Meta-analysis. Arch Phys Med Rehabil [Internet]. Arch Phys Med Rehabil; 2024 [cited 2024 Aug 7];105. Available from: https://pubmed-ncbi-nlm-nih-gov.mu.idm.oclc.org/37875170/
124. Chang YK, Etnier JL, Li RH, Ren FF, Ai JY, Chu CH. Acute Exercise Effect on Neurocognitive Function Among Cognitively Normal Late-Middle-Aged Adults With/Without Genetic Risk of AD: The Moderating Role of Exercise Volume and APOE Genotype. J Gerontol A Biol Sci Med Sci [Internet]. J Gerontol A Biol Sci Med Sci; 2024 [cited 2024 Aug 7];79:glad179. Available from: https://pubmed-ncbi-nlm-nih-gov.mu.idm.oclc.org/37526237/
125. Lefferts WK, DeBlois JP, White CN, Heffernan KS. Effects of Acute Aerobic Exercise on Cognition and Constructs of Decision-Making in Adults With and Without Hypertension. Front Aging Neurosci [Internet]. 2019 [cited 2019 Mar 21];11. Available from: https://www.frontiersin.org/article/10.3389/fnagi.2019.00041/full
126. Mattiuzzi C, Lippi G. Worldwide disease epidemiology in the older persons. Eur Geriatr Med [Internet]. Springer; 2020 [cited 2022 Jan 3];11:147–53. Available from: https://link.springer.com/article/10.1007/s41999-019-00265-2
127. World Health Organization W. Integrated care for older people: Guidelines on community-level interventions to manage declines in intrinsic capacity. Geneva; 2017.
128. López-Ortiz S, Lista S, Peñín-Grandes S, Pinto-Fraga J, Valenzuela PL, Nisticò R, et al. Defining and assessing intrinsic capacity in older people: A systematic review and a proposed scoring system. Ageing Res Rev. Elsevier; 2022;79:101640.
129. Boa Sorte Silva NC, Barha CK, Erickson KI, Kramer AF, Liu-Ambrose T. Physical exercise, cognition, and brain health in aging. Trends Neurosci [Internet]. Elsevier; 2024 [cited 2024 Jun 26];47:402–17. Available from: http://www.cell.com.mu.idm.oclc.org/article/S0166223624000626/fulltext
130. Stern Y, Arenaza-Urquijo EM, Bartrés-Faz D, Belleville S, Cantilon M, Chetelat G, et al. Whitepaper: Defining and investigating cognitive reserve, brain reserve, and brain maintenance. Alzheimer's Dement [Internet]. John Wiley & Sons, Ltd; 2020 [cited 2024 Aug 7];16:1305–11. Available from: https://onlinelibrary-wiley-com.mu.idm.oclc.org/doi/full/10.1016/j.jalz.2018.07.219
131. Giskeødegård GF, Lydersen S. Measurements below the detection limit. Tidsskr Den Nor legeforening [Internet]. Norwegian Medical Association; 2022 [cited 2024 Jul 11];142. Available from: https://tidsskriftet.no/en/2022/09/medicine-and-numbers/measurements-below-detection-limit
132. Begliuomini S, Casarosa E, Pluchino N, Lenzi E, Centofanti M, Freschi L, et al. Influence of endogenous and exogenous sex hormones on plasma brain-derived neurotrophic factor. Hum Reprod [Internet]. Hum Reprod; 2007 [cited 2024 Jul 11];22:995–1002. Available from: https://pubmed.ncbi.nlm.nih.gov/17251358/
133. Pluchino N, Cubeddu A, Begliuomini S, Merlini S, Giannini A, Bucci F, et al. Daily variation of brain-derived neurotrophic factor and cortisol in women with normal menstrual cycles, undergoing oral contraception and in postmenopause. Hum Reprod [Internet]. Oxford Academic; 2009 [cited 2024 Jul 16];24:2303–9. Available from: https://dx.doi.org/10.1093/humrep/dep119

134. Piccinni A, Marazziti D, Del Debbio A, Bianchi C, Roncaglia I, Mannari C, et al. Diurnal variation of plasma brain-derived neurotrophic factor (BDNF) in humans: an analysis of sex differences. Chronobiol Int [Internet]. Chronobiol Int; 2008 [cited 2024 Jul 16];25:819–26. Available from: https://pubmed.ncbi.nlm.nih.gov/18780207/
135. Choi SW, Bhang S, Ahn JH. Diurnal variation and gender differences of plasma brain-derived neurotrophic factor in healthy human subjects. Psychiatry Res [Internet]. Psychiatry Res; 2011 [cited 2024 Jul 16];186:427–30. Available from: https://pubmed.ncbi.nlm.nih.gov/20826006/
136. Molendijk ML, Haffmans JPM, Bus BAA, Spinhoven P, Penninx BWJH, Prickaerts J, et al. Serum BDNF Concentrations Show Strong Seasonal Variation and Correlations with the Amount of Ambient Sunlight. PLoS One [Internet]. PLOS; 2012 [cited 2024 Jul 16];7:e48046. Available from: /pmc/articles/PMC3487856/
137. Li S, Weinstein G, Zare H, Teumer A, Völker U, Friedrich N, et al. The genetics of circulating BDNF: towards understanding the role of BDNF in brain structure and function in middle and old ages. Brain Commun [Internet]. Oxford University Press (OUP); 2020 [cited 2021 Jan 23];2. Available from: /pmc/articles/PMC7734441/?report=abstract
138. Ieraci A, Madaio AI, Mallei A, Lee FS, Popoli M. Brain-Derived Neurotrophic Factor Val66Met Human Polymorphism Impairs the Beneficial Exercise-Induced Neurobiological Changes in Mice. Neuropsychopharmacol 2016 4113 [Internet]. Nature Publishing Group; 2016 [cited 2024 Jul 16];41:3070–9. Available from: https://www.nature.com/articles/npp2016120
139. Brown BM, Frost N, Rainey-Smith SR, Doecke J, Markovic S, Gordon N, et al. High-intensity exercise and cognitive function in cognitively normal older adults: a pilot randomised clinical trial. Alzheimers Res Ther [Internet]. Alzheimers Res Ther; 2021 [cited 2024 Jul 16];13. Available from: https://pubmed.ncbi.nlm.nih.gov/33522961/
140. Gejl AK, Enevold C, Bugge A, Andersen MS, Nielsen CH, Andersen LB. Associations between serum and plasma brain-derived neurotrophic factor and influence of storage time and centrifugation strategy. Sci Reports 2019 91 [Internet]. Nature Publishing Group; 2019 [cited 2021 Nov 25];9:1–9. Available from: https://www.nature.com/articles/s41598-019-45976-5
141. Gong Y, Liang S, Zeng L, Ni Y, Zhou S, Yuan X. Effects of blood sample handling procedures on measurable interleukin 6 in plasma and serum. J Clin Lab Anal [Internet]. John Wiley & Sons, Ltd; 2019 [cited 2021 Nov 25];33:e22924. Available from: https://onlinelibrary.wiley.com/doi/full/10.1002/jcla.22924
142. Boström P, Wu J, Jedrychowski MP, Korde A, Ye L, Lo JC, et al. A PGC1-α-dependent myokine that drives brown-fat-like development of white fat and thermogenesis. Nature [Internet]. NIH Public Access; 2012 [cited 2020 Nov 13];481:463–8. Available from: /pmc/articles/PMC3522098/?report=abstract
143. Li H, Wang F, Yang M, Sun J, Zhao Y, Tang D. The Effect of Irisin as a Metabolic Regulator and Its Therapeutic Potential for Obesity. Int J Endocrinol [Internet]. Int J Endocrinol; 2021 [cited 2024 Jul 11];2021:6572342. Available from: https://pubmed.ncbi.nlm.nih.gov/33790964/
144. Islam MR, Valaris S, Young MF, Haley EB, Luo R, Bond SF, et al. Exercise hormone irisin is a critical regulator of cognitive function. Nat Metab [Internet]. Nature Publishing Group; 2021 [cited 2023 Apr 16];3:1058–70. Available from: https://www.nature.com/articles/s42255-021-00438-z
145. Sanchis-Gomar F, Alis R, Pareja-Galeano H, Romagnoli M, Perez-Quilis C. Inconsistency in circulating irisin levels: What is really happening? Horm Metab Res [Internet]. Georg Thieme Verlag; 2014 [cited 2024 Jul 11];46:591–6. Available from: http://www.thieme-connect.de/products/ejournals/html/10.1055/s-0033-1363283
146. Albrecht E, Norheim F, Thiede B, Holen T, Ohashi T, Schering L, et al. Irisin – a myth rather than an exercise-inducible myokine. Sci Reports 2015 51 [Internet]. Nature Publishing Group; 2015 [cited 2024 Jul 11];5:1–10. Available from: https://www.nature.com/articles/srep08889

147. Montes-Nieto R, Martínez-García MÁ, Luque-Ramírez M, Escobar-Morreale HF. Differences in analytical and biological results between older and newer lots of a widely used irisin immunoassay question the validity of previous studies. Clin Chem Lab Med [Internet]. Walter de Gruyter GmbH; 2016 [cited 2024 Jul 11];54:e199–201. Available from: https://www.degruyter.com/document/doi/10.1515/cclm-2015-1071/html
148. Albrecht E, Schering L, Buck F, Vlach K, Schober HC, Drevon CA, et al. Irisin: Still chasing shadows. Mol Metab [Internet]. Mol Metab; 2020 [cited 2024 Jul 11];34:124–35. Available from: https://pubmed.ncbi.nlm.nih.gov/32180552/
149. Lind A, Boraxbekk CJ, Petersen ET, Paulson OB, Andersen O, Siebner HR, et al. Do glia provide the link between low-grade systemic inflammation and normal cognitive ageing? A 1H magnetic resonance spectroscopy study at 7 tesla. J Neurochem [Internet]. John Wiley & Sons, Ltd; 2021 [cited 2021 Nov 7];159:185–96. Available from: https://onlinelibrary.wiley.com/doi/full/10.1111/jnc.15456
150. Li X, Strasser B, Jafari-Khouzani K, Thapa B, Small J, Cahill DP, et al. Super-Resolution Whole-Brain 3D MR Spectroscopic Imaging for Mapping D-2-Hydroxyglutarate and Tumor Metabolism in Isocitrate Dehydrogenase 1–mutated Human Gliomas. Radiology [Internet]. Radiological Society of North America Inc.; 2020 [cited 2024 Aug 26];294:589–97. Available from: https://pubs-rsna-org.mu.idm.oclc.org/doi/10.1148/radiol.2020191529
151. Chowdhury F, Williams A, Johnson P. Validation and comparison of two multiplex technologies, Luminex® and Mesoscale Discovery, for human cytokine profiling. J Immunol Methods. Elsevier; 2009;340:55–64.
152. Tighe PJ, Ryder RR, Todd I, Fairclough LC. ELISA in the multiplex era: Potentials and pitfalls. PROTEOMICS – Clin Appl [Internet]. John Wiley & Sons, Ltd; 2015 [cited 2024 Aug 26];9:406–22. Available from: https://onlinelibrary.wiley.com/doi/full/10.1002/prca.201400130
153. Gubert C, Hannan AJ. Exercise mimetics: harnessing the therapeutic effects of physical activity. Nat Rev Drug Discov 2021 [Internet]. Nature Publishing Group; 2021 [cited 2021 Sep 16];20:862–79. Available from: https://www-nature-com.kuleuven.e-bronnen.be/articles/s41573-021-00217-1
154. Zhu Y, Song G. Molecular origin and biological effects of exercise mimetics. J Exerc Sci Fit. Elsevier; 2024;22:73–85.
155. Findeisen M, Allen TL, Henstridge DC, Kammoun H, Brandon AE, Baggio LL, et al. Treatment of type 2 diabetes with the designer cytokine IC7Fc. Nat 2019 5747776 [Internet]. Nature Publishing Group; 2019 [cited 2024 Aug 26];574:63–8. Available from: https://www.nature.com/articles/s41586-019-1601-9
156. Kurz E, Hirsch CA, Dalton T, Shadaloey SA, Khodadadi-Jamayran A, Miller G, et al. Exercise-induced engagement of the IL-15/IL-15Rα axis promotes anti-tumor immunity in pancreatic cancer. Cancer Cell [Internet]. Cell Press; 2022 [cited 2024 Aug 26];40:720-737.e5. Available from: http://www.cell.com/article/S1535610822002173/fulltext

CHAPTER 13

IMPACT / VALORIZATION OF THE DISSERTATION

IMPACT / VALORIZATION OF THE DISSERTATION

This chapter starts with a description of the scientific impact, explaining in which sense this dissertation has enhanced our knowledge on the mechanisms underlying exercise effects on the brain and cognition. However, outside the scientific community a more practical answer may be desired [1]. Therefore, we added a paragraph describing the societal impact of the new scientific evidence, the clinical impact, and noted how this new knowledge may impact an individual person's life.

1. Scientific impact

This dissertation provides new insights into the mechanisms underlying the exercise-cognition relationship. The studies included in this dissertation pioneered by their multimodal approach, forming a bridge between the humeral, cerebral and cognitive responses to physical exercise. Using a hypothesis-driven approach, we explored several proposed relationships that had little or no previous evidence. For instance, there is novelty of our research studies since they assess the effects of resistance exercise on brain health and cognitive function. The amount of studies on resistance training is limited compared to studies on cardiovascular exercise [2]. Additionally, only one study [3] recently published about the relationship between chronic inflammation and neurometabolites prior to publication of our own findings [4]. No prior studies have examined the influence of sarcopenia or the impact of resistance exercise training on ^{1}H-MRS metabolites, and there is very limited knowledge on how participant characteristics such as obesity, sarcopenia or cognitive status affect brain health and the exercise-induced effects on exerkines, brain health and cognitive function. More specifically, our research uncovered several key findings that should guide future researchers in advancing the field. For example, we summarized evidence from animal studies that reported brain-derived neurotrophic factor (BDNF) and irisin facilitate while tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) impede the exercise-induced enhancement of long-term synaptic potentiation [2]. Furthermore, insulin-like growth factor-1 (IGF-1), β-hydroxybutyrate and irisin can induce the cerebral release of BDNF following physical exercise, exerting similar indirect effects [2]. Although this knowledge is derived from animal studies, it currently provides the strongest evidence that these six exerkines influence neuroplasticity directly or indirectly following exercise. However, the actions of many of the existing exerkines remain unclear. We compiled findings from secretome and transcriptome studies, counting 1125 secretory proteins that are released from muscle tissue alone [5]. This does not imply that all of these putative myokines enter the bloodstream, nor that they can cross the blood-brain barrier. However, our findings

underscore the significant knowledge gap in this area. Subsequently, we made a small step forward in the field by discovering that circulating levels of kynurenine related to metabolic alterations associated with neuroinflammation and neurodegeneration [4] and that older adults with a high risk of mild cognitive impairment (MCI) have elevated kynurenine levels [6]. Higher levels of kynurenine were also associated with decreased handgrip force [7], which is considered a marker of global muscular strength and physical health in general [8][9]. Additionally, handgrip strength was found in our studies not only to be associated with the inflammatory marker kynurenine, but also with total brain volume and neurometabolic indices of neurodegeneration [7]. Our findings suggest that kynurenine and handgrip strength may be surrogates of neurodegeneration. Longitudinal research may prove if they may also serve as predictors, which will be a topic of our future investigations (e.g., see PROSPERO registration number CRD42024536276). Another novelty suggested by our findings is that the effect of resistance exercise on brain and cognitive outcomes may be larger in older adults with MCI compared to cognitively healthy older adults [6]. Furthermore, we explored the effect of high-intensity resistance exercise, in line with the finding from previous studies that moderate to high-intensity cardiovascular exercise has larger effects than low-intensity exercise. Since we found only marginal effects of resistance exercise in our study, it can be hypothesized that this association reported in previous studies exists by the influence of lactate rather than the muscle work itself. The high relative load we used in our resistance exercise studies will likely not have caused a large release in lactate, as would high-intensity cardiovascular exercise. A promising muscle training approach to elevate lactate levels is neuromuscular electrical stimulation (NMES, see below) or blood flow restriction exercise, which will be the topic of our future research (see ClinicalTrials.gov: NCT05744167). I advocate for the development of additional hypothesis-driven studies to deepen our understanding of the underlying mechanisms linking exercise and cognition.

The work included in this dissertation related to individuals with spinal cord injury (SCI) was particularly novel. Our findings underscore the importance of evaluating cognitive deficits, which are a widely underrecognized problem in this population [10]. We helped the research field forward by summarizing SCI-specific risk factors related to cognitive impairments and describing the current knowledge of the role of chronic inflammation in a process of accelerated cognitive decline [10]. Moreover, we were the first to propose physical exercise as a promising intervention to combat age-related cognitive decline in this population and test the effects of neuromuscular electrical stimulation on cognitive function in SCI [11][12]. Many questions remain, such as when and who should be screened for cognitive deficits, the influence of cognitive impairments on prognosis and effectiveness of rehabilitation treatment and how to combat cognitive decline in the chronic phase after SCI.

2. Societal impact

In 2019, the global societal costs of dementia were estimated to be about US $ 1313.4 billion annually. Approximately 50% of these costs are associated with informal care provided by family members, friends or others, highlighting its impact beyond the health care system and the individual [13]. It has been predicted that the prevalence of cognitive decline and dementias will further increase due to population aging and increases in the prevalence of risk factors related to cognitive decline [14]. Indeed, epidemiological studies report worldwide increases in the number of persons with risk factors such as obesity [15], sarcopenia [16] and sedentary behavior [17] over the last decades. Building on the existing knowledge about risk factors of cognitive aging, we discovered that obese and underweight older adults have reduced brain volumes. Furthermore, decreased handgrip strength, a marker for sarcopenia, was associated with increased kynurenine levels in the blood circulation, as well as reduced total brain volume and altered neurometabolic indices indicative of neurodegeneration [7]. In general, physical activity programs and services for older adults are effective and cost-effective [18]. These findings may stimulate health policy makers to promote healthy lifestyles by implementing programs for the prevention of obesity, sarcopenia (especially muscle strength loss) and sedentary behavior, and with it prevent or delay the onset of age-related cognitive deficits.

3. Clinical impact

Age-related cognitive decline affects 99% of the population at a certain age [19]. Yet, still no successful medical treatment exists to reverse cognitive decline. Given its high prevalence, cognitive decline is often neglected or considered normal. However, cognitive impairments can importantly impact older adults' self-reliance and pose a burden to their caregivers and relatives [20]. In 2013, a study was published predicting that without a substantial reorganization, the current health care system would be inadequate to meet the demand of the growing disease burden, resulting in reduced access to care and reduced patients' quality of life by 2025 [21]. Evidence included in this dissertation underscores the potential role of physical exercise to prevent or delay age-related neurodegeneration and cognitive decline. Community services should therefore continue to take a role in promoting physical exercise as a preventive strategy against (cognitive) aging. Furthermore, general practitioners may prescribe physical exercise as a treatment strategy against cognitive decline and cognitive aging risk factors. Our findings show preliminary evidence that blood assessments of kynurenine or handgrip strength assessments may be used to monitor neurodegeneration, and screen for older adults at risk of developing cognitive impairments [4][7]. Additionally, our results suggest

that high-risk populations, such as those with obesity, sarcopenia and SCI, may need more attention when it comes to screening for cognitive impairments [7][10]. Clinicians should encourage these patients to remain physically active to counteract accelerated cognitive aging. Moreover, measuring 'myokine profiles' specific to cognitive aging or certain muscular diseases could become a useful tool for assessing disease severity and predicting treatment responses, as recently proposed for individuals with myasthenia gravis [22]. Similarly, also research in the development of '^{1}H-MRS profiles' may provide future prospects in staging and follow-up of diseases associated with neuroinflammation or neurodegeneration.

Another potential advent, could be the development of an exercise pill. The insights gained from this dissertation have the potential to advance this research trajectory. Given the unique characteristics of specific exerkines, it is likely that a series of pills will be developed with each their specific effects [23]. Our findings may suggest that possible targets to combat neurodegeneration are enhancers or agonists related to BDNF, IGF-1, β-hydroxybutyrate and irisin, or antagonists of TNF-α and IL-1β, given their exercise-induced (in)direct effects on neuroplasticity were shown in animal studies [2]. In addition to age-related neurodegeneration, these pills may be relevant in populations will neurological injury, such as stroke, multiple sclerosis, spinal cord injury and traumatic brain injury. I would refer to these exerkines as 'makers', while I would consider kynurenine or IL-6 more promising as 'markers' with respect to neural health. Kynurenine and IL-6 are indicators of overall inflammatory status, and therefore valuable to be measured for screening older adults at risk of cognitive decline. One of the main effects of IL-6, however, is the regulation of metabolic processes [24]. It could therefore be considered a 'maker' with respect to metabolic health, and its characteristics might therefore be included in exercise pills related to metabolic syndrome. However, more research will be needed before exercise pills will become available on the market.

4. Individual impact

For the individual person, we should discuss the most optimal physical exercise program to preserve cognitive abilities throughout life. There is a growing need to develop exercise programs designed to take into account individual variation in responses to physical exercise interventions on brain health and cognitive function. However, the large heterogeneity of population and exercise characteristics in studies makes it presently impossible to provide individualized training recommendations. Large scale studies or meta-analyses are needed to move the field forward. This will be a goal of an ongoing meta-analysis of our research group [5]. In this meta-analysis, the main objective is to compile a sufficient amount of studies to prove if a mediating effect of myokines on the

beneficial effect of physical exercise on cognitive function in older adults is present or absent. Specifically for individuals with spinal cord injury, our systematic and narrative review may offer a theoretical framework for the onset of accelerated cognitive aging, including risk factors related to this process [10]. Individuals with SCI can strive to mitigate these risk factors as effectively as possible. In conclusion, the papers included and reviewed in this dissertation may suggest that any type of physical exercise can have benefit on cognitive function of any individual person [2][25]. At present, some evidence suggests that multimodal approaches, possibly targeting several beneficial mechanisms at once, would have the strongest effect. This means that the combination of several exercise modes, but also the addition of a healthy diet, cognitive training and social engagement would be the best approach for the individual to combat age-related cognitive decline [26][27]. Notably, it appears that physical exercise primarily delays or prevents age-related declines in brain health and cognitive performance, whereas evidence for improvements in cognitive performance is less clear from studies by our research group and others [6][28][29][30][31]. Therefore, I believe that it is most important that physical exercise is started early and continued for a longer period of time, at best throughout life. Indeed, one study showed that cognitive gains tended to decay after cessation of the physical intervention [32]. While increasing your physical activity levels in late adulthood will have added benefit, cognitive decline already starts at the age of 20 and middle-aged adults implementing physical exercise were found to build resilience against neurodegeneration and cognitive aging in late adulthood [25][33][34]. I would therefore, recommend the individual person to stay physically active and choose an exercise program of their own choice, preferably a multimodal program, but especially one that offers the person pleasure and/or motivation so that the chances are highest to be maintained over time.

References

1. Voss MW, Vivar C, Kramer AF, van Praag H. Bridging animal and human models of exercise-induced brain plasticity. Trends Cogn Sci [Internet]. NIH Public Access; 2013 [cited 2024 Jun 19];17:525–44. Available from: /pmc/articles/PMC4565723/
2. Vints WAJ, Levin O, Fujiyama H, Verbunt J, Masiulis N. Exerkines and long-term synaptic potentiation: Mechanisms of exercise-induced neuroplasticity. Front Neuroendocrinol [Internet]. Academic Press; 2022 [cited 2022 Mar 15];66:100993. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0091302222000164
3. Lind A, Boraxbekk CJ, Petersen ET, Paulson OB, Andersen O, Siebner HR, et al. Do glia provide the link between low-grade systemic inflammation and normal cognitive ageing? A 1H magnetic resonance spectroscopy study at 7 tesla. J Neurochem [Internet]. John Wiley & Sons, Ltd; 2021 [cited 2021 Nov 7];159:185–96. Available from: https://onlinelibrary.wiley.com/doi/full/10.1111/jnc.15456
4. Vints WAJ, Kušleikienė S, Sheoran S, Šarkinaitė M, Valatkevičienė K, Gleiznienė R, et al. Inflammatory blood biomarker kynurenine is linked with elevated neuroinflammation and neurodegeneration in older adults: evidence from two 1H-MRS post-processing analysis methods. Front Psychiatry [Internet]. Frontiers; 2022 [cited 2022 Mar 17];13:859772. Available from: https://www.frontiersin.org/articles/10.3389/fpsyt.2022.859772/abstract
5. Vints WAJ, Gökçe E, Langeard A, Pavlova I, Çevik ÖS, Ziaaldini MM, et al. Myokines as mediators of exercise-induced cognitive changes in older adults: protocol for a comprehensive living systematic review and meta-analysis. Front Aging Neurosci. Frontiers Media SA; 2023;15:1213057.
6. Vints WAJ, Gökçe E, Šeikinaitė J, Kušleikienė S, Česnaitienė VJ, Verbunt J, et al. Resistance training's impact on blood biomarkers and cognitive function in older adults with low and high risk of mild cognitive impairment: a randomized controlled trial. Eur Rev Aging Phys Act [Internet]. BMC; 2024 [cited 2024 Jul 4];21:9. Available from: /pmc/articles/PMC11005144/
7. Vints WAJ, Kušleikienė S, Sheoran S, Valatkevičienė K, Gleiznienė R, Himmelreich U, et al. Body fat and components of sarcopenia relate to inflammation, brain volume, and neurometabolism in older adults. Neurobiol Aging [Internet]. 2023;127:1–11. Available from: https://doi.org/10.1016/j.neurobiolaging.2023.02.011
8. Tolea MI, Galvin JE. Sarcopenia and impairment in cognitive and physical performance. Clin Interv Aging [Internet]. Clin Interv Aging; 2015 [cited 2022 Feb 20];10:663–71. Available from: https://pubmed.ncbi.nlm.nih.gov/25878493/
9. Sui SX, Holloway-Kew KL, Hyde NK, Williams LJ, Leach S, Pasco JA. Muscle strength and gait speed rather than lean mass are better indicators for poor cognitive function in older men. Sci Rep [Internet]. Sci Rep; 2020 [cited 2022 Feb 20];10:10367. Available from: https://doi.org/10.1038/s41598-020-67251-8
10. Vints WAJ, Levin O, Masiulis N, Verbunt J, van Laake-Geelen CCM. Myokines may target accelerated cognitive aging in people with spinal cord injury: A systematic and topical review. Neurosci Biobehav Rev [Internet]. Pergamon; 2023 [cited 2023 Jan 31];146:105065. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0149763423000349
11. Vints WAJ, Mazuronytė U, Qipo O, Levin O, Verbunt J, van Laake-Geelen CCM, et al. Acute neuromuscular electrical stimulation effects on lactate, insulin-like growth factor-1 and processing speed in spinal cord injured individuals: a randomized cross-over study. unpublished.
12. Vints WAJ, Levin O, van Griensven M, Vlaeyen JWS, Masiulis N, Verbunt J, et al. Neuromuscular electrical stimulation to combat cognitive aging in people with spinal cord injury: protocol for a single case experimental design study. BMC Neurol. 2024;24:197.

13. Wimo A, Seeher K, Cataldi R, Cyhlarova E, Dielemann JL, Frisell O, et al. The worldwide costs of dementia in 2019. Alzheimer's Dement [Internet]. John Wiley & Sons, Ltd; 2023 [cited 2024 Aug 28];19:2865–73. Available from: https://onlinelibrary.wiley.com/doi/full/10.1002/alz.12901
14. Mattiuzzi C, Lippi G. Worldwide disease epidemiology in the older persons. Eur Geriatr Med [Internet]. Springer; 2020 [cited 2022 Jan 3];11:147–53. Available from: https://link.springer.com/article/10.1007/s41999-019-00265-2
15. Blüher M. Obesity: global epidemiology and pathogenesis. Nat Rev Endocrinol 2019 155 [Internet]. Nature Publishing Group; 2019 [cited 2022 Mar 6];15:288–98. Available from: https://www-nature-com.mu.idm.oclc.org/articles/s41574-019-0176-8
16. Ethgen O, Beaudart C, Buckinx F, Bruyère O, Reginster JY. The Future Prevalence of Sarcopenia in Europe: A Claim for Public Health Action. Calcif Tissue Int [Internet]. Springer; 2017 [cited 2022 Mar 6];100:229. Available from: /pmc/articles/PMC5313588/
17. López-Valenciano A, Mayo X, Liguori G, Copeland RJ, Lamb M, Jimenez A. Changes in sedentary behaviour in European Union adults between 2002 and 2017. BMC Public Health [Internet]. BioMed Central; 2020 [cited 2022 Mar 6];20:1–10. Available from: https://bmcpublichealth.biomedcentral.com/articles/10.1186/s12889-020-09293-1
18. Pinheiro MB, Howard K, Oliveira JS, Kwok WS, Tiedemann A, Wang B, et al. Cost-effectiveness of physical activity programs and services for older adults: a scoping review. Age Ageing [Internet]. Age Ageing; 2023 [cited 2024 Sep 18];52:afad023. Available from: https://pubmed.ncbi.nlm.nih.gov/36934340/
19. Petersen RC. Mild Cognitive Impairment. N Engl J Med [Internet]. 2011 [cited 2019 May 11];364:2227–61. Available from: https://www-nejm-org.kuleuven.ezproxy.kuleuven.be/doi/pdf/10.1056%2FNEJMcp0910237
20. Kasper JD. Cognitive Impairment among Functionally Limited Elderly People in the Community: Future Considerations for Long-Term Care Policy. Milbank Q. JSTOR; 1990;68:109.
21. Dall TM, Gallo PD, Chakrabarti R, West T, Semilla AP, Storm M V. An Aging Population And Growing Disease Burden Will Require A Large And Specialized Health Care Workforce By 2025. Health Aff [Internet]. Health Affairs; 2013 [cited 2019 Jul 23];32:2013–20. Available from: http://www.healthaffairs.org/doi/10.1377/hlthaff.2013.0714
22. Seok JW, Lee J, Kim M, Kim MJ, Shin HY, Kim SW. Plasma Myokine Profiles in Patients With AChR- and MuSK-Ab-Positive Myasthenia Gravis. J Clin Neurol [Internet]. J Clin Neurol; 2023 [cited 2024 Aug 26];19:469–77. Available from: https://pubmed.ncbi.nlm.nih.gov/37455510/
23. Hawley JA, Joyner MJ, Green DJ. Mimicking exercise: what matters most and where to next? J Physiol [Internet]. John Wiley & Sons, Ltd; 2021 [cited 2021 Sep 16];599:791–802. Available from: https://onlinelibrary.wiley.com/doi/full/10.1113/JP278761
24. Febbraio MA, Pedersen BK. Muscle-derived interleukin-6: mechanisms for activation and possible biological roles. FASEB J [Internet]. Wiley; 2002 [cited 2020 Nov 11];16:1335–47. Available from: https://onlinelibrary.wiley.com/doi/abs/10.1096/fj.01-0876rev
25. Boa Sorte Silva NC, Barha CK, Erickson KI, Kramer AF, Liu-Ambrose T. Physical exercise, cognition, and brain health in aging. Trends Neurosci [Internet]. Elsevier; 2024 [cited 2024 Jun 26];47:402–17. Available from: http://www.cell.com.mu.idm.oclc.org/article/S0166223624000626/fulltext
26. Lee KJ, Bae HJ. What have clinical trials taught us about brain health? Cereb Circ - Cogn Behav [Internet]. Elsevier; 2024 [cited 2024 Aug 7];6:100199. Available from: /pmc/articles/PMC10792690/
27. Zhang YR, Xu W, Zhang W, Wang HF, Ou YN, Qu Y, et al. Modifiable risk factors for incident dementia and cognitive impairment: An umbrella review of evidence. J Affect Disord [Internet]. J Affect Disord; 2022 [cited 2023 Jan 22];314:160–7. Available from: https://pubmed.ncbi.nlm.nih.gov/35863541/
28. Sujkowski A, Hong L, Wessells RJ, Todi S V. The protective role of exercise against age-related neurodegeneration. Ageing Res Rev. Elsevier; 2022;74:101543.

29. Wilckens KA, Stillman CM, Waiwood AM, Kang C, Leckie RL, Peven JC, et al. Exercise interventions preserve hippocampal volume: A meta-analysis. Hippocampus. 2021;31:335–47.
30. Thomas BP, Yezhuvath US, Tseng BY, Liu P, Levine BD, Zhang R, et al. Life-long aerobic exercise preserved baseline cerebral blood flow but reduced vascular reactivity to CO_2. J Magn Reson Imaging [Internet]. V C 2013 Wiley Periodicals, Inc; 2013 [cited 2021 Feb 24];38:1177–83. Available from: http://doi.wiley.com/10.1002/jmri.24090
31. Vints WAJ, Šeikinaitė J, Gökçe E, Kušleikienė S, Šarkinaite M, Valatkeviciene K, et al. Resistance exercise effects on hippocampus subfield volumes and biomarkers of neuroplasticity and neuroinflammation in older adults with low and high risk of mild cognitive impairment: a randomized controlled trial. GeroScience [Internet]. Geroscience; 2024 [cited 2024 Jun 26]; Available from: https://pubmed.ncbi.nlm.nih.gov/38478179/
32. Kaufman M, Dyrek P, Fredericson M, Oppezzo M, Roche M, Frehlich L, et al. The Role of Physical Exercise in Cognitive Preservation: A Systematic Review. Am J Lifestyle Med [Internet]. SAGE Publications Inc.; 2023 [cited 2024 Aug 28];18:574–91. Available from: https://journals.sagepub.com/doi/full/10.1177/15598276231201555
33. Zamroziewicz MK, Barbey AK. The mediterranean diet and healthy brain aging: Innovations from nutritional cognitive neuroscience. Role Mediterr Diet Brain Neurodegener Dis. Elsevier; 2017. p. 17–33.
34. Stern Y, Arenaza-Urquijo EM, Bartrés-Faz D, Belleville S, Cantilon M, Chetelat G, et al. Whitepaper: Defining and investigating cognitive reserve, brain reserve, and brain maintenance. Alzheimer's Dement [Internet]. John Wiley & Sons, Ltd; 2020 [cited 2024 Aug 7];16:1305–11. Available from: https://onlinelibrary-wiley-com.mu.idm.oclc.org/doi/full/10.1016/j.jalz.2018.07.219

CHAPTER 14

SUMMARY OF THE DISSERTATION IN THREE LANGUAGES

1. Summary

Cognitive decline is inherent to aging [1]. It can manifest in various ways, including concentration difficulties, disorientation, slower information processing, frequently being unable to recall things, absent-mindedness, or forgetfulness, while the ability to use previously acquired knowledge, skills, and experiences generally remains intact [2]. Depending on individual and environmental factors, cognitive aging will sooner or later lead to a need for assistance [3]. Some major risk factors include educational level, physical inactivity, obesity, type 2 diabetes mellitus, smoking, high blood pressure, alcohol use, brain trauma, depression, air pollution, hearing loss, and social isolation. It is estimated that the prevalence of dementia could be reduced by 40% at the population level by eliminating these risk factors [4]. However, worldwide, the opposite trend is occurring. For example, the prevalence of physical inactivity increased by 5% between 2005 and 2017 [5], type 2 diabetes mellitus increased by 50% between 1990 and 2015 [6], and the prevalence of obesity rose by 300% between 1975 and 2014 [7]. A 2013 research article estimated that by 2025, the current healthcare system would no longer be able to provide necessary care due to the aging population and an increase in individuals living with chronic diseases if no additional efforts are made in prevention [8].

Exercise plays an important role in maintaining good physical and mental health. It has positive effects on nearly every body system, including the brain [9]. An improvement in cognitive functions has been confirmed in an impressive number of studies involving participants of all ages [10][11][12]. Despite this knowledge, which has been around for decades, there remains uncertainty and a lack of consensus on the underlying mechanisms [13]. An interesting finding was made in 2003 by Bente Pedersen's research group in Denmark. They discovered that the cytokine interleukin-6 (IL-6) was released by muscle cells during contractions, triggering signaling cascades in other organs, and they called it a 'myokine' [14]. It later became clear that during exercise, thousands of factors enter the bloodstream from almost all body systems, each with its own local and/or systemic effects. These exercise-related factors were called 'exerkines' [15]. Only a limited number of these exerkines are known to have effects on the brain or to be associated with cognitive changes after exercise [16]. The function of most exerkines remains unknown.

The primary goal of this dissertation was to gain more knowledge and understanding of the role of exerkines in promoting brain health and cognitive function after exercise in older adults. Research into the role of exerkines, and the mechanism underlying the effect of exercise on cognitive functioning in general, is essential for developing evidence-based exercise programs aimed at preventing age-related cognitive decline. For our intervention studies, we chose resistance training, as more research has been conducted on endurance training up till now, while some researchers argued that myokines might be released to a greater extent after resistance training compared to

endurance training [17]. In addition, research was conducted on individuals with spinal cord injuries. In this population, neuromuscular electrical stimulation was chosen as an intervention, as it may release even higher amounts of myokines than resistance training [18][19].

In **Part I** of this dissertation, a literature review was conducted on the effects of exerkines on neuroplasticity. In the first study (*Chapter 1*), we detailed the signaling cascades activated by 16 exerkines with known (in)direct effects on long-term synaptic potentiation (LTP) [20]. LTP is a form of neuroplasticity at the level of the synaptic connection between two nerve cells. In these synaptic connections, a chemical reaction occurs that leads to the transmission of a nerve impulse from one nerve cell to the next. To reach the threshold at which a new nerve signal is generated in the following nerve cell, the chemical signal must be sufficiently strong. LTP increases the chemical signal released by the pre-synaptic nerve cell and lowers the threshold for transmitting the signal in the post-synaptic nerve cell [21]. Although the described signaling cascades are based on animal research, they can be seen as an indication of the neurobiological effects of exerkines at the molecular level. Therefore, this knowledge forms an important theoretical background for research into the effects of exerkines. Additionally, we described changes in exerkine levels in circulation after exercise from human studies and in the brain, primarily from animal studies. Overall, we can state that exerkines with neurotrophic or anti-inflammatory effects increased after a single session of exercise (acute exercise) and after exercise over several weeks (chronic exercise). Exerkines with pro-inflammatory effects also increased after acute exercise but decreased after chronic exercise. Thus, sustained exercise appears to have a neurotrophic and anti-inflammatory effect, mediated by changes in exerkines. It is important to note that the effect of exercise depended on various influencing factors, such as the type of exercise (resistance training, endurance training, multimodal training, mind-body training, balance exercises, etc.), the intensity and duration of the training, or the volume of the exercise program, as well as weight loss associated with the intervention, age, gender, or comorbidities of the study participants. In a second article (*Chapter 2*), we described the protocol for a future literature review with meta-analysis [16]. In this review, we aim to systematically map the current state of knowledge regarding the role of myokines in cognitive functioning in older adults and, if possible, conduct an analysis of the mediating role of these myokines on cognitive functioning. We plan to update this article every six months over a minimum of five years post-publication. To be as comprehensive as possible, we developed a list of 1,126 potential myokines derived from various secretome and transcriptome studies on human skeletal muscle. After an initial literature analysis, we included 33 studies in the meta-analysis. The results are currently being analyzed.

Part II of this dissertation contains the results of a cross-sectional analysis we conducted to investigate the relationship between baseline exerkine levels and signs of brain aging [22][23]. More specifically, we assessed the levels of inflammatory (interleukin-6, IL-6; kynurenine) and neurotrophic (insulin-like growth factor-1, IGF-1) factors in the blood of older adults and looked for associations with neurometabolic signs of neuroinflammation and neurodegeneration, as well as gray matter atrophy in the brain (*Chapter 3*). We then analyzed the influence of participants' personal characteristics on blood factors and markers of brain aging (*Chapter 4*). Based on previous research, we specifically tested whether age, global cognition, body fat percentage, or characteristics of sarcopenia (muscle strength, muscle volume, and physical performance) in older adults were related to the levels of neurotrophic or inflammatory factors in the blood, total gray matter volume in the brain, and neurometabolic status and gray matter volumes of five selected brain regions [23]. We found the following: Older adults with underweight or obesity had lower total brain volumes. Additionally, lower handgrip strength was associated with lower total brain volumes. Furthermore, older adults with lower handgrip strength had lower levels of N-acetylaspartate in two of the five measured brain regions, the dorsal posterior cingulate cortex and the dorsolateral prefrontal cortex. Lower levels of this neurometabolite may indicate that fewer nerve cells are present per volume, a sign of neurodegeneration. Finally, lower handgrip strength was associated with higher levels of kynurenine in blood serum [23]. Higher kynurenine levels in blood serum were also associated with neurometabolic changes consistent with neuroinflammation and neurodegeneration [22]. This suggests that handgrip strength and kynurenine may potentially serve as proxy measures for assessing brain health [22][23].

Part III of this dissertation presents the results of our intervention studies in older adults. We investigated whether a lower-body resistance training intervention could influence circulating blood factors (IL-6, kynurenine, and IGF-1), neurometabolites related to neurodegeneration and neuroinflammation, subregional gray matter volume in the hippocampus, or cognitive performance in older adults, and whether there were relationships between changes in these outcomes in the intervention group. In the first intervention study (*Chapter 5*), we compared cognitive changes immediately after a single high-load resistance training session with a control group [24]. Cognition was tested using three computerized cognitive tasks and a balance-cognition dual-task [24]. In the dual-task, participants were asked to maintain balance while standing in tandem Romberg position on a force plate, while simultaneously solving a math problem. In this study, we confirmed that even a single session of resistance training led to immediate improvements in working memory. This acute effect of exercise has been found in other studies and generally lasts for 15-60 minutes. In a second intervention study (results are presented in *Chapters 6 and 7*), we evaluated the effect of a twelve-week moderate-to-high-intensity resistance training intervention in seventy older adults with either intact

cognitive function or an elevated risk of mild cognitive impairment (MCI) [25][26]. We discovered that older adults at higher risk of MCI had higher kynurenine levels and lower subiculum volumes (a part of the hippocampus) compared to cognitively healthy adults. We observed a non-significant increase in IL-6 levels and in total N-acetylaspartate levels in the hippocampus and a reduction in age-related decline of the gray matter volume of the dentate gyrus of the hippocampus, with a moderate effect size. The findings for hippocampus volume suggested that the intervention group experienced prevention of further volumetric loss rather than improvement. We can speculate that the effects might have been significant if the intervention had lasted longer. It is estimated that an intervention period of at least 6 months is necessary. This will need to be confirmed in future research. Finally, our results showed improvements in the Go/No-go test in the intervention group compared to the control group, but this effect depended on the cognitive status of the older adults and was only significant in those at high risk for MCI. The Go/No-go test is a cognitive inhibition test in which participants must respond as quickly as possible to a 'Go' signal, but withhold a response to a 'No-go' signal. It is a component of executive functioning.

The twelve-week intervention study with older adults took place during the COVID-19 pandemic, which caused additional challenges in recruiting participants and continuing the experiments. Some older participants decided to withdraw from the experiment or had to stop due to illness. It is important to note that an infection with the COVID-19 virus (SARS-CoV-2) may have neurological consequences, which could have potentially affected our results. We had the opportunity to make a unique comparison of pre- and post-COVID-19 structural and neurometabolic brain measurements in three participants (*Chapter 8*) [27]. In this case series, we discovered neurometabolic changes in the hippocampus that could indicate neuroinflammation immediately after recovery from COVID-19. Finally, our research findings showed increased hippocampus volume in the experimental participants with COVID-19. In contrast with our statement above that hippocampal volume did not change in experimental group, at group level, but rather decreased in controls, this may indicate that on an individual basis some participants did show increases in volume following resistance exercise and this was not influenced by COVID-19.

Part IV of this dissertation contains all studies related to individuals with spinal cord injuries. *Chapter 9* consists of a literature review, in which we suggested that there is accelerated age-related cognitive decline in this population, likely caused at least in part by a chronic neuroinflammatory response originating from the location of the spinal cord injury [28]. Based on our previous findings and knowledge from older adults, we hypothesized that the anti-inflammatory and neurotrophic effects of exercise could prevent or delay cognitive decline in individuals with spinal cord injuries. However, a systematic review of this topic did not yield any intervention studies evaluating the relationship

between exercise and cognition in this population [28]. Therefore, we conducted our first intervention study (*Chapter 10*) aimed at evaluating the acute effects of muscle training with low- or high-intensity neuromuscular electrical stimulation on lactate levels, IGF-1 levels, and information processing speed [29]. The study had a crossover design. We found that lactate increased significantly after both interventions. Lactate has previously been shown to have positive direct and indirect effects on neuroplastic processes in the brain [30][31]. However, we did not find a significant increase in IGF-1 or significant improvement in information processing speed. Additional findings showed that a longer time since the spinal cord injury was associated with smaller changes in IGF-1 in the low-intensity group, and a higher injury level was associated with smaller improvements in information processing speed. Using this information, we ultimately designed a new intervention study with a chronic (12-week) intervention using neuromuscular electrical stimulation (*Chapter 11*). This research is currently ongoing at Maastricht University, Netherlands, but the protocol for this study is included in this dissertation [32].

In conclusion, there is neuroscientific evidence for an effect of exerkines on synaptic plasticity in animal studies [1]. This suggests that exerkines may, at least in part, mediate the positive effects of exercise on cognitive functions. Elevated serum kynurenine levels and decreased handgrip strength are potential markers of brain aging in older adults. Older adults should aim for a healthy body fat percentage, as both underweight and obesity were associated with brain volume loss. Both a single session and a twelve-week resistance training intervention have positive effects on executive functioning in older adults, as demonstrated by a working memory task in healthy older adults and a cognitive inhibition task in older adults with high MCI risk, respectively. Increases in IGF-1 in the exercise group and IL-6 in the total group were associated with improvements in working memory. However, these findings need to be confirmed in larger and longer-duration studies. From the final part of this dissertation, we can conclude that individuals with spinal cord injuries experience accelerated cognitive aging, which may be partly caused by chronic neuroinflammation. To date, there are no studies that have examined the effects of exercise on cognitive functions or brain health in this population. We conducted a pilot study where the intervention consisted of a single session of neuromuscular electrical stimulation. We found an increase in lactate but no changes in IGF-1 or cognitive performance on an information processing speed test. Based on all that I have learned during the preparation of this dissertation, I would advise everyone to choose multimodal physical exercise in a motivating and enjoyable setting, so that exercise can be sustained throughout life and help prevent or mitigate cognitive aging.

2. Samenvatting

Cognitieve achteruitgang is inherent aan het ouder worden [1]. Cognitieve achteruitgang kan zich onder andere uiten in de vorm van concentratiestoornissen, desoriëntatie, het vertraagd verwerken van informatie, regelmatig ergens niet op kunnen komen, verstrooidheid of vergeetachtigheid, terwijl het vermogen om eerder verworven kennis, vaardigheden en ervaringen te gebruiken gewoonlijk intact blijft [2]. Afhankelijk van individuele en omgevingsfactoren leidt cognitieve veroudering vroeg of laat tot hulpbehoevendheid [3]. Enkele belangrijke risicofactoren voor dementie zijn opleidingsniveau, fysieke inactiviteit, obesitas, diabetes mellitus type 2, roken, hoge bloeddruk, alcoholgebruik, hersentraumata, depressie, luchtvervuiling, gehoorverlies en sociale isolatie. Er wordt aangenomen dat de prevalentie van dementie met 40% zou kunnen worden verminderd op populatieniveau door deze risicofactoren weg te nemen [4]. Wereldwijd zien we echter het omgekeerde gebeuren. De fysieke inactiviteit, bijvoorbeeld, nam met 5% toe tussen 2005 en 2017 [5], type 2 diabetes mellitus steeg met 50% tussen 1990 en 2015 [6] en obesitas is met 300% toegenomen tussen 1975 en 2014 [7]. In een onderzoekartikel uit 2013 schreef men dat men verwachtte dat het huidige zorgsysteem tegen 2025 niet meer in staat zou zijn om de nodige zorg te bieden vanwege de vergrijzing van de populatie en vanwege het toenemend aantal personen die leven met chronische ziektes als er niet extra zou ingezet worden op preventie [8].

Lichaamsbeweging heeft een belangrijke rol in het behouden van een goede fysieke en mentale gezondheid. Lichaamsbeweging heeft positieve effecten op vrijwel elk lichaamssysteem, inclusief op de hersenen [9]. Een verbetering in cognitieve functies geassocieerd aan lichaamsbeweging werd bevestigd in een impressionant aantal onderzoekstudies met deelnemers van alle leeftijden [10][11][12]. Ondanks dat dit al tientallen jaren bekend is, blijft er onduidelijkheid en is er gebrek aan consensus over het onderliggend mechanisme van deze verbetering in cognitieve functies door lichaamsbeweging [13]. Een interessante bevinding werd gedaan in 2003 door de onderzoeksgroep van Bente Pedersen in Denemarken. Zij ontdekten dat cytokine interleukine-6 (IL-6) wordt vrijgegeven door spiercellen tijdens spiercontracties waarop het signaalcascades activeert in andere organen. Ze noemden IL-6 een ‘myokine’ [14]. Later werd duidelijk dat tijdens lichaamsbeweging duizenden factoren, met elk hun eigen locale en/of systemische effecten, in het bloed terechtkomen vanuit vrijwel alle lichaamssystemen. Deze inspannings-gerelateerde factoren werden ‘exerkines’ genoemd [15]. Van een beperkt aantal van deze exerkines is ondertussen bekend dat ze effecten hebben op de hersenen of gerelateerd zijn met cognitieve veranderingen na lichaamsbeweging [16]. De meeste exerkines hebben een onbekende functie.

Het primaire doel van dit proefschrift was om meer kennis en begrip te verwerven over de rol van exerkines in het bevorderen van de gezondheid van de hersenen en van de cognitieve functies na lichaamsbeweging bij oudere volwassenen. Onderzoek naar de rol van exerkines, en het mechanisme onderliggend aan het effect van lichaamsbeweging op het cognitief functioneren in het algemeen, is essentieel voor het kunnen ontwikkelen van op wetenschappelijk bewijs gebaseerde beweegprogramma's voor de preventie van ouderdomsgerelateerde cognitieve achteruitgang. Voor onze interventiestudies werd gekozen voor krachttraining, gezien er voornamelijk onderzoek was gedaan met duurtraining, dit terwijl sommige onderzoekers argumenteerden dat myokines in toegenomen mate zouden kunnen worden vrijgesteld na krachttraining in vergelijking met duurtraining [17]. Daarnaast werd onderzoek verricht bij personen met een ruggenmergletsel. In deze populatie werd als interventie gekozen voor neuromusculaire elektrische stimulatie. We verwachtten dat neuromusculaire elektrische stimulatie mogelijks nog hogere hoeveelheden aan myokines zou vrijgeven dan krachttraining [18] [19].

Deel I van dit proefschrift bevat literatuuronderzoek naar de effecten van exerkines op neuroplasticiteit. In een eerste studie (*hoofdstuk 1*) beschrijven we in detail de signaalcascades die worden geactiveerd door 16 exerkines met bekende (in)directe effecten op lange termijn synaptische potentiëring (LTP) [20]. LTP is een vorm van neuroplasticiteit op het niveau van de synaptische verbinding tussen twee zenuwcellen. In deze synaptische verbindingen vindt een chemische reactie plaats die leidt tot de overdracht van een zenuwprikkel van de ene zenuwcel naar de volgende. Om de drempel te bereiken waarop een nieuw zenuwsignaal gegenereerd wordt in deze volgende zenuwcel moet het chemisch signaal voldoende groot zijn. Door LTP wordt het chemisch signaal dat wordt vrijgegeven door de pre-synaptische zenuwcel, vergroot en de drempel om het signaal door te zetten in het post-synaptische zenuwcel verlaagd [21]. Hoewel de beschreven signaalcascades gebaseerd zijn op dierenonderzoek, kunnen ze gezien worden als een aanwijzing voor de neurobiologische effecten van exerkines op moleculair niveau en daarom vormt deze kennis een belangrijke theoretische achtergrond voor het onderzoek naar de effecten van exerkines. Verder beschrijven we in onze studie de veranderingen die plaats vinden na inspanning zowel voor wat betreft de hoeveelheden exerkines in de bloedsomloop (voornamelijk op basis van onderzoek op mensen) als in de hersenen (voornamelijk vanuit dierenonderzoek). Over het algemeen kunnen we stellen dat de hoeveelheden exerkines met neurotrofe of anti-inflammatoire (anti-ontsteking) effecten toenemen na een enkele sessie lichaamsbeweging (acute lichaamsbeweging) en na lichaamsbeweging voor meerdere weken (chronische lichaamsbeweging). De hoeveelheden exerkines met pro-inflammatoire effecten nemen echter ook toe na acute lichaamsbeweging, maar nemen wel af na chronische lichaamsbeweging. Volgehouden lichaamsbeweging lijkt dus een neurotroof en anti-inflammatoir effect te hebben dat

gemedieerd wordt door veranderingen in exerkines. Daarbij moet worden opgemerkt dat het in de studie gemeten effect van lichaamsbeweging afhankelijk was van verschillende beïnvloedende factoren zoals het soort lichaamsbeweging (krachttraining, duurtraining, multimodale training, mind-body training, evenwichtsoefeningen, enz.), de intensiteit van de training, de duur van de training of het volume van het oefenprogramma, de hoeveelheid gewichtsverlies geassocieerd met de interventie, de leeftijd, het geslacht of de co-morbiditeiten van de studiedeelnemers. In een tweede artikel (*hoofdstuk 2*) beschrijven we het protocol voor een toekomstig literatuuronderzoek met meta-analyse [16]. In dit literatuuronderzoek willen we op systematische wijze de laatste stand van zaken betreffende de rol van myokines op het cognitief functioneren bij oudere volwassenen in kaart brengen en willen we, voor zover als mogelijk, de mediërende rol van deze myokines op het cognitief functioneren analyseren. Dit onderzoek plannen we aan te houden voor een periode van minimaal vijf jaar na publicatie van het artikel, door halfjaarlijks het artikel up te daten. Om zo volledig mogelijk te zijn, ontwikkelden we een lijst met 1126 mogelijke myokines die we konden afleiden uit verschillende secretoom- en transcriptoomstudies op menselijke skeletspieren. Na een eerste literatuuranalyse konden we 33 studies includeren in de meta-analyse. De resultaten worden momenteel geanalyseerd.

Deel II van dit proefschrift bevat resultaten van een cross-sectionele analyse die we hebben uitgevoerd om de relatie tussen basale exerkinewaarden en tekenen van veroudering in de hersenen te onderzoeken [22][23]. We beoordeelden meer bepaald de hoeveelheid inflammatoire (interleukine-6, IL-6; kynurenine) en neurotrofe (insuline-achtige groeifactor-1, IGF-1) factoren in het bloed van oudere volwassenen en zochten naar associaties met neurometabole tekenen van neuro-inflammatie en neurodegeneratie, alsook naar atrofie van de grijze stof in de hersenen (*hoofdstuk 3*). Vervolgens deden we een analyse waarbij we de invloed van persoonlijke karakteristieken van de deelnemers op de bloedfactoren en markers van hersenveroudering konden beoordelen (*hoofdstuk 4*). Op basis van eerder onderzoek besloten we specifiek te testen of leeftijd, en/of globale cognitie, en/of lichaamsvetpercentage en/of kenmerken van sarcopenie (spierkracht, spiervolume en fysieke prestaties) van de oudere volwassenen gerelateerd waren aan de hoeveelheid neurotrofe of ontstekingsfactoren in het bloed, aan het grijze stofvolume in de totale hersenen en aan de neurometabole status en grijze stofvolumes van vijf gekozen hersengebieden [23]. We kwamen tot de volgende bevindingen: oudere volwassenen met een ondergewicht of met obesitas hadden lagere totale hersenvolumes. Ook een lagere handknijpkracht kon worden geassocieerd met lagere totale hersenvolumes. Bovendien hadden oudere volwassenen met lagere handknijpkracht lagere hoeveelheden N-acetylaspartaat in twee van de vijf gemeten hersenregio's, namelijk in de dorsale achterste cingulate cortex en in de dorsolaterale prefrontale cortex. Lagere hoeveelheden van deze neurometaboliet kunnen erop wijzen dat er per volume ook nog eens minder

zenuwcellen aanwezig zijn, wat een teken is van neurodegeneratie. Tot slot kon een lagere handknijpkracht worden gerelateerd aan een grotere hoeveelheid kynurenine in het bloedserum [23]. Hogere kynurenineniveaus in het bloedserum bleken zelf ook nog gerelateerd te zijn aan neurometabole veranderingen die passen bij neuroinflammatie en neurodegeneratie [22]. Dit duidt erop dat handknijpkracht en kynurenine mogelijk kunnen dienen als afgeleide metingen om een uitspraak te doen over de gezondheid van de hersenen [22][23].

In **deel III** van dit proefschrift zijn de resultaten van onze interventiestudies bij oudere volwassenen beschreven. Er werd onderzocht of een interventie met krachttraining van de onderste ledematen de hoeveelheid circulerende bloedfactoren (IL-6, kynurenine en IGF-1), de neurometabolieten gerelateerd aan neurodegeneratie en neuro-inflammatie, het subregionale grijze stofvolume in de hippocampus of de cognitieve prestaties van de oudere volwassenen kon beïnvloeden en of er relaties bestonden tussen veranderingen in deze uitkomsten in de interventiegroep. In een eerste interventiestudie (*hoofdstuk 5*) vergeleken we cognitieve veranderingen onmiddellijk na een enkele sessie krachttraining met hoge relatieve belasting met een controlegroep [24]. Cognitie werd getest met drie computergestuurde cognitieve taken en een balans-cognitie dubbeltaak [24]. Bij de dubbeltaak moesten de deelnemers hun balans bewaren terwijl ze op een krachtplaat stonden met de voeten achter elkaar geplaatst (tandem Romberg positie). Ondertussen werden ze gevraagd om een rekensom te maken. Bij dit onderzoek konden we bevestigen dat zelfs één enkele sessie krachttraining al tot een onmiddellijke verbetering in werkgeheugen leidde. Dit acuut effect van lichaamsbeweging is ook gevonden in andere studies en betreft meestal een kortdurend effect van 15 tot 60 minuten. In een tweede interventiestudie (resultaten zijn weergegeven in *hoofdstuk 6 en 7*) evalueerden we het effect van een twaalf weken durende interventie met krachttraining aan matige tot hoge intensiteit bij zeventig oudere volwassenen met ofwel intacte cognitieve functie ofwel een verhoogd risico op milde cognitieve stoornissen (MCI) [25][26]. We ontdekten dat oudere volwassenen met een verhoogd risico op MCI hogere kynureninespiegels en lagere subiculumvolumes (een onderdeel van de hippocampus) hadden vergeleken met cognitief gezonde volwassenen. We zagen een niet-significante toename in IL-6-niveaus en de totale N-acetylaspartaat-niveaus in de hippocampus en een afname van de leeftijdsgebonden achteruitgang in het grijze stofvolume van de dentate gyrus van de hippocampus, met een matige effectgrootte. De bevindingen betreffende het volume van de hippocampus wezen eerder op preventie van verdere veroudering met de tijd in de interventiegroep, dan dat er een verbetering optrad. We kunnen speculeren dat de effecten mogelijk wel significant geweest zouden zijn als de interventie van langere duur was geweest. Naar schatting is een interventieperiode van minimaal 6 maanden noodzakelijk. Dit zal in toekomstig onderzoek moeten worden bevestigd; Ten slotte lieten onze resultaten verbeteringen zien op de Go/No-go-test in de interventiegroep vergeleken

met de controlegroep. Dit effect was echter afhankelijk van de cognitieve status van de oudere volwassenen en was alleen significant bij oudere volwassenen met een hoog risico op MCI. De Go/No-go test is een cognitieve inhibitietest, waarbij personen zo snel mogelijk moeten reageren op een 'Go' signaal, maar een reactie moeten onderdrukken bij een 'No-go' signaal. Het is een component van het executief functioneren.

Het twaalf weken durende interventieonderzoek met oudere volwassenen vond plaats tijdens de COVID-19-pandemie, wat extra problemen veroorzaakte bij het rekruteren van deelnemers en het voortzetten van de experimenten. Een deel van de deelnemende ouderen besloot het experiment te verlaten of moest vanwege ziekte stoppen. Belangrijk te vermelden is dat een infectie met het COVID-19 virus (SARS-COV-2) ook mogelijke neurologische gevolgen heeft, die onze resultaten mogelijk hebben beïnvloed. We hadden de mogelijkheid om bij drie deelnemers een unieke vergelijking te maken tussen pre- en post-COVID-19 structurele en neurometabole hersenmetingen (*hoofdstuk 8*) [27]. In deze case-seriestudie ontdekten we neurometabole veranderingen in de hippocampus, die mogelijk kan wijzen op neuro-inflammatie onmiddellijk na het herstel van COVID-19. Tot slot lieten onze onderzoeksresultaten zien dat bij de experimentele deelnemers met COVID-19 het volume van de hippocampus toenam. In tegenstelling tot onze eerdere stelling dat het volume van de hippocampus in de experimentele groep niet veranderde op groepsniveau, maar wel afnam in de controlegroep, kan dit erop wijzen dat sommige deelnemers op individueel niveau een toename in volume vertoonden na krachttraining, en dat dit niet werd beïnvloed door COVID-19.

Deel IV van dit proefschrift bevat alle studies met betrekking tot personen met een dwarslaesie. *Hoofdstuk 9* bestaat uit een literatuuronderzoek, waarin we suggereerden dat er een versnelde leeftijdsgebonden cognitieve achteruitgang is in deze populatie, waarschijnlijk op zijn minst gedeeltelijk veroorzaakt door een chronische neuro-inflammatoire respons die voortkomt vanuit de locatie van de dwarslaesie [28]. Gebaseerd op onze eerdere bevindingen en kennis bij oudere volwassenen, gingen we uit van de hypothese dat de ontstekingsremmende en neurotrofe effecten van lichaamsbeweging de cognitieve achteruitgang bij personen met een dwarslaesie zouden kunnen voorkomen of vertragen. Een systematische review van dit onderwerp leverde echter geen interventiestudies op waarin de relatie tussen inspanning en cognitie in deze populatie werd geëvalueerd [28]. Daarom hebben we zelf een eerste interventiestudie uitgevoerd (*hoofdstuk 10*), bedoeld om de acute effecten van spiertraining met neuromusculaire elektrische stimulatie van lage of hoge intensiteit op lactaatniveaus, IGF-1-niveaus en informatieverwerkingssnelheid te evalueren [29]. De studie had een cross-over design. We ontdekten dat het lactaat na beide interventies aanzienlijk toenam. Er was eerder aangetoond dat lactaat positieve directe en indirecte effecten heeft op neuroplastische processen in de hersenen [30][31]. We vonden echter geen significante toename in IGF-1 en ook geen significante verbetering in de snelheid van informatieverwerking.

Bijkomende bevindingen waren dat een langere tijd sinds dwarslaesie kon worden geassocieerd met kleinere veranderingen in IGF-1 in de lage intensiteitsgroep en een hoger letselniveau kon worden geassocieerd met kleinere verbeteringen in de snelheid van informatieverwerking. Met behulp van deze informatie hebben we uiteindelijk een nieuwe interventiestudie ontworpen met een chronische (12 weken durende) interventie met behulp van neuromusculaire elektrische stimulatie (*hoofdstuk 11*). Dit onderzoek loopt momenteel nog aan de Universiteit Maastricht, Nederland, maar het protocol van dit onderzoek is opgenomen in dit proefschrift [32].

Concluderend kunnen we stellen dat er neurowetenschappelijke aanwijzingen zijn voor een effect van exerkines op synaptische plasticiteit bij dierenstudies [20]. Dit suggereert dat exerkines mogelijk, op zijn minst voor een deel, het positief effect van lichaamsbeweging op de cognitieve functies mediëren. Verhoogde serum kynurenineniveaus en verminderde handknijpkracht zijn mogelijk afgeleide maten voor hersenveroudering bij oudere volwassenen. Tevens dienen oudere volwassenen een gezond lichaamsvetpercentage na te streven, gezien zowel ondergewicht als obesitas kunnen worden gerelateerd aan hersenvolumeverlies. Zowel één enkele sessie als een 12 weken durende interventie met krachttraining resulteerde in onze studies in positieve effecten op het executief functioneren van oudere volwassenen. Dit kon respectievelijk met een werkgeheugentaak en een cognitieve inhibitietaak worden aangetoond. Een toename in IGF-1 in de interventiegroep en in IL-6 in de totale groep kon worden geassocieerd met werkgeheugen verbeteringen. Deze bevindingen dienen echter nog bevestigd te worden in grotere studies met een langere duur. Uit het laatste deel van dit proefschrift kunnen we concluderen dat individuen met een dwarslaesie een versnelde cognitieve veroudering doormaken, die mogelijks deels veroorzaakt wordt door chronische neuroinflammatie. Tot hiertoe zijn er geen studies die het effect van lichaamsbeweging op cognitieve functies of hersengezondheid hebben onderzocht. We hebben een eerste pilotstudie uitgevoerd waarbij de interventie bestond uit één enkele sessie met neuromusculaire elektrische stimulatie. We vonden een toename in lactaat, maar geen veranderingen in IGF-1 of cognitieve prestaties op een verwerkingssnelheid test. Gebaseerd op al wat ik heb geleerd tijdens het maken van dit proefschrift, zou ik iedereen willen adviseren om een multimodale lichaamsbeweging te kiezen in een motiverende en plezierige setting, zodat de lichaamsbeweging kan worden volgehouden gedurende de volledige loop van het leven en kan bijdragen tot het voorkomen of beperken van cognitieve veroudering.

3. Santrauka

Senstant būdingas kognityvinių funkcijų silpnėjimas [1]. Jis gali pasireikšti įvairiais būdais, įskaitant sunkumą susikaupti, dezorientaciją, lėtesnį informacijos apdorojimą, dažną nesugebėjimą prisiminti dalykų, išsiblaškymą ar užmaršumą, tačiau gebėjimas naudotis anksčiau įgytomis žiniomis, įgūdžiais ir patirtimi paprastai lieka nepakitęs [2]. Priklausomai nuo individualių ir aplinkos veiksnių, dėl senstant silpnėjančių kognityvinių funkcijų anksčiau ar vėliau kyla pagalbos poreikis [3]. Kai kurie pagrindiniai rizikos veiksniai yra išsilavinimo lygmuo, fizinio aktyvumo stoka, nutukimas, antrojo tipo cukrinis diabetas, rūkymas, aukštas kraujospūdis, alkoholio vartojimas, smegenų trauma, depresija, oro tarša, klausos praradimas ir socialinė izoliacija. Apskaičiuota, kad pašalinus šiuos rizikos veiksnius, demencijos rizika galėtų sumažėti 40 % [4]. Tačiau pasaulyje vyksta priešinga tendencija. Pavyzdžiui, fizinis pasyvumas nuo 2005 iki 2017 metų padidėjo 5 % [5], antrojo tipo cukrinio diabeto atvejų nuo 1990 iki 2015 metų padaugėjo 50 % [6], o nutukimo paplitimas nuo 1975 iki 2014 metų išaugo 300 % [7]. Dar 2013 metais atliekant mokslinį tyrimą buvo apskaičiuota, kad iki 2025 metų dabartinė sveikatos priežiūros sistema nebepajėgs teikti reikiamos sveikatos priežiūros dėl senėjančios visuomenės ir didėjančio lėtinėmis ligomis sergančių asmenų skaičiaus, jeigu nebus imtasi papildomų prevencijos priemonių [8].

Fizinis aktyvumas vaidina svarbų vaidmenį palaikant gerą fizinę ir psichinę sveikatą. Jis teigiamai veikia beveik visas žmogaus kūno sistemas, įskaitant smegenis [9]. Daugybe tyrimų patvirtinta, kad dėl taikyto fizinio krūvio kognityvinės funkcijos gerėjo visų amžiaus grupių tiriamiesiems [10][11][12]. Nepaisant šių per kelis dešimtmečius sukauptų žinių, vis dar išlieka neaiškumų ir trūksta sutarimo dėl pagrindinio šio reiškinio mechanizmo [13]. Įdomų atradimą 2003 metais padarė Bente'ės Pedersen tyrimų grupė iš Danijos. Jie nustatė, kad susitraukimų metu raumenų ląstelės išskiria citokiną interleukiną-6 (IL-6), kuris aktyvina signalinius kelius kituose organuose, ir pavadino jį miokinu (gr. *mys* – raumuo + *o* + gr. *kinein* – judėti = angl. *myokine*) [14]. Vėliau paaiškėjo, kad fizinio krūvio metu iš beveik visų kūno sistemų į kraują patenka tūkstančiai veiksnių, kiekvienas turėdamas savo vietinį ir (arba) sisteminį poveikį. Šie su fiziniu krūviu susiję veiksniai buvo pavadinti ekserkinais (angl. *exercise* – treniruoti + gr. *kinein* – judėti = angl. *exerkine*) [15]. Nedidelė dalis šių ekserkinų daro poveikį smegenims arba yra susiję su kognityviniais pokyčiais po fizinio krūvio [16]. Daugumos ekserkinų funkcijos vis dar nežinomos.

Pagrindinis šios disertacijos tikslas buvo įgyti daugiau žinių ir suprasti ekserkinų poveikį vyresnio amžiaus suaugusiųjų smegenų sveikatai bei kognityvinėms funkcijoms po fizinio krūvio. Ekserkinų vaidmens ir mechanizmo, kuriuo grindžiamas fizinių pratimų poveikis kognityvinėms funkcijoms, tyrimai labai svarbūs rengiant įrodymais pagrįstas fizinių pratimų programas, kuriomis siekiama užkirsti kelią su amžiumi susijusiam kognityvinių funkcijų silpnėjimui. Šios disertacijos intervenciniuose tyrimuose taikytos

jėgos treniruotės, nes iki šiol daugiau tyrimų atlikta su ištvermės treniruotėmis. Be to, kai kurie mokslininkai teigia, kad miokinai didesniu mastu gali būti išskiriami po jėgos treniruočių, palyginti su ištvermės treniruotėmis [17].

Tyrimai taip pat buvo atlikti su asmenimis, patyrusiais stuburo smegenų traumą. Šioje populiacijoje kaip intervencija buvo pasirinkta neuroraumeninė elektros stimuliacija, nes ji gali aktyvinti dar didesnį miokinų kiekį nei jėgos treniruotės [18][19].

Pirmojoje šios disertacijos dalyje pateikiama literatūros apie ekserkinų poveikį neuroplastiškumui apžvalga. Pirmajame skyriuje išsamiai aprašomi signaliniai keliai, kuriuos aktyvina 16-ka ekserkinų, darančių (ne)tiesioginį poveikį ilgalaikei sinapsės potenciacijai, (angl. *long-term potentiation*, LTP) [20]. LTP yra neuroplastiškumo forma, atsirandanti tarp dviejų nervinių ląstelių, kurias jungia sinapsė. Dėl šios sinapsės funkcijos įvyksta cheminė reakcija, kuri lemia nervinio impulso perdavimą iš vienos nervinės ląstelės kitai. Kad kitoje nervinėje ląstelėje susidarytų naujas signalas, cheminės reakcijos signalas turi būti pakankamai stiprus. LTP padidina presinapsinės nervinės ląstelės išskiriamą cheminės reakcijos signalą ir sumažina slenkstį signalui posinapsinėje nervinėje ląstelėje perduoti [21]. Nors aprašyti signaliniai keliai grindžiami tyrimais su gyvūnais, jie gali būti laikomi neurobiologinio ekserkinų poveikio molekuliniu lygiu indikacija. Todėl šios žinios sudaro svarbų teorinį pagrindą ekserkinų poveikio tyrimams.

Be to, pirmajame skyriuje aprašomi po fizinio krūvio nustatyti ekserkinų koncentracijos pokyčiai (remiantis tyrimais su žmonėmis – kraujotakoje; daugiausia remiantis tyrimais su gyvūnais – smegenyse). Bendrai galima teigti, kad neurotrofinį arba priešuždegiminį poveikį turinčių ekserkinų koncentracija padidėja ir po vienkartinio fizinio krūvio, ir po kelias savaites trunkančio fizinio krūvio. Ekserkinų, turinčių uždegiminį poveikį, taip pat padaugėja po vienkartinio fizinio krūvio, bet sumažėja po ilgalaikio fizinio krūvio. Taigi, galima manyti, kad tarpininkaujant ekserkinams ilgalaikis fizinis krūvis pasižymi neurotrofiniu ir priešuždegiminiu poveikiu. Svarbu pažymėti, kad fizinio krūvio poveikis priklauso nuo įvairių veiksnių, tokių kaip fizinio krūvio tipas (jėgos, ištvermės, daugiakomponentės kūno ir proto treniruotės, pusiausvyros pratimai ir kt.), treniruotės intensyvumas ir trukmė arba treniruočių programos apimtis, taip pat nuo svorio netekimo, susijusio su intervencija, amžiaus, lyties ar tiriamųjų gretutinių ligų.

Antrajame skyriuje aprašomas būsimosios literatūros apžvalgos su metaanalize protokolas [16]. Šioje apžvalgoje siekiama susisteminti dabartines žinias apie miokinų vaidmenį vyresnio amžiaus žmonių kognityvinėms funkcijoms ir, esant galimybei, atlikti miokinų tarpininkavimo reikšmės kognityvinei funkcijai analizę. Šį skyrių planuojama atnaujinti kas šešis mėnesius, mažiausiai penkerius metus po disertacijos publikavimo. Siekiant pateikti kuo tikslesnius ir išsamesnius duomenis, buvo sudarytas 1126 potencialių miokinų sąrašas, apie kuriuos informacija gauta iš įvairių žmonių griaučių raumenų sekretomų ir transkriptomų tyrimų. Po pirminės literatūros analizės, į metaanalizę įtraukti 33 tyrimai. Šiuo metu rezultatai analizuojami.

Antrojoje dalyje (3–4 skyriai) pristatomi atliktos skerspjūvio analizės, kurioje tirtas ryšys tarp pradinio ekserkinų lygio ir smegenų senėjimo požymių, rezultatai [22][23]. Tiksliau tariant, įvertinta uždegiminių (IL-6; kinurenino) ir neurotrofinių (į insuliną panašaus augimo faktoriaus-1, IGF-1) veiksnių koncentracija vyresnio amžiaus žmonių kraujyje ir ieškota sąsajų su neurometaboliniais neuroužd egimo ir neurodegeneracijos požymiais bei pilkosios smegenų medžiagos atrofija (trečiasis skyrius). Po to analizuota tiriamųjų asmeninių savybių įtaka kraujo veiksniams ir smegenų senėjimo požymiams (ketvirtasis skyrius). Remiantis ankstesniais darbais, buvo tiriama, ar vyresnio amžiaus žmonių amžius, kognityvinė funkcija, kūno riebalų procentas ar sarkopenijos požymiai (raumenų jėga, raumenų tūris ir fizinis pajėgumas) yra susiję su neurotrofiniais arba uždegiminiais kraujo veiksniais, bendru pilkosios medžiagos tūriu smegenyse bei neurometaboliniais ir pilkosios medžiagos tūrio pokyčiais penkiose pasirinktose smegenų srityse [23]. Nustatyta, kad: vyresnio amžiaus žmonės, turintys per mažą ar per didelį kūno svorį, turėjo mažesnį bendrą smegenų tūrį, su kuo buvo susijusi ir mažesnė plaštakos suspaudimo jėga. Be to, vyresnio amžiaus žmonės, turintys mažesnę plaštakos suspaudimo jėgą, turėjo mažesnį N-acetilaspartato lygį dviejose iš penkių išmatuotų smegenų sričių: užpakalinėje juostinėje žievėje ir dorsolateralinėje prefrontalinėje žievėje. Mažesnis šio neurometabolito lygis gali rodyti mažesnį nervinių ląstelių skaičių tūrio vienete, o tai yra neurodegeneracijos požymis. Galiausiai mažesnė plaštakos suspaudimo jėga buvo susijusi su didesne kinurenino koncentracija kraujo serume [23]. Didesnė kinurenino koncentracija kraujo serume taip pat buvo susijusi su neurometaboliniais pokyčiais, atspindinčiais neuroužd egimą ir neurodegeneraciją [22]. Tai leidžia manyti, kad plaštakos suspaudimo jėga ir kinureninas gali būti potencialūs smegenų sveikatos vertinimo rodikliai [22][23].

Trečiojoje dalyje (5–8 skyriai) pristatomi su vyresnio amžiaus žmonėmis atliktų intervencinių tyrimų rezultatai. Buvo tiriama, ar apatinių galūnių jėgos treniruotės gali daryti poveikį vyresnio amžiaus žmonių cirkuliuojantiems kraujo veiksniams (IL-6, kinureninui ir IGF-1), su neurodegeneracija ir neuroužd egimu susijusiems neurometabolitams, subregioniniam pilkosios medžiagos tūriui hipokampe arba kognityvinei funkcijai. Taip pat siekta nustatyti, ar yra ryšys tarp šių pokyčių eksperimentinėje grupėje.

Pirmajame intervenciniame tyrime (penktasis skyrius) kognityviniai pokyčiai lyginti iš karto po vienos didelio intensyvumo jėgos treniruotės su kontrolinės grupės rezultatais [24]. Kognityvinėms funkcijoms tirti buvo naudojamos trys kompiuterizuotos kognityvinės užduotys bei bendra pusiausvyros ir kognityvinė užduotis (dviguba užduotis) [24]. Dvigubos užduoties metu tiriamieji turėjo išlaikyti pusiausvyrą stovėdami Rombergo testo padėtyje ant jėgos plokštės ir tuo pat metu spręsti matematikos uždavinį. Šiuo tyrimu patvirtinta, kad ir vienkartinė jėgos treniruotė pagerina darbinę atmintį. Panašus fizinio krūvio poveikis, trunkantis maždaug 15–60 minučių, buvo pastebėtas ir kituose tyrimuose.

Antrajame intervenciniame tyrime (rezultatai pateikiami šeštajame ir septintajame skyriuose) vertintas 12-kos savaičių trukmės vidutinio ir didelio intensyvumo jėgos treniruočių poveikis

70-čiai vyresnio amžiaus žmonių, turinčių arba nepažeistas kognityvines funkcijas, arba padidėjusią lengvojo kognityvinio sutrikimo (LKS) riziką [25][26]. Nustatyta, kad vyresnio amžiaus žmonės, turintys padidėjusią LKS riziką, turėjo aukštesnį kinurenino lygį ir mažesnį ramsčio (hipokampo dalis) tūrį, palyginti su nepažeistas kognityvines funkcijas turinčiais asmenimis. Pastebėtas nereikšmingas

IL-6 kiekio ir bendro N-acetilaspartato kiekio padidėjimas hipokampe ir su amžiumi susijęs hipokampo dantytosios klostės pilkosios medžiagos tūrio sumažėjimas, poveikio dydis – vidutinis. Remiantis hipokampo tūrio tyrimo rezultatais, eksperimentinės grupės tiriamieji veikiau išvengė tolesnio tūrio mažėjimo, nes bendro padidėjimo nenustatyta. Galima spėti, kad tyrimo rezultatai būtų reikšmingi, jeigu intervencija būtų trukusi ilgiau. Manoma, kad būtina bent šešių mėnesių trukmės intervencija, ką reikėtų patvirtinti kitais tyrimais. Be to, eksperimentinėje grupėje, palyginti su kontroline grupe, stebėtas

„Go/No-go“ testo rezultatų pagerėjimas, bet buvo reikšmingas tik tiems, kuriems buvo nustatyta didelė LKS rizika. „Go/No-go“ testas yra atsako slopinimo testas, kurio metu tiriamieji turi kuo greičiau sureaguoti į „Go“ signalą, bet nereaguoti į „No-go“ signalą. Tai vykdomųjų funkcijų sudedamoji dalis.

12-kos savaičių intervencinis tyrimas vyresnio amžiaus žmonėms buvo vykdomas ir prasidėjus COVID-19 ligos pandemijai, kas sukėlė papildomų sunkumų įtraukiant tiriamuosius ir tęsiant tyrimus. Kai kurie vyresnio amžiaus tiriamieji nusprendė pasitraukti iš tyrimo arba buvo priversti pasitraukti dėl ligos. Svarbu paminėti, kad COVID-19 liga, kurią sukelia koronavirusas SARS-CoV-2, gali turėti neurologinių pasekmių, o tai galėjo paveikti tyrimų rezultatus. Tačiau dėl to atsirado unikali galimybė palyginti trijų tiriamųjų struktūrinius ir neurometabolinius smegenų pokyčius prieš ir po COVID-19 ligos (aštuntasis skyrius) [27]. Šioje atvejo analizėje nustatyti neurometaboliniai pokyčiai hipokampe, kurie gali rodyti neurouždegimą iškart po pasveikimo nuo COVID-19 ligos. Galiausiai nustatyta, kad eksperimentinėje grupėje tiriamųjų, sirgusių COVID-19 liga, hipokampo tūris padidėjo. Tai prieštarauja ankstesniam teiginiui, kad eksperimentinėje grupėje, vertinant bendrai, hipokampo tūris nepasikeitė. Taigi po jėgos treniruočių kai kurių tiriamųjų hipokampo tūris vis dėlto padidėjo, bet manoma, kad COVID-19 liga tam įtakos neturėjo.

Ketvirtojoje disertacijos dalyje (9–11 skyriai) pateikiami visi tyrimai, susiję su asmenimis, patyrusiais stuburo smegenų traumą. Devintąjį skyrių sudaro literatūros apžvalga, kur daroma išvada, kad šioje populiacijoje pasireiškia pagreitėjęs su amžiumi susijęs kognityvinis silpnėjimas, kurį bent iš dalies lemia lėtinė neurouždegiminė reakcija, kylanti iš stuburo smegenų traumos vietos [28]. Remiantis ankstesniais tyrimais ir žiniomis apie vyresnio amžiaus žmones, darbe keliama hipotezė, kad priešuždegiminis ir neurotrofinis fizinio krūvio poveikis galėtų užkirsti kelią arba atitolinti kognityvinį silpnėjimą asmenims, patyrusiems stuburo smegenų traumą. Tačiau atlikus sisteminę šios temos apžvalgą nerasta intervencinių tyrimų, kuriuose būtų vertinamas ryšys tarp fizinio krūvio ir kognityvinių funkcijų šioje populiacijoje [28]. Todėl atliktas pirmasis intervencinis

tyrimas (10-asis skyrius), kurio tikslas buvo įvertinti trumpalaikį raumenų treniravimo su mažo arba didelio intensyvumo neuroraumenine elektros stimuliacija poveikį laktato, IGF-1 koncentracijai ir informacijos apdorojimo greičiui [29]. Tyrimas vykdytas kryžminiu būdu. Nustatyta, kad po abiejų intervencijų laktato lygis tiriamiesiems padidėjo reikšmingai. Anksčiau buvo įrodyta, kad laktatas turi teigiamą tiesioginį ir netiesioginį poveikį neuroplastiškumo procesams smegenyse [30][31]. Tačiau atlikus tyrimą nenustatyta reikšmingo IGF-1 padidėjimo ar reikšmingo informacijos apdorojimo greičio padidėjimo. Papildomai nustatyta, kad nuo stuburo smegenų traumos praėjęs ilgesnis laikotarpis buvo susijęs su mažesniais

IGF-1 pokyčiais mažo intensyvumo fizinio krūvio grupėje, o aukštesnis traumos vietos lygis buvo susijęs su mažesniu informacijos apdorojimo greičio padidėjimu. Remiantis šiais rezultatais sukurtas naujas intervencinis tyrimas, kurio metu atliekama 12-kos savaičių trukmės neuroraumeninė elektros stimuliacijos intervencija (11-asis skyrius). Šis tyrimas vis dar vykdomas Mastrichto universitete, Nyderlanduose, tačiau tyrimo protokolas įtrauktas į disertaciją [32].

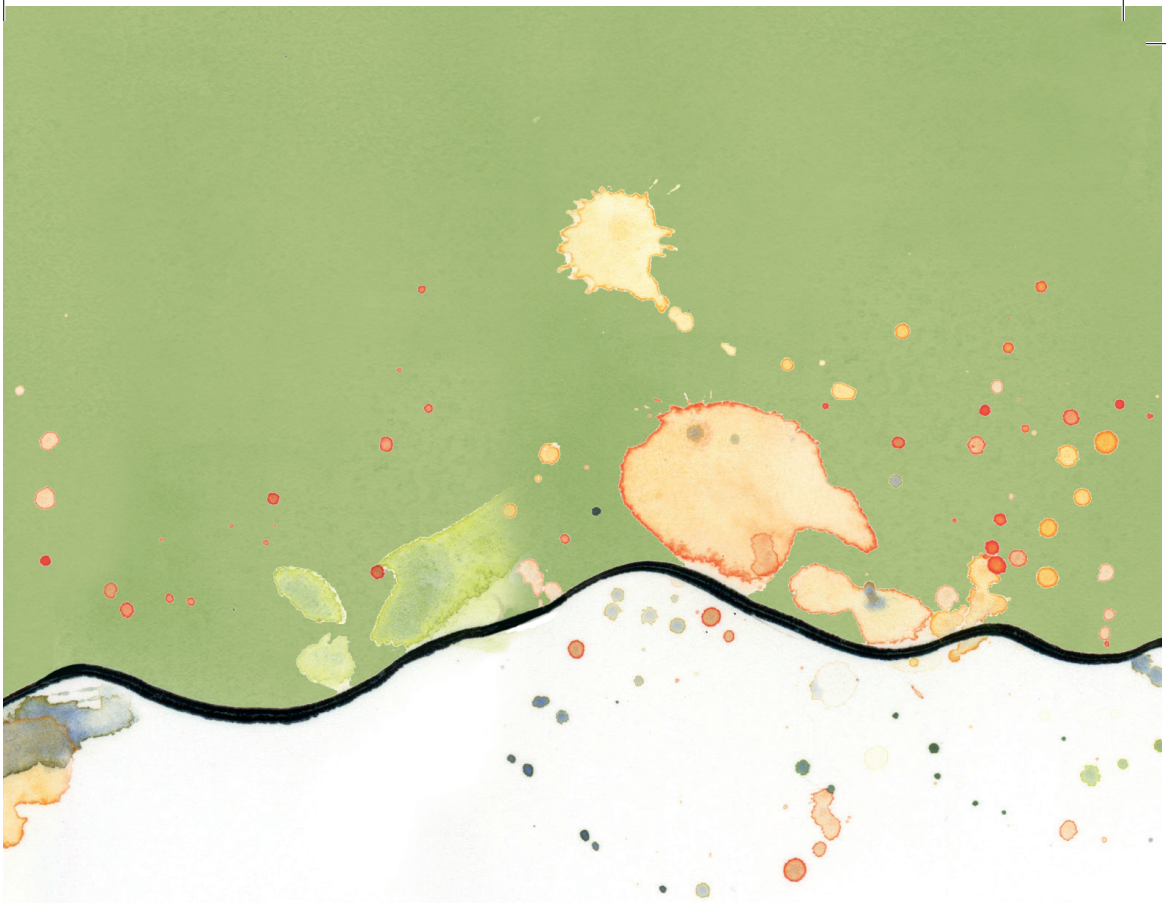
Apibendrinant, neuromoksliniais tyrimais su gyvūnais nustatytas ekserkinų poveikis sinapsiniam plastiškumui [1]. Tai rodo, kad ekserkinai galėtų bent iš dalies prisidėti prie teigiamo fizinio krūvio poveikio kognityvinėms funkcijoms. Padidėjęs kinurenino lygis kraujo serume ir sumažėjusi plaštakos suspaudimo jėga gali būti smegenų senėjimo požymiai vyresnio amžiaus žmonėms. Jie turėtų siekti palaikyti sveiką kūno riebalų procentą, nes tiek nepakankamas svoris, tiek nutukimas yra susiję su smegenų tūrio mažėjimu. Ir vienkartinė treniruotė, ir 12-kos savaičių jėgos treniruočių intervencija turi teigiamą poveikį vyresnio amžiaus žmonių vykdomosioms funkcijoms, tai įrodyta per darbinės atminties sveikiems vyresniems žmonėms ir kognityvinio slopinimo padidėjusią LKS riziką turintiems vyresniems žmonėms užduotis. IGF-1 (fizinio aktyvumo grupėje) ir IL-6 (visiems tiriamiesiems) padidėjimas yra susijęs su darbinės atminties pagerėjimu. Tačiau šie rezultatai turi būti patvirtinti didesnės apimties, ilgesniuose tyrimuose.

Iš paskutiniosios šios disertacijos dalies galima daryti išvadą, kad asmenims, patyrusiems stuburo smegenų traumą, pasireiškia pagreitėjęs kognityvinių funkcijų senėjimas, kurį galimai iš dalies lemia lėtinis neuroužd egimas. Iki šiol nebuvo tyrimų, kuriuose būtų nagrinėjamas fizinio aktyvumo poveikis kognityvinėms funkcijoms ar smegenų sveikatai šioje populiacijoje. Dėl to atliktas pilotinis tyrimas, kurio metu intervencija susidėjo iš vienos neuroraumeninės elektros stimuliacijos sesijos. Nustatytas laktato padidėjimas, tačiau IGF-1 ar kognityvinių pokyčių atliekant informacijos apdorojimo greičio testą nerasta. Remiantis žiniomis, įgytomis rengiant disertaciją, visiems rekomenduotina rinktis daugiakomponentį fizinį aktyvumą motyvuojančioje, malonioje aplinkoje ir jį palaikyti visą gyvenimą, kad būtų galima išvengti arba atitolinti kognityvinį senėjimą.

References

1. Petersen RC. Mild Cognitive Impairment. N Engl J Med. 2011;364:2227–61.
2. Zamroziewicz MK, Barbey AK. The mediterranean diet and healthy brain aging: Innovations from nutritional cognitive neuroscience. In: Role of the Mediterranean Diet in the Brain and Neurodegenerative Diseases. Elsevier; 2017. p. 17–33.
3. Tucker-Drob EM. Cognitive Aging and Dementia: A Life-Span Perspective. Annu Rev Dev Psychol. 2019;1:177–96.
4. Livingston G, Huntley J, Sommerlad A, Ames D, Ballard C, Banerjee S, et al. Dementia prevention, intervention, and care: 2020 report of the Lancet Commission. Lancet Comm. 2020;396:413–46.
5. López-Valenciano A, Mayo X, Liguori G, Copeland RJ, Lamb M, Jimenez A. Changes in sedentary behaviour in European Union adults between 2002 and 2017. BMC Public Health. 2020;20:1–10.
6. Khan MAB, Hashim MJ, King JK, Govender RD, Mustafa H, Kaabi J Al. Epidemiology of Type 2 Diabetes – Global Burden of Disease and Forecasted Trends. J Epidemiol Glob Health. 2020;10:107–11.
7. Blüher M. Obesity: global epidemiology and pathogenesis. Nat Rev Endocrinol 2019 155. 2019;15:288–98.
8. Dall TM, Gallo PD, Chakrabarti R, West T, Semilla AP, Storm M V. An Aging Population And Growing Disease Burden Will Require A Large And Specialized Health Care Workforce By 2025. Health Aff. 2013;32:2013–20.
9. Pedersen BK, Saltin B. Exercise as medicine – evidence for prescribing exercise as therapy in 26 different chronic diseases. Scand J Med Sci Sports. 2015;25 Suppl. 3:1–72.
10. Erickson KI, Hillman C, Stillman CM, Ballard RM, Bloodgood B, Conroy DE, et al. Physical Activity, Cognition, and Brain Outcomes: A Review of the 2018 Physical Activity Guidelines. Med Sci Sports Exerc. 2019;51:1242–51.
11. Haverkamp BF, Wiersma R, Vertessen K, van Ewijk H, Oosterlaan J, Hartman E. Effects of physical activity interventions on cognitive outcomes and academic performance in adolescents and young adults: A meta-analysis. J Sports Sci. 2020;38:2637–60.
12. Zhang J, Tam WWS, Hounsri K, Kusuyama J, Wu VX. Effectiveness of Combined Aerobic and Resistance Exercise on Cognition, Metabolic Health, Physical Function, and Health-related Quality of Life in Middle-aged and Older Adults With Type 2 Diabetes Mellitus: A Systematic Review and Meta-analysis. Arch Phys Med Rehabil. 2024;105.
13. Ciria LF, Román-Caballero R, Vadillo MA, Holgado D, Luque-Casado A, Perakakis P, et al. An umbrella review of randomized control trials on the effects of physical exercise on cognition. Nat Hum Behav 2023 76. 2023;7:928–41.
14. Pedersen BK, Steensberg A, Keller P, Keller C, Fischer C, Hiscock N, et al. Muscle-derived interleukin-6: lipolytic, anti-inflammatory and immune regulatory effects. Pflugers Arch-Eur J Physiol. 2003;446:9–16.
15. Liang YY, Zhang LD, Luo X, Wu LL, Chen ZW, Wei GH, et al. All roads lead to Rome - a review of the potential mechanisms by which exerkines exhibit neuroprotective effects in Alzheimer's disease. Neural Regen Res. 2022;17:1210–27.
16. Vints WAJ, Gökçe E, Langeard A, Pavlova I, Çevik ÖS, Ziaaldini MM, et al. Myokines as mediators of exercise-induced cognitive changes in older adults: protocol for a comprehensive living systematic review and meta-analysis. Front Aging Neurosci. 2023;15:1213057.
17. Kim S, Choi JY, Moon S, Park DH, Kwak HB, Kang JH. Roles of myokines in exercise-induced improvement of neuropsychiatric function. Pflugers Archiv European Journal of Physiology. 2019;471:491–505.
18. Kimura T, Kaneko F, Iwamoto E, Saitoh S, Yamada T. Neuromuscular electrical stimulation increases serum brain-derived neurotrophic factor in humans. Exp Brain Res. 2019;237:47–56.

19. Dalise S, Cavalli L, Ghuman H, Wahlberg B, Gerwig M, Chisari C, et al. Biological effects of dosing aerobic exercise and neuromuscular electrical stimulation in rats. Sci Rep. 2017;7.
20. Vints WAJ, Levin O, Fujiyama H, Verbunt J, Masiulis N. Exerkines and long-term synaptic potentiation: Mechanisms of exercise-induced neuroplasticity. Front Neuroendocrinol. 2022;66:100993.
21. Lisman JE. Three Ca2+ levels affect plasticity differently: The LTP zone, the LTD zone and no man's land. J Physiol. 2001;532:285.
22. Vints WAJ, Kušleikienė S, Sheoran S, Šarkinaitė M, Valatkevičienė K, Gleiznienė R, et al. Inflammatory blood biomarker kynurenine is linked with elevated neuroinflammation and neurodegeneration in older adults: evidence from two 1H-MRS post-processing analysis methods. Front Psychiatry. 2022;13:859772.
23. Vints WAJ, Kušleikienė S, Sheoran S, Valatkevičienė K, Gleiznienė R, Himmelreich U, et al. Body fat and components of sarcopenia relate to inflammation, brain volume, and neurometabolism in older adults. Neurobiol Aging. 2023;127:1–11.
24. Vints WAJ, Ghafelzadeh Ahwaz F, Westhof-Jacobs C, Drozdova-Statkevičienė M, Česnaitienė VJ, Ziv G, et al. The effects of a single bout of high-intense strength exercise on cognitive function and postural dual-task control in older adults. Int J Sport Exerc Psychol. 2023. https://doi.org/10.1080/1612197X.2023.2255604.
25. Vints WAJ, Šeikinaitė J, Gökçe E, Kušleikienė S, Šarkinaite M, Valatkeviciene K, et al. Resistance exercise effects on hippocampus subfield volumes and biomarkers of neuroplasticity and neuroinflammation in older adults with low and high risk of mild cognitive impairment: a randomized controlled trial. GeroScience. 2024. https://doi.org/10.1007/S11357-024-01110-6.
26. Vints WAJ, Gökçe E, Šeikinaitė J, Kušleikienė S, Česnaitienė VJ, Verbunt J, et al. Resistance training's impact on blood biomarkers and cognitive function in older adults with low and high risk of mild cognitive impairment: a randomized controlled trial. Eur Rev Aging Phys Act. 2024;21:9.
27. Vints WAJ, Valatkevičienė K, Levin O, Weerasekera A, Jesmanas S, Kušleikienė S, et al. Hippocampal neurometabolic and structural changes from pre-to post-COVID-19: A case-series study. Magn Reson Imaging. 2024;109:249–55.
28. Vints WAJ, Levin O, Masiulis N, Verbunt J, van Laake-Geelen CCM. Myokines may target accelerated cognitive aging in people with spinal cord injury: A systematic and topical review. Neurosci Biobehav Rev. 2023;146:105065.
29. Vints WAJ, Mazuronytė U, Qipo O, Levin O, Verbunt J, van Laake-Geelen CCM, et al. Acute neuromuscular electrical stimulation effects on lactate, insulin-like growth factor-1 and processing speed in spinal cord injured individuals: a randomized cross-over study. unpublished.
30. Yang J, Ruchti E, Petit JM, Jourdain P, Grenningloh G, Allaman I, et al. Lactate promotes plasticity gene expression by potentiating NMDA signaling in neurons. Proc Natl Acad Sci U S A. 2014;111:12228–33.
31. Proia P, di Liegro CM, Schiera G, Fricano A, Di Liegro I. Lactate as a Metabolite and a Regulator in the Central Nervous System. Int J Mol Sci 2016, Vol 17, Page 1450. 2016;17:1450.
32. Vints WAJ, Levin O, van Griensven M, Vlaeyen JWS, Masiulis N, Verbunt J, et al. Neuromuscular electrical stimulation to combat cognitive aging in people with spinal cord injury: protocol for a single case experimental design study. BMC Neurol. 2024;24:197.
33. Novkovic T, Mittmann T, Manahan-Vaughan D. BDNF contributes to the facilitation of hippocampal synaptic plasticity and learning enabled by environmental enrichment. Hippocampus. 2015;25:1–15.
34. Lourenco M V., Frozza RL, de Freitas GB, Zhang H, Kincheski GC, Ribeiro FC, et al. Exercise-linked FNDC5/irisin rescues synaptic plasticity and memory defects in Alzheimer's models. Nat Med. 2019;25:165–75.
35. Sun L, Li X, Wang F, Zhang J, Wang D, Yuan L, et al. High-intensity treadmill running impairs cognitive behavior and hippocampal synaptic plasticity of rats via activation of inflammatory response. J Neurosci Res. 2017;95:1611–20.



APPENDICES

APPENDIX

WORD OF GRATITUDE

WORD OF GRATITUDE

This work is the result of a team effort
by both visible and invisible contributors.
This work is for all of you and by all of you.
It is thanks to you!

I present this dissertation to Prof. Diana Rėklaitienė, Rector at the Lithuanian Sports University and Prof. Dr. Pamela Habibović, Rector at Maastricht University. Thank you for granting me the opportunity to defend my doctoral thesis and to conduct scientific research at your universities.

A special word of gratitude goes to my scientific supervisors Prof. Nerijus Masiulis and Prof. Dr. Jeanine Verbunt and my secondary supervisors Prof. Oron Levin and Dr. Charlotte van Laake-Geelen. Both content-related as well as personal I soon experienced a strong match between us. You have cultivated my enthusiasm for research and given me the opportunity to individualize my own trajectory. Thank you for introducing me to interesting world of scientific research. And thank you for allowing me to further explore this world on my own. You have guided me towards becoming an independent researcher, listened to my ideas and treated me as a colleague instead of a student. Without you, I would not be where I am today.

A special thanks goes to Prof. Nerijus Masiulis and Prof. Oron Levin, who have offered me this PhD position. You have always been flexible and available for me, even in late evenings or weekends. Your enthusiasm and kindness are beyond measure. Thank you for the inspiring, motivating and pleasant time we have had. I hope we will be able to continue this in the future.

For my promotor Prof. Dr. Jeanine Verbunt and my daily supervisor Dr. Charlotte van Laake-Geelen, I would like express my gratitude for always proposing options to stimulate my personal growth. Conducting research while managing a busy medical clinic can be challenging, but you have shown me a strong example, and you have always been available for advice or a word of kindness. I look forward to collaborating more in future projects.

Further, I would like to thank the members of the Dissertation Defence Council both in Maastricht and in Kaunas for the critical evaluation of my doctoral thesis.

Thank you to Prof. Vida Česnaitienė, drs. Simona Kušleikienė, Prof. Gal Ziv, Prof. Dr. Rymantė Gleiznienė, Dr. Kristina Valatkevičienė, drs. Milda Šarkinaite, Orgesa Qipo, Samrat Sheoran, Dr. Margarita Drozdova-Statkevičienė, Prof. Ivan Bautmans, Prof. Mati Pääsuke and all other colleagues who helped with the recruitment, training and measurement of participants or the analysis of collected data. Being part of this multidisciplinary research group made it possible to cross previous boundaries, applying a multimodal approach.

Combining both my research ambitions and my training in rehabilitation medicine would not have been possible without the support and flexibility of my trainers Drs. Wilbert Bakx and Dr. Erik de Klerk. You have made it possible for me to pursue this dream while still complying to all the demands of my residency. Thank you for your enthusiasm and trust.

Thank you to all the colleagues of the professional group in Lithuanian Sports University and Maastricht University. Special thanks to Margareth van Krevel and Pužas, Kęstutis for being my first contact person in case of logistical questions. You have made my life so much easier. Thank you to all my colleagues in healthcare, the other trainees, the rehabilitation specialists and the internship supervisors for your genuine interest in my research topic and for giving me time and space to complete my doctoral thesis.

A special word of gratitude goes to all the study participants. You have been indispensable for the successful completion of this doctoral thesis. Thank you for dedicating your time and effort.

Dear family and family in law, thank you for always being there and for being an example. Your support has created the optimal environment for my ambitions. Thank you mum and dad for your continued guidance.

Dear Evelien, I love you more than anything. Thank you for allowing me my regular visits to Lithuania even though you prefer me to be home with you. Thank you for always being there when I need it, for giving up your time, for standing next to me in the easy and the difficult times. Nobody understands me like you do, nobody supports me like you do. Dearest Eden, your unstoppable joy and love can make any dark cloud disappear. I take an example of your eagerness to learn.

APPENDIX

CURRICULUM VITAE

About the author

Wouter Vints was born on September 24th, 1994 in Bonheiden, Belgium. He earned his Bachelor's and Master's degree in Medicine (2012-2018) and Advanced Master's degree in Sports Medicine (2018-2019) with magna cum laude from the Catholic University of Leuven. While he persued his Advanced Master's degree in Sports Medicine, he worked consecutively as a resident in Rehabilitation Medicine at University Hospitals Leuven and Brussels, Belgium and at the rehabilitation clinic of Adelante in Hoensbroek, the Netherlands. Subsequently, he worked as a resident in Neurology in Zuyderland Hospital Heerlen, the Netherlands. In 2020, he began his training in Rehabilitation Medicine at Adelante and commenced his PhD project at the Lithuanian Sports University and Maastricht University (CAPHRI school) which resulted in this dissertation. He will finish his training in Rehabilitation Medicine by April 2025 and will continue to work in the rehabilitation clinic of Adelante in Hoensbroek. He lives together with his wife Evelien and their son Eden (2023). In his free time, he enjoys engaging in sports acitivities, including running, cycling and fitness, playing with his son, traveling and spending quality time with his family and friends.

Contact: w.vints@maastrichtuniversity.nl

List of publications

1. **Vints WAJ**, Matricali G, Geusens E, Nijs S, Hoekstra H. *Long-term outcome after operative management of talus fractures: what have we learned?* Foot Ankle International. 2018;39(12):1432-1443. Doi 10.1177/1071100718790242.
2. Baadjou V, **Vints WAJ**, Erens T, van Horn Y, Verbunt J. *Ervaringen in de COVID-19 revalidatiezorg.* Tijdschrift voor Bedrijfs- en Verzekeringsgeneeskunde. 2021;29(1):22-25. Doi 10.1007/s12498-020-1314-x
3. Wiertz C, **Vints WAJ**, Maas G, Rasquin S, van Horn Y, Dremmen M, Hemmen B, Verbunt J. *COVID-19: patient characteristics in the first phase of post-intensive care rehabilitation.* Archives of Rehabilitation Research & Clinical Translation. 2021;3(2):100108. Doi 10.1016/j.arrct.2021.100108
4. **Vints WAJ.** *4PM&R: Een gratis service om op de hoogte te blijven van wetenschappelijk revalidatieonderzoek.* Nederlands Tijdschrift voor Revalidatiegeneeskunde. 2022;44(4):34-35
5. **Vints WAJ**, Levin O, Fujiyama H, Verbunt J, Masiulis N. *Exerkines and long-term synaptic potentiation: Mechanisms of exercise-induced neuroplasticity. Frontiers in Neuroendocrinology.* 2022;66:100993. Doi 10.1016/j.yfrne.2022.100993
6. **Vints WAJ,** Kušleikienė S, Sheoran S, Šarkinaitė M, Valatkevičienė K, Gleiznienė R, Kvedaras M, Pukėnas K, Himmelreich U, Česnaitienė V, Levin O, Verbunt J, Masiulis N. *Inflammatory Blood Biomarker Kynurenine Is Linked With Elevated Neuroinflammation and Neurodegeneration in Older Adults: Evidence From Two 1H-MRS Post-Processing Analysis Methods.* Frontiers in Psychiatry. 2022;13. Doi 10.3389/fpsyt.2022.859772
7. Levin O, **Vints WAJ**, Ziv G, Katkutė G, Kušleikienė S, Valatkevičienė K, Sheoran S, Drozdova-Statkevičienė M, Gleiznienė R, Pääsuke M, Dudonienė V, Himmelreich U, Česnaitienė V, Masiulis N. *Neurometabolic correlates of posturography in normal aging and older adults with mild cognitive impairment: evidence from a 1H-MRS study*. NeuroImage: Clinical. 2023:103304. Doi 10.1016/j.nicl.2022.103304
8. Sheoran S, **Vints WAJ**, Valatkevičienė K, Kušleikienė S, Gleiznienė R, Česnaitienė V, Himmelreich U, Levin O, Masiulis N. *Strength gains after 12 weeks of resistance training correlate with neurochemical markers of brain health in older adults: a randomized control 1H-MRS study.* GeroScience 2023:1-19 Doi 10.1007/s11357-023-00732-6
9. **Vints WAJ**, Levin O, Masiulis N, Verbunt J, van Laake-Geelen C. *Myokines may target accelerated cognitive aging in people with spinal cord injury: A systematic and topical review.* Neuroscience and Biobehavioral Reviews 2023;146:105065 Doi 10.1016/j.neubiorev.2023.105065

10. Valatkevičienė K, Levin O, Šarkinaitė M, **Vints WAJ**, Kunickaitė R, Danylė G, Kušleikienė S, Sheoran S, Česnaitienė V, Masiulis N, Himmelreich U, Gleiznienė R. *N-acetyl-aspartate and myo-inositol as markers of white matter microstructural organization in mild cognitive impairment: evidence from a DTI-1H-MRS pilot study.* Diagnostics 2023;13(4):654 Doi 10.3390/diagnostics13040654
11. **Vints WAJ**, Kušleikienė S, Sheoran S, Valatkevičienė K, Gleiznienė R, Himmelreich U, Pääsuke M, Česnaitienė V, Levin O, Verbunt J, Masiulis N. *Body fat and components of sarcopenia relate to inflammation, brain volume and neurometabolism in older adults.* Neurobiology of Aging 2023; 127(7):1-11 Doi 10.1016/j.neurobiolaging.2023.02.011
12. **Vints WAJ**, Gökçe E, Langeard A, Pavlova I, Çevik ÖS, Ziaaldini MM, Todri J, Lena O, Sakkas GK, Jak S, Zorba I, Karatzaferi C, Levin O, Masiulis N, Netz Y. *Myokines as mediators of exercise-induced cognitive changes in older adults a protocol for a comprehensive living systematic review and meta-analysis*. Frontiers in Aging Neuroscience 2023; 15:1213057 Doi 10.3389/fnagi.2023.1213057
13. **Vints WAJ**, Ghafelzadeh Ahwaz F, Westhof-Jacobs C, Drozdova-Statkevičienė M, Česnaitienė V, Ziv G, Pauwels L, Levin O, Verbunt J, Masiulis N. *The effects of a single bout of high-intense strength exercise on cognitive function and postural dual-task control in older adults*. International Journal of Sport and Exercise Psychology 2023; 1-21 Doi 10.1080/1612197X.2023.2255604
14. **Vints WAJ**, Šeikinaitė J, Gökçe E, Kušleikienė S, Šarkinaite M, Valatkeviciene K, Česnaitienė VJ, Verbunt J, Levin O, Masiulis N. *Resistance exercise effects on hippocampus subfield volumes and biomarkers of neuroplasticity and neuroinflammation in older adults with low and high risk of mild cognitive impairment: a randomized controlled trial*. Geroscience 2024; 46(4):3971-3991 Doi 10.1007/s11357-024-01110-6
15. Gökçe E, Kaushal N, Fontanille T, Vrinceanu T, Saillant K, **Vints WAJ**, Freret T, Gauthier A, Bherer L, Langeard A. *The mediating role of lower body muscle strength and IGF-1 level in the relationship between age and cognition. A MIDUS substudy*. Experimental Gerontology 2024; 189:112399 Doi 10.1016/j.exger.2024.112399
16. **Vints WAJ,** Valatkevičienė K, Levin O, Weerasekera A, Jesmanas S, Kušleikienė S, Česnaitienė VJ, Himmelreich U, Verbunt JA, Ratai E, Gleiznienė R, Masiulis N. *Hippocampal neurometabolic and structural changes from pre-to post-COVID-19: A case-series study*. Magnetic Resonance Imaging 2024; 109:249-255 Doi 10.1016/j.mri.2024.03.032
17. **Vints WAJ**, Gökçe E, Šeikinaitė J, Kušleikienė S, Česnaitienė VJ, Verbunt J, Levin O, Masiulis N. *Resistance Training's Impact on Blood Biomarkers and Cognitive Function in Older Adults with Low and High Risk of Mild Cognitive Impairment: A Randomized Controlled Trial*. European Review of Aging and Physical Activity 2024; 21(1):9 Doi 10.1186/s11556-024-00344-9

18. **Vints WAJ,** van Laake-Geelen C, Verbunt J. *Bewegen als behandeloptie voor cognitieve veroudering: Focus op personen met een dwarslaesie*. Nederlands Tijdschrift voor Revalidatiegeneeskunde – NTR 2024; April:41-43
19. **Vints WAJ**, Levin O, van Griensven M, Vlaeyen JWS, Masiulis N, Verbunt J, van Laake-Geelen CCM. *Neuromuscular electrical stimulation to combat cognitive aging in people with spinal cord injury: protocol for a single case experimental design study*. BMC Neurology 2024; 24:197 Doi 10.1186/s12883-024-03699-9

Editorial Boards

Founder and Editor-in-Chief of 4PM&R: For Physical Medicine & Rehabilitation https://www.4abstracts.com/spin-offs/4pm-r

Conference presentations

1. **Vints W**, Kvedaras M, Levin O, Masiulis N. Resistance training and cognitive aging: muscle-brain crosstalk. European Group for Research into Elderly and Physical Activity (EGREPA) conference, Krakow, Poland, 05-2021
2. **Vints W**, Levin O, Valatkeviciene K, Weerasekera A, Kvedaras M, Jesmanas S, Kušleikiene S, Cesnaitiene V, Pukenas K, Himmelreich U, Verbunt J, Ratai E, Gleizniene R, Masiulis N. Pre versus post- COVID-19 neurometabolic and structural alterations in human brain: preliminary observations from MRS/MRI neuroimaging in 3 older adults. European College of Sport Science (ECSS) conference, virtual, 09-2021
3. **Vints W**. Resistance exercise and muscle-brain crosstalk. Finalist of Pitch your PhD, CAPHRI Research Meeting, Maastricht, the Netherlands, 11-2021
4. **Vints W**, Levin O, Valatkeviciene K, Kvedaras M, Kušleikiene S, Cesnaitiene V, Himmelreich U, Verbunt J, Gleizniene R, Masiulis N. Successful aging: the association of body composition, muscular fitness and physical exercise with cognition, brain volume, neurodegeneration and neuroinflammation. European College of Sport Science (ECSS) conference, Sevilla, Spain, 09-2022
5. **Vints W**. Resistance exercise and muscle-brain crosstalk in older adults. Invited symposium, Leuven, Belgium, 11-2022
6. **Vints W**, Kvedaras M, Kielė D, Ziv G, Pääsuke M, Česnaitienė VJ, Levin O, Masiulis N. Blood Flow Restriction Training and Eccentric Training affect different Cognitive Function Subdomains in Older Adults: preliminary results from the BRAIN-M trial. European College of Sport Science (ECSS) conference, Paris, France, 7-2023

7. **Vints W**, Pääsuke M, Česnaitienė V, Levin O, Verbunt J,Masiulis N. Cognitieve functieverbeteringen na een sessie krachttraining met hoge metabole of hoge mechanische trainingsstimulus in oudere mannen: de rol van lactaat. Sportmedisch Wetenschappelijk Jaarcongres, Vianen, the Netherlands, 11-2023
8. **Vints W.** Unraveling exerkines' molecular pathways to enhance cognitive function. Symposium presentation. European Group for Research into Elderly and Physical Activity (EGREPA) conference, Kaunas, Lithuania, 04-2024

Poster presentations

1. **Vints W**. 4PM&R: Stay informed about scientific rehabilitation research. Dutch Congress of Rehabilitation Medicine (DCRM), virtual, the Netherlands, 11-2021
2. **Vints W**, Levin O, Masiulis N, Verbunt J, van Laake C. Physical exercise is a promising strategy to combat accelerated cognitive aging in people with spinal cord injury. Beweegdag MUMC+, Maastricht, the Netherlands, 11-2022
3. **Vints W**, Levin O, Masiulis N, Verbunt J, van Laake C. Myokines may target accelerated cognitive aging in people with spinal cord injury: A systematic and topical review. Dutch Congress of Rehabilitation Medicine (DCRM), the Netherlands, 11-2023
4. **Vints W**, Gökçe E, Langeard A, Pavlova I, Selin Çevik Ö, Mosaferi Ziaaldini M, Todri J, Lena O, Sakkas G, Jak S, Zorba I, Karatzaferi C, Levin O, Masiulis N, Netz Y. Myokines as mediators of cognitive improvements in older adults: living systematic review and meta-analysis. European Group for Research into Elderly and Physical Activity (EGREPA) conference, Kaunas, Lithuania, 04-2024
5. **Vints W**, Mazuronytė U, Qipo O, Levin O, Verbunt J, van Laake-Geelen C, Pokvytytė V, Masiulis N. A randomized cross-over study to examine the effect of a single bout of neuromuscular electrical stimulation on cognitive function in spinal cord injury. International Spinal Cord Society Annual Scientific Meeting (ISCOS), Antwerp, Belgium, 09-2024

Awards

1. Young Researcher Award for best oral presentation. European Group for Research into Elderly and Physical Activity (EGREPA) conference, Krakow, Poland, 2021
2. LIVIT Trophy, annual price for resident in training in rehabilitation medicine who has made a special contribution to the scientific field. Livit Ottobock Care, online VRA Colloquium, the Netherlands, 2023

3. Doctoral student award for productivity and outstanding study results. Research Council of Lithuania (Lietuvos mokslo taryba), Lithuania, 2023
4. Young researcher of the year. Lithuanian Sports University, Lithuania, 2023
5. Doctoral student award for productivity and outstanding study results. Research Council of Lithuania (Lietuvos mokslo taryba), Lithuania, 2024
6. Young researcher award for best poster presentation. European Group for Research on Aging and Physical Activity (EGRAPA) conference, Kaunas, Lithuania, 04-2024