Neuroprotective mechanisms of exercise and the importance of fitness for healthy brain ageing

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Ageing is a scientifically fascinating and complex biological occurrence characterised by morphological and functional changes due to accumulated molecular and cellular damage impairing tissue and organ function. Ageing is often accompanied by cognitive decline but is also the biggest known risk factor for Alzheimer's disease, the most common form of dementia. Emerging evidence suggests that sedentary and unhealthy lifestyles accelerate brain ageing, while regular physical activity, high cardiorespiratory fitness (CRF), or a combination of both, can mitigate cognitive impairment and reduce dementia risk. The purpose of this Review is to explore the neuroprotective mechanisms of endurance exercise and highlight the importance of CRF in promoting healthy brain ageing. Key findings show how CRF mediates the neuroprotective effects of exercise via mechanisms such as improved cerebral blood flow, reduced inflammation, and enhanced neuroplasticity. We summarise evidence supporting the integration of endurance exercise that enhances CRF into public health initiatives as a preventive measure against age-related cognitive decline. Additionally, we address important challenges such as lack of long-term studies with harmonised study designs across preclinical and clinical settings, employing carefully controlled and repeatable exercise protocols, and outline directions for future research.

Lancet 2025; 405: 1093–118

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Introduction

Ageing is associated with cognitive decline and an increased risk of neurodegenerative diseases, such as Alzheimer's disease.[1] Normative ageing does not necessarily result in considerable structural damage to the brain but rather a gradual decline in memory and reasoning abilities from early adulthood up until around age 65 years, after which the decline accelerates. Perceptual speed declines more consistently starting at age 30–40 years, while vocabulary knowledge increases from early adulthood, followed by a modest decline from age 60 years.[2] However, a transition to pathological ageing can accelerate cognitive deterioration, considerably impairing daily functioning and quality of life. Therefore, understanding non-pathological cognitive ageing is pivotal for accurately defining and understanding accelerated ageing.[2] Genetic factors (eg, *APOE4* genotype) and environmental influences (eg, sedentary lifestyles, cardiometabolic risk factors, psychosocial factors, and chronic health conditions) can exacerbate this decline, potentially progressing from normal age-related cognitive decline to mild cognitive impairment, and ultimately, to dementia.[3] Despite these changes, the brain retains a capacity that is a cognitive reserve built from a lifetime of intellectual, social, and physical activities, which enhances the brain's resilience to neurological damage.[4] This cognitive reserve might explain individual differences in resilience, enabling some individuals to maintain cognitive function longer and delay clinical symptoms.[4]

The rapidly ageing global population poses considerable public health challenges with age being a major non-modifiable risk factor for cognitive decline, underscoring the urgent need for optimised prevention, diagnostics, and treatment.[1] Physical inactivity, however, is a key modifiable risk factor for accelerated ageing and dementia.[5] Conversely, physical activity helps counteract structural and functional decline during ageing (figure 1),[6] and is inversely linked to other dementia risk factors, including hypertension, obesity, diabetes, and depression. A meta-analysis revealed that high levels of physical activity reduce the risk of neurodegenerative dementia by 28%.[7] Moreover, superagers (ie, individuals with remarkable resistance to cognitive decline)[8] are considerably more physically active than their peers, emphasising physical activity as a cornerstone for successful cognitive ageing.[9] Starting physical activity at any age can provide cognitive benefits, but engaging in regular physical activity early in life offers the most long-term benefits for reducing the risk of cognitive decline. These cognitive benefits are due to the cumulative effects of improved cardiovascular health, neuroplasticity, and the establishment of lifelong healthy habits.[5]

Search strategy and selection criteria

The literature search focused on identifying influential and highly relevant studies on exercise and brain health that substantially contribute to understanding key mechanisms. Data were sourced from MEDLINE and PubMed, and the references within relevant articles using the search terms: "exercise in ageing", "neuronal effect of exercise in the ageing brain", "physical activity and brain ageing", "exerkines", "neuroinflammation", "brain metabolism during ageing", "blood brain barrier and exercise", "role of cardiorespiratory fitness in brain ageing", "adult neurogenesis and exercise", "neural plasticity and exercise", "gut and oral microbiota and exercise", "acute effects of exercise training and brain", "endurance training and brain ageing", "maximal oxygen uptake and cognitive function", and "peak oxygen uptake and ageing". Only English-language articles addressing brain ageing and neuroprotective mechanisms were included.

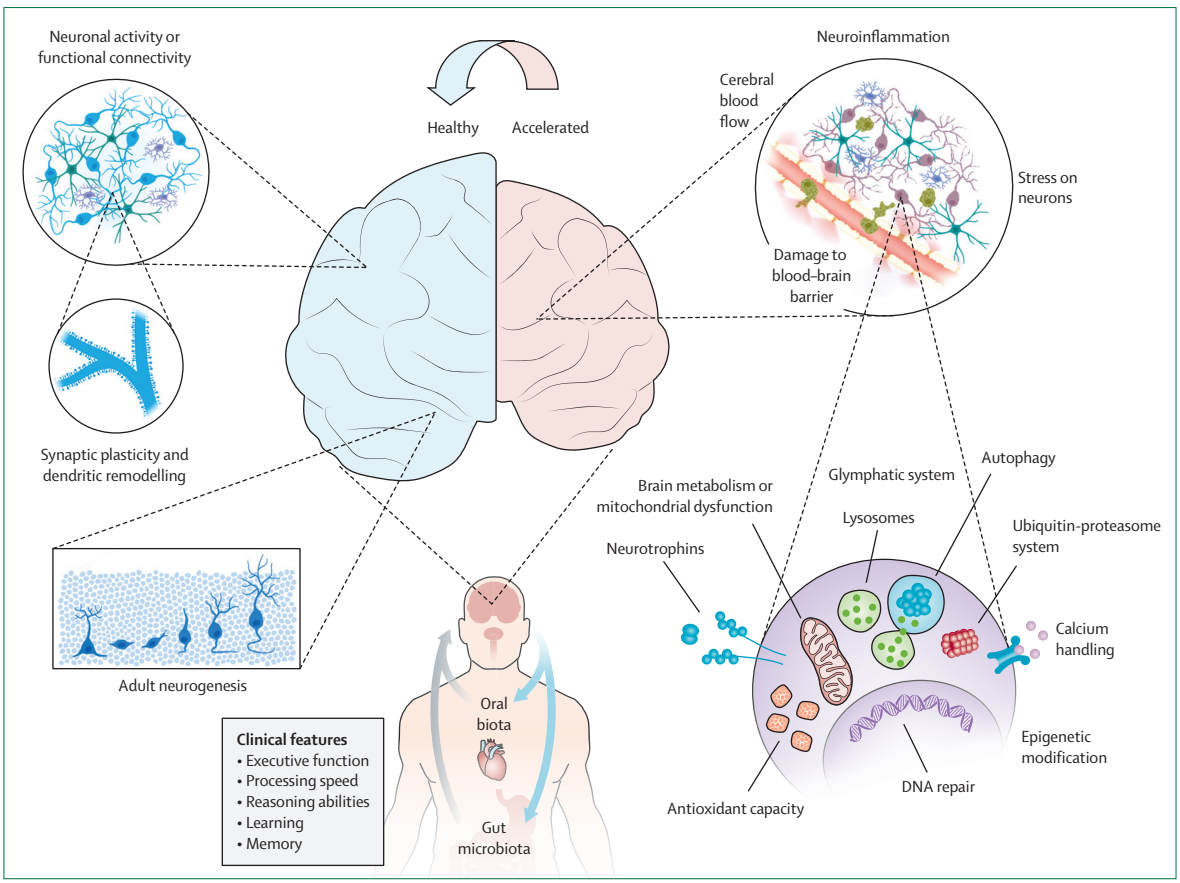

Figure 1: **Clinical, tissue, and molecular differences between healthy ageing and accelerated ageing**
The brain health of the older population can be simplified into two categories; namely: accelerated brain ageing and healthy brain ageing. Differences between healthy brain ageing and accelerated ageing are explained at three levels. At the clinical level, the major differences include reduced capacities in executive function, processing speed, reasoning abilities, learning, and memory in accelerated ageing. At the organ, tissue, and cellular levels, accelerated brain ageing is exemplified by impaired neuronal network activity and compromised functional connectivity, reduced synaptic plasticity and dendritic remodelling, decreased adult neurogenesis, and damage to the blood–brain barrier. Furthermore, there is evidence of a vicious cycle between biota dysbiosis (ie, oral biota and gut microbiota) and brain health. At the molecular level, accelerated ageing is the result of compromised brain repair (eg, reduced capacities in anti-oxidation, DNA repair, epigenetic modifications, and Ca^{2+} homoeostasis) and damaged waste clearance systems (eg, the glymphatic system, autophagy, lysosome, and the ubiquitin–proteasome system). Exercise has the potential to reverse or slow several of the factors underlying accelerated brain ageing.

Cardiorespiratory fitness (CRF), a clinical vital sign reflecting the maximal capacity of cardiovascular and respiratory systems to deliver oxygen and nutrition to organs (including the brain),[10] is a more robust predictor of longevity than physical activity alone.[11] Although CRF declines with age and inactivity, it can be improved with exercise, and is inversely associated with dementia risk. For example, a prospective study of 30 375 people showed that maintaining or improving CRF over time was associated with a reduced risk of developing dementia by 40% and dementia-related mortality by 44%, underscoring its crucial role in healthy brain ageing.[12]

A recent WHO position paper regarding optimising brain health across the life course, highlights the importance of maintaining good brain health to enable individuals to optimise their cognitive potential throughout life.[13] Physical activity and CRF possess great potential as the most accessible prevention strategies in the global effort to combat age-related cognitive decline,[14–16] with well documented benefits for cognitive function and quality of life in ageing populations. However, there is an incomplete understanding of the determinants of these effects. This Review summarises the neuroprotective mechanisms of physical activity, particularly endurance exercise, emphasises the importance of CRF in promoting healthy brain ageing, highlights knowledge gaps, and advocates for more precise evidence-based physical activity recommendations to preserve brain health.

Brain volume and cerebral blood flow

Adequate cerebral blood flow (CBF) is important for delivering oxygen, nutrients, neurotrophins, and other factors necessary for brain function. Systematic reviews show that both acute (ie, a single bout of exercise) and

chronic (ie, several days per week on a regular basis) exercise increase CBF in several brain regions, including the hippocampus.[17,18] Exercise-induced increases in CBF have been observed in both young adults[19] and older adults,[20] correlating with changes in CRF. Endurance training in healthy middle-aged to older adults (age 55–80 years), including in those with amnestic mild cognitive impairment, improved CBF and cognitive functions.[21] Proposed mechanisms include increased production of endothelial nitric oxide synthase, which supports vascular health,[22] and increased permeability of the human blood–brain barrier (BBB),[23] allowing more efficient delivery of oxygen and nutrients to the brain.

Ageing is associated with a shrinkage in brain regions, such as the hippocampus, which is crucial for learning and memory, and enlargement of the cerebral ventricles.[24,25] Higher atrophy rates are linked to an increased risk of accelerated ageing.[26] Reduced CBF with ageing[27] contributes to brain shrinkage and compromised function, as even brief disruptions can cause neuronal cell death and cognitive deficits.[28]

Brain atrophy during normal ageing can be mitigated by exercise,[29,30] but not uniformly throughout all brain regions. The hippocampus is particularly sensitive to exercise-induced enhancement. A systematic review and meta-analysis found no significant effect of exercise on total hippocampal volume, but identified a significant enhancement in left hippocampal volume,[31] whereas another meta-analysis reported substantial effects of exercise on the right hippocampus.[32] A more recent meta-analysis found a considerable effect of aerobic exercise on total hippocampal volume, which was not constrained to one hemisphere.[33] Although these inconsistencies might be due to the limited sample size and different exercise protocols, the analysis unanimously found that hippocampal volume is maintained by exercise. Of note, sex differences in brain volume have been observed, with higher levels of daily activity correlating with larger hippocampal volumes among older females, but not males.[34] The brain responds quickly to exercise training, with one study reporting that even short periods of exercise (ie, 6 weeks) were sufficient to enhance hippocampal volume in sedentary adults.[35] These volume gains returned to baseline 6 weeks after aerobic exercise ceased.[35]

Endurance exercise refers to activities that involve dynamic work with large muscle groups and increases heart rate over prolonged time, such as walking, jogging, swimming, etc. Endurance exercise is neuroprotective even when initiated late in life. A randomised controlled trial showed that 1 year of moderate-intensity endurance exercise increased hippocampal size and improved spatial memory in older adults.[29] Similarly, a 3-month intervention that improved CRF correlated with increased hippocampal head volume and cognitive improvements in healthy older adults.[20] Regular endurance exercise also induces structural changes beyond the hippocampus. A 12-week period of moderate-intensity activity resulted in widespread increases in cortical thickness in both cognitively impaired and healthy older adults.[36] Furthermore, a 6-month walking intervention in sedentary older adults increased the volume of several brain regions.[37] The major caveats of most intervention studies are that they are short (<1 year), and vary in exercise regimens and populations. Longer studies are required to conclusively establish whether exercise influences hippocampal volume long term. Towards this goal, positive associations between CRF and brain structure have been reported in older adults participating in a 5-year exercise intervention trial (the Generation 100 Study), suggesting that maintaining high CRF protects against loss of brain volume and structural integrity in ageing-sensitive brain regions.[38,39]

Neuroinflammation, mitochondrial function, calcium handling, and oxidative stress

As we age, the brain's immune system undergoes considerable changes that influence healthy or accelerated brain ageing.[40] A hallmark of accelerated ageing is chronic, low-grade inflammation, often termed inflammaging. In the brain, this process involves several types of cells, including microglia, the primary immune cells of the brain that when activated can release pro-inflammatory cytokines.[41] In mice, activated microglia also produce excessive levels of nitric oxide, which can damage neurons with reactive oxidative stress. Toll-like receptors (TLRs), key in immune responses against pathogens, are increasingly linked to neuroinflammation during accelerated ageing.[42] Of note, female rodents exhibit a greater density of TLRs than age-matched male rodents, potentially underlying the sex differences in immune system composition and function.[43] Experimental activation of a specific TLR in microglia (TLR4) exacerbates neuronal degeneration in age-related neurodegenerative conditions, whereas inhibition of microglial activation shows neuroprotective effects.[44]

Endurance exercise broadly affects immune function—ie, both immune cell mobilisation and pro-inflammatory cytokine release. Moderate exercise in adult humans helps reduce chronic, low-grade inflammation by stabilising inflammatory cytokines and improving immune function.[45] In mice, endurance exercise has been shown to decrease inflammation by reducing adipose tissue, which is known to release pro-inflammatory cytokines.[46] Similarly, in a randomised, double-masked, clinical trial, exercise reduced visceral adipose tissue via mechanisms involving the inflammatory cytokine interleukin 6 (IL-6).[47] Although strenuous or prolonged aerobic exercise in humans can cause oxidative stress,[48] it also enhances antioxidant capacity.[49] Numerous studies have shown an inverse association between increased physical activity and CRF and inflammatory biomarkers in older adults.[50]

Pre-clinical data show that mitochondrial dysfunction and impaired intracellular calcium handling are other key features of brain ageing.[51] As neurons are highly dependent on mitochondrial function for energy, oxidative damage to mitochondria impairs neuronal function and viability, contributing to cognitive decline.[51] Calcium ions are essential for numerous neuronal functions, including neurotransmitter release, signal transmission between neurons,[52] and neuronal survival.[53] As neurons age, their ability to regulate intracellular calcium levels becomes less efficient. Changes in calcium channels, pumps, and buffering systems can cause excessive intracellular calcium build-up, leading to overstimulation of neuronal signalling pathways that result in excitotoxicity, cell damage, or death.[54] Of interest, neuronal mitochondrial dysfunction is exacerbated by calcium dysregulation, as mitochondria are crucial for buffering calcium. Hence, impaired mitochondria can lead to further calcium accumulation to trigger a harmful cycle of damage.[55] Direct evidence for the effects of exercise on calcium homoeostasis in the context of the ageing brain is scarce. However, exercise is known to increase brain-derived neurotrophic factor (BDNF) expression in rodents,[56] which can reduce neurotoxic calcium influx.[57] The beneficial effects of exercise might include enhanced capacity of mitochondria to regulate calcium levels, thereby protecting neurons against calcium-related damage.[58] Systemic biomarkers, such as S100 calcium-binding protein B, show promise not only as it is an indicator of brain ageing and neurodegeneration, but also due to its regulation by aerobic exercise training.[59]

Autophagic and proteasomal degradation

Neurons maintain cellular homoeostasis by clearing damaged molecules and organelles that can be disrupted by ageing-related changes, such as neuroinflammation and mitochondrial dysfunction. This process relies on mechanisms such as autophagy, which directs damaged molecules to lysosomes for degradation, and the proteasomal system, which targets and degrades defective proteins.[60] Ageing impairs these processes, leading to intracellular accumulation of autophagosomes containing undegraded cargos, dysfunctional mitochondria, and misfolded proteins.[60] Endurance exercise in older adults has been shown to counteract these declines by promoting autophagy.[61]

Epigenetic modifications

Age-related impairments in autophagic and proteasomal degradation contribute to genomic instability in neurons by compromising DNA repair mechanisms, leading to the accumulation of DNA damage. Ageing is associated with pronounced epigenetic changes, including histone modifications, DNA methylation alterations, and shifts in microRNA levels. MicroRNAs—non-coding single-stranded RNA molecules that regulate gene expression by repressing translation or degrading target transcripts—are influenced by exercise, which transiently increases hippocampal levels of more than 20 microRNAs, and decreases several others,[62] including miR-135a-5p, which is known to promote generation of new neurons.[63] Additionally, exercise alters microRNA expression in the blood,[64] potentially mediating communication between the periphery and the brain. Studies have also identified sex differences in microRNA expression after exercise.[65] MicroRNAs associated with CRF also differ between female and male individuals.[66] However, further research is required to identify their role in sex-specific exercise adaption.

DNA methylation is the process by which the enzyme DNA methyltransferase adds a methyl group to a cytosine moiety of a genomic CpG dinucleotide at its 5□ carbon position. DNA methylation plays a crucial role in regulating gene expression, maintaining genomic stability, and influencing cellular differentiation. With age, shifts in DNA methylation patterns, known as epigenetic drift, alter gene expression and contribute to accelerated ageing.[67] Exercise, both acute and chronic, modifies DNA methylation in a gene-specific manner. A meta-analysis revealed greater changes in female than male individuals after exercise, suggesting sex differences in the epigenetic response to exercise.[68] Studies also suggest that exercise-induced epigenetic benefits could be transferred across generations.[69]

Brain plasticity

Neuroplasticity is the brain's ability to adapt structurally and functionally to changing demands, supporting learning, cognitive enhancement, recovery from injury, and adaptation to new information. Structural plasticity includes processes, such as the generation and integration of new neurons (ie, adult neurogenesis) and changes in the number and shape of dendritic spines—that form connections with axons of neighbouring neurons. Functional plasticity involves the strengthening, weakening, or remodelling of existing synapses to optimise neural communication.[70]

Structural and functional connectivity

The brain's organisation is defined by both structural and functional connectivity within and across its regions. Structural connectivity refers to the physical networks that connect different regions of the brain. Functional connectivity reflects the synchronisation of activity between different brain regions, indicating how well they coordinate and communicate. Ageing weakens local connectivity and reduces network efficiency, impairing communication and cognitive performance.[71] Regular endurance exercise and increased CRF have been shown to improve both structural and functional connectivity enhancing cognitive and motor function.[72]

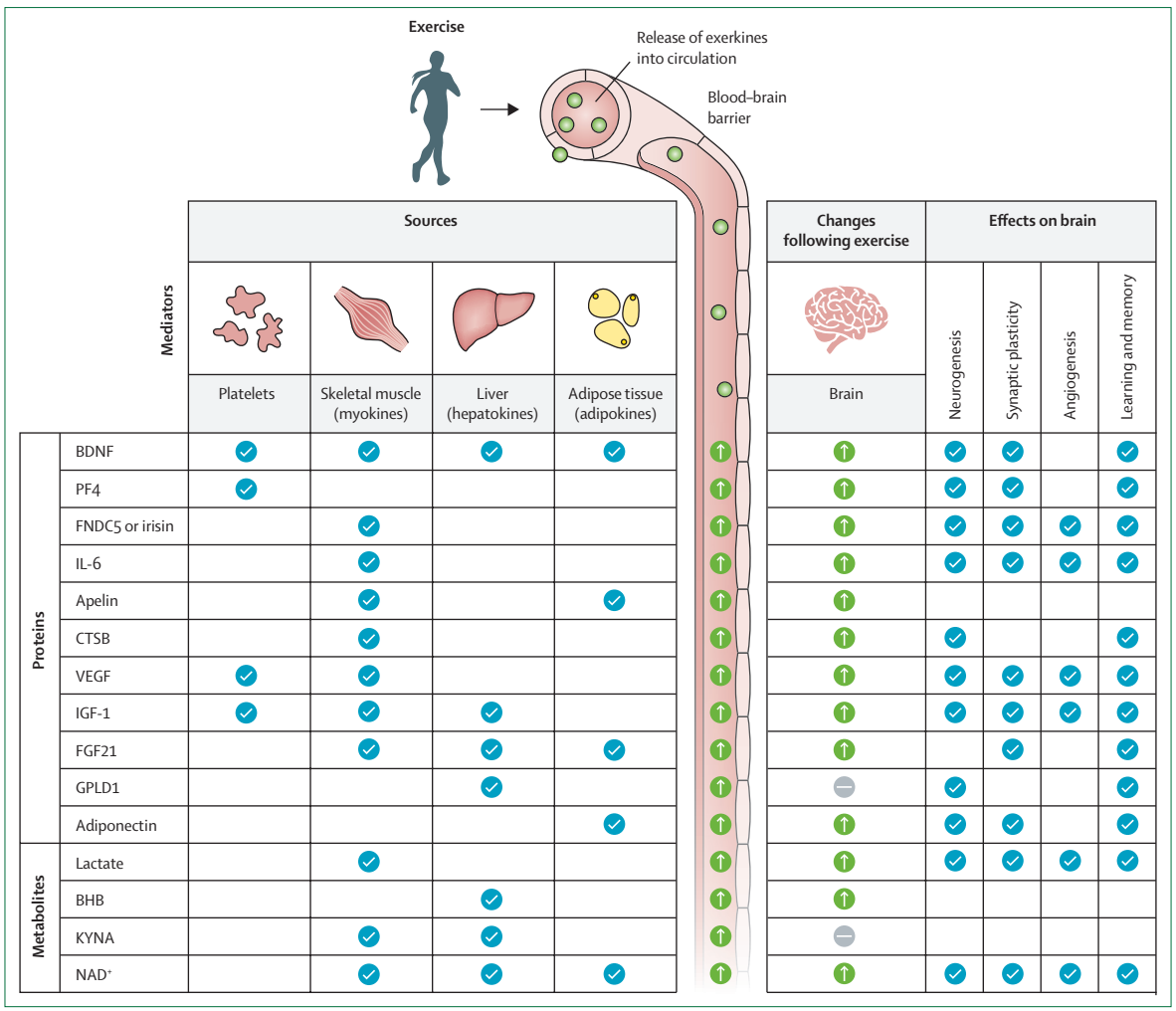

Figure 2: **Molecular mechanisms whereby exercise safeguards brain health with a focus on exerkines**
Exerkines include any proteins and metabolites that are generated and released by source organs or tissues (eg, platelets, skeletal muscles, liver, and adipose tissue) in response to acute or chronic exercise. Depending on their ability to pass through the blood–brain barrier, exerkines could benefit the brain indirectly (a non-autonomous effect) or directly (an autonomous effect) by enhancing the activities of various pathways, such as adult neurogenesis, synaptic plasticity, and angiogenesis. This increase in exerkine concentration might result in improved learning and memory function. BDNF=brain-derived neurotrophic factor. BHB=ketone body β-hydroxybutyrate. CTSB=cathepsin B. FGF21=fibroblast growth factor 21. FNDC5=fibronectin type III domain-containing protein 5. GPLD1=glycosylphosphatidylinositol phospholipase D1. IGF-1=insulin-like growth factor 1. IL-6=interleukin 6. KYNA=kynurenic acid. NAD^+=nicotinamide adenine dinucleotide, oxidised form. PF4=platelet factor 4. VEGF=vascular endothelial growth factor.

Synaptic plasticity and dendritic remodelling

Synaptic plasticity—the strengthening, weakening, and addition or removal of synapses—is a process crucial for learning and memory. The high degree of plasticity in the hippocampus supports cognitive functions, but also increases vulnerability to ageing and pathological conditions.[73] During ageing, although neuron numbers and dendritic structures remain somewhat stable, there is a decline in synaptic connections and alterations in the mechanisms that support long-term potentiation—a process that strengthens synapses over time. This impairment in synaptic plasticity diminishes the capacity of the hippocampus to adapt and sustain cognitive functions, thereby contributing to age-related cognitive decline.[74] Both acute and chronic exercise is shown to enhance synaptic plasticity, with acute exercise in humans transiently altering key long-term potentiation mediators, including the neurotransmitters γ-aminobutyric-acid (GABA) and glutamate.[75] Voluntary wheel running for 2–8 weeks in mice improves dendritic complexity and spine formation, while physically active adults exhibit enhanced synaptic plasticity in the motor cortex.[76]

Adult neurogenesis

Adult neurogenesis, the lifelong addition of new functional neurons into the existing circuitry, occurs in the hippocampus and is crucial for various cognitive functions, including pattern separation[77] and spatial learning and memory.[78] First reported in humans in 1998,[79] hippocampal

neurogenesis is now widely accepted, although its rate declines with age and in conditions such as Alzheimer's disease. The reduction of adult neurogenesis has been implicated as one of the cellular mechanisms contributing to age-related cognitive decline. Several factors contribute to this process. With age, neural stem cells exhibit reduced proliferative capacity, characterised by declines in both proliferation rate and cell density, resulting in a diminished pool of cells available for neuronal differentiation.[80] Age-related changes in CBF, characterised by vascular deterioration and reduced blood supply to the neurogenic niche, considerably impair neurogenesis in the ageing brain.[81] Moreover, increased microglial activation and overproduction of pro-inflammatory cytokines,[82] and the age-related disruption in trophic factors, such as BDNF, might negatively affect neurogenesis.[83] Exercise is one of the strongest physiological enhancers of adult hippocampal neurogenesis in experimental models.[84] Importantly, exercise can enhance neurogenesis even in later life, suggesting that it could help preserve or enhance cognitive function during ageing.[85] However, whether exercise stimulates hippocampal neurogenesis in humans remains to be determined.

Neurotrophins

Neurotrophins are proteins crucial for the growth, development, and survival of neurons. Neurotrophins are essential for maintaining the health of the nervous system throughout life and support processes, such as neurogenesis, the growth of neuronal connections (synaptogenesis), and protect neurons from damage. Neurotrophins exert their effects by binding to specific receptors on the surface of neurons, triggering signalling pathways that promote cell survival, growth, and differentiation. Although essential for nervous system development during embryonic and early life stages, neurotrophins also maintain and repair the nervous system in adulthood.

BDNF is the most studied neurotrophin and a key mediator of synaptic plasticity and neuronal development. BDNF levels decline with age, contributing to synaptic loss,[86] hippocampal atrophy,[87] and cognitive decline.[88] Voluntary running increases BDNF levels in the rodent brain,[56] whereas acute exercise elevates circulating BDNF in humans, with effects varying by exercise duration and possibly sex.[88] A similar sex difference was observed in rodent studies with male mice showing a greater increase in exercise-induced BDNF concentrations than female mice.[89] However, due to its large size, BDNF has restricted ability to cross the BBB,[90] meaning its peripheral effects on the brain are indirect. Notably, many genes upregulated by exercise interact with BDNF, emphasising its central role in exercise-induced brain plasticity. For instance, exercise promoted mitochondrial function in neurons via a pathway involving BDNF and the transcription factor peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α), a key regulator of mitochondrial biogenesis.[91] Genetic factors also play an important role in the function of BDNF. The Val66Met polymorphism in the human *BDNF* gene, present in 20–30% of White people,[92] reduces activity-dependent BDNF secretion[93] attenuating exercise-induced hippocampal volume increases in humans[94] and dampens running-induced BDNF elevation and neurogenesis in animal models.[95]

Cellular senescence

In addition to age-related decline in both structural and functional neuronal connectivity and adult neurogenesis, senescent cells accumulate in the ageing brain and are recognised as a contributing factor to many age-related neurodegenerative diseases.[96] Senescence is characterised by irreversible cell cycle arrest, a pro-inflammatory secretory phenotype, and resistance to programmed cell death. Exercise has been shown to reduce the concentration of circulating biomarkers of cellular senescence in humans.[97] Similarly, chronic exercise training in healthy middle-aged marathon runners and triathletes reduced the systemic concentrations of widely studied senescence markers.[98]

Exerkines—molecular elixir for healthy brain ageing?

Growing evidence suggests that peripherally released factors play a key role in mediating the effects of exercise on the brain. Exerkines are signalling molecules, including hormones, metabolites, proteins, lipids, and nucleic acids that are released in response to exercise and exert their effects via endocrine, paracrine, or autocrine pathways. These molecules are produced by various tissues, cells, or organs, including muscle (myokines), liver (hepatokines), and adipose tissue (adipokines; figure 2). Exercise has been shown to increase the permeability of the human BBB[23] allowing more efficient delivery of exerkines to the brain. Different modes of physical exercise might activate distinct pathways; for instance, resistance exercise predominantly triggers the release of myokines, whereas endurance exercise influences other exerkines.[99] Exerkines are secreted following both acute and chronic exercise, with differing effects. Acute exerkines typically support the maintenance of immediate metabolic homoeostasis and include anti-inflammatory mediators to counteract acute inflammation.[100] In contrast, chronic exerkines are more typically linked to long-term metabolic adaptions.[101]

Hepatokines

The liver secretes over 2500 secreted proteins,[102] many of which function as exerkines, including the well studied insulin-like growth factor 1 (IGF-1). IGF-1 supports brain development and synaptic plasticity, enhances neuronal excitability, and protects against cell death. IGF-1 levels peak at puberty, decline sharply between age 30–40 years, and decreases gradually thereafter.[103] In rodents, IGF has been shown to be upregulated during exercise and to have an effect in neurons, but its role in mediating the

effects of exercise in humans is inconclusive. A meta-analysis of 115 studies reported no change in circulating IGF-1 levels following exercise in 50% of the studies, increases in 37%, and decreases in 13%,[104] with participant age being the primary influencing factor rather than exercise modality and intensity. In contrast, another study in athletes found varying IGF-1 responses depending on the type of endurance exercise performed.[105] Notably, although circulating IGF-1 can mimic some exercise benefits, reduced IGF-1 has been linked to dramatically increased lifespan in many model systems.[106]

Other key hepatokines include fibroblast growth factor 21 (FGF21) and glycosylphosphatidylinositol phospholipase D1 (GPLD1). FGF21, regulated by PGC-1α, is involved in the regulation of energy metabolism by improving insulin sensitivity and glucose and lipid homoeostasis. FGF21 is also expressed in the brain where it acts on dopaminergic neurons to enhance mitochondrial activity and decrease brain oxidative stress.[107] FGF21 has been shown to enhance cognition by increasing hippocampal synaptic plasticity, reducing systemic inflammation, and decreasing brain cell death.[108] Circulating FGF21 levels increase with age in healthy individuals,[109] and have been linked to extended lifespan in mice with transgenic overexpression.[110] However, conflicting findings report lower FGF21 levels in centenarians compared with controls.[111] Exercise influences FGF21 levels, with increases observed after exhaustive treadmill running in mice and intensity-dependent changes in healthy men.[112] In contrast, endurance exercise in older adults has been shown to reduce FGF21 concentrations[113] (in contrast to acute exercise-induced increases). GPLD1, another liver-derived protein, is involved in multiple biological processes. Although its expression does not change with age, it increases following exercise, and overexpression in ageing mice promotes neurogenesis and cognition.[114] Similarly, higher GPLD1 expression has been observed in exercising older humans.[114] However, as GPLD1 is not able to cross the BBB, its effect on the brain could be mediated indirectly.

Myokines

In 2000, it was reported that exercise induces the release of IL-6 from skeletal muscle. This discovery initiated the concept of myokines.[115] IL-6 has dual roles, acting in a pro-inflammatory manner in injury and infection and in an anti-inflammatory manner following acute[116] and chronic[47] exercise. Systemic IL-6 concentrations increase with age and low expression is a marker of successful ageing.[117] Acute exercise increases systemic IL-6 concentration in an intensity-dependent manner,[118] and prolonged intense exercise, such as marathon running, can keep concentrations elevated for hours post-exercise.[118] Women exhibit a greater IL-6 response to maximal exercise, suggesting a sex difference in the immune response to stress.[119] Experiments involving acute exercise[120] or IL-6 infusion[121] suggest that IL-6 functions as a metabolic coordinator during acute exercise by promoting lipolysis in adipose tissue,[121] and maintaining glucose homoeostasis with enhanced glucose-mediated insulin secretion.[120] Pharmacological blocking of IL-6 signalling has been shown to prevent exercise training-induced reduction of visceral fat in people with abdominal obeseity,[47] underscoring the importance of IL-6 signalling in the metabolic adaptations to exercise.

Exercise activates PGC-1α. In muscle, PGC-1α stimulates the production of fibronectin type III domain containing 5 (FNDC5), a protein that is cleaved and secreted as irisin. Irisin is a myokine that plays an important role in oxygen consumption, converting white fat into brown fat, and supporting heat production (thermogenesis).[122] In muscle, levels of FNDC5 and irisin concentrations decreased with age,[123] and irisin deficiency accelerated muscle loss (sarcopenia) in mice, reversable by systemic irisin delivery.[123] Exercise increases irisin concentrations in human plasma,[124] which can cross the BBB to mediate the beneficial effects of exercise on synaptic plasticity, cognition, and the formation of new neurons.[125] Irisin concentrations increase more in women than in men after acute exercise,[126] although it is unclear whether this reflects true sex differences or other variables, such as baseline fitness levels or genetics.

Cathepsin B, a myokine regulated by PGC-1α, plays a key role in autophagy and lysosomal pathways essential for brain waste clearance. Cathepsin B concentrations increase with age in both serum[127] and brain microglia.[128] Exercise elevates cathepsin B in muscle and plasma of rodents, and in plasma of healthy adults, where higher concentrations correlate with improved CRF and better hippocampus-dependent memory.[129] Supporting its role in exercise-enhanced brain benefits, cathepsin B deletion in mice abolished exercise-induced hippocampal neurogenesis and spatial memory.[129] Conversely, long-term exercise training has been reported to improve memory while decreasing cathepsin B in young (age 17–25 years) and middle-aged men (age 46–68 years), suggesting a complex relationship that might depend on exercise duration and age.[130]

Vascular endothelial growth factor (VEGF) is a key growth factor that promotes blood vessel formation, and its decrease could drive physiological ageing in mice.[131] Data from rodents suggest that exercise increases expression of VEGF in the brain, stimulating the proliferation, survival, and migration of endothelial cells, which supports angiogenesis and vessel remodelling.[132] VEGF is also an important mediator of the exercise-induced increase in adult hippocampal neurogenesis, as blocking peripheral VEGF prevents this response.[133]

Klotho

Klotho, an anti-ageing protein, is a transmembrane glycoprotein predominantly expressed in the kidneys and

brain choroid plexus that plays a protective role in brain health. *Klotho* knockout mice exhibit notable cognitive impairments and premature death,[134] whereas overexpression improves cognition, extends lifespan, and reduces age-related decline.[135] In humans, the common KL-VS haplotype, which includes six missense variants, is linked to higher Klotho expression, greater cortical volume, and better global cognition in older adults. A study showed that KL-VSHET genotype is associated with reduced Alzheimer's disease risk in cognitively normal individuals with APOE4.[136] Exercise training increases circulating Klotho, with acute and single bouts of aerobic high-intensity exercise boosting the concentration in young and middle-aged adults.[137,138]

Adipokines

Similar to liver and muscle, adipose tissue-derived cytokines are affected by exercise.[139] Adiponectin, the most abundant cytokine secreted by adipose tissue, regulates glucose and lipid metabolism. Although serum adiponectin concentrations increase with age in healthy adults,[140] they are paradoxically higher in frail individuals.[141] This finding contrasts with experimental models where the absence of adiponectin accelerates brain ageing[141] whereas the overexpression of adiponectin prolongs health and increases lifespan in mice.[142] In rodents, exercise raises adiponectin levels, enhancing fatty acid oxidation and glucose uptake in skeletal muscle.[143] Furthermore, adiponectin crosses the BBB where it modulates hippocampal neurogenesis and mood regulation,[143] and stimulates the release of anti-inflammatory cytokines, such as interleukin-10 (IL-10), and contributes to improved cardiovascular health.[144]

Platelet-derived exerkines

Platelets are emerging as important mediators of brain function. Ageing reduces platelet counts and is associated with dysregulated platelet functions, leading to platelet hyper-reactivity, contributing to a pro-thrombotic and pro-inflammatory systemic milieu linked to age-related diseases.[145] Acute exercise activates platelets in mice[146] and humans[147] leading to the release of humoral factors, such as platelet factor 4 (PF4). Moreover, platelets are required for exercise-induced hippocampal neurogenesis observed in both young[146] and older mice as their depletion abolishes this effect.[148] Exercise also alters the platelet proteome,[146] enabling protein uptake from the plasma via endocytosis. Regular endurance exercise in women[149] and men[150] has been shown to reduce basal platelet reactivity, adhesiveness, and aggregability, suggesting endurance exercise to be protective against thrombotic events.

Metabolites

Exercise increases the expression of proteins called monocarboxylate transporters, which facilitate the uptake of ketones, such as β-hydroxybutyrate into neurons.[151] In addition to ketones, the brain uses lactate as an energy source. Lactate is produced within the brain by astrocytes[152] and systemically by skeletal muscle during intense exercise. Systemic lactate crosses the BBB via monocarboxylate transporters, enhancing hippocampal mitochondrial function in mice.[153] Exercise also affects tryptophan metabolism—an essential amino acid that is the precursor for serotonin, and a neurotransmitter involved in mood regulation. Tryptophan can be converted into kynurenic acid (KYNA), a compound with neuroprotective properties, and NAD^+, a coenzyme essential for energy metabolism. Acute endurance exercise increases the expression of the enzymes responsible for converting tryptophan into KYNA in both peripheral immune cells and skeletal muscle. This conversion reduces the transport of kynurenine (a potentially toxic intermediate) into the brain, thereby providing a neuroprotective effect.[154]

Gut and oral microbiota

In addition to the exerkines, the gut microbiota (ie, the community of microorganisms of the digestive tract) produces a multitude of metabolites crucial for maintaining brain health. These metabolites support digestion, nutrient absorption, and the immune system, and act as hormone-like signalling molecules. Given the presence of these metabolites, the gut microbiota is considered an endocrine-like organ with systemic influence. Ageing disrupts the delicate balance between the host and gut microbiota, leading to changes in mucus secretion, bowel contractility, and gut barrier integrity that can lead to dysbiosis. Dysbiosis is characterised by an imbalance in the composition and function of the gut microbiota that facilitates the translocation of bacterial metabolites and toxins into the bloodstream, promoting systemic inflammation or directly interacting with neural pathways to affect brain function.[155] Notably, high gut microbiota diversity is associated with health, resilience, and youth, whereas low diversity is linked to ageing and disease.[156]

A systematic review shows that regular moderate exercise enhances gut microbiota diversity, stability, and function,[157] promoting both metabolic and cognitive health in humans[158] and mice.[159] Physically active individuals harbour a higher abundance of beneficial bacterial species, particularly from the *Firmicutes* phylum, and fewer pro-inflammatory bacterial species from the *Clostridium* genus.[160] However, the effect of exercise intensity on gut health are inconsistent, with some studies suggesting negative effects of high-intensity exercise, whereas others find no such correlation.[161] CRF levels strongly correlate with gut microbiota composition and diversity, with differences in maximum oxygen uptake accounting for approximately 20% of the variation in taxonomic diversity, and are directly linked to the functional capacity of microbiota.[162] However, the effect of exercise on the ageing gut microbiota remains less understood. Age-related differences in metabolic and inflammatory states and other

	Study type	Context	Participants	Intervention, treatment, and key method	Control and comparison	Tissue	Exerkine	Main findings	Limitations
Bailey et al (2011)[23]	Experimental	Blood–brain barrier	Humans (n=8, all male, mean age 35 years)	Incremental bout of semi-recumbent cycling exercise until exhaustion (mean 8·5 min)	Within-subjects repeated measurements	Blood, brain	..	Exercise might alter blood–brain barrier permeability	Small sample size; only male individuals performing just one type of exercise; measurements only immediately after, not during, exercise
Wedell-Neergaard et al (2019)[47]	RCT	Acute exercise; exercise training	Humans (n=53, 75% female, mean age in the groups ~44 years [SD 12])	Interval training on bicycle ergometer, 45 min, with progressive increase in intensity (50–85% VO_2max) for 12-week intervention period plus tocilizumab (IL-6 receptor antagonist, intravenous infusions every 4 weeks) or exercise plus placebo (saline infusion)	No exercise plus tocilizumab or no exercise plus placebo	Blood	IL-6	IL-6 is necessary for exercise-induced reduction in visceral fat	Heterogeneous population in terms of age and sex; sex-dependent differences in fat distribution not accounted
Neeper et al (1995)[56]	RCT	Exercise training	Rats (n=16, adult rats; strain, sex, and age not specified)	Access to running wheel for 2, 4, or 7 nights (n=4 in each)	No access to running wheel (n=4)	Brain	BDNF	Exercise promotes BDNF expression in the brain	Small sample of poorly defined participants; protein concentrations not measured; timing of tissue collection after last exercise unclear
Davis et al (2015)[99]	RCT	Exercise training	Humans (n=24, all male, mean age 22 years)	Resistance training, three times per week for 12 weeks, 40–50 min sessions (n=12); aerobic exercise assessed via questionnaire	No resistance training (n=12)	Blood	Adiponectin	Aerobic exercise might boost strength training-induced adiponectin response	Only young male individuals; uneven distribution of participants with a parent having type 2 diabetes between groups; aerobic exercise assessed via questionnaire; adiponectin levels measured by Western blot; exact timing of blood collection after last exercise unclear
Ostrowski et al (1999)[100]	Experimental	Acute exercise	Humans (n=10, male, age range 24–37 years, median age 28 years)	Marathon running (median finish time 3 h 26 min), blood collection before, immediately after, and every 30 min until 4 h after finish	Within-subjects repeated measurements	Blood	IL-6, IL-10	During recovery, anti-inflammatory cytokines and cytokine inhibitors are released to balance the acute inflammatory response from high-intensity exercise	Small sample size; only young male participants; single (extreme) mode of exercise; carbohydrate intake was not controlled; origin of cytokines not assessed
Oh et al (2016)[86]	Observational	Ageing	Humans (n=209 post-mortem brains, n=26 independent validation cohort, male and female, age range 16–96 years)	BDNF expression in post-mortem brain samples	Cross-sectional analysis of correlation between BDNF expression and age	Brain	BDNF	BDNF expression in the brain is decreased with age	BDNF expression was only measured at mRNA level and from a homogenate with all cortical layers
Oh et al (2016)[86]	Experimental	Ageing	Mice (n=24, male, age 9–10 weeks)	Transgenic mice fed with 1NMPP1 for 3 weeks to block NTRK2-mediated BDNF signalling (n=12)	Mice receiving vehicle (0·0003% DMSO) in drinking water (n=12)	Brain	BDNF	Reduced BDNF signalling might cause synaptic alterations	Possible contribution of systemic effects or blockage of other neurotrophin-interaction with NTRK2 could not be excluded

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	Study type	Context	Participants	Intervention, treatment, and key method	Control and comparison	Tissue	Exerkine	Main findings	Limitations
(Continued from previous page)									
Mizoguchi et al (2020)[87]	Observational	Ageing; exercise training (physical activity)	Human (n=256, 53% female, 60–89 years)	Serum BDNF levels analysed using ELISA, physical activity assessed using a questionnaire and cognitive function assessed using Rivermead Behavioural Memory Test and modified Stroop test	Cross-sectional analysis of associations between cognitive function and BDNF, age, sex, education, physical activity, and hippocampal atrophy	Blood, brain	BDNF	Lower levels of BDNF, physical inactivity, and hippocampal atrophy are independently associated with memory impairment	Cross-sectional design limits the ability to infer causality; limited selection of cognitive tests
Venezia et al (2016)[89]	Experimental	Exercise training	Mice (n=40, C57BL/6, 1:1 male and female, age 8 weeks)	Individual housing with running wheel for 20 weeks (n=20); hippocampal BDNF mRNA and protein expression assessed with qRT-PCR and ELISA	Individual housing without running wheel for 20 weeks (n=20); hippocampal BDNF mRNA and protein expression assessed with qRT-PCR and ELISA	Brain	BDNF	While BDNF IV mRNA expression was increased with exercise in both males and females, total BDNF mRNA and protein levels were only significantly increased in male mice	Control mice had no immobilised running wheel, introducing a potential additional effect from environmental enrichment for exercising mice
Wrann et al (2013)[91]	Experimental	Exercise training	Mice (n not specified, C57/Bl6, male, age 6 weeks)	Running wheel access for 30 days; tissue collection approximately 10 h after last exercise	No running wheel access	Brain	FNDC5	Exercise promotes PGC-1α and FNDC5 expression in hippocampus	Number of mice not stated; only young males; only mRNA levels were measured; exercise not measured or controlled
Wrann et al (2013)[91]	Experimental	Exercise training	Mice (n not specified, BALB/C, male, age 5 weeks)	Adenoviral delivery of FNDC5 intravenously; tissue collection 7 days later	Adenoviral delivery of GFP intravenously; tissue collection seven days later	Blood, brain	FNDC5	Systemic administration of FNDC5 promotes hippocampal BDNF expression	Number of mice not stated; only young male mice; only mRNA expression was measured
Wrann et al (2013)[91]	Experimental	Exercise training	Primary cortical and hippocampal neurons (n not specified)	Various: adenoviral transduction of FNDC5, lentiviral inhibition of FNDC5, neurotrophins and growth factors	Various: GFP, luciferase, PBS	Brain	FNDC5, BDNF	PGC-1α regulates FNDC5, which in turn might regulate BDNF in a feedback loop	Number of replicates not stated; only mRNA expression measured; effects on neuronal viability or mitochondrial biogenesis not assessed
Shimizu et al (2004)[92]	Observational	Genotype	Humans (n=395, male and female: n=151 Japanese, n=111 Italian, n=133 American)	Genotyping for BDNF Val66Met polymorphism	Comparison between genotypes	Brain	BDNF	BDNF Val66Met polymorphism varies in different populations	Low sample size
Egan et al (2003)[93]	Observational	Genotype	Humans (total n=641, male and female, age 18–60 years: n=133 healthy individuals, n=203 patients with schizophrenia, n=305 healthy siblings)	Cognitive test battery (n=641); functional MRI in two cohorts (cohort 1: n=8 Val/Val, n=5 Val/Met, cohort 2: n=12 Val/Val, n=5 Val/Met)	Comparison between genotypes	Brain	BDNF	BDNF genotype might affect cognitive function and hippocampal activity	Cross-sectional design limits the ability to infer causality; small sample size particularly in functional MRI
Egan et al (2003)[93]	Experimental	Genotype	Hippocampal neurons (n=50 neurons per genotype analysed)	Neurons were infected with virus containing BDNF-GFP constructs (Val variant)	Neurons were infected with virus containing BDNF-GFP constructs (Met variant)	Brain	BDNF	The BDNF Val66Met polymorphism might impair activity-dependent secretion of BDNF	Only immunocytochemical quantification; absence of in vivo experiments
Brown et al (2014)[94]	Observational	Genotype; exercise training (physical activity)	Humans (n=114, 47% female, age >60 years: n=78 Val/Val homozygotes and n=36 Met carriers)	Genotyping for BDNF Val66Met polymorphism, physical activity questionnaire, brain MRI	Cross-sectional analysis of interaction effect of physical activity and BDNF genotype on brain volumes	Brain	BDNF	BDNF genotype might affect changes in brain volume associated with physical activity	Cross-sectional design; physical activity only assessed via questionnaire

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	Study type	Context	Participants	Intervention, treatment, and key method	Control and comparison	Tissue	Exerkine	Main findings	Limitations
(Continued from previous page)									
Ieraci et al (2016)[95]	Experimental	Genotype; exercise training	Mice (n=6–9 per group, male, age 3–4 months, BDNF Met/Met and BDNF Val/Val knock-in homozygotes)	Access to running wheel for 4 weeks	No access to running wheel	Brain	BDNF	BDNF genotype might affect exercise-induced effects on behaviour and hippocampal plasticity	Behavioural testing restricted to assessing anxiety-like and depression-like behaviour; exercise not measured or controlled
Trejo et al (2001)[167]	Experimental	Exercise training	Rats (assumedly n=3 per group, Wistar, male, adult)	Subcutaneous infusion of IGF-1 (50 µg/kg per day) for 7 days; tissue collection at 24 h (n=3) or 3 weeks (n=3) after last infusion	Saline infusion; tissue collection 24 h (n=3) or 3 weeks (n=3) after last infusion	Blood	IGF-1	Peripheral IGF-1 promotes hippocampal neurogenesis	Small sample size; only male rats; IGF-1 or protein expressions in the brain not assessed
Trejo et al (2001)[167]	Experimental	Exercise training	Rats (assumedly n=3 per group, Wistar, male, adult)	Treadmill running for 2 weeks (1 h/day, 17 metres/min) combined with subcutaneous anti-IGF-1 antiserum infusions (20% in saline, 0·25 µL/h) for 15 days; tissue collection at 24 h or 2 weeks after last infusion	Treadmill running (1 h/day, 17 metres/min) or no-exercise combined with non-immune normal rabbit serum infusions (20% in saline, 0·25 µL/h); tissue collection 24 h or 2 weeks after last infusion	Blood	IGF-1	IGF-1 mediates exercise-induced hippocampal neurogenesis	Small sample size; only male rats; IGF-1 expression or protein expression in the brain not assessed
Carro et al (2000)[168]	Experimental	Acute exercise; blood–brain barrier	Rats (n=3 per group, Wistar, male, adult)	1 h of treadmill running plus IGF-1 injected intracarotid (10 µg in 100 µL saline) or intracerebroventricularly, in blocking experiments together with excess unlabelled IGF (100 µg) or insulin (2 mg)	No exercise, saline injections	Brain, CSF	IGF-1	Exercise might induce uptake of circulating IGF-1 in the brain	Small sample size; only male rats; IGF-1 mRNA expression in the brain not assessed
Nguyen et al (1998)[105]	Experimental	Acute exercise	Humans (n=25, sex not specified, mean age 26 years, range 21–38 years)	Incremental ergometer cycling until exhaustion test (n=14), cross-country ski race (n=14), treadmill exercise simulating a soccer match (n=11); single bouts of exercise, ranging from mean 21 min for cycling to 3 h 12 min for cross-country skiing	Pre-exercise to post-exercise comparison, samples collected before and either immediately after (VO_2max test and both periods of the simulated match) or 15 min after (ski race)	Blood	IGF-1	Duration and intensity of exercise might affect IGF-1 responses	Sex not specified; pre-exercise dietary intake and status were not addressed
Mäkelä et al (2014)[107]	Experimental	Ageing	Human dopaminergic neurons (n=4)	50 ng/mL of FGF21 added in medium, effects assessed 24 h later	No additional FGF21	Brain	FGF21	FGF21 protects neurons against oxidative stress and enhances mitochondrial function	Absence of in vivo experiments
Sa-Nguanmoo et al (2016)[108]	Experimental	Ageing	Rats (n=18, Wistar, male, age not specified)	High-fat diet for 12 weeks plus human recombinant FGF21 (0·1 mg/kg per day) for 4 weeks (n=6)	Standard diet plus saline vehicle (n=6) and high-fat diet plus saline vehicle (n=6)	Blood	FGF21	FGF21 can protect neurons against inflammation and oxidative stress	Only male rats; absence of standard diet plus FGF21 group limits findings to obese-insulin resistant conditions
Hanks et al (2015)[109]	Observational	Ageing	Humans (n=184, 55% female, age 5–80 years)	Fasting serum samples from healthy children (age 5–12 years), young adults (age 20–29 years), adults (age 30–50 years), older adults (age 55–64 years), and older people (age 65–80 years)	Cross-sectional analysis of FGF21 levels across age groups	Blood	FGF21	FGF21 serum levels increase with age	Cross-sectional design limits the ability to infer causality; while all adults did not have obesity, the children were classified to have obesity

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	Study type	Context	Participants	Intervention, treatment, and key method	Control and comparison	Tissue	Exerkine	Main findings	Limitations
(Continued from previous page)									
Zhang et al (2012)[110]	Experimental	Ageing	Mice (total n=77 FGF21-transgenic, n=67 wildtype C57Bl/6J, approximately 1:1 male and female, mainly age 26–27 months at the time of analysis)	Transgenic mice with FGF21 expression under the control of apolipoprotein E promoter; previously shown to have 50-fold higher hepatic FGF21 mRNA expression and 5–10-fold higher plasma FGF21 than control	Wildtype mice	Blood	FGF21	Overexpression of FGF21 increases insulin sensitivity, lifespan in mice	Experiments only in mice; FGF21 transgenic mice had bone loss, and the use of transgenic gain of function might have also other side-effects left unexplored
Sanchis-Gomar et al (2015)[111]	Observational	Ageing	Humans (total n=127 female and male in 1:1 ratio: n=81 age ≥100 years and n=46 age 70–80 years)	Analysis of serum levels of 15 proteins, including FGF21, FGF19, chemerin, fetuin-A	Cross-sectional comparison of protein levels between centenarians and older age 70–80-year controls	Blood	FGF21	FGF21, FGF19, chemerin, and fetuin-A expression are independently associated with successful ageing	Small sample size; cross-sectional design limits the ability to infer causality between the protein levels and successful ageing
Kim et al (2013)[112]	Experimental	Acute exercise	Humans (n=8–13 per group, male, mean age 22 years)	Single bout of treadmill running, 30 min at 50% VO_2max (n=13) or 80% VO_2max (n=8); blood collected before, immediately after, and 1 h after exercise	Comparison between pre-exercise, post-exercise, and 1 h-post-exercise levels within intensity groups and 1 h-post-exercise levels between the groups	Blood	FGF21	Intensity of exercise might affect FGF21 responses	Small sample size; only young male participants; within-subject correlation possibly not accounted for in the comparisons within the 50% and 80% VO_2max intensity groups
Kim et al (2013)[112]	Experimental	Acute exercise	Mice (n=4–8 per analysis, male, age 12 weeks)	Single bout of incremental treadmill running; warm-up at 5 metres/min, then increased by 5 metres/min every 5 min until 25 miles/min, which was kept until reaching total 1 h or at exhaustion; blood collected before and immediately after	Pre-exercise to post-exercise comparison	Blood	FGF21	Exercise increases FGF21 mRNA expression in liver and protein level in serum	Small sample size; only male mice; absence of uniform endpoint: differences in duration and relative intensity of exercise
Taniguchi et al (2016)[113]	Randomised crossover trial	Exercise training	Humans (n=33, male, age 62–76 years)	Supervised exercise training on cycle ergometer three times per week for 5 weeks with gradual increase in duration and intensity over time; duration: first 2 weeks 30 min, last 3 weeks 45 min; intensity: first week at 60% VO_2max, next 2 weeks at 70%, then 75%; fasting serum for FGF21 analysis derived minimum 3 days after last exercise	Crossover design, 5 weeks exercise or no exercise, followed by the other	Blood	FGF21	Exercise training might reduce circulating FGF21 in correlation with a reduction in hepatic fat content	Only older male participants; absence of a separate no-exercise control group; short duration of intervention
Horowitz et al (2020)[114]	Experimental	Exercise training; ageing	Mice (recipients: n=3–16 per group per experiment, C57BL/6, male, age 18–23 months; donors: n=30 of both exercised and sedentary, C57BL/6, male, age 6–7 and 18–23 months)	Tail vein injections of blood plasma collected from exercised (6 weeks voluntary wheel running) adult or aged mice, 100 µL administered eight times for 3 weeks (saline as an additional control in experiments with adult donors and aged recipients)	Tail vein injections of blood plasma collected from sedentary adult or aged mice, 100 µL administered eight times for 3 weeks	Blood	Gpld1	Systemic administration of exercised plasma mimics the beneficial effects of exercise in ageing brain	Small sample size; only male mice; exercise not measured or controlled; timing of donor plasma collection after last exercise unclear
Horowitz et al (2020)[114]	Experimental	Exercise training; ageing	Mice (n=3–25 per group per analysis, C57BL/6, male, age 18–23 months)	Tail vein injections of Gpld1-expression constructs	Tail vein injections of GFP	Blood	Gpld1	Liver-derived Gpld1 might mediate beneficial effects of exercise in ageing brain in an indirect manner	Small sample size; only male mice

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	Study type	Context	Participants	Intervention, treatment, and key method	Control and comparison	Tissue	Exerkine	Main findings	Limitations
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Horowitz et al (2020)[114]	Observational	Exercise training (physical activity)	Humans (n=20, 50% female, age 66–78 years)	High physical activity level (≥7100 steps); average daily steps for approximately 1 month measured by using an activity tracker	Low physical activity level (<7100 steps); average daily steps for approximately one month measured with an activity tracker	Blood	Gpld1	Physical activity might increase circulating Gpld1 concentrations in humans	Small sample size; no exercise intervention, activity level determined only by number of steps taken; timing of blood collection after last exercise unclear
Steensberg et al (2000)[115]	Experimental	Acute exercise	Humans (n=6, male, mean age 26 years, range 22–33 years)	One-leg knee extension (from 90° to 30° angle) exercise for 5 h on a modified ergometer at 40% of maximum power output; blood collected just before and after every 1 h of exercise	Within-subjects repeated measurements	Blood	IL-6	Contraction of skeletal muscle induces secretion of IL-6	Small sample size; only young male participants; only one type of exercise
Starkie et al (2003)[116]	Randomised crossover trial	Acute exercise	Humans (n=8, male, age range 23–52 years)	A single 3 h bout of cycling, recombinant human IL-6 infusion during rest for 3 h, or resting for 3 h; each condition included infusion of *Escherichia coli* endotoxin (0·06 ng/kg bodyweight)	Crossover design; rest and exercise in randomised order; minimum 1 week washout period between conditions; comparisons between timepoints and conditions	Blood	IL-6	Exercise and IL-6 attenuate the inflammatory (TNF) response induced by low-grade endotoxemia	A small number of only male participants with a wide age range
Puzianowska-Kuźnicka et al (2016)[117]	Observational	Ageing	Humans (n=3750: n=3496 for IL-6 and n=3632 for CRP analysis, 48% female, age ≥65 years)	IL-6 and CRP concentrations analysed in serum, cognitive function assessed with mini-mental state examination and physical performance in activities of daily living	Cross-sectional analysis of associations between IL-6 and CRP levels and age, successful ageing, and mortality	Blood	IL-6	IL-6 and CRP concentrations increase with age, and higher levels associate with poorer physical and cognitive performance and increased risk of mortality in older people	Cross-sectional design limits the ability to infer causality between IL-6 and CRP concentrations and ageing and cognitive function
Cullen et al (2016)[118]	Experimental	Acute exercise	Humans (n=10, 50% female, mean age 24 years)	Three 35-min exercise sessions on a cycle ergometer at varying intensity: high (five times for 4-min intervals at 80% VO_2max, each followed by 3 min at 50% VO_2max), moderate (five times for 5 min at 50% VO_2max, each followed by 2 min at 80% VO_2max), and low (continuous 50% VO_2max); blood collection immediately before and after exercise	Within-subjects repeated measurements, exercise sessions completed in a counterbalanced order	Blood	IL-6, IL-10	Exercise-induced increase in IL-6 might be dependent on exercise intensity	Small sample size; only young adults; only one mode of exercise; origin of IL-6 not assessed; cytokines measured only immediately before and after exercise
Edwards et al (2006)[119]	Experimental	Acute exercise	Humans (n=24, 1:1 female and male, mean age 24 years)	After a 20 min rest, cycling for 45 min, including a gradual increase in intensity to either maximum or sub-maximum level, followed by 60 min rest; blood collected immediately before and after exercise, and 30 min and 1 h after exercise	Crossover design; non-exercise control task included otherwise same protocol, but participants remained sitting still on bicycle ergometer	Blood	IL-6	Exercise increases circulating IL-6 concentrations in both sexes, but the increase in IL-6 after intense exercise might be greater in females than in males	Small sample size; only young participants
Febbraio et al (2004)[120]	Experimental	Acute exercise	Humans (n=6, male, mean age 24 years)	Three bicycle exercise sessions, each lasting 120 min: one at 70% VO_2peak, two at 40% VO_2peak of which one had human recombinant IL-6 infusion (mimicking 70% VO_2peak exercise levels)	Bicycle exercise at 40% VO_2peak with albumin sham infusion	Blood	IL-6	IL-6 regulates glucose homoeostasis during exercise	Small sample size; only young male participants; only aerobic exercise; IL-6 infusion rate was slightly overestimated early during exercise

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	Study type	Context	Participants	Intervention, treatment, and key method	Control and comparison	Tissue	Exerkine	Main findings	Limitations
(Continued from previous page)									
van Hall et al (2003)[121]	Experimental	Acute exercise	Humans (n=18, male, mean age in each group 23–26 years)	Low level (n=6, mimicking intense exercise) or high level (n=6, mimicking infection) infusion of recombinant human IL-6 for 3 h; blood samples taken before and 30 min, 1 h, 2 h, 3 h, 4 h, 5 h, and 6 h after the start of infusion	Saline infusion (n=6)	Blood	IL-6	IL-6 regulates lipolysis	Small sample size; only young male participants
Bostrom et al (2012)[122]	Experimental	Exercise training	Mice (n=7–10 per group, B6, sex not specified, age 12 weeks)	Access to running wheel for 3 weeks or swimming on 7 days	No access to running wheel or swimming	Blood	Irisin	Exercise training-induced irisin could stimulate subcutaneous fat browning	Exercise not measured or controlled (voluntary wheel running); timing of tissue collection after last exercise unclear
Bostrom et al (2012)[122]	Experimental	Exercise training	Mice (n=7 per group, C57/Bl6J, sex not specified, age 26 weeks and BALB/C, sex not specified, age 8–12 weeks old)	Adenoviral delivery of FNDC5 (10^{10} particles) intravenously, analyses 10 days after; C57/Bl6J mice fed high-fat diet for 20 weeks before the injection	Adenoviral delivery of GFP (10^{10} particles) intravenously	Blood	FNDC5 or irisin	FNDC5 stimulates fat browning in vitro	FNDC5 concentrations only measured by Western blot
Bostrom et al (2012)[122]	Experimental	Exercise training	Humans (n=8, male, age not specified)	Endurance exercise training for 10 weeks, including 20–35 min cycling sessions, 4–5 times per week, at 65% VO_2max	Within-subjects comparison, pre-training and post-training	Blood	FNDC5 or irisin	Exercise training increases irisin levels in blood plasma	Small sample size; only male participants; FNDC5 concentrations only measured by Western blot; timing of tissue collection after last exercise unclear
Guo et al (2023)[123]	Observational	Ageing	Mice (n=3–6 per group per analysis, C57BL6J, sex unspecified, age 2 months and 24 months)	Analysis of FNDC5 or irisin concentration in skeletal muscle and blood	Comparisons between young and older mice	Blood, skeletal muscle	FNDC5 or irisin	FDNC5 and irisin expression is reduced in aged skeletal muscle both at mRNA and protein levels; irisin levels are reduced in aged serum	Small sample size; sex of the mice not specified; only two age groups
Guo et al (2023)[123]	Experimental	Ageing	Mice (C57BL6J with or without FNDC5 or irisin knockout, sex unspecified, age 22 months)	Knockout of FNDC5 or irisin expression	Wildtype mice	Skeletal muscle	FNDC5 or irisin	FNDC5 or irisin deficiency might exacerbate skeletal muscle atrophy and strength loss	Small sample size; sex of the mice not specified
Guo et al (2023)[123]	Experimental	Ageing	Mice (C57BL6J, n=5–8, sex unspecified, age 14 months and 22 months)	Intraperitoneal injections of recombinant irisin (2 mg/kg), three times per week for 4 months (age 14 months) or 1 month (age 22 month mice)	Intraperitoneal injections of control His-tag, three times per week for 4 months (age 14 month mice) or 1 month (age 22 month mice)	Adipose tissue, blood, skeletal muscle	Irisin	Irisin administration might improve metabolic dysfunction and alleviate skeletal muscle atrophy and strength loss in ageing	Small sample size; sex of the mice not specified
Guo et al (2023)[123]	Observational	Ageing	Humans (n=25, sex unspecified, age 20–80 years)	Vastus lateralis biopsies collected from patients who had orthopaedic surgery	Age versus FNDC5 expression correlation analysis within the single cohort of patients	Skeletal muscle	FNDC5	FNDC5 expression in skeletal muscle decreases with age	Small sample of orthopaedic surgery patients, whose sex is left unspecified; only mRNA expression analysed; no blood samples analysed for irisin levels

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	Study type	Context	Participants	Intervention, treatment, and key method	Control and comparison	Tissue	Exerkine	Main findings	Limitations
(Continued from previous page)									
Jedrychowski et al (2015)[124]	Experimental	Exercise training	Humans (n=10, male, mean age 25 and 26 years in each group)	High-intensity interval training; four 4-min intervals >90% VO_2peak with 3 min rest, three times a week plus walking 45 min at 70% VO_2peak, 2 times a week for 12 weeks (n=6); blood collected after at least 3 days without exercise	No exercise (n=4)	Blood	Irisin	Exercise training increases irisin concentration in blood plasma	Small sample size; only young male participants
Islam et al (2021)[125]	Experimental	Exercise training	Mice (n=5–14 per group per analysis, C57BL/6J with or without FNDC5 knockout, male and female, age 6–13 weeks, sex and age-matched for each experiment)	Access to running wheel, total duration not specified, but daily mean running distance around 4 km for both wildtype and knockout mice	No access to running wheel	Blood	Irisin	FNDC5 or irisin is an important mediator of exercise effects on the brain	Exercise not controlled and of uncertain total quantity; embryonical knockout of FNDC5 and developmental defects might have influenced the outcomes
Islam et al (2021)[125]	Experimental	Alzheimer's disease	Mice (n=2–15 per group per experiment, APP/PS1 with or without FNDC5 knockout, male, age 6–7 months)	Tail vein injection of adenoviral vector to induce overexpression of irisin in liver, or no treatment	Tail vein injection of adenoviral vector with GFP	Brain	Irisin	FNDC5 or irisin is important for cognitive function	Small sample size; only male mice; embryonical knockout of FNDC5 and developmental defects might have influenced the outcomes
Islam et al (2021)[125]	Experimental	Blood–brain barrier	Mice (n=6 per group, C57BL/6J, male, age 7 weeks)	Tail vein injection of adenoviral vector to induce overexpression of irisin in liver	Tail vein injection of adenoviral vector with GFP	Blood, brain	Irisin	Irisin crosses the blood–brain barrier	Only young male mice
Zügel et al (2016)[126]	Experimental	Acute exercise	Humans (n=16 lean participants, nine female, seven male, mean age 26 years)	Cycling with gradually increasing intensity until exhaustion; blood collected before, immediately after, and 10 and 60 min after exercise	Within-subjects repeated measurements	Blood	Irisin	Intensive exercise causes an acute increase in circulating irisin levels in both male and female subjects, but the effect is greater in females	Small sample size
Zügel et al (2016)[126]	Experimental	Acute exercise	Humans (n=12 obese participants, seven female, five male, mean age 53–54 years)	Walking with gradually increasing intensity until exhaustion for approximately 10 min; blood collected before and immediately after exercise	Within-subjects repeated measurements	Blood	Irisin	Exercise effects on circulating irisin levels might be diminished in people with obesity	Small sample size; irisin levels only measured before and immediately after exercise
Wyczałkowska-Tomasik and Pączek (2012)[128]	Observational	Ageing	Humans (n=20 per age group: young age 20–22 years, middle age 49–52 years, older age 77–82 years, 1:1 female and male)	Fasting serum samples analysed for CTSB and CTSL	Cross-sectional comparisons between age groups	Blood	CTSB	Active CTSB expression increase with age	Small sample size; cross-sectional design limits the ability to infer causality
Moon et al (2016)[129]	Experimental	Exercise training	Mice (total n=64, n=6–8 per group, C57BL/6, male, age 1 month)	Access to running wheel for 3, 14, or 30 days	No access to running wheel	Blood	CTSB	Longer-term exercise training increases CTSB expression in mouse plasma and mRNA expression in hippocampus	Only young male mice; exercise not controlled; timing of tissue collection after last exercise unclear; cerebral CTSB only measured at mRNA expression in the hippocampus

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	Study type	Context	Participants	Intervention, treatment, and key method	Control and comparison	Tissue	Exerkine	Main findings	Limitations
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Moon et al (2016)[129]	Experimental	Acute exercise	Cultured skeletal myoblast cells (n=4 replicates for each condition, rat-derived L6)	Treatment with 5-Aminoimidazole-4-carboxamide ribonucleotide (100 μM) to activate AMP-activated protein kinase, and thereby mimic effect of acute exercise in vitro	Treatment with vehicle, distilled water (0·1% DMSO)	Skeletal muscle	CTSB	Exercise might induce an acute increase in CTSB expression in skeletal muscle	In vitro experiment might not accurately reflect the AMP kinase activation during exercise; acute time-course investigation not applied in vivo
Moon et al (2016)[129]	Experimental	Blood-brain barrier	Mice (n=3 per group, C57BL/6 with CTSB knockout, male, age 6–7 weeks)	Single intravenous injection of recombinant CTSB, 50 μg (n=3)	Intravenous injection of vehicle (distilled water; n=3)	Blood, brain	CTSB	CTSB might cross the blood–brain barrier	Only few young male mice used; cerebral CTSB only measured at mRNA level; possibility that CTSB knockout might affect blood–brain barrier cannot be excluded
Moon et al (2016)[129]	Experimental	Exercise training	Monkeys (n=3 female and n=10 male, Rhesus, age 7–21 years)	Treadmill walking (average 0·25 miles/day), 5 days a week, for 4 months	Within-subject comparison	Blood	CTSB	Exercise training increases CTSB concentration in monkey plasma	Heterogeneous population in terms of age and sex; timing of blood collection after last exercise unclear
Moon et al (2016)[129]	RCT	Exercise training	Humans (n=43, 56% female, age range 19–34 years)	Exercise training, 45–75 min at 70–90% HRmax, three times a week for 4 months	Short walk (10–15 min at 50% HRmax), twice a week	Blood	CTSB	Exercise training increases CTSB concentration in human plasma, correlating with change in fitness	Timing of blood collection after last exercise unclear; changes in fitness measured as change in VO_2 ventilatory threshold instead of VO_2max
De la Rosa et al (2019)[130]	Observational	Exercise training	Humans (n=37 young age 17–25 years, n=49 middle-age 46–68 years, all male)	Trained young had participated in varying sports last 7 years; middle-aged trained had all practiced rugby for mean 35 years; those defined as sedentary reported less than 150 min low-intensity physical activity the previous week; cognitive test battery; blood samples collected 24 h after last exercise	Cross-sectional analysis of association between self-reported exercise training, cognitive function, and BDNF and CTSB concentrations	Blood	BDNF, CTSB	Long-term exercise training might improve immediate recall ability in males age 46–68 years and reduce circulating concentrations of BDNF and CTSB in young and middle-aged males	Cross-sectional design restricts the ability to infer causality; only male participants; no controlled exercise, only self-reported data; single timepoint for blood collection
Grunewald et al (2021)[131]	Experimental	Ageing	Mice (total n=100 bi-transgenic VEGF gain-of-function, n=56 with only driver transgene, n=44 with only responder transgene, of which 50% female)	Transgenic, liver-specific, VEGF gain-of-function (tetracycline-off system with a driver line and a responder line) induced by withdrawal of tetracycline from drinking water; bimonthly measurement of VEGF levels in blood	Two control groups, each with one of the transgenes, either the driver or the responder; bi-monthly measurement of VEGF in blood	Blood	VEGF	Counteracting age-related VEGF signalling insufficiency by long-term elevation of systemic VEGF level increases health span and lifespan in mice	Only mouse models used; the use of transgenic gain-of-function might have off-target effects that were not fully explored
Grunewald et al (2021)[131]	Experimental	Ageing	Mice (n=5 per group per analysis, C57BL/6JRccHsd, age 2 months or 8 months)	Adenoviral VEGF delivery intraperitoneally for a mild increase of systemic levels	Adenoviral delivery of scrambled control sequence intraperitoneally	Blood	VEGF	Counteracting age-related VEGF signalling insufficiency by long-term elevation of systemic VEGF level increases health span and lifespan in mice	Only mouse models used; the use of viral delivery system might have off-target effects that were not fully explored

(Table continues on next page)

	Study type	Context	Participants	Intervention, treatment, and key method	Control and comparison	Tissue	Exerkine	Main findings	Limitations
(Continued from previous page)									
Grunewald et al (2021)[131]	Experimental	Ageing	Mice (n=5–16 per analysis, bi-transgenic VEGF loss-of-function)	Transgenic VEGF loss-of-function by induction of VEGFR1 (decoy receptor of VEGF) in endothelial cells induced by withdrawal of tetracycline from drinking water; monthly measurements of VEGFR1 levels in blood	No induction of VEGFR1	Blood	VEGF	Loss of VEGF function accelerates development of ageing hallmarks	Only mouse models used; the use of transgenic loss-of-function might have off-target effects not fully explored
Ding et al (2006)[132]	Experimental	Exercise training	Rats (n=4 per group per analysis, Fisher 344, female, age 22 months)	Treadmill exercise, 30 min/day (15 miles/min) for 3 weeks (n=8)	No exercise training (n=8)	Brain	VEGF	Exercise training increases expression of VEGF and induces angiogenesis in motor cortex and striatum	Small sample size; only older female rats; short training period; restricted selection of brain regions analysed
Fabel et al (2003)[133]	Experimental	Exercise training	Mice (n=4–8 per group, C57/BL6, male, age 8 weeks)	Access to running wheel for 1 week plus either no injection, intravenous injection of adenovirus encoding Flt1 (a decoy receptor for VEGF), or intravenous injection of vehicle	Housed with immobilised running wheel plus either no injection, intravenous injection of adenovirus encoding Flt1 (a decoy receptor for VEGF), or intravenous injection of vehicle	Blood	VEGF	VEGF is an essential factor in exercise-induced hippocampal neurogenesis	Only young male mice; exercise not controlled or measured; short duration of exercise; only short-term effects on cell proliferation assessed, leaving effects on maturation or integration of new neurons undetermined
Kuro-o et al (1997)[134]	Experimental	Ageing	Mice (n=6–10 per age at analysis, wildtype mice two transgenic mouse lines with defective klotho expression, sex-matched and age-matched)	Transgenic mice with defective expression of klotho characterised per se and following exogenous induction of klotho expression	Wildtype mice and mice heterozygous to mutated klotho	Brain, kidney	Klotho	Identification of klotho, which is highly expressed in the brain and kidney and involved in the suppression of several ageing phenotypes	While a resemblance to human ageing was noted, all analyses were restricted to mice; the mechanisms underlying the observed development of premature ageing were not investigated in detail
Kurosu et al (2005)[135]	Experimental	Ageing	Mice (n=17–29 per group in lifespan analyses, n=6–12 per group in physiological analyses, two transgenic lines overexpressing klotho and wildtype C3H mice, sex-matched and age-matched)	Two transgenic lines of mice generated to overexpress klotho	Wildtype mice	Blood	Klotho, IGF-1	Klotho overexpression extends lifespan but leads to insulin and IGF-1 resistance	Global alterations to klotho expression limit the ability to decipher the role of the transmembrane and secreted forms of klotho; health span effects of klotho overexpression not extensively covered
Tan et al (2018)[138]	Experimental	Acute exercise	Humans (n=10, 1:1 male and female, age range 44–51 years)	Bruce protocol exercise test, where speed and incline of treadmill are gradually increased every 3 min until volitional exhaustion; blood collected 1 h before, immediately before, immediately after, and 30 min, 60 min, 120 min, 240 min, 1 day, and 1 week after exercise	Comparison of serum klotho concentrations between timepoints and sexes	Blood	Klotho	High-intensity exercise induces an acute increase in circulating klotho concentrations	Small sample size; only middle-aged participants

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	Study type	Context	Participants	Intervention, treatment, and key method	Control and comparison	Tissue	Exerkine	Main findings	Limitations
(Continued from previous page)									
Muratsu et al (2022)[140]	Observational	Ageing	Humans (n=2206 female, mean age 67 years, n=3467 male, mean age 66 years, all age >60 years)	Measurements of serum adiponectin levels and HOMA-IR	Cross-sectional analyses of the relationship between serum adiponectin levels, HOMA-IR, age, and BMI	Blood	Adiponectin	Ageing might be associated with higher adiponectin expression and insulin resistance	Restricted age range, as only participants older than age 60 years were included; cross-sectional design limits the ability to infer causality
Li et al (2021)[142]	Experimental	Ageing	Mice (total n=3–10 per group per analysis, C57BL/6J with or without adiponectin knockout, male, age 8–140 weeks)	Adiponectin knockout mice fed a chow diet or high-fat diet	Wildtype mice fed on chow diet or high-fat diet	Blood, adipose tissue, kidney, liver	Adiponectin	Adiponectin knockout impairs glucose and lipid homoeostasis, increases tissue inflammation and fibrosis and shortens lifespan, particularly in combination with a high-fat diet	Small sample size; only mouse models used; the use of transgenic loss of function might have off-target effects not fully explored
Li et al (2021)[142]	Experimental	Ageing	Mice (total n=3–10 per group per analysis, transgenic ΔGly and wildtype, male, age 8–140 weeks)	ΔGly mice with increased circulating adiponectin fed a chow diet	Wildtype mice fed on chow diet	Blood, adipose tissue, liver	Adiponectin	Increased circulating adiponectin improves insulin sensitivity, reduces tissue inflammation and fibrosis, and lengthens lifespan	Small sample size; only mouse models used; the use of transgenic gain of function might have off-target effects not fully explored
Yau et al (2014)[143]	Experimental	Exercise training	Mice (n=4–8 per group per experiment, C57/BL6J, male, age 8–9 weeks)	Access to running wheel for 14 days or intracerebroventricular injection of adenovirus expressing adiponectin	Access to running wheel or intracerebroventricular injection of adenovirus expressing luciferase	Brain	Adiponectin	Exercise increases adiponectin expression in the hippocampus	Small sample size; only young male mice; exercise not controlled or measured; timing of tissue collection after last exercise unclear
Yau et al (2014)[143]	Experimental	Exercise training	Mice (n=8–10 per group, adiponectin knockout C57BL6J, male, age 8–9 weeks)	Access to running wheel for 14 days or a single intravenous injection of recombinant adiponectin with analyses 3 h after	Access to running wheel or intravenous injection of PBS	Brain	Adiponectin	Adiponectin is an important mediator of exercise effects on depression-like behaviour and hippocampal neurogenesis; adiponectin might pass the blood–brain barrier	Only young male mice; exercise not controlled or measured; possibility that adipokine knockout might affect the blood–brain barrier cannot be excluded
Leiter et al (2019)[146]	Experimental	Acute exercise; exercise training	Mice (n=4–13 per group per experiment, C57BL/6JRj, female, age 8–10 weeks)	Access to running wheel for 1–21 days	No access to running wheel	Blood	PF4	Exercise-induced platelet activation is a key mediator of exercise effects on hippocampal neurogenesis	Only young female mice; exercise not controlled or measured; effects on maturation or integration of new neurons undetermined
Leiter et al (2019)[146]	Experimental	Acute exercise; exercise training	Mice (n=8, C57BL/6JRj, female, age 8–10 weeks)	PF4 hippocampal infusion for 7 days (100 mg/mL)	Vehicle hippocampal infusion for 7 days	Blood	PF4	PF4 promotes hippocampal neurogenesis	Only young female mice; effects on maturation and integration of new neurons undetermined
Leiter et al (2019)[146]	Experimental	Acute exercise; exercise training	Mice (n=14–15 per group, C57BL/6JRj, female, age 8 weeks)	Anti-platelet serum injection every second day for 13 days, access to running wheel	Normal rabbit serum injection every second day for 13 days, access to running wheel	Blood	PF4	Platelets regulate exercise-induced hippocampal neurogenesis	Only young female mice; effects on maturation or integration of new neurons undetermined
Röcker et al (1986)[147]	Experimental	Acute exercise	Humans (n=16, male, mean age 31·8 years)	Marathon running (mean finish time 2 h 44 min), blood collection 15 min before, within 1 min after, 1 h, and 22 h post-run	Comparison between timepoints	Blood	PF4	Prolonged intense exercise induces an acute activation of platelets and release of PF4	Small sample size (considerable inter-individual variability); only well-trained male participants

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	Study type	Context	Participants	Intervention, treatment, and key method	Control and comparison	Tissue	Exerkine	Main findings	Limitations
(Continued from previous page)									
Leiter et al (2023)[148]	Experimental	Exercise training; ageing	Mice (n=9–10 per group, C57BL/6J, female, age 8 weeks)	Intravenous injection of recombinant PF4 (500 ng) every other day for 1 week	Intravenous injection of saline every other day for 1 week	Blood	PF4	Systemic PF4 enhances hippocampal neurogenesis	Effects on complete maturation or integration of new neurons undetermined
Leiter et al (2023)[148]	Experimental	Exercise training; Ageing	Mice (n=8–12 per group, C57BL/6J PF4-knockout, male, 8 weeks old)	Access to running wheel for 10 days	No access to running wheel	Blood	PF4	PF4 is necessary for exercise-induced hippocampal neurogenesis	Effects on complete maturation or integration of new neurons undetermined
Leiter et al (2023)[148]	Experimental	Exercise training; ageing	Mice (n=9–23, C57BL/6J, female, age 8–10 weeks and 18–20 months)	Access to running wheel for 4, 10, 21, or 28 days	No access to running wheel	Blood	PF4	Exercise stimulates platelet activation associated with proteomic changes and enhances hippocampal neurogenesis in older mice	Effects on complete maturation or integration of new neurons undetermined
Leiter et al (2023)[148]	Experimental	Exercise training; ageing	Mice (n=13–14 per group, C57BL/6J, female, age 18 months)	Anti-platelet serum plus access or no access to running wheel for 28 days	Control serum plus access or no access to running wheel	Blood	PF4	Platelets are necessary for exercise-induced hippocampal neurogenesis in older mice	Effects on complete maturation or integration of new neurons undetermined
Leiter et al (2023)[148]	Experimental	Exercise training; ageing	Mice (n=6–9 per group in neurogenesis or dendritic analyses, n=18–23 per group in behavioural testing, C57BL/6J, female, age 20 months)	Intravenous injection of recombinant mouse PF4 (500 ng) every third day for 24 days	Intravenous injection of saline (0·9% sodium chloride) every third day for 24 days	Blood	PF4	Systemic PF4 recapitulates the effects of exercise on cognitive function in older mice	Effects on complete maturation or integration of new neurons undetermined
Leiter et al (2023)[148]	Experimental	Exercise training; ageing	Mice (n=9–12 per group, transgenic DCXdtr, male and female, age 20 months)	Recombinant mouse PF4 (500 ng/mL), every third day for 24 days (n=24 of which 12 received DTR for immature neuron ablation induction four times between days 18–24)	Saline, every third day for 24 days (n=20 of which nine received DTR for immature neuron ablation induction four times between days 18 and 24)	Blood	PF4	PF4 improves cognitive function in aged mice by promoting hippocampal neurogenesis	Effects of PF4 on cognitive function only assessed in aged mice; selectivity of DTR-induced effects on hippocampal immature neurons not verified
Wang et al (1997)[149]	RCT	Exercise training	Humans (n=16, female, mean age 22 years)	Bicycle ergometer exercise at 50% VO_2max for 30 min/day, 5 days/week for 8 weeks (n=8); training period followed by 12 weeks of deconditioning; blood samples collected before and immediately after VO_2max test, performed in the midfollicular phase of each menstrual cycle	No exercise training, only a VO_2max test performed at baseline and after 8 weeks (n=8)	Blood	Platelets	While acute exercise increases platelet reactivity (adhesiveness and aggregability), the response is diminished by exercise training, which reduces both resting and post-exercise reactivity; training effect is lost by around 12 weeks of deconditioning	Small sample size; only young, previously sedentary, female participants
Wang et al (1995)[150]	RCT	Exercise training	Humans (n=23, male, mean age 21 years)	Bicycle ergometer exercise at 60% VO_2max for 30 min/day, 5 days/week for 8 weeks (n=11); training period followed by 12 weeks of deconditioning; blood samples collected before and immediately after VO_2max test, performed every 4 weeks	No exercise training, only a VO_2max test performed at baseline and after 8 weeks (n=12)	Blood	Platelets	While acute high-intensity exercise increases platelet reactivity (adhesiveness and aggregability), exercise training reduces both resting and post-exercise reactivity; training effect is lost by 12 weeks of deconditioning	Small sample size; only young, previously sedentary, male participants

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	Study type	Context	Participants	Intervention, treatment, and key method	Control and comparison	Tissue	Exerkine	Main findings	Limitations
(Continued from previous page)									
Takimoto and Hamada (2014)[151]	Experimental	Acute exercise	Rats (n=4–7 per group, Sprague-Dawley, sex and age not specified)	Single bout of treadmill exercise (30, 60, 90, or 120 min at 20 miles/min, 8% incline, equivalent to 50–70% VO_2max); samples collected immediately after exercise, for the analysis of monocarboxylate transporters also 5, 10, 18, and 24 h post exercise	No exercise	Blood, brain	Lactate, β-hydroxy-butyrate	Exercise induces acute increases in lactate and β-hydroxybutyrate concentration in blood and brain and upregulates the expression of their transporters in the brain	Small sample size; unclear sex and age of rats; monocarboxylate transporter expression only measured by Western blot
Park et al (2021)[153]	Experimental	Acute exercise	Mice (n=8–10 per group, ICR, male, age 8 weeks)	After treadmill habituation and 3 days of rest, mice performed single bouts of exercise at low (n=8–10, 10 miles/min) or moderate (n=8–10, 20 miles/min) intensity for 30 min, or at high-intensity (n=8-10, 1 min intervals separated by 10 s rest, speed progressively increased from 20 miles/min by 5 miles/min after every three intervals) until exhaustion	No exercise (n=9); comparisons between groups; lactate measured before and immediately after exercise; brain collection at 12, 24, 48, and 72 h post exercise	Blood, brain	Lactate	High-intensity exercise increases hippocampal PGC-1α expression 12 h post-exercise, and mitochondrial DNA copy number 48 h post-exercise, suggesting promoted mitochondrial biogenesis	Mitochondrial number and morphology were not visually assessed
Park et al (2021)[153]	Experimental	Acute exercise	Mice (n=8–10 per group, ICR, male, age 8 weeks)	Intraperitoneal injection of vehicle saline followed by lactate 30 min later	Intraperitoneal injection of monocarboxylate transporter inhibitor followed by lactate 30 min later	Blood, brain	Lactate	Circulating lactate (similar to high-intensity exercise) crosses the blood–brain barrier via monocarboxylate transporters and stimulates hippocampal mitochondrial biogenesis	Mitochondrial number and morphology were not visually assessed
Park et al (2021)[153]	Experimental	Acute exercise	Mice (n=8–10 per group, ICR, male, age 8 weeks)	Intraperitoneal injection of vehicle saline followed by high-intensity exercise (1 min intervals separated by 10 s rest, speed progressively increased from 20 miles/min by 5 miles/min after every three intervals until exhaustion) 30 min after	Intraperitoneal injection of monocarboxylate transporter inhibitor followed by high-intensity exercise 30 min after	Blood, brain	Lactate	High-intensity exercise-induced lactate crosses the blood–brain barrier via monocarboxylate transporters and stimulates hippocampal mitochondrial biogenesis	Mitochondrial number and morphology were not visually assessed
Joisten et al (2020)[154]	Randomised crossover trial	Acute exercise	Humans (n=24, male, age range 20–35 years)	Single sessions; endurance: 5 min warm-up followed by 45 min cycling at 60% of peak power output; strength: 5 min warm-up followed by 45 min, four 8–10 repetitions at 70% of one repetition maximum in five exercises; samples collected before, immediately after, and 1 h after exercise	Crossover design; comparisons between timepoints and exercise types	Blood	Kynurenic acid, IL-6	Exercise induces changes in circulating levels of proteins involved in the kynurenine pathway	Only young male participants

BDNF=brain-derived neurotrophic factor. CSF=cerebrospinal fluid. CTSB=cathepsin B. CTSL=cathepsin L. DMSO=dimethyl sulfoxide. DTR=diphtheria toxin receptor. ELISA=enzyme-linked immunosorbent assay. FGF19=fibroblast growth factor 19. FGF21=fibroblast growth factor 21. FNDC5=fibronectin type III domain-containing protein 5. GFP=green fluorescent protein. Gpld1=glycosylphosphatidylinositol-specific phospholipase D1. HOMA-IR=homeostasis model assessment for insulin resistance. HRmax=heart rate maximum. ICR=Institute of Cancer Research. IGF-1=insulin-like growth factor 1. IL-6=interleukin 6. IL-10=interleukin 10. PF4=platelet factor 4. PGC-1α=peroxisome proliferator-activated receptor gamma coactivator 1α. RCT=randomised controlled trial. VEGF=vascular endothelial growth factor. VO_2max=maximum oxygen uptake.

Table: **Summary of research articles on exerkines**

confounding conditions, such as sex, might contribute to the variability in findings. Overall, the relationship between exercise and gut health is complex, and further research is required to fully understand the underlying mechanisms, particularly in the context of ageing.

The oral microbiome is even more understudied than the gut microbiome. Nonetheless, emerging evidence suggests that the oral microbiota could also influence brain health.[163] The oral cavity harbours a diverse community of bacteria, but age-related changes in microbial diversity can lead to dysbiosis, increasing susceptibility to periodontal disease.[164] Dysbiosis of the oral microbiome is associated with higher amounts of pathogenic periodontal bacteria, which can influence the CNS via both direct and indirect pathways. Directly, these bacteria can enter the bloodstream or reach areas where the BBB is compromised, facilitating their interaction with the brain.[165] Indirectly, the bacteria can trigger the production of pro-inflammatory cytokines, resulting in neuroinflammation. Age-related increases in these pathogenic bacteria might, therefore, exacerbate the risk of accelerated brain ageing or neurodegenerative diseases. Maintaining a healthy oral microbiome could, therefore, help to mitigate these risks. Exercise has been shown to improve oral microbiome health: a 16-week study in older adults found benefits from both high-intensity interval training and moderate-intensity continuous training.[166] These changes were opposite to those observed in neurological disorders and brain ageing, suggesting a potential protective effect. Despite these findings, the oral–microbiome–brain axis remains underexplored, and further research is needed to better understand the mechanisms linking exercise, oral health, and brain ageing.

Challenges and future perspectives

The need for streamlined study protocols

Different types of endurance exercise varying in duration, intensity, and frequency yield distinct changes as shown in the table and figure 3. This variability complicates the interpretation of findings and limits the ability to draw definitive conclusions on molecular changes underpinning beneficial effects of exercise on the brain. To address this knowledge gap, future studies should prioritise harmonised study designs across preclinical and clinical settings, using carefully controlled and repeatable exercise protocols. We propose that future research should use precisely defined exercise regimens (eg, percentages of maximal heart rate, heart rate reserve, or maximal oxygen uptake) and clearly state the timing of biological sample collection relative to the last exercise session. This precision will also help distinguish between the acute and chronic effects of endurance training on brain health enabling more targeted interventions.

The need for modified advice for physical activity

Although the mechanistic links are not fully understood, strong evidence connects exercise to improved cognitive outcomes and healthy brain ageing, which should be emphasised in public policies and incorporated into global physical activity guidelines.

Today, WHO recommends all adults to engage in at least 150 min of moderate-intensity aerobic activity or 75 min of vigorous-intensity aerobic activity per week, along with muscle-strengthening activities on two or more days.[169] However, 70–90% of the global population do not meet these guidelines. Importantly, several landmark studies show that less physical activity can yield substantial health benefits, provided the intensity is high,[12,170–175] and that small amounts of high-intensity activity are more effective than hours of low-to-moderate exercise training for health outcomes.[176,177] Exercising at a higher intensity has been shown to be superior to improve CRF in various populations, also reflecting superior health outcomes.[170,172,174–176]

For example, large studies have clearly shown that a total of about 30 min/week (divided over as many days as

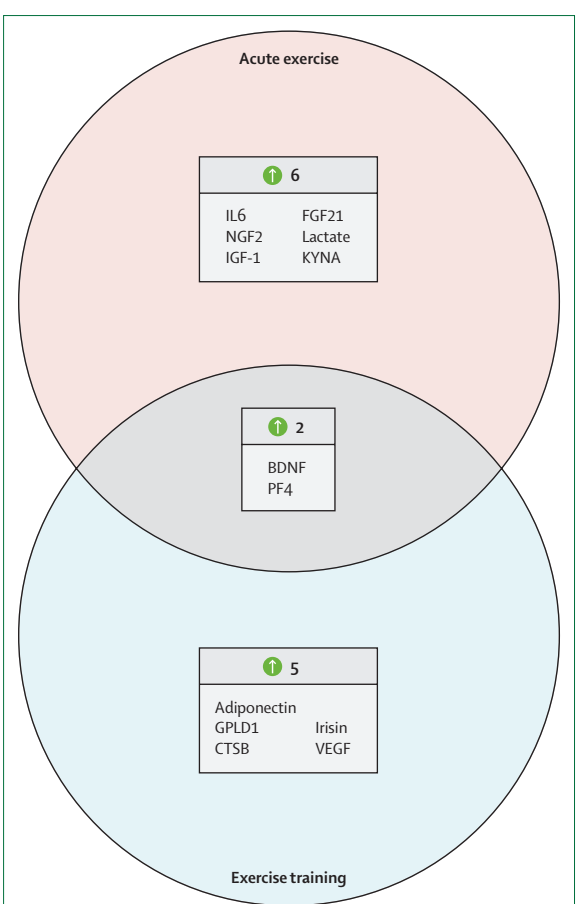

Figure 3: **Exerkines related to ageing that alter in response to acute and chronic exercise**
BDNF=brain-derived neurotrophic factor. CTSB=cathepsin B. FGF21=fibroblast growth factor 21. GPLD1=glycosylphosphatidylinositol specific phospholipase D1. IGF-1=insulin-like growth factor 1. IL-6=interleukin 6. KYNA=kynurenic acid. NGF2=nerve growth factor 2. PF4=platelet factor 4. VEGF=vascular endothelial growth factor.

preferred) of high intensity physical activity (~85% of maximal heart rate—ie, people could have a short conversation, but were not able to sing) leads to about 40% risk reduction from all-cause mortality[171,174] and about 30% and 40% reduced risk of developing or dying from dementia.[173] This effect in risk reduction is notably greater than for those exercising many more minutes per week at low-to-moderate intensity.[171,173,174]

We propose that physical activity recommendations for healthy brain ageing should immediately emphasise this information, as it could be crucial for motivating individuals to transition from inactivity to even modest levels of physical activity. Therefore, we suggest that undertaking a total of 30 min of high intensity physical activity accumulated over a week should be the immediate advice to help ensure healthy brain ageing across the globe. This target is probably a more achievable goal for most people compared with the general physical activity advice worldwide. Such training has been shown to be both safe and feasible even in older adults,[172] including those with established cardiovascular disease.[178]

Future personalised physical activity advice

Advances in high-throughput technologies, such as multiomics, and the application of artificial intelligence (AI) in health monitoring present exciting opportunities for more personalised physical activity recommendations.[179] AI can analyse diverse health data, including biometric measurements, lifestyle factors, blood biomarkers, and genetic predispositions to detect early signs of accelerated ageing. By identifying individuals at risk of early cognitive decline, proactive measures can be implemented to mitigate risks and prevent age-related conditions. Future research should address key knowledge gaps to optimise exercise interventions for brain health in the global ageing population. First, longitudinal studies in well defined populations from different ethnicities and across sexes are needed to explain the bidirectional relationship between physical activity and cognitive decline, clarifying the mechanisms by which exercise enhances neuroplasticity and mitigates neurodegenerative processes over time. Second, comprehensive investigations should establish the optimal exercise modalities—specifically the intensity, duration, and frequency—that most effectively promote cognitive function among diverse demographic groups, including those with varying genetic backgrounds, pre-existing health conditions, and socioeconomic status. Finally, more studies are required to develop and validate personalised exercise prescriptions tailored to individual phenotypic variations and health profiles, ensuring accessibility and effectiveness for enhancing cognitive resilience against age-related decline. Such knowledge will provide valuable insights and contribute to evidence-based public health strategies designed to support brain health across the lifespan.

Contributors

All authors contributed to the overall paper structure and concepts and provided input and expertise to the relevant sections. All authors reviewed the final manuscript and had final responsibility for the decision to submit for publication.

Declaration of interests

We declare no competing interests.

Acknowledgments

We thank Evandro F Fang (University of Oslo, Oslo, Norway) for his constructive comments on parts of the manuscript. This work was supported by the Research Council of Norway, the Norwegian University of Science and Technology (NTNU), the Liaison Committee between the Central Norway Regional Health Authority and NTNU, the Central Norway Regional Health Authority, St Olavs hospital, Trondheim, Norway, the DAM Foundation via the Norwegian Brain Council, National (Australia) Institutes of Health RO1 grant (RO1AG077816–2022-2027), and the Brazil Family Program for Neurology (Philanthropic research funding to TLW's laboratory).

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