The effect of long-term lactate and high-intensity interval training (HIIT) on brain neuroplasticity of aged mice

Doctoral dissertation

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List of Abbreviations

HIIT	High-Intensity Interval Training
VEGF	Vascular Endothelial Growth Factor
AKT	Protein Kinase B
eNOS	Endothelial Nitric Oxide Synthase
SDHA	Succinate Dehydrogenase Subunit A
CREB	cAMP Response Element-Binding Protein
HSL	Hormone-Sensitive Lipase
LDH	Lactate Dehydrogenase
PGC-1α	Peroxisome Proliferator-Activated Receptor Gamma Coactivator 1-Alpha
SIRT1	Sirtuin 1
BDNF	Brain-Derived Neurotrophic Factor
mTOR	Mechanistic Target of Rapamycin Kinase
iNOS	Inducible Nitric Oxide Synthase
nNOS	Neuronal Nitric Oxide Synthase
HIF-1α	Hypoxia-Inducible Factor 1-Alpha
SIRT3	Sirtuin 3
NAMPT	Nicotinamide Phosphoribosyltransferase
CS	Citrate Synthase
FNDC5	Fibronectin Type III Domain-Containing Protein 5
WHO	World Health Organization
DNA	Deoxyribonucleic Acid
AD	Alzheimer's Disease
CDC	Centers for Disease Control and Prevention
ACSM	American College of Sports Medicine
VO2max	Maximum Oxygen Consumption
MRI	Magnetic Resonance Imaging
IGF-1	Insulin-Like Growth Factor 1
PI3K	Phosphoinositide 3-Kinase

TrkB	Tropomyosin Receptor Kinase B
AMPK	AMP-Activated Protein Kinase
ATP	Adenosine Triphosphate
CNS	Central Nervous System
NAD	Nicotinamide Adenine Dinucleotide
PPAR	Peroxisome Proliferator-Activated Receptor
ERR	Estrogen-Related Receptor
FOXO1	Forkhead Box O1
NRF	Nuclear Respiratory Factor
ATF	Activating Transcription Factor
MEF2	Myocyte Enhancer Factor 2
AβOs	Amyloid-Beta Oligomers
MCT	Monocarboxylate Transporter
GPR81	G-Protein-Coupled Receptor 81
BBB	Blood-Brain Barrier
HRP	Horseradish Peroxidase
TBS-T	Tris-Buffered Saline with Tween
PVDF	Polyvinylidene Difluoride
ANOVA	Analysis of Variance
SD	Standard Deviation

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1. Introduction

The prevalence of age-related memory declines and age-related neurodegenerative diseases is likely to escalate in the years to come, in pace with the rapid aging of the population. Our prior research has specifically shown how age negatively impacts brain health, revealing significant impairments in mitochondrial function and increased oxidative stress (L. Zhou et al., 2025). In recent decades, numerous studies have confirmed the effects of exercise on attenuating the symptoms of neurodegenerative diseases, such as Alzheimer's (AD) and Parkinson's disease (Sujkowski et al., 2022). These positive changes observed in the brain after exercise are proposed to be partly mediated by the induction of brain-derived neurotrophic factor (BDNF) and vascular endothelial growth factor (VEGF) expression in the hippocampus (Müller et al., 2020). Several experiments using rodent models have highlighted the beneficial impact of various types of exercise on the mRNA and protein expression of BDNF and VEGF. In these models, enhanced BDNF and VEGF expression has been associated with improved neurogenesis, synaptic plasticity, and cognitive performance (Reddy et al., 2023; Y. Yang et al., 2021). However, the molecular pathways responsible for exercise-mediated BDNF and VEGF induction remain elusive. Our previous study demonstrated that iron supplementation with vitamin B6 enhanced VO2 max and promoted skeletal muscle mitochondrial biogenesis, and although the effects were muscle-specific, such findings highlight that metabolic modulators can influence tissue function and may extend to brain health (L. Zhou et al., 2024). Therefore, identifying exercise-induced molecules—such as myokines, adipokines, and other metabolic factors—that mediate these changes and support learning and memory may be key to unlocking the therapeutic potential of exercise.

In recent years, an increased understanding of lactate, the by-product of exercise, has suggested a close relationship between lactate and brain health (M. Cai et al., 2022;

Hashimoto et al., 2021; Müller et al., 2020). As early as 2013, one study discovered that lactate administration reproduces specific brain and liver exercise-related changes (E et al., 2013). Besides, one study reported that exercise induces cerebral VEGF and angiogenesis via the lactate receptor, and this effect is hypoxia-inducible factor 1-Alpha (HIF-1α) independent (Morland et al., 2017). In addition, another research proposed that lactate partially mediates the effect of physical exercise on adult rodents' neurogenesis but without changes in cognition tests (Lev-Vachnish et al., 2019). Furthermore, using inhibitors, one important rodent study discovered that lactate mediates the effects of exercise on learning and memory through the Sirtuin 1 (SIRT1)/ Peroxisome Proliferator-Activated Receptor Gamma Coactivator 1-Alpha (PGC-1α)/ Fibronectin Type III Domain-Containing Protein 5 (FNDC5)/BDNF pathway. In this study, lactate intervention significantly increased adult rodents' performance in the Morris water maze test (El Hayek et al., 2019). In recent years, a new study noticed that lactate induces neurogenesis in the mouse ventricular-subventricular zone via the lactate receptor (Lambertus et al., 2021, p. 1), and one study proposed that even acute exercise-induced lactate can mediate mitochondrial biogenesis in the hippocampus of mice (Park et al., 2021). Altogether, current knowledge has verified that exercise and its by-product, lactate, have positive effects on the brain function of rodents. However, there are still some unrecognized questions. One study proposed that the dose of lactate concentrations is a critical factor in lactate's effects because chronic lactate accumulation damages the brain activity of rodents' brain (Wang et al., 2019). Current lactate studies use a variety of lactate injection protocols, and the results are not in accordance with each other. In addition to the dose-dependent effects of lactate, current lactate studies only use adult animals and lack a comparison with the exercise effect. To further explore these undetermined areas of lactate functions, this study first tested the effects of three different doses of chronic lactate injection on aging mice (20-22 months) to select the best

intervention protocol. And then use this concentration (500 mg/kg) on aged mice (25-27 months) to check the effects of lactate on aged rodents, thereby providing insight into potential clinical uses of lactate to provide beneficial effect on the aging brain.

2. Literature Review

2.1 Impact of Aging on the Brain

Report from World Health Organization (WHO) indicates that at the end of 2030, the age of 1 in 6 people in the world will reach 60 years and over. In this decade, the population aged over 60 years old will increase from 1 billion in 2020 to 1.4 billion. As estimated by the current birth rate, by the end of 2050, the population around the world of people aged over 60 years old will double reaching 2.1 billion. Meanwhile, the quantity of persons aged over 80 years old is estimated to triple from 2020 to 2050 reaching 426 million (Ageing and Health, n.d.). The increasing number of elderly people is undeniably a global challenge. As improvements in quality of life have extended human life expectancy, age-related diseases have also become more prevalent.

Aging is a complex and inevitable biological process influenced by a combination of environmental and genetic factors. As a result of advancements in healthcare and living conditions, humans are now living longer than ever before. However, this increased lifespan is accompanied by various age-related changes, including reduced autophagy, diminished immune function, mitochondrial dysfunction, stem cell exhaustion, accumulation of somatic and mitochondrial deoxyribonucleic acid (DNA) mutations, telomere attrition, and altered nutrient metabolism (Guo et al., 2022; Palmer & Jensen, 2022; Rebelo-Marques et al., 2018). These changes contribute to a decline in physiological functions and the onset of age-related diseases, such as Alzheimer's disease (AD), which severely impact health and longevity (López-Otín et al., 2023). The extension of life expectancy has not been matched by an equivalent increase in healthy life expectancy, significantly diminishing the quality of life for the elderly. Consequently, with the global population aging, there is an urgent need to address the challenges associated with aging and age-related diseases. This issue has become a focal point of research and public health initiatives worldwide.

As we age, our bodies experience numerous changes that can be both physiological and pathological. The brain, like other organs, is particularly susceptible to aging and undergoes a series of structural and functional changes over time (López-Otín et al., 2023). In the context of brain biology, aging is a major risk factor for cognitive impairments, such as AD, which is the most common form of dementia and affects approximately 55 million people worldwide (Jia et al., 2020). Therefore, a comprehensive understanding of the biological events underlying brain aging is essential for developing effective drugs or treatments to prevent or reverse cognitive impairment.

2.1.1 Aging-Induced Brain Structure Changes

The aging process exerts distinct effects on various brain regions and networks distributed across the cortex. Structural alterations in the brain attributed to aging encompass diminished gray matter volume and cortical thickness. Notably affected regions include the prefrontal cortex, hippocampus, medial temporal lobe, and association areas within the parietal lobes (Allen et al., 2005; Peters, 2006). Furthermore, age-related declines in white matter microstructural integrity manifest initially in anterior cortices and progressively extend to posterior regions, as evidenced by diminishing fiber coherence and organization (Carmichael & Lockhart, 2011). Gray matter atrophy correlates with declines in memory performance, while white matter hyperintensities are linked to diminished executive function and compromised white matter integrity is associated with disrupted network connectivity. These factors collectively contribute to slower processing speed and reduced executive control consistently observed in older adults (Carmichael & Lockhart, 2011; Madden et al., 2009).

2.1.2 Aging-Induced Brain Physiology Changes

In addition to regional age-related structural gray and white matter alterations, aging also induces physiological changes with a global impact across the brain. These global changes not only affect the trajectory of brain volume and white matter integrity during aging but also have detrimental consequences for cognitive performance.

Energy metabolism changes

Aging leads to notable changes in brain energy metabolism, contributing to cognitive and functional decline. Glucose metabolism, which is critical for neuronal activity, decreases with age due to reduced expression of glucose transporters and impaired mitochondrial efficiency. This decline is particularly evident in the hippocampus and prefrontal cortex, regions essential for memory and executive function (Camandola & Mattson, 2017). Mitochondrial dysfunction, a hallmark of aging, reduces ATP production while increasing oxidative stress through excessive reactive oxygen species (ROS) generation. Additionally, the brain's ability to utilize alternative substrates like lactate and ketone bodies diminishes with age due to reduced expression of key transporters and enzymes (Camandola & Mattson, 2017). These metabolic changes consequently exacerbate synaptic loss and neuronal vulnerability, contributing to structural alterations such as decreased dendritic complexity and gray matter volume, ultimately impairing cognitive functions like learning and memory (Camandola & Mattson, 2017). Iron, crucial for the electron transport chain in mitochondria, plays a pivotal role in energy metabolism. However, inefficient binding of iron during aging leads to mitochondrial dysfunction, reduced energy output, and generation of damaging reactive oxygen species that harm neural cell membranes (Raz & Daugherty, 2018). Diminished energy sources also hinder cellular repair mechanisms, ultimately causing loss of neuropil and myelin (Raz & Daugherty, 2018). This results in atrophy in peripheral brain vasculature and regions such as the dorsolateral prefrontal and inferior parietal cortices (Kennedy & Raz, 2015),

leading to reduced information processing capacity of brain networks and impairment of cognitive processes. Complex, less automated processes are particularly susceptible to this system-wide noise (Raz & Daugherty, 2018).

Calcium homeostasis

Aging disrupts calcium homeostasis, impacting its movement through plasma membranes, intercellular concentrations, and its role as metabolic buffers and sensors crucial for neuronal function. This dysregulation disrupts neurotransmitter release, neuronal excitability, synaptic plasticity, gene expression, programmed cell death, and other metabolic processes in the brain (Nikoletopoulou & Tavernarakis, 2012). These disruptions have implications for cognitive functions such as learning and memory, which depend on molecular mechanisms activated by calcium signaling.

Neuronal dysfunction

Brain aging is marked by maladaptive alterations in neuronal function, characterized by reduced synaptic plasticity-related gene expression, diminished synaptic density, and abnormal electrophysiological processes (Fan et al., 2017). Certain regions, such as the hippocampus, are particularly vulnerable to these effects. Age-related changes in molecular and cellular functions lead to both decreased and increased neuronal excitability, depending on cell type and brain region (Mattson & Arumugam, 2018). These alterations indicate a complex dysregulation of excitatory and inhibitory inputs, resulting in broader maladaptive circuit changes and driving cognitive decline, especially in spatial learning and memory, associative memory, and episodic/working memory (Fan et al., 2017).

Regenerative decline

Brain aging involves a significant decline in regenerative capacity, particularly affecting adult neuronal stem cells and oligodendrocyte progenitor cells (Bieri et al., 2023). Adult

neurogenesis, responsible for generating new neurons, markedly decreases in key neurogenic regions such as the hippocampal dentate gyrus, the subventricular zone, and the hypothalamus (Moreno-Jiménez et al., 2019; Navarro Negredo et al., 2020). While the impact of reduced neurogenesis on cognitive decline in old age remains uncertain, the rate of oligodendrocyte differentiation and myelin renewal declines with age. Age-related regenerative decline is influenced by both cell-intrinsic mechanisms and changes in the neurogenic niche and systemic environment (Fan et al., 2017; Navarro Negredo et al., 2020).

Neuroinflammatory changes

As individuals age, pro-inflammatory proteins increase while anti-inflammatory proteins decrease (Garaschuk et al., 2018), resulting in higher levels of oxidative stress due to lipid peroxidation. If left unchecked, inflammation and free radicals can damage neurons and synapses, profoundly affecting cognitive function. Longitudinal studies have correlated inflammatory proteins with processing speed, attention, and memory measures in older adults (Teunissen et al., 2003). Additionally, older adults with low low-density ipoprotein cholesterol and high levels of inflammation exhibited lower scores for general cognition and memory tests (Van Den Kommer et al., 2012).

Neuroinflammation, primarily mediated by microglia, the brain's resident macrophages, increases with age, leading to production of reactive oxygen species, pro-inflammatory cytokines, complement system components, morphological changes, and impaired phagocytosis (Grabert et al., 2016). Microglial dysfunction has been linked to age-related cognitive decline (Stephan et al., 2013). Microglia may influence astrocyte reactivity through the secretion of pro-inflammatory factors. Astrocytes, in turn, become more reactive with age, expressing gene sets associated with synapse elimination, neurotoxicity, and oligodendrocyte toxicity (Clarke et al., 2018). Both microglia and astrocytes may co-regulate synaptic pruning during aging through molecular signals like complement. The

functional heterogeneity of microglia and astrocytes across brain regions and developmental time points is increasingly recognized. Additionally, other cell types, including border-associated macrophages and immune cells at brain borders, may contribute to neuroinflammatory changes, raising questions about their roles in driving brain aging and cognitive decline (Buckley & McGavern, 2022; Mrdjen et al., 2018).

Vascular and blood-brain barrier changes

The brain's vasculature forms a specialized blood-brain barrier that regulates the transport of nutrients, molecules, and cells. Aging affects the morphology of brain vasculature, leading to increased stiffness and dysregulation in cerebral blood flow and tissue oxygenation (De Silva & Faraci, 2020). Traditionally, it was believed that the blood-brain barrier breaks down with age, resulting in cognitive dysfunction (Nation et al., 2019). However, recent advances in single-cell RNA sequencing have revealed nuanced age-related changes in brain endothelial cells and other vascular components (A. C. Yang et al., 2020). Brain endothelial cells exhibit region- and segment-specific increases in inflammatory markers, as well as reduced density, volume, and pericyte coverage with age (Bieri et al., 2023). Age-related alterations in brain endothelial cell transcytosis affect the uptake of plasma proteins, shifting from receptor-mediated to nonspecific caveolar transcytosis. These vascular changes fundamentally alter the relay of blood signals to the brain. Furthermore, new insights into the choroid plexus blood-cerebrospinal fluid barrier offer novel avenues for understanding the mechanisms driving brain aging (A. C. Yang et al., 2020).

2.2 The Effects of Exercise on the Aging Brain

As we have summarized in the last section, aging has risen as a critical health concern in contemporary society, particularly regarding its impact on brain health. The imperative to investigate innovative pharmaceuticals or therapeutic strategies to address the health consequences of brain aging is becoming increasingly necessary. In recent years, there

has been a notable shift towards recognizing the beneficial effects of exercise in both disease management and the aging process. Substantial advancements in scientific inquiry have highlighted the affirmative influence of exercise on brain aging.

The body of evidence substantiating the favorable impact of exercise on human health continues to grow, with physical inactivity identified as a foundational factor underlying numerous chronic diseases prevalent in contemporary society. Beyond its well-documented cardioprotective effects, a spectrum of exercise modalities has been found to confer health benefits to diverse organ systems, contributing significantly to overall resilience, health span, and longevity. Of particular significance is the wealth of studies underscoring the positive correlation between physical exercise and healthy aging, particularly among the elderly (Zia et al., 2021). A comprehensive analysis of 127 observational studies elucidated a notable reduction in the relative risk of cognitive decline among individuals aged 50 and above in economically developed countries with regular physical exercise (Plassman et al., 2010). Furthermore, a systematic review encompassing 33,816 non-demented individuals aged over 65, observed over 1 to 12 years across 13 prospective studies, revealed a robust association between high levels of physical exercise and diminished cognitive decline (Huuha et al., 2022).

Crucially, exercise plays a pivotal role in augmenting brain functions and exerting positive influences on mental health, neurodegenerative diseases, and acquired brain injuries. Serving as a non-pharmacological intervention, exercise proves efficacious in preventing and alleviating neurological conditions and age-related cognitive decline, exerting its impact on both the physiological and structural facets of the brain. Recognizing the substantial effect of cognitive dysfunction on physical and mental well-being, leading to diminished quality of life and imposing a significant economic burden on society, exercise emerges as a prominent non-pharmacological therapeutic avenue characterized by its non-toxic, cost-effective, and universally applicable nature.

The neuroprotective effects of exercise manifest through a multifaceted interplay of mechanisms, including the release of neurotrophins, heightened adult hippocampal neurogenesis, attenuation of neuroinflammation, modulation of cerebral blood flow, and structural reorganization. Furthermore, exercise fosters social interactions, contributing positively to cognitive outcomes (Lu et al., 2023). Doses and types of exercise may yield diverse effects, with research elucidating optimal regimens to enhance cognition in older adults, thereby providing practical recommendations for promoting optimal cognitive health. A recent systematic review intriguingly indicated the absence of a minimal threshold for exercise-induced cognitive benefits, suggesting that any exercise dose surpasses the absence of exercise. Noteworthy is the emerging evidence underscoring the better effects of resistance exercises over other modalities (Gallardo-Gómez et al., 2022). Despite the predominant focus on aerobic exercise in existing literature, resistance exercise emerges as a promising avenue for enhancing cognition and brain outcomes in older adults.

2.2.1 Endurance Exercise and Cognition

Endurance exercise involves prolonged physical exertion primarily fueled by endurance metabolism. Public health recommendations from organizations such as the World Health Organization (WHO), Centers for Disease Control (CDC), and American College of Sports Medicine (ACSM) advise older adults to engage in at least 150 minutes of moderate-intensity endurance exercise per week (46–63% of VO2max) to maintain health and fitness (Garber et al., 2011). Numerous studies have demonstrated that endurance exercise yields beneficial physiological adaptations in older adults, including increased cardiorespiratory fitness, metabolic improvements benefiting glycemic control and lipid profiles, and reduced body fat (Chodzko-Zajko et al., 2009).

Most studies examining the impact of exercise on brain health focus on endurance exercise or physical activity, which primarily encompasses endurance-type activities. Observational studies have shown a positive association between self-reported physical activity and cognitive differences at baseline or longitudinal gains over time (Buchman et al., 2012; Key & Szabo-Reed, 2023; Larson et al., 2006). Magnetic resonance imaging (MRI) studies suggest that exercise and associated endurance fitness levels may mitigate age- and AD-related brain changes, with higher endurance fitness levels linked to less age-related brain volume decline (Colcombe et al., 2003). Randomized controlled trials have also investigated the effects of endurance exercise on cognition. While results vary, the overall evidence suggests that endurance exercise in healthy older adults may enhance cognitive performance, promote brain plasticity, attenuate hippocampal atrophy, and improve visual attention and memory (Colcombe et al., 2004; Erickson et al., 2011; Kramer et al., 1999).

2.2.2 Resistance Training and Cognition

Resistance training is an essential component of a comprehensive exercise program for older adults, utilizing muscular contraction against resistance to counteract the effects of aging on neuromuscular function and functional capacity (Borde et al., 2015; Peterson et al., 2010). Although fewer large, well-designed, randomized controlled trials have assessed resistance training's impact on brain health outcomes, available literature shows promising results. Randomized clinical trials have demonstrated that participation in resistance training improves executive function, memory, verbal fluency, and global cognition (Liu-Ambrose et al., 2008; T. Suzuki et al., 2012; Tarazona-Santabalbina et al., 2016).

2.2.3 High-Intensity Interval Training (HIIT) and Cognition

HIIT has emerged as a time-efficient strategy to enhance health-related fitness compared to traditional training methods. HIIT involves alternating exercises at high intensity with short periods of lower-intensity movements. One systematic review (Leahy et al., 2020) presented compelling evidence in their recent review of 22 studies, suggesting that engaging in HIIT holds promise for enhancing cognitive function and mental health in children and adolescents. Recent evidence also supports the contention that HIIT elicits higher fat oxidation in skeletal muscle than other forms of exercise and is an excellent stimulus to increase maximal oxygen uptake (VO2 max). HIIT also seems to be an excellent stimulus to enhance BDNF (Jiménez-Maldonado et al., 2018). HIIT has also enhanced cognitive flexibility in older adults. Findings in older mice suggest HIIT can improve physical function and reduce frailty, decreasing the risk of disability and loss of independence with age (Mekari et al., 2020; Seldeen et al., 2018). However, more research on HIIT is needed before solid conclusions can be drawn. Overall, the literature suggests that exercise and physical activity positively affect cognitive function and brain health, although it remains unclear which exercise modalities are most effective for maintaining and potentially enhancing cognition and brain health with age.

2.3 The Molecular Mechanism of How Exercise Affects Brain Health: The Exercise Responsome

Accumulating evidence from both animal and human studies strongly supports the idea that physical exercise can enhance neuroplasticity, thus lowering the risk of various neurodegenerative diseases. The neurobiological mechanisms underlying exercise-induced neuroplasticity have been the focus of significant attention. Over the past decade, a wealth of new data has confirmed the beneficial effects of exercise on hippocampal neurogenesis and the maintenance or improvement of cognitive function. Investigations utilizing animal and human models have yielded novel insights into the mechanisms by

which exercise influences a wide range of signaling pathways, both acutely and chronically. During physical exercise, our body undergoes numerous cellular and molecular changes orchestrated by the exercise responsome, facilitating tissue and organ crosstalk across nearly every system in our body. Cytokines released from skeletal muscles (myokines), adipose tissues (adipokines), liver (hepatokines), and bone (osteokines) exert autocrine and paracrine effects, regulating whole-body metabolism, including the brain. These cytokines, along with microRNAs (miRNAs) and other metabolites released from various organs in response to exercise, are collectively termed as exerkines. Exerkines play a significant role in mediating the overall benefits of exercise on multiple physiological systems (Reddy et al., 2023). Certain exerkines can cross the blood-brain barrier and activate beneficial signaling pathways in the central nervous system, promoting cell survival and longevity. Exercise boosts key pathways like Insulin-like growth factor 1 (IGF-1)/ Phosphoinositide 3-Kinase (PI3K)/Protein Kinase B (AKT) and AMP-activated protein kinase (AMPK)/SIRT1/PGC1α (J.-Y. Lin et al., 2020), offering neuroprotective effects. Understanding these pathways is crucial for grasping the broad benefits of exercise. Researchers are also exploring pharmaceutical options to mimic exercise effects. Exercise mimetics are drugs that activate similar pathways, leading to cellular changes akin to exercise (Zhao, 2024). These drugs could enhance endurance and trigger physiological adaptations, like muscle remodeling and increased mitochondrial activity, similar to exercise-induced effects.

The exercise responsome refers to changes in tissues and organs when the body exercises, triggering beneficial adaptations through organ communication. It's categorized into direct and indirect effectors. Indirect effectors, known as exerkines, include substances like cytokines and miRNAs released into the bloodstream from various tissues, mediating exercise benefits. Myokines, for instance, are muscle-secreted molecules with widespread effects, potentially improving lifestyle disorders. Direct effectors are molecules and

factors that undergo changes initiated by exerkines, leading to metabolic shifts like increased glucose uptake and mitochondrial activity. These changes, orchestrated by pathways like AMPK/SIRT1/PGC1α and IGF-1/PI3K/AKT, contribute to neurogenesis and synaptic plasticity.

2.3.1 Exercise-Induced Signaling Molecules in the Brain

BDNF

Exercise triggers the release of growth factors like BDNF, which is crucial for brain health. BDNF levels increase temporarily after exercise, primarily from cells in blood vessels, muscles, and immune cells. In mice, BDNF can cross the blood-brain barrier, influencing brain functions (Jaberi & Fahnestock, 2023). BDNF plays a vital role in neuron survival and function, especially in brain regions like the hippocampus and cortex. It enhances synaptic connections and may alleviate symptoms in conditions like Parkinson's disease. Reduced BDNF levels are linked to various neurodegenerative diseases (Cefis et al., 2023). Animal studies demonstrate that exercise boosts BDNF expression in the brain, particularly in regions related to memory and learning. Blocking BDNF diminishes exercise-related benefits, further indicating its importance. Some exerkines can stimulate BDNF production, contributing to neurogenesis and memory enhancement (Cefis et al., 2023). BDNF enhances cognition by binding to tropomyosin receptor kinase B (TrkB) receptors in the brain, initiating multiple signaling pathways crucial for neuron function and communication (Singh & Bhatti, 2024). While peripheral BDNF can cross the blood-brain barrier in mice, its significance in human brain health and metabolism remains uncertain. Further research is needed to understand its role fully.

AMPK

AMPK was initially discovered as a kinase that regulates enzymes involved in energy metabolism. It responds to AMP: Adenosine Triphosphate (ATP) ratio changes,

activating catabolic pathways to generate ATP and inhibiting anabolic pathways. AMPK exists as a complex with different subunits, and studies show that its activity varies depending on exercise intensity (Lantier et al., 2014). Research on human skeletal muscle found that high-intensity exercise activates α2-AMPK more significantly than α1-AMPK. This suggests that α2-AMPK is preferentially activated during exercise, particularly in response to high AMP levels. Knock-out mouse studies confirmed the importance of AMPK in maintaining muscle function and glucose uptake during exercise (Z.-P. Chen et al., 2003; Jørgensen et al., 2006).

In the central nervous system (CNS), AMPK regulates glucose metabolism, mitochondrial function, and neuronal processes like neurodevelopment and autophagy (Rakshe et al., 2024; Zia et al., 2021). While AMPK activation is protective in some brain disorders like Alzheimer's disease (AD), excessive activation can contribute to disease progression. Maintaining optimal AMPK activity is crucial for brain function, as both under- and over-activation can be harmful. It's essential to understand how different exercise regimens affect AMPK activation in the brain to prevent adverse effects (Rakshe et al., 2024; Zia et al., 2021).

SIRT1

Sirtuins (SIRTs) are a group of enzymes crucial for cellular functions like senescence, proliferation, and stress response, depending on nicotinamide adenine dinucleotide (NAD+) levels for activity (Herskovits & Guarente, 2014). Among them, SIRT1 acts as a metabolic sensor, influencing glucose metabolism and insulin sensitivity. It collaborates with AMPK, and their interaction enhances metabolic processes in the body. SIRT1 regulates various processes like gluconeogenesis and insulin secretion in the brain and other tissues. It interacts with AMPK, and their activation mutually benefits glucose homeostasis and lipid metabolism. SIRT1's deacetylase activity in the CNS influences neuroprotection by acting on substrates like FoxO and PGC1α (J. Xu et al., 2018).

Resveratrol, a SIRT1 activator, shows promise in improving cognitive function and delaying neurodegeneration by activating SIRT1 and its downstream targets. Exercise also boosts SIRT1 expression, with high-intensity training and sprint exercises particularly effective (Sarubbo et al., 2018). Exercise-induced lactate levels contribute to SIRT1 activation, impacting gene expression in the hippocampus and promoting neuro-metabolism. SIRT1's role in regulating vital metabolic pathways, including those involving AMPK, PGC1α, and BDNF, underscores its significance, especially in the CNS. Research into its therapeutic potential for neurodegenerative disorders is ongoing (Sarubbo et al., 2018).

PGC-1α

PGC-1α is a crucial regulator of mitochondrial biogenesis and energy metabolism, influencing various physiological processes. It acts on multiple substrates, including peroxisome proliferator-activated receptors (PPARs), estrogen-related receptors (ERRs), forkhead box o1 (FOXO1), and nuclear respiratory factor (NRFs), and is influenced by metabolic sensors like AMPK and SIRT1 (Halling & Pilegaard, 2020). Exercise triggers PGC-1α activation, leading to mitochondrial biogenesis and metabolic adaptations in tissues like skeletal muscle. Studies suggest that p38 MAPK and transcription factors like activating transcription factor2 (ATF2) and myocyte enhancer factor 2 (MEF2) contribute to PGC-1α's response to exercise, orchestrating mitochondrial adaptation (Kong et al., 2022).

In the brain, PGC-1α plays a protective role against oxidative stress and contributes to synaptic plasticity and neuroprotection. It interacts with the SIRT1-FNDC5/irisin axis, promoting BDNF expression in the hippocampus, which is crucial for cognitive function (Steiner et al., 2011). As a metabolic hub, PGC-1α holds therapeutic potential for metabolic disorders and neurodegenerative diseases by modulating mitochondrial

function and energy metabolism. Its role in mediating exercise-induced responses underscores its significance in promoting overall health.

FNDC5/Irisin

When first discovered in 2012, irisin was hailed as an exercise-induced myokine with the potential for browning adipose tissues, improving glucose homeostasis, and boosting energy expenditure. Initial doubts about its existence were dispelled with advancements in detection methods, confirming its presence in the bloodstream, especially after exercise (Waseem et al., 2022). Irisin's secretion is dependent on PGC1α, a key regulator of exercise-induced effects on various organs. Studies show that PGC1α activates FNDC5 gene expression in skeletal muscle, leading to irisin release. Irisin's impact extends beyond muscle, potentially positively influencing brain health by upregulating BDNF expression in hippocampal neurons (Pignataro et al., 2021).

Research suggests that irisin may hold therapeutic promise for neurodegenerative disorders like Alzheimer's disease (AD). It blocks the activity of Amyloid β oligomers (AβOs) associated with AD and activates pathways linked to memory function. Additionally, irisin shows potential in reducing brain damage in cerebral ischemia and exerting anti-inflammatory effects in AD-related neuropathology (Lourenco et al., 2019). Overall, irisin's role in mediating exercise-induced benefits underscores its potential as a therapeutic agent for neurological disorders and highlights the broader impact of exercise on brain health.

VEGF

Vascular endothelial growth factors play a crucial role in stimulating the growth of blood vessels by binding to two tyrosine kinase receptors, VEGFR-1 and VEGFR-2. Platelets are a significant source of circulating VEGF (Ferrara, 2009; Rosenstein, 2004). Various forms of exercise have been found to boost VEGF levels. For instance, acute sprint

training and resistance exercises with vascular occlusion have shown increases in circulating VEGF. Interestingly, exercise has also been linked to increased cerebral blood flow, particularly in the hippocampus, which could be beneficial, especially for individuals at risk of Alzheimer's (Vital et al., 2014).

Studies have demonstrated that VEGF receptors are expressed in neurons and that VEGF plays roles in brain functions like regulating blood-brain barrier permeability and promoting neurogenesis, particularly in the hippocampus. Both aerobic and resistance exercises have been shown to elevate VEGF levels along with brain-derived neurotrophic factor (BDNF), potentially influencing neurogenesis positively (Karakilic et al., 2021; Vital et al., 2014).

VEGF-R1 is abundant in postnatal neurons but declines with age, while VEGF-R2 signaling is associated with neuron proliferation, migration, and differentiation, persisting into adulthood. Animal studies have shown that altering VEGF levels through exercise can lead to increased angiogenesis and neural stem cell proliferation, which may have implications for conditions like AD (Pedrinolla et al., 2020). Overall, the evidence suggests that exercise-induced changes in VEGF levels could contribute to neurogenesis, offering potential benefits for brain health.

To summarize, different types of exercise lead to increased levels of various genes, proteins and hormones in multiple organs and circulation, as indicated in Table 1. Research has consistently shown the importance of these changes in stimulating neurogenesis, which can positively impact cognitive function. Factors such as exercise mode, duration, intensity, as well as age, training status, and gender, can influence the levels of these hormones. Future investigations should focus on understanding how these changes interact with each other and identify the most effective exercise strategies to enhance neurogenesis, particularly in individuals with traumatic brain injury or

neurodegenerative conditions. Such insights will be valuable in tailoring exercise recommendations aimed at promoting neurogenesis.

Signaling Molecule	Role in Exercise	Effects on Brain Health
BDNF	Elevated after exercise; released from vascular, muscle, and immune cells	Promotes neuronal survival, synaptic plasticity, memory; protective in neurodegeneration
AMPK	Activated by AMP:ATP ratio; intensity-dependent	Regulates energy metabolism, mitochondria, autophagy; essential for cognitive function
SIRT1	Upregulated by exercise and lactate; linked with AMPK	Metabolic sensor; deacetylates FoxO/PGC-1α; supports neuroprotection and hippocampal metabolism
PGC-1α	Activated by AMPK/SIRT1; drives mitochondrial biogenesis	Counters oxidative stress; induces BDNF via FNDC5/Irisin; promotes neuroplasticity
FNDC5/Irisin	Myokine secreted by muscle; PGC-1α-dependent	Upregulates BDNF; enhances memory pathways; antagonizes amyloid-β
VEGF	Elevated with exercise; lactate–GPR81 induced	Stimulates angiogenesis, neurogenesis; improves hippocampal blood flow

Table 1 Exercise-induced signaling molecules and their effects on brain health

2.4 The rising “star” of the molecular signaling effects of lactate

2.4.1 Research Progress of Lactate

In 1808, Swedish chemist Berzelius first described the accumulation of lactic acid in the bodies of living animals (Gladden, 2008). For over a century after that, lactic acid was considered as an energy supplier for muscle contraction. It wasn't until 1926, with the

discovery of ATP and the elucidation of the entire glycolytic pathway, that people realized lactic acid is an inert byproduct produced through anaerobic glycolysis in the absence of oxygen in the organism (Kresge et al., 2005). Consequently, for much of the 20th century, lactic acid was perceived as the primary cause of muscle fatigue and a critical factor in acidosis-induced tissue damage, primarily considered as a metabolic waste product detrimental to the body (Wasserman, 1984). However, research in recent decades has shown that lactic acid plays a crucial role in cellular signaling through its transporters (MCTs) and receptors (GPR81). It is involved in various biological processes such as energy regulation, immune tolerance, wound healing, as well as the growth and metastasis of cancer (Sun et al., 2017).

2.4.2 Lactate Metabolism

Lactate was once considered a byproduct of anaerobic metabolism in the body; however, recent research indicates that even in aerobic conditions, lactate continues to be produced, transported, and circulated (Dienel, 2012). Lactate production occurs as a result of glycolysis, regardless of whether glucose originates from blood or is derived from glycogen via glycogenolysis. During glycolysis, glucose is metabolized to pyruvate, which can be converted to lactate under conditions of insufficient oxygen or rapid energy demand. Importantly, lactate is not merely a byproduct of anaerobic metabolism but can also be produced and utilized under fully aerobic conditions. Acting as an intermediate compound, lactate serves as a substrate for mitochondrial respiration, linking glycolysis and aerobic metabolism (Brooks, 2018). Under normal oxygen conditions, approximately 1,500mM of lactic acid enters circulation daily in adults. Major contributors to lactate production include muscles (~25%), skin (~25%), brain (~25%), red blood cells (~20%), and intestines (~10%). The liver primarily clears the circulating lactic acid through

gluconeogenesis (Cori cycle) and ATP generation (oxidative phosphorylation, Krebs cycle), as illustrated in Figure 1.

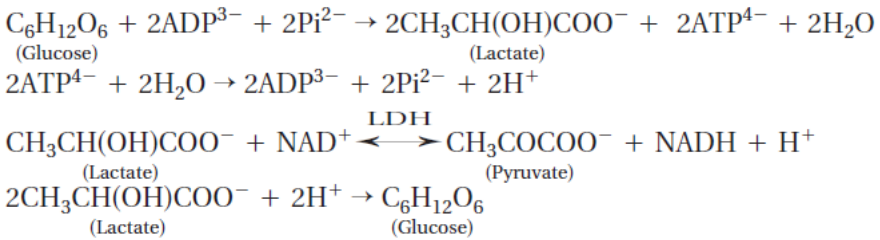

Figure 1 Metabolic equation of lactate generation and metabolism.

This schematic illustrates the key biochemical pathways involved in lactate generation and metabolism. Glucose is converted into lactate through glycolysis, producing ATP and releasing proton

Figure 1 depicts a simplified metabolic equation for lactic acid generation and metabolism. Reaction 1 illustrates the simplified conversion of glucose to lactic acid, while Reaction 2 shows the consumption of two ATP in Reaction 1, releasing 2H+ and causing acid accumulation. Reaction 3 demonstrates the mutual conversion between lactic acid and pyruvic acid catalyzed by lactate dehydrogenase (LDH), where, under normal physiological conditions, the concentration of lactic acid is approximately 10 times that of pyruvic acid. Reaction 4 illustrates lactic acid gluconeogenesis, predominantly occurring in the liver and, to a lesser extent, in the kidneys.

The metabolism of lactic acid undergoes alterations in different organs and under various physiological stress conditions. During exercise, the liver's lactic acid uptake can increase tenfold (Ahlborg et al., 1974). Simultaneously, during exercise, low blood sugar, increased adrenaline, and fasting, renal gluconeogenesis can contribute significantly to circulating glucose, increasing from approximately 5-16% to 40% (Meyer et al., 2002). Myocardial cells also utilize oxidative lactic acid as an energy source (Wisneski et al., 1985). At rest, lactic acid constitutes 10-15% of the heart's energy supply, which increases

to 30% during moderate-intensity exercise (Gertz et al., 1988). Studies with rodents have indicated that eliminating circulating lactic acid impairs heart function (Levy et al., 2007), highlighting the crucial role of lactic acid in normal cardiac physiology.

In recent years, research focused on lactic acid has centered on its role in brain energy metabolism and molecular signal transmission. Numerous studies, both domestic and international, have found that lactic acid is an indispensable factor in the normal physiological activity of the brain. Under the influence of neuronal activity signals, lactic acid is primarily formed from glucose or glycogen in astrocytes, subsequently transferring to neurons. This process ensures an ample energy supply for the brain, regulating neuronal excitability, adapting and shaping the brain, and maintaining memory consolidation. Lactic acid serves as a key signaling factor to maintain the "steady state" of the nervous system (Magistretti & Allaman, 2018). Additionally, changes in lactic acid metabolism occur in the diseased state of the body, leading to the accumulation of lactic acid in circulation, a condition known as lactic acidosis. Research has linked elevated lactic acid levels to various diseases such as shock, cardiac arrest, trauma, ischemia, diabetic ketoacidosis, liver dysfunction, and sepsis. Lactic acidosis exacerbates the condition and increases mortality in critically ill patients (Andersen et al., 2013).

In summary, despite extensive research exploring the metabolic characteristics of lactic acid in different organs and stress states, the specific mechanisms by which lactic acid functions in various organs and stress states are not yet clear due to its transmembrane shuttle properties, warranting further investigation.

2.4.3 Lactate Transport and Signal Transduction

Monocarboxylate Transporters (MCTs)

Lactate is transported across membranes through several Monocarboxylate Transporters (MCTs), which belong to the SLC16 gene family. Human genome analysis has identified

at least 14 members of the monocarboxylate transporter proteins, playing crucial roles in the transport of intercellular nutrients, cellular metabolism, and pH regulation. Among these 14 members, only four (MCT1-4) have been confirmed to transport monocarboxylates such as lactate, pyruvate, and ketone bodies (Halestrap & Meredith, 2004). Limited research has been conducted on MCTs 5-14, and ongoing studies suggest that MCT6 transports exogenous substances like bumetanide, nateglinide, and sulpiride (Murakami et al., 2005). MCT7 has been found to transport ketone bodies (Hugo et al., 2012), while MCT8 and MCT10 function in transporting thyroid hormones, with MCT10 also being associated with transporting aromatic amino acids (Halestrap & Meredith, 2004). Recent studies propose MCT9 as a key transporter for carnitine efflux, and MCT12 is considered a creatine transporter. Besides their roles in metabolite transport, MCT8, widely expressed at the blood-brain barrier, is linked to the Allan-Herndon-Dudley syndrome (Felmlee et al., 2020). Additionally, various MCT subtypes are associated with hormones, lipids, and glucose homeostasis (Felmlee et al., 2020). Due to their diverse functions, recent research has focused on the potential roles of MCTs in diseases, hoping to identify them as novel therapeutic targets (Felmlee et al., 2020).

MCTs responsible for lactate transport, namely MCT 1-4, are encoded by SLC16A1, SLC16A7, SLC16A8, and SLC16A3, respectively (Halestrap & Meredith, 2004). Among them, MCT1 has a moderate affinity for lactate (KM 3.5–10 mM) and is involved in the entry and efflux of monocarboxylates within cells, widely expressed in the human body (Fishbein et al., 2002; Manoharan et al., 2006). MCT2 primarily regulates the entry of monocarboxylates, with a higher affinity for lactate (KM 0.5 mM). It is less distributed in tissues but is mainly expressed in organs utilizing lactate as an energy substrate, such as the brain, heart, kidneys, liver, and red skeletal muscles (Halestrap, 2013; R.-Y. Lin et al., 1998). Research on MCT3 is limited, showing moderate substrate affinity for lactate (KM 6.0 mM) (Halestrap, 2012). MCT4 exhibits a low affinity for lactate (KM value of

22 mM) and is expressed in highly glycolytic tissues like white skeletal muscles, astrocytes, and leukocytes, primarily facilitating lactate efflux (Dimmer et al., 2000). The diverse functions and distribution of MCT subtypes result in varying impacts of lactate on different tissues and organs. The specific distribution of other MCT subtypes is illustrated in Figure 2 (Felmlee et al., 2020).

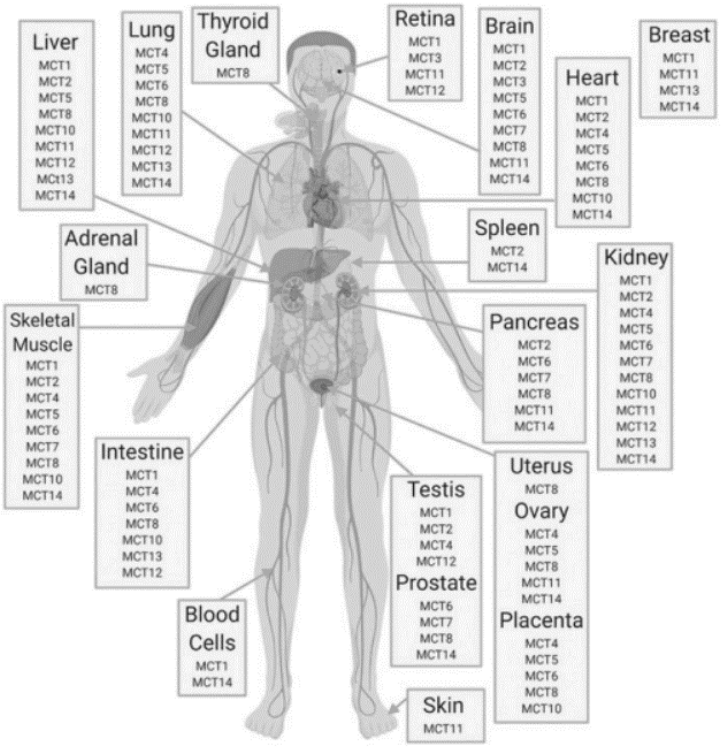

Figure 2 Tissue distribution of MCTs in the human body

This diagram illustrates the tissue-specific expression patterns of MCTs (MCT1–MCT14) in various human organs and systems.The diverse distribution reflects the varying metabolic needs and roles of MCTs across different tissues, highlighting their significance in physiological and pathological conditions.

The discovery and study of MCTs have provided clues for the "lactate shuttle" theory, further supporting the role of lactate in energy substance transfer and cellular signaling. The lactate shuttle theory includes extracellular and intracellular lactate shuttles. As shown in Figure 3 (Brooks, 2018), examples of intercellular lactate shuttles involve

lactate exchange between glycolytic and oxidative fibers during exercise, as well as lactate exchange between skeletal muscles and organs like the heart, brain, liver, kidneys, astrocytes, and neurons. Examples of intracellular lactate shuttles include lactate exchange between the cytoplasm and mitochondria, as well as between the cytoplasm and peroxisomes. Studies suggest that most intercellular and intracellular lactate shuttles are driven by lactate concentration, pH gradients, or redox states, with MCTs playing a crucial role in this process (Brooks, 2018). However, due to the complexity and uncertainty of lactate shuttling, there is still controversy over the specific mechanisms of action of MCTs in existing research.

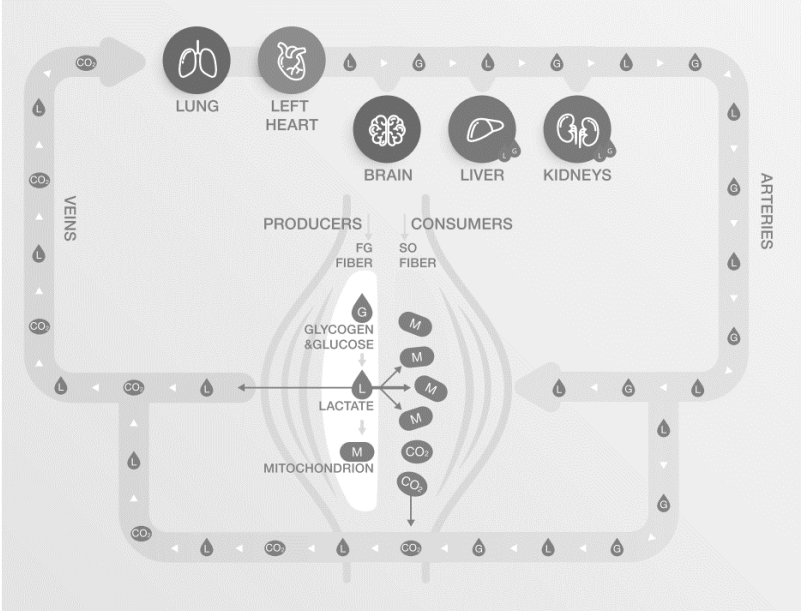

Figure 3 Exercise-induced lactate shuttle

This schematic illustrates the lactate shuttle mechanism, highlighting the roles of lactate as both a substrate and signaling molecule. Fast-glycolytic fibers in skeletal muscle produce lactate from glycogen and glucose, which is then released into circulation. Lactate can be taken up by slow-oxidative fibers, converted back into pyruvate, and used for mitochondrial respiration. Additionally, lactate is transported to various organs such

as the liver, kidneys, heart, and brain for utilization as an energy source and signaling molecule.

Recent studies have found that MCTs themselves play a significant role in various metabolic diseases in addition to lactate transport. For example, experiments on human subjects have revealed increased expression of MCT4 in the muscles of obese patients, decreasing with weight loss (Fisel et al., 2018). Additionally, in animal experiments, the deletion of MCT1 expression in mice resulted in obesity induced by an immune excess diet (Fisel et al., 2018). As lactate and MCTs are crucial components of body metabolism, understanding their roles can enhance our knowledge of the pathogenesis of various metabolic diseases. However, the specific mechanisms by which MCTs affect metabolism are still unclear and require further exploration.

Lactate Receptor: G-Protein-Coupled Receptor 81 (GPR81)

Over the past decade, GPR81 has been identified as a Gi protein-coupled receptor with high homology to GPR109A and GPR109B, with a molecular weight of approximately 40 kDa. While primarily located on the cell surface, recent studies suggest that GPR81 is also expressed in intracellular organelles such as mitochondria, indicating its potential role in lactate transport between the plasma membrane and intracellular compartments (T. P. Brown & Ganapathy, 2020; C. Liu et al., 2009). GPR81 is reported to be expressed in various human cells and tissues, including adipose tissue, skeletal muscle, liver, kidneys, and the brain, with its most significant expression observed in adipose tissue. The reason for the correlation between GPR81 and adipose tissue expression in different tissues is still under investigation (T.-Q. Cai et al., 2008). Moreover, the widespread expression of GPR81 in different tissues indicates its potential involvement in various physiological functions. Currently, GPR81 expression has been confirmed in humans and rodents, and genomic sequence observations suggest its expression in most mammals and fish (Offermanns et al., 2011).

Research on the molecular signaling of GPR81 is relatively limited, as it was initially considered an orphan receptor until the discovery in 2008 that lactate acts as a ligand activating GPR81. This realization gradually led to the understanding of the molecular signaling function between GPR81 and lactate (Offermanns et al., 2011). Existing studies suggest that GPR81 serves as a metabolic sensor, with physiological functions including the regulation of lipid metabolism in adipose tissue, influencing neuronal excitability changes, participating in cell development and survival, and modulating inflammatory responses. These findings provide new clues for unraveling the molecular signaling functions of GPR81 (Hu et al., 2020).

Lactate at concentrations ranging from 0.1 to 30.0 mM can activate GPR81, with experimental evidence suggesting an EC50 for lactate around 1-5 mM. GPR81 can be partially activated even at lactate concentrations as low as 0.2-1.0 mM (Dienel, 2012). Therefore, physiological lactate concentrations are sufficient to activate or partially activate GPR81. In addition to lactate, known ligands for GPR81 include 3,5-dihydroxybenzoic acid (3,5-DHBA; EC50: 0.15 mM), trichloro-5-hydroxybenzoic acid (EC50: 0.02 mM), and compound 2 (EC50: 50 nM) (Dvorak et al., 2012; C. Liu et al., 2012; Sakurai et al., 2014)

However, the physiological and pharmacological effects of GPR81 are still unclear for several reasons. Firstly, the rapid metabolism of lactate limits in vivo experiments on GPR81 using lactate. Secondly, despite the ongoing development of specific GPR81 agonists, no clinical studies have reported the application and efficacy of GPR81 agonists. Finally, existing experiments and studies do not clearly elucidate how GPR81 regulates downstream molecular signaling pathways. Despite these challenges, exploring the mechanisms by which GPR81 regulates metabolism and inflammatory signaling offers the potential for identifying new targets for the prevention and treatment of metabolic and

inflammatory diseases. Consequently, research on the lactate receptor GPR81 has attracted attention from scholars in various disciplines (Hu et al., 2020).

2.5 The Effect of Lactate on Brain Function

2.5.1 Lactate Metabolism in the Brain

Lactate metabolism in the brain primarily occurs through two pathways: blood lactate circulation and the conversion of glycogen to lactate in astrocytes. Monocarboxylic acid transporters (MCTs), specifically MCT1 to MCT4, facilitate the transport of lactate across cell membranes, including through the blood-brain barrier (BBB) (Huang et al., 2021). During exercise, as muscles contract, lactate is produced and released into the bloodstream. Some of this blood lactate can cross the BBB with the help of MCTs and enter the brain (Huang et al., 2021).

Previously, the accumulation of lactate in the brain was thought to contribute to central fatigue during exercise. However, studies on lactate kinetics have shown that circulating blood lactate serves as a primary energy source for the brain during exercise. This is evidenced by increased lactate utilization and decreased glucose utilization rates (Quistorff et al., 2008). Additionally, lactate derived from astrocytes is crucial for maintaining various brain functions. Normally, glucose is the brain's primary energy source, entering neurons and astrocytes through the BBB. Astrocytes store some of this glucose as glycogen (Proia et al., 2016). During periods of low glucose availability and increased metabolic demand, neuronal activity triggers the rapid breakdown of glycogen into lactate in astrocytes. This lactate is then released into the extracellular space through MCTs, providing neurons with an alternative energy source to sustain their function (A. M. Brown et al., 2004).

2.5.2 The Effects of Lactate on the Brain

The influence of lactate on the brain is multifaceted and has been extensively studied. Suzuki discovered that during learning tasks in rats, hippocampal astrocytes release lactate into the extracellular space, crucial for maintaining long-term potentiation (LTP), a process vital for learning and memory. Injection of L-lactate into the hippocampus rescued LTP impairment, indicating its role in neuronal signal enhancement (A. Suzuki et al., 2011). Furthermore, astrocyte-derived lactate is essential for sustaining brain function during exhaustive exercise (Matsui et al., 2017). Following experimental cerebral ischemia, lactate accumulation around the hematoma enhances neurogenesis and angiogenesis, contributing to recovery (J. Zhou et al., 2018). Exogenous lactate administration has shown neuroprotective effects after cerebral ischemia and traumatic brain injury (TBI) (Huang et al., 2021). Alvarez found that lactate-releasing biomaterial scaffold promotes the continuous production of neurons and glial cells, fostering angiogenesis (Song et al., 2018).

Method	Species	Significance	Reference
Subcutaneous injection of lactate	Adult C57BL/6 Mice	Increased brain VEGF-A protein and capillary density	(Morland et al., 2017)
Intraperitoneal injection of lactate	Adult C57BL/6 Mice	Promoted neurogenesis	(Lev-Vachnish et al., 2019)
Intraperitoneal injection of lactate	Adult C57BL/6 Mice	Upregulated the expression of BDNF-PGC1α-SIRT1 in the hippocampus and improved cognitive function	(El Hayek et al., 2019)
Intraperitoneal injection of lactate	Mice (AlCl3/D-gal treatment induced AD)	Improved cognitive function (AD mice)	(Han et al., 2023)
Intraperitoneal injection of lactate	Adult C57BL/6 Mice	Inhibited neuroinflammation in the prefrontal cortex and anti-depression (LPS)	(Hong et al., 2023)

Prefrontal cortex injection of lactate	Mice (CSDS induced depression)	Anti-depression (CSDS induced depression)	(Yao et al., 2023)
Intravenous injection of lactate	Adult Human	Increased BDNF content in the blood (Human)	(Schiffer et al., 2011)

Table 2 Effects of lactate on the brain (In vivo; Rodents and Human)

Plenty of other studies also proposed that exogenous lactate administration can promote neuroplasticity in rodents (Lev-Vachnish et al., 2019; J. Yang et al., 2014). Surprisingly, lactate may serve as a novel antidepressant, as seen in animal models where L-lactate administration reduces depression-like behaviors (Carrard et al., 2018; Karnib et al., 2019). However, conflicting results exist regarding lactate's cognitive effects, possibly due to dosing discrepancies.

In summary, lactate has diverse effects on the brain, impacting learning, memory, neuroprotection, and even mood regulation. Further elucidation of its molecular mechanisms could pave the way for targeted treatments.

Cell Type (Lactate treatment)	Significance	Reference
Primary cultured microglia (LPS treatment)	Inhibit neuroinflammation and promote elongation by activating AKT	(Hong et al., 2023)
BV2 microglia (OGD)	Inhibit neuroinflammation by inhibiting NF-κB	(Y. Zhang et al., 2023)
Human microglia cell line	Promoted M2 polarization in microglia	(Longhitano et al., 2023)
Hippocampal precursor cells	Promoted proliferation	(Pötzsch et al., 2021)
BV2 microglia	Promoted proliferation	(Y. Liu et al., 2020)

SH–SY5Y cells	Promoted neurite outgrowth and differentiation	(Y. Xu et al., 2023)

Table 3 Effects of lactate on the brain (In vitro)

2.5.3 The Involvement of Lactate in Exercise-Induced Brain Plasticity

Most researchers agree that exercise-induced brain plasticity involves increased expression or downstream signaling of neurotrophic factors such as BDNF and VEGF. Blocking BDNF signaling in the hippocampus attenuates exercise-induced neuroplasticity in rodents (Griesbach et al., 2009), while inhibition of circulating VEGF prevents exercise-induced hippocampal neurogenesis (Fabel et al., 2003).

Additionally, irisin, a myokine derived from FNDC5, has gained attention for its role in mediating exercise benefits (Young et al., 2019). FNDC5, regulated by PGC-1α, increases during exercise, leading to elevated circulating irisin. The PGC-1α/FNDC5/BDNF pathway is considered crucial for neuroprotection. Irisin is expressed in various brain regions and regulates essential brain functions (Boström et al., 2012). Studies indicate that exercise-induced irisin production in the hippocampus increases BDNF expression, promoting spatial learning and memory (Wrann et al., 2013). Reduced hippocampal irisin levels are observed in Alzheimer's disease (AD), and blocking irisin/FNDC5 impairs exercise-induced synaptic plasticity and memory in AD models (Lourenco et al., 2019). Similarly, irisin blockade attenuates exercise-induced neuroprotection after cerebral ischemia (Li et al., 2017). However, the mechanism of irisin action is not fully elucidated. The debate continues regarding whether peripheral irisin reaches the brain or triggers brain irisin increase through specific mechanisms. Additionally, little is known about the initial molecular signals of exercise-induced neuroplasticity and their transmission from muscles to the brain. Lactate and its receptors are implicated in various

exercise effects on the brain and may serve as initial molecular signals mediating beneficial brain adaptations. Studies have shown that exogenous lactate administration mimics some adaptive changes induced by exercise (E et al., 2013). Morland demonstrated that both HIIT and lactate injection increase brain lactate levels, VEGF, and capillary density in mice (Morland et al., 2017). They found a high concentration of the lactate receptor GPR81 in perivascular cells, suggesting its involvement in cerebrovascular remodeling. El Hayek showed lactate activates the PGC-1α/FNDC5/BDNF pathway in a SIRT1-dependent manner, enhancing spatial learning and memory (El Hayek et al., 2019). Lambertus demonstrated that lactate-induced GPR81 activation promotes adult neurogenesis in the subventricular zone (Lambertus et al., 2021). Human studies also support the link between lactate and BDNF, suggesting lactate's role in neuroplasticity (Huang et al., 2021).

In summary, lactate may act as a molecular signal via specific neurotrophic factors to activate neurogenesis or cerebrovascular plasticity, as described in Table 2 and Table 3. However, controversy exists regarding the precise mechanisms of lactate action in the brain, with some studies implicating PGC1-α/FNDC5/BDNF. Further research is needed to clarify these mechanisms and understand lactate's role in exercise-induced brain changes.

3. Objectives

3.1 Aim of the Study:

The primary aim of this research is to investigate the impact of long-term lactate intervention and HIIT on the brain neuroplasticity of aged wild-type mice.

3.2 Objectives

Objective 1: Determine how different doses of lactate affect brain health and biomarkers in aging mice.

- Administer three lactate doses to aged mice (20–22 months) and measure changes in blood lactate levels.
- Assess brain biomarkers such as SIRT1, VEGF, and AKT to evaluate the impact of varying lactate doses.
- Evaluate behavioral outcomes using the open field, novel object recognition, and passive avoidance tests.

Objective 2: Compare the effects of long-term lactate treatment and HIIT on brain health in aged mice.

- Use the most effective lactate dose from Objective 1 for extended treatment in older mice (25–27 months) to assess its effects on angiogenesis signaling, BDNF signaling, mitochondrial biomarkers, and hippocampal metabolism.
- Conduct long-term HIIT on aged mice and compare the outcomes with lactate treatment, focusing on behavioral tests and key brain biomarkers.

4. Methods

4.1 Grouping

Experiment 1: twenty 20-22 months old wild-type mice were randomly divided into five groups (n=4), including 1. Control group, which receives PBS injections. 2. High-intensity interval training group. 3. High-dose lactate injection group. 4. Medium-dose lactate injection group 5. Low-dose lactate injection group.

Experiment 2: twenty-one 25-27 months old wild-type mice were randomly divided into three groups (n=7) including 1. Control group, which receives PBS injections. 2. High-intensity interval training group. 3. Low-dose lactate injection group (Figure 4).

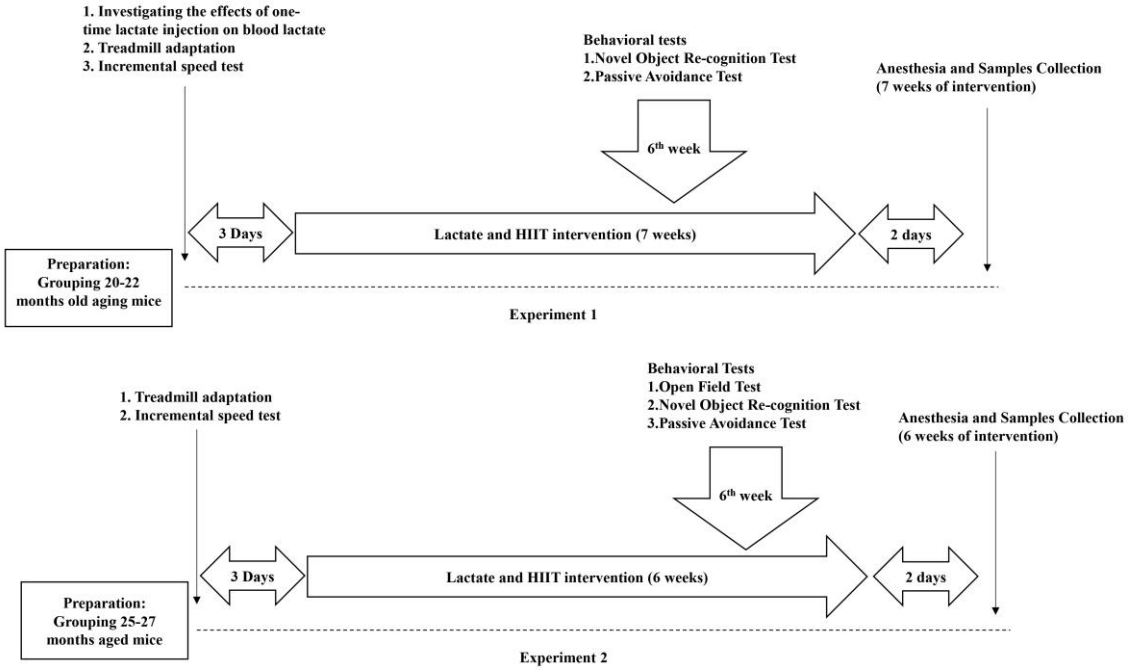

Figure 4 Timeline of the experiment.

This figure outlines the timeline and methodology for two experiments investigating the effects of lactate and HIIT interventions on brain health in aging and aged mice.

During the experiment, all animals were maintained in a thermoneutral room on a 12:12 h photoperiod and provided standard laboratory

chow and water ad libitum. All experiments were approved by the animal ethics committee of Sichuan University (K2020004) and conform with all the applicable institutional and governmental regulations on the ethical use of animals.

4.2 Lactate Intervention Protocol

High-dose lactate group mice received 2000 mg lactate per 1kg body weight of sodium lactate (Sigma-Aldrich, 71718) dissolved in phosphate-buffered saline (PBS; Sigma, 806552)-pH 7.4, sterile-filtered. Medium-dose lactate group received 1000 mg/kg of sodium lactate in PBS. Low-dose lactate group received 500 mg/kg of sodium lactate in PBS. The control for the lactate groups, the PBS group, and the HIIT groups were administered PBS at a volume that approximated the average volume administered to the lactate mice, around 0.2-0.3 ml per animal. Injections were performed intraperitoneally five times/per week for 7 weeks in experiment1 and 6 weeks in experiment2. Two days after the intervention, all animals were anesthetized with intraperitoneal injections of ketamine (50 mg/kg) perfused by saline. The brain was quickly removed and weighed, and the hippocampus was dissected, frozen in liquid nitrogen, and stored at -80 °C. A section of the hippocampus was homogenized in a buffer containing 137 mM NaCl, 20 mM Tris-HCl pH8.0, 2% NP 40, 10% glycerol, and protease inhibitors (PMSF, aprotinin, leupeptin, orthovanadate). Protein levels were determined using Bradford techniques.

4.3 Training Protocol

Mice from HIIT groups first participated in 3 days of adaptation training on a motorized treadmill to limit training stress. After the adaptation, mice in the HIIT group underwent a modified incremental speed test at a 10% incline. The test includes a 5-minute warm-up at 6 m/min and then gradually increases the speed for 1 m/min every 2 min until the mice cannot maintain the original movement speed on the running machine and resist moving after stimulation for 10s. We removed those who refused to run and recorded the

speed. Eventually, the average max speed is used to calculate the HIIT protocol speed in the formal test. The average max-speed in the aging mice group (20-22 months) is 22 m/min, while the max-speed in the aged mice group (25-27 months) is 18 m/min. In the formal test, the high-intensity interval training consists of 3 minutes of 85% max speed and 2 min of 45% max speed for ten cycles at a 10% incline, starting with a 5-minute warm-up at 50% max speed. The training was conducted five days per week for 7 weeks in experiment 1 and 6 weeks in experiment 2.

4.4 Open Field Tests

The open-field test is widely used to study the reaction to novelty and provides some insight into the state of anxiety in rodents (Toldy et al., 2009). Mice were positioned in the center of an open-field box consisting of a cylindrical arena of 80 cm diameter, divided into 20 sectors by concentric and radial lines, and surrounded by a 35 cm-high wall. The exploration time for each mouse is 5 min. The video is recorded to evaluate the time mice spent in inner and outer zones, which is widely used to indicate the animals' stress level and exploration of novelty.

4.5 Novel Object Recognition Tests

The novel object recognition test is used to study rodents' memory functions (Antunes & Biala, 2012). This test was performed in an open-field box (62.5x34.5x32 cm). Three trials were conducted per session. In the first trial, each mouse is placed in the middle of the empty open area and allowed for free exploration for 5 min. After 24 hours, each mouse was placed in the same area with two identical objects in the opposite quadrants of the area (NE and SW corners) and allowed free exploration for 5 min. The last test is conducted 3 hours after the second trial. A new object with a different size, color, shape, and material replaced the one used in the second test. The object's location remained the same to avoid any influence of spatial memory. The box and objects were cleaned after

each animal to remove any scent. The interaction time was recorded with the object (e.g., touching, climbing, and sniffing with the nose at a 2 cm distance). Memory's recognition index was calculated by the formula: time to investigate the new object/time to investigate both objects.

4.6 Passive Avoidance Tests

The Passive Avoidance task is a fear-aggravated test used to evaluate learning and memory in rodent models (Torma et al., 2014). The apparatus comprised two equally sized compartments, a dark one and a well-lit white compartment (20×25×25 cm each), separated by a small sliding door. All mice were brought to the testing room at least 30 minutes before testing, and the lights were turned off to allow the animals to acclimate to the darkened room. For the experiment, each mouse was picked up gently by its tail, removed from the home cage, and placed onto the grid floor in the illuminated area of the box, facing away from the door. The timer was initiated when the mouse was placed into the chamber. After 5 seconds, the door to the darkened area of the chamber was opened. Once the mouse entered the darkened area of the chamber, the door was closed, and scrambled footshock (0.8 mA) was delivered for 2 seconds. The animal remains in the dark compartment for an additional 20 seconds after terminating the aversive stimulus before being removed and placed back into its home cage. After 24 hours, the test was conducted again, and the latency of the entrance into the dark compartment was recorded. If a mouse failed to walk into the dark chamber within 300 seconds, then the timer was stopped, a maximum 300-second latency was recorded, and the mouse was returned to its home cage.

4.7 Western Blot

Proteins were electrophoresed on 8-12% v/v polyacrylamide SDS-PAGE gels and were transferred onto polyvinylidene difluoride PVDF membranes. The membranes were

subsequently washed, and after blocking, PVDF membranes were incubated at 4°C with antibodies (Table 4) including Tubulin (T6199), β-Actin (sc69879), VEGF (sc152),p-mTOR/mTOR (cst5536,2983), p-AKT/AKT (cst9271,4691), nNOS (bd610309), eNOS (ab76198), iNOS (cst13120), SIRT3 (cst#2627), Pan-Kla (STJ11101522), HIF-1α (H6536), BDNF (sc546), PGC-1α (nbp1-04676), SIRT1 (ab110304), CS (ab96600), SDHA (sc98253), FNDC5 (ab174833), p-HSL/HSL (cst18381s, 4139), p-CREB/CREB (cst9198,9197s), LDH (sc33781), Visfatin/Nampt (ab45890), and Synapsin (cst2312). After incubation with primary antibodies, membranes were washed 3x10 min in TBS-Tween-20 (TBS-T) and incubated with horseradish peroxidase (HRP)–conjugated secondary antibodies at 4°C for 1 hr. After incubation with a secondary antibody, membranes were repeatedly washed and developed by HRP reagent (Super Signal West Pico Chemiluminescent Substrate, Thermo Scientific #34080). The bands were quantified by ImageJ software and normalized to β-actin or tubulin, which served as an internal control. For all the Phosphorylated proteins, the phosphorylated one is developed at first, and then the same PVDF was striped and repeated for the first antibody of the total protein. The ratio is calculated based on the protein expression from the same PVDF.

Antibody	Source	Catalog Number	Dilution	Incubation Conditions
Tubulin	Sigma-Aldrich	T6199	1:15000	Overnight at 4°C
β-Actin	Santa Cruz Biotechnology	sc-69879	1:10000	Overnight at 4°C
VEGF	Santa Cruz Biotechnology	sc-152	1:300	Overnight at 4°C
p-mTOR (Ser2448)	Cell Signaling Technology	#5536	1:1000	Overnight at 4°C
mTOR	Cell Signaling Technology	#2983	1:1000	Overnight at 4°C
p-AKT (Ser473)	Cell Signaling Technology	#9271	1:1000	Overnight at 4°C
AKT	Cell Signaling Technology	#4691	1:1000	Overnight at 4°C

nNOS	BD Biosciences	610309	1:1000	Overnight at 4°C
eNOS	Abcam	ab76198	1:1000	Overnight at 4°C
iNOS	Cell Signaling Technology	#13120	1:1000	Overnight at 4°C
SIRT3	Cell Signaling Technology	#2627	1:1000	Overnight at 4°C
Pan-Kla	St John's Laboratory	STJ11101522	1:1000	Overnight at 4°C
HIF-1α	Sigma-Aldrich	H6536	1:500	Overnight at 4°C
BDNF	Santa Cruz Biotechnology	sc-546	1:200	Overnight at 4°C
PGC-1α	Novus Biologicals	NBP1-04676	1:1000	Overnight at 4°C
SIRT1	Abcam	ab110304	1:1000	Overnight at 4°C
Citrate Synthase (CS)	Abcam	ab96600	1:3000	Overnight at 4°C
SDHA	Santa Cruz Biotechnology	sc-98253	1:1000	Overnight at 4°C
FNDC5	Abcam	ab174833	1:1000	Overnight at 4°C
p-HSL (Ser563)	Cell Signaling Technology	#4139	1:1000	Overnight at 4°C
HSL	Cell Signaling Technology	18381S	1:1000	Overnight at 4°C
p-CREB (Ser133)	Cell Signaling Technology	#9198	1:500	Overnight at 4°C
CREB	Cell Signaling Technology	#9197	1:1000	Overnight at 4°C
LDH	Santa Cruz Biotechnology	sc-33781	1:500	Overnight at 4°C
Visfatin/NAMPT	Abcam	ab45890	1:1000	Overnight at 4°C
Synapsin	Cell Signaling Technology	#2312	1:3000	Overnight at 4°C
Secondary Antibody (Anti-Mouse IgG HRP)	Jackson ImmunoResearch	115-035-003	1:5000	1 hour at RT
Secondary Antibody (Anti-Rabbit IgG HRP)	Jackson ImmunoResearch	111-035-003	1:5000	1 hour at RT

Table 4 List of primary and secondary antibodies used in western blot analysis

4.8 Blood Lactate Measurement

Portable electrochemical devices Lactate Scout and Sensors (Nova Biomedical, 40828/40813) were used to determine the blood lactate level in a previously described protocol (L. Zhou et al., 2021; L. Zhou et al., 2024). A drop of blood was excised from the aseptically treated wound of the mice's tail. After resting for 20 min, resting blood lactate concentration was determined. Then, the mice were placed in a separate cage to avoid external stimuli for the subsequent tests. After different doses of lactate injection, the blood lactate level is tested at several time points until the blood lactate level recovers to resting concentration.

4.9 Tissue Lactate and Pyruvate Concentration Measurement

Lactate and pyruvate concentration of the hemisphere after long-term exercise and lactate intervention was assayed using pyruvate and lactate assay kits (Abcam; ab65330, ab65342). About 40±2 mg of the homogenized left hemisphere of seven mice of each group were weighed and lysed on ice. After deproteination, the sample was analyzed according to the protocol provided by the kit.

4.10 Statistical Analysis

The statistical analysis was performed using one-way analysis of variance (ANOVA) analysis followed by Dunnett's multiple comparisons tests using Graph pad prism version 9.1 software. Data were reported as mean ± standard deviation (SD). Statistical significance was denoted as *$p < 0.05$.

5. Results

5.1 The Dose-Dependent Effects of Lactate and Exercise on Brain Function of Aging Mice (Pilot study)

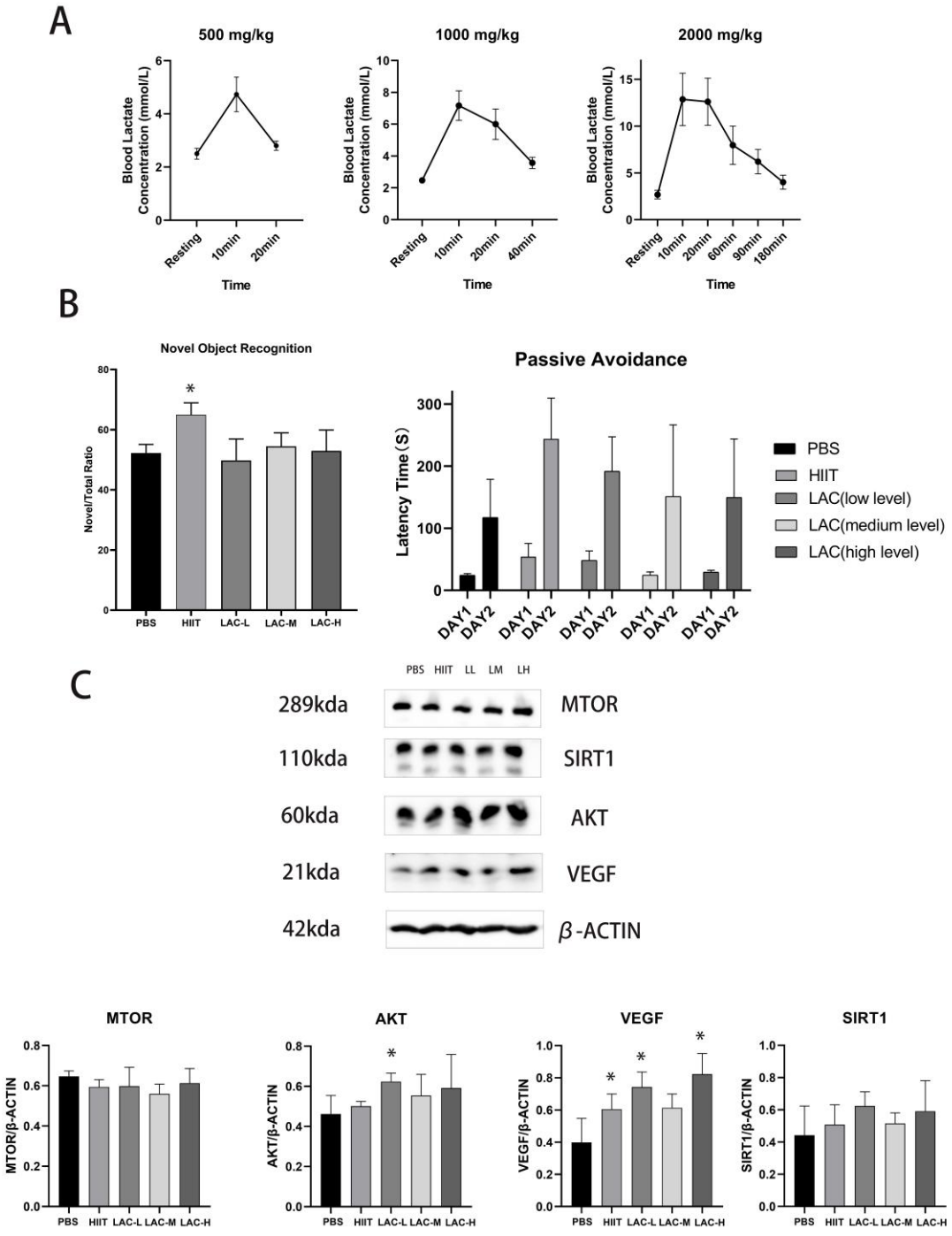

Figure 5 The dose-dependent effects of lactate and exercise on brain function of aging mice (Pilot study)

(A) The effect of different doses of lactate interventions on blood lactate level. (B) The effect of different doses of lactate injections and exercise on cognition tests performance of aging mice. (C) The effects of lactate and exercise on brain plastic biomarkers of aging mice's hippocampus. Data are presented as means ± SD (n = 4). Western blot data is based on each animal's protein expression. Low-level lactate injection (Lac-L), Medium-level lactate injection (Lac-M), High-level lactate injection (Lac-H). One-way ANOVA followed by Dunnett's test was used to compare experimental groups with the control. P < 0.05 was considered significant.

The primary aim of this pilot study is to test the dose-dependent effect of lactate and select the best volume to conduct the formal experiment. Three days before the start of the intervention, we confirmed the effects of lactate injection on blood lactate concentration. As shown in Figure 5 (A), the peak blood lactate concentration of the low-level lactate group reached 4.73±0.65 mmol/L 10 min after lactate injection and decreased to baseline levels at 20 min following injection. In the medium-level lactate group, peak lactate concentration reached 7.16±0.93 mmol/L 10 min after injection and decreased to baseline levels at 40 min following injection. As for the high-level lactate group, peak lactate concentration reached 13.85±2.8 mmol/L 10 min after intervention and decreased to baseline levels at 180 min following injection. In summary, higher injection volume will increase the blood lactate level and the recovery time. After five weeks of intervention, we tested the animal's performance in the novel object recognition and passive avoidance tests. As shown in Figure 5 (B), five weeks of HIIT significantly increased ($p=0.014$) the novel/total ratio in the novel object recognition test compared with the control group. While low, medium and high-level lactate injection does not affect novel object recognition tests. Furthermore, five weeks of HIIT ($p=0.0949$) and low-level lactate injection ($p=0.416$) slightly increased the latency time in the passive avoidance test, but the result was not significant. After seven weeks of intervention, we examined several

neuro-plastic biomarkers in the hippocampus, including SIRT1, VEGF, mTOR (Mechanistic Target of Rapamycin Kinase), and AKT, as indicated in Figure 5 (C). The result shows that both low (p=0.009) and high doses (p=0.034) of lactate injection significantly increase the protein expression of VEGF compared with the control group. In addition, SIRT1 and AKT are slightly increased in the low and high lactate injection groups; however, only low-level lactate injection (p=0.043) significantly increased AKT, while the others are not significant. Besides, we noticed that the animals' movement was slowed immediately after the high-dose lactate injection, and they behaved lethargy, including being immobile and staying in the corner. We hypothesized that 13.85±2.8 mmol/L blood lactate concentration might cause animal discomfort. Altogether, this pilot study verified that both low and high-dose lactate positively affect the brain biomarkers of aging mice. Since the low-dose protocol is comparatively safer and as effective as the high dose, we used the low-dose lactate protocol in subsequent experiments.

5.2 The Effect of Lactate and Exercise on Cognition Tests Performance of Aged Mice

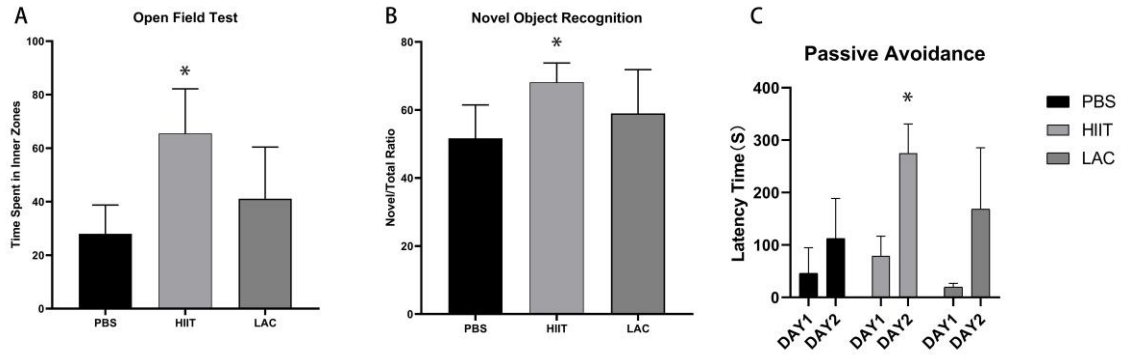

Figure 6 The effect of lactate and exercise on cognition tests performance of aged mice

*(A) Open field test (B) Novel object recognition test (C) Passive avoidance test. Data are presented as means ± SD (n = 7). Statistical significance was denoted as *p <0.05.*

After five weeks of intervention, animals in all groups underwent cognition tests in a sequence of open-field tests, novel object tests, and passive avoidance tests. As shown in

Figure 6 (A), compared with the control group, HIIT significantly increased ($p < 0.001$) the time (S) the animal spent in the inner area, while lactate did not affect ($p=0.263$) the performance of aged mice in the open field test. Figure 6 (B) indicates that five weeks of HIIT significantly increased ($p=0.021$) the investigation time of the novel object compared to the control group, while lactate slightly increased ($p=0.397$) the ratio, but the result is not significant. In the end, Figure 6 (C) indicates that HIIT significantly increased ($p=0.044$) the latency time the animal entered the dark chamber, while lactate did not affect ($p=0.599$) the aged mice's performance on the passive avoidance test. These results show that five weeks of HIIT effectively improves aged mice's performance in the open field, novel object recognition, and passive avoidance tests. Conversely, five weeks of lactate injection does not affect these tests.

5.3 The Effect of Lactate and Exercise on Hippocampus Angiogenesis Signaling Pathway in Aged Mice

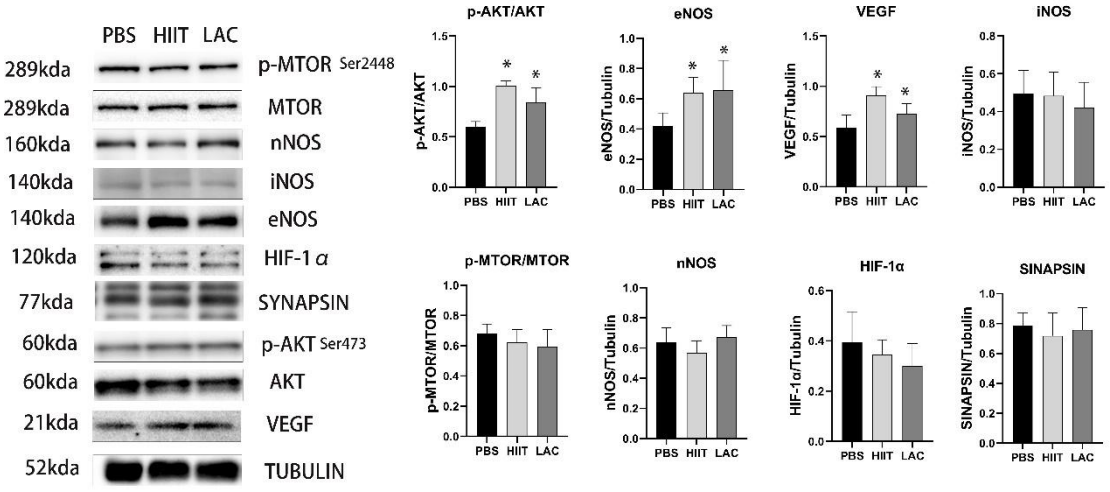

Figure 7 The effect of lactate and exercise on hippocampus angiogenesis signaling pathway in aged mice

*The effect of lactate and HIIT on hippocampus angiogenesis signaling pathway in aged mice. Data are presented as means ± SD (n = 5). Statistical significance was denoted as *p < 0.05. The western blot statistics are calculated by each animal's data, and the*

highest and lowest in each group are excluded from the statistics to remove extreme data due to technical issues (membrane/transfer artifacts).

As shown in Figure 7, compared with the control group, after six weeks of intervention, both HIIT ($p < 0.001$) and lactate injection ($p=0.002$) significantly increased the activity of AKT signaling (ser473), indicating elevated angiogenesis in the hippocampus of aged mice. However, the activity of its downstream signal, mTOR (ser2448), has not changed after the intervention. As a result of the elevated AKT pathway, the Endothelial Nitric Oxide Synthase (eNOS) protein expression is significantly increased in both HIIT ($p=0.048$) and lactate ($p=0.042$) groups. Furthermore, the protein expression of VEGF is also significantly increased in the HIIT ($p < 0.001$) and lactate group ($p=0.045$). In addition, we also tested several brain angiogenesis-related proteins, including nNOS and HIF-1α. Our result discovered that exercise and lactate do not affect the protein expression of nNOS and HIF-1α in the hippocampus of aged mice. Besides, our result shows that exercise and lactate do not affect the protein expression of SYNAPSIN.

5.4 The Effect of Lactate and Exercise on Hippocampus BDNF Signaling and Mitochondrial Biomarkers in Aged Mice

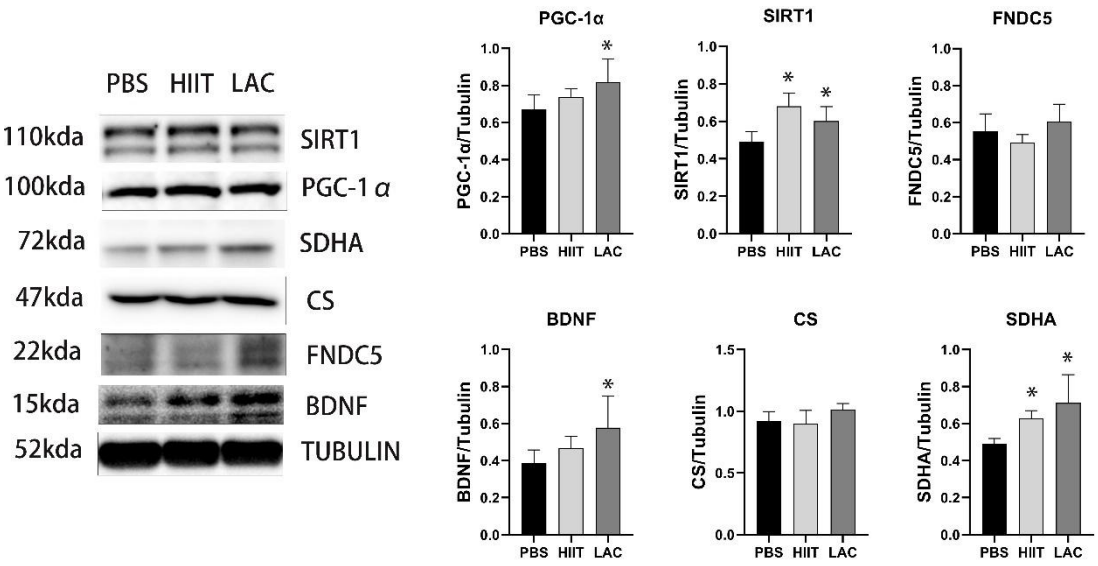

Figure 8 The effect of lactate and exercise on hippocampus BDNF signaling and mitochondrial biomarkers in aged mice

*The effect of lactate and HIIT on hippocampus BDNF signaling and mitochondrial biomarkers in aged mice. Data are presented as means ± SD (n = 5). Statistical significance was denoted as *p <0.05. The western blot statistic is calculated by each animal's data, and the highest and lowest in each group are excluded from the statistic to remove extreme data.*

Recent lactate and exercise studies have proposed that the elevation of BDNF is achieved through the PGC-1α-SIRT1-FNDC5-BDNF signaling. As shown in Figure 8, compared with the control group, lactate significantly increased (p=0.035) the BDNF protein expression in the hippocampus of aged mice. The upper signaling proteins, including PGC-1α (p=0.04), SIRT1 (p=0.034), and FNDC5 (p=0.226), also show an increasing tendency. On the contrary, the effect of HIIT on BDNF signaling in the hippocampus of aged mice is minimal. Six weeks of HIIT slightly increased the protein expression of

BDNF ($p=0.407$) and PGC-1α ($p=0.403$); however, the result is not significant. Only the SIRT1 protein expression is significantly elevated ($p<0.001$) by HIIT. Furthermore, we also involved two mitochondrial biomarkers, CS and SDHA. Our result shows that both HIIT ($p=0.047$) and lactate ($p=0.005$) significantly increased the protein expression of SDHA but without the increase of CS (HIIT $p=0.866$; Lactate $p=0.076$).

5.5 The Effect of Lactate and Exercise on Metabolic Content and Related Signaling in Aged Mice

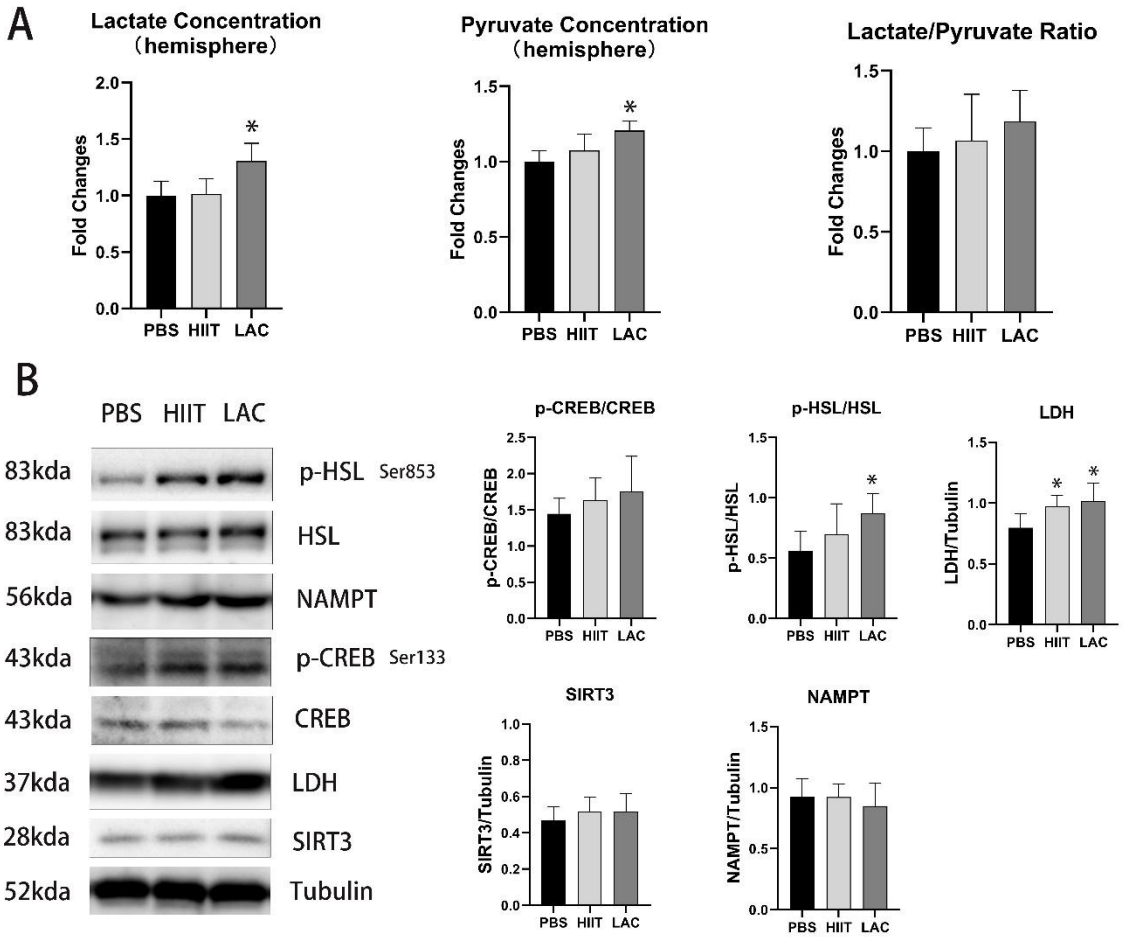

Figure 9 The effect of lactate and exercise on metabolic content and related signaling in aged mice

*(A) The effect of chronic lactate and exercise interventions on pyruvate and lactate concentration in the hemisphere of aged mice. (B) The effect of lactate and HIIT on hippocampus metabolism-related signaling in aged mice. Data are presented as means ± SD (n = 5). Statistical significance was denoted as *p <0.05. The western blot statistics are calculated by each animal's data, and the highest and lowest in each group are excluded from the statistic to remove extreme data.*

Recent studies have proposed that lactate is crucial in regulating energy metabolism. However, whether lactate affects metabolic pathways in the brains of aged mice is still unclear. In Figure 9 (A), compared with the control group, the lactate group shows a significant increase in pyruvate and lactate levels in the hemisphere of aged mice. However, the lactate/pyruvate ratio does not change after the intervention. As presented in Figure 9 (B), chronic HIIT slightly increased the signaling pathway of CREB (cAMP Response Element-Binding Protein) ($p=0.542$) and HSL (Hormone-Sensitive Lipase) ($p=0.356$) but without significant changes. On the other hand, lactate induced more prominent elevation. Six weeks of lactate intervention significantly increased ($p=0.047$) the HSL activity but failed to elevate ($p=0.225$) pCREB/CREB protein expression. Furthermore, both HIIT ($p=0.037$) and lactate ($p=0.009$) significantly elevated LDH protein expression while not affecting the protein expression of sirtuin 3 (SIRT3) and nicotinamide phosphoribosyltransferase (NAMPT).

5.6 The Effect of Lactate and Exercise on Pan Lactic acid-Lysine Protein Expression

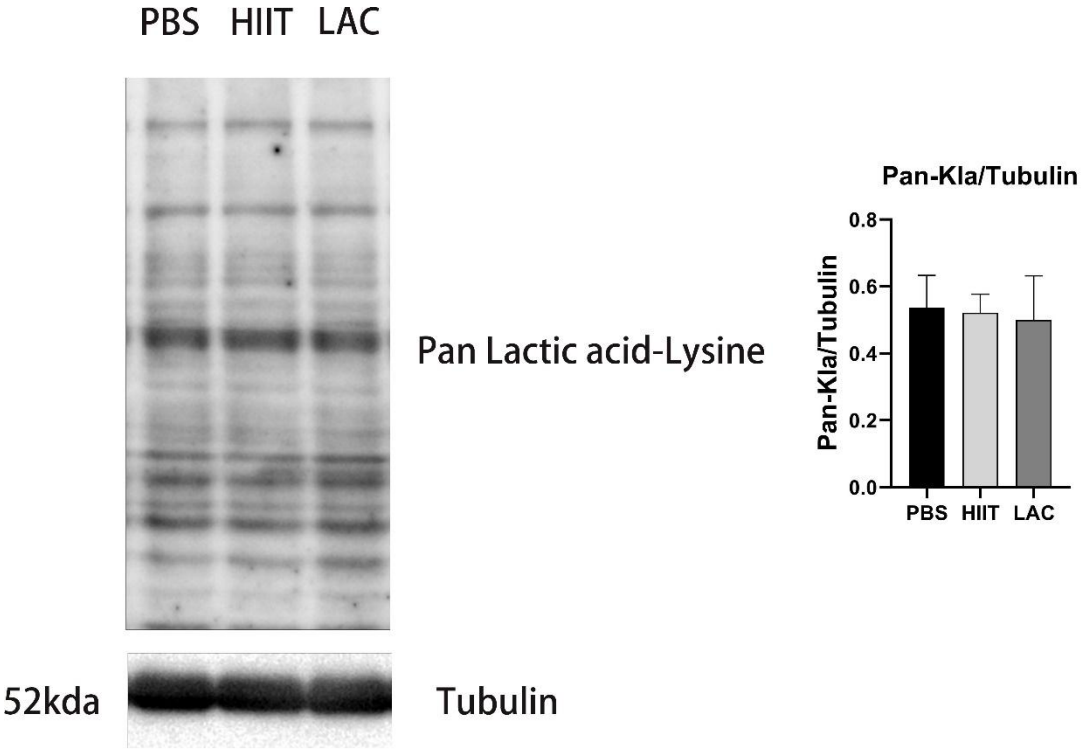

Figure 10 The effect of lactate and exercise on Pan Lactic acid-Lysine protein expression

*The effect of lactate and HIIT on Pan Lactic acid-Lysine protein expression in aged mice. Data are presented as means ± SD (n = 5). Statistical significance was denoted as *p < 0.05. The western blot statistics are calculated by each animal's data, and the highest and lowest in each group are excluded from the statistics to remove extreme data.*

Studies have proposed that lactate-derived lactylation of histone lysine residues serves as an epigenetic modification that directly stimulates gene transcription. Our result (Figure 10) shows that chronic lactate and HIIT intervention does not affect the Pan Lactic acid-Lysine protein expression in the hippocampus of aged mice.

6. Discussion

To our knowledge, this is the first study investigating the dose-dependent effects of lactate on the brain health of aging rodents, as well as exploring the impact of lactate on aged rodents' brain health. In the pilot study, our result demonstrates that both high (2000 mg/kg) and low (500 mg/kg) doses of chronic lactate injection have a positive influence on brain health biomarkers, including SIRT1, VEGF, and AKT in aging mice. However, no changes were observed in behavior tests. Conversely, long-term HIIT significantly improves performance in novel-object recognition tests for aging mice. In the subsequent formal study, our findings reveal that long-term lactate treatment (500 mg/kg) has beneficial effects on angiogenesis signaling pathway (p/t-AKT, eNOS, and VEGF), BDNF signaling (PGC-1α, SIRT1, and BDNF), mitochondrial biomarkers (SDHA), metabolism-related proteins (p/t-HSL and LDH) in the hippocampus of aged mice. However, chronic lactate treatment does not improve aged mice's performance in the open field, novel-object recognition tests, and passive avoidance tests. Conversely, chronic HIIT significantly improved aging mice's performance in these behavior tests. In summary, the present study proved that chronic HIIT and lactate treatment positively impact the brain functions of aged mice.

Research to date has provided novel insights into lactate's positive role in multiple brain functions and several brain diseases. Reviews from Cai, Hashimoto, and Muller have systematically summarized the function of lactate in the brain (M. Cai et al., 2022; Hashimoto et al., 2021; Müller et al., 2020). Based on current knowledge, this study tries to provide evidence of the dose-dependent effect of lactate and the effect of lactate on aged rodents. In our pilot study, we selected three doses of lactate concentration: 1. 2000 mg/kg, resulting in the blood lactate level reaching 13.85±2.8 mmol/L 10 min after intervention and decreasing to baseline levels at baseline 180 min following injection. 2. 1000 mg/kg resulting in the blood lactate level reaching 7.16±0.93 mmol/L 10 min after

injection and decreasing to baseline levels at 40 min following injection.3. 500 mg/kg resulting in the blood lactate level reaching 4.73±0.65 mmol/L 10 min after lactate injection and decreasing to baseline levels at 20 min following injection. In summary, our results show that the effect of lactate injection on blood lactate concentration is dose dependent. Higher injection volume will increase the blood lactate level and recovery time. Our data is in accordance with the previous studies. Morland et al. discovered that the 2500 mg/kg level of lactate injection elevates the blood lactate concentration to 14 mM, and after 7 weeks of intervention, the cerebral VEGF expression and angiogenesis are increased in adult rodents (Morland et al., 2017). Yaeli et al. suggested that a 1750 mg/kg level of lactate injection elevated the blood lactate level to 15.2 ± 1.94 mM and 2 weeks after the intervention, hippocampal neurogenesis is increased in adult rodents (Lev-Vachnish et al., 2019). One rodent study (El Hayek et al., 2019) proposed that 180 mg/kg and 117 mg/kg lactate injections can increase hippocampal lactate concentration, and after lactate injection, the BDNF signaling is activated in adult rodents. Here, this study concluded previous injection protocols and made a comparison of the dose-dependent effect of lactate. Our results show that both low and high-dose lactate have the same effects on biomarkers of brain function. Interestingly, the middle dose of lactate injection is less effective than the low and high doses. Because of our limited animal number, we cannot verify but further hypothesize that the dose of lactate intervention is a critical factor in lactate's effects. As a result, we used the low-level lactate injection protocol for the follow-up formal study. It is also important to mention that after different doses of chronic lactate intervention, the aging animals' performance in the novel object recognition test and passive avoidance test did not change. On the contrary, chronic HIIT significantly increased the performance of aging mice in novel object recognition tests and passive avoidance tests.

Current research has well-established that exercise delays brain aging preserves memory and cognition, and improves symptoms of neurodegenerative pathologies (Sujkowski et al., 2022). In our formal study, we verified that long-term HIIT has a positive effect on the brain health of aged mice, indicated by the significant increase in the performance in the open-field test, novel object recognition test, and passive avoidance test. This result is in line with previous studies. Several experiments have discovered that long-term exercise can increase the aged mice's performance in passive avoidance tests, novel object recognition tests, and open field tests (Amirazodi et al., 2020; Samorajski et al., 1985). Regarding lactate's effect, our data show that lactate intervention alone can slightly increase the mice's performance in the passive avoidance test and novel object recognition test, but the result is not significant. According to previous studies conducted on adult animals, some researchers proposed that lactate can increase the mice's performance in the Morris maze test (Amirazodi et al., 2020), while other studies suggest that lactate cannot increase the performance (Lev-Vachnish et al., 2019). Our results prove that lactate alone is less effective than HIIT in improving aged mice's learning and memory ability and exploration habits, as indicated by behavior tests.

In addition to cognition tests, this study also investigated the brain function-related signaling pathway. Numerous studies have concluded that exercise and lactate intervention can increase the angiogenesis signaling pathway in adult rodents, and the brain's angiogenesis is closely related to brain health (Morland et al., 2017). However, whether lactate affects aged animals is still unclear. Investigating this question will provide evidence for the clinical usage of lactate in neurodegenerative diseases. Our results show that both chronic HIIT and lactate intervention significantly increased the activity of AKT signaling, indicating elevated angiogenesis in the hippocampus of aged mice (Karar & Maity, 2011). However, the downstream signal, mTOR, has not changed after the intervention. As a result of the elevated AKT pathway, the eNOS and VEGF

protein expression is significantly increased in both HIIT and lactate groups. In addition, we also tested several brain angiogenesis-related proteins, including neuronal nitric oxide synthase (nNOS), inducible nitric oxide synthase (iNOS), and HIF-1α. Our result discovered that exercise and lactate do not affect the protein expression of these proteins in the hippocampus of aged mice. Altogether, our results show that both long-term exercise and lactate intervention can activate hippocampus angiogenesis in aged mice.

Furthermore, this study investigated the effect of lactate and exercise on BDNF signaling and mitochondrial biomarkers, including PGC-1α, SIRT1, FNDC5, BDNF, CS, and SDHA. As early as 2013, one study (Wrann et al., 2013) discovered that exercise induces hippocampal BDNF through a PGC-1α/FNDC5 pathway, and the elevation of BDNF is associated with improved cognitive function. In recent years, one rodent (El Hayek et al., 2019) proposed that lactate promotes BDNF through PGC-1α, SIRT1, FNDC5, and BDNF signaling. Also, studies have suggested that lactate intervention has a positive effect on mitochondria in the brain (Park et al., 2021), skeletal muscle (S. Chen et al., 2021; L. Zhou et al., 2021), and white adipose tissues (Qu et al., 2022). However, data regarding aged rodents is unclear. Our results show that lactate significantly increased the BDNF protein expression in the hippocampus of aged mice. The upper signaling protein, including PGC-1α, SIRT1, and FNDC5, also shows an increasing tendency. On the contrary, the effect of HIIT on BDNF signaling in the hippocampus of aged mice is minimal. Six weeks of HIIT slightly increased the protein expression of BDNF and PGC-1α; however, the result is not significant. Only the SIRT1 protein expression is significantly elevated by HIIT. Previous studies also reported that the effect of exercise on BDNF expression is smaller in aged animals compared to young ones (Adlard et al., 2005). Here, we prove that long-term lactate intervention is more effective than exercise in promoting BDNF signaling. Furthermore, we also involved two mitochondrial biomarkers, CS and SDHA. Our result shows that both HIIT and lactate significantly

increased the protein expression of SDHA but without the increase of CS. Altogether, our results show that lactate effectively improves BDNF signaling and mitochondrial biomarkers in the hippocampus of aged mice, and this effect is more substantial than exercise.

Recent studies have suggested a close relationship between lactate and metabolism. One research proposed the underestimated physiological potential of lactate (Rabinowitz & Enerbäck, 2020). However, whether long-term lactate intervention affects metabolic pathways in the brains of aged mice is still unclear. Previous studies have reported that the increased lactate level in the brain is related to the increased brain blood flow (Mintun et al., 2004). On the contrary, lactate content in the brain decreases during memory impairment in mouse models of AD (M. Zhang et al., 2018). Furthermore, recent studies have proposed that pyruvate, in addition to its well-recognized function in energy metabolism, has a unique combination of neuroprotective effects (Zilberter et al., 2015). As presented in this study, our data suggest that long-term lactate intervention significantly increased the lactate and pyruvate concentration in the hemisphere of aged mice without changing the lactate and pyruvate ratio. While compared with the control group, long-term HIIT does not alter the pyruvate and lactate concentration. Brain LDH is responsible for the interconversion of pyruvate and lactate. Previous studies have reported that blood LDH levels can be triggered by both resistance and aerobic exercise (Callegari et al., 2017). To our knowledge, this is the first study examining the effect of HIIT and lactate intervention on aged rodents' hippocampus LDH expression. Our results show that chronic lactate and HIIT can elevate LDH and thereby may positively improve the capacity to process lactate and pyruvate. Also, our results show that chronic HIIT slightly increased the signaling pathway of CREB/HSL but without significant changes. On the other hand, lactate induced more prominent elevation. Long-term lactate intervention significantly increased the HSL activity with nonsignificant elevated p/t-

CREB/CREB expression. Previous studies have indicated that the activation of CREB can promote BDNF expression (Amidfar et al., 2020). In our study, long-term lactate intervention slightly increased CREB activity with elevated BDNF protein expression. As a result of CREB elevation, the phosphorylation of the HSL ratio also increased. The functional role of this enzyme in the brain remains unexplored. This study first proposes that lactate intervention can significantly increase the activity of HSL in a way that might be related to CREB. Moreover, this study reports that HIIT and lactate do not affect the protein expression of SIRT3 in the hippocampus of aged mice. Our previous study (L. Zhou et al., 2022) has indicated that the effect of exercise on SIRT3 expression is time-dependent; thus, the duration of our training protocol is not enough to trigger SIRT3 elevation. While NAMPT/VISFATIN is the rate-limiting enzyme in NAD+ salvage pathways (Ji & Yeo, 2022). Our data show that chronic exercise and lactate intervention do not change the protein expression of NAMPT/VISFATIN. Altogether, our results show that lactate is effective in elevating the pyruvate and lactate concentration, CREB/HSL signaling pathway, and LDH protein expression in the brain of aged mice.

Recent studies have proposed that lactate-derived lactylation of histone lysine residues serves as an epigenetic modification that directly stimulates gene transcription (D. Zhang et al., 2019). Several studies have identified the pivotal role of protein lactylation in cell fate determination, embryonic development, inflammation, cancer, and neuropsychiatric disorders, offering key breakpoints for further functional and mechanistic research (X. Liu et al., 2022). Our result shows that lactate and HIIT intervention does not affect the Pan Lactic acid-Lysine protein expression in the hippocampus of aged mice. It is important to mention that we harvested the sample 48 hours after the last intervention. Further time-dependent and age-dependent studies are needed to further explore the mechanisms of lactate-induced lactylation.

To our knowledge, this is the first study systematically evaluate the dose-dependent effects of long-term exogenous lactate administration on brain in aged mice and to compare these effects with HIIT. While previous studies have largely focused on adult animal models and acute interventions, our work expands the current understanding by providing critical insights into how chronic lactate exposure influences key molecular pathways related to angiogenesis, neurotrophic signaling (e.g., BDNF, PGC-1α, SIRT1), and brain metabolism in aged models. Moreover, this study highlights the differential impact of lactate versus HIIT on behavioral outcomes and molecular markers, demonstrating that while lactate enhances molecular markers of neuroplasticity more robustly, HIIT yields superior improvements in cognitive performance. This dual approach not only validates lactate as a potential therapeutic agent for age-related cognitive decline but also sets the stage for further mechanistic investigations into its signaling role and possible synergy with exercise in neurodegeneration and healthy brain aging.

7. Conclusions

In summary, our study provides evidence that both long-term exercise and lactate interventions provide beneficial effects to brain health in aging rodents. Through an analysis of various biomarkers and behavior tests, we observed distinct yet complementary impacts of these two interventions on the aging brain.

Firstly, the exercise group exhibited better cognitive performance in novel object recognition tests and passive avoidance tests, indicative of enhanced learning and memory abilities. This improvement was accompanied by upregulated angiogenesis signaling and improved mitochondrial biomarkers in the hippocampus, suggesting a multifaceted enhancement of brain health through exercise.

Conversely, lactate intervention yielded its own array of positive effects on brain function. While not as pronounced in behavior tests compared to exercise, lactate intervention significantly improved angiogenesis signaling, BDNF signaling, mitochondrial biomarkers, and metabolic content and signaling in the hippocampus of aged mice. These findings highlight the potential of lactate as a therapeutic agent for addressing neurodegenerative diseases, particularly given its ability to modulate critical pathways involved in brain health and function.

Nevertheless, it's important to mention the limitations of this study, including the need for further investigation into the long-term effects of lactate intervention and its potential interactions with other physiological processes. Additionally, future research should investigate the specific mechanisms underlying lactate's effects on brain function, including its role in epigenetic modifications such as lactylation of histone lysine residues.

In conclusion, our study underscores the promising potential of both exercise and lactate interventions in preserving brain health during aging. These findings suggest that lactate and HIIT may contribute to promoting brain health during aging. However, further

research is necessary to fully understand the underlying mechanisms and to explore the potential therapeutic applications of lactate in neurodegenerative diseases.

8. Summary

Extensive research has confirmed numerous advantages of exercise for promoting brain health. More recent studies have proposed the potential benefits of lactate, the by-product of exercise, in various aspects of brain function and disorders. However, there remains a gap in understanding the effects of lactate dosage and its impact on aged rodents. The present study first examined the long-term effects of three different doses of lactate intervention (2000 mg/kg, 1000 mg/kg, and 500 mg/kg) and HIIT on aging mice (20-22 months) as a pilot investigation. Subsequently, we investigated the long-term effects of 500 mg/kg lactate intervention and HIIT on brain neuroplasticity in aged mice (25-27 months).

The results of the pilot study demonstrated that both HIIT and different doses of lactate intervention (500 mg/kg and 2000 mg/kg) positively impacted the neuroplasticity biomarker VEGF in the hippocampus of aging mice. Subsequently, the follow-up study revealed that long-term HIIT significantly improved the performance of mice in open-field, novel object recognition, and passive avoidance tests. However, lactate intervention did not significantly affect these behavioral tests. Moreover, compared to the control group, both HIIT and lactate intervention positively influenced the angiogenesis signaling pathway (p/t-AKT/eNOS/VEGF), mitochondrial biomarker (SDHA), and metabolic protein (p/t-CREB, p/t-HSL, and LDH) in the hippocampus of aged mice. Notably, only lactate intervention significantly elevated the BDNF (PGC-1α, SIRT1, and BDNF) signaling pathway and metabolic content (lactate and pyruvate). In the end, long-term HIIT and lactate intervention failed to change the protein expression of p/t-mTOR, iNOS, nNOS, HIF-1α, SYNAPSIN, SIRT3, NAMPT, CS, FNDC5, and Pan Lactic Acid-Lysine in the hippocampus of aged mice.

In summary, the present study proved that long-term HIIT and lactate treatment have positive effects on the brain health of aged mice, suggesting the potential usage of lactate as a therapeutic strategy in neurodegenerative diseases in the elderly population.

Keywords: Lactate intervention; Exercise; Aging; Brain function; Lactylation; Hippocampus

Összefoglaló

Kiterjedt kutatások megerősítették a testmozgás számos előnyét az agy egészségének elősegítésében. Az újabb tanulmányok javasolták a laktát, a testmozgás melléktermékének, potenciális előnyeit az agyműködés és az agyi rendellenességek különböző aspektusaiban. Azonban még mindig van egy hiányosság a laktát adagolásának hatásainak megértésében és annak öregedő rágcsálókon való hatásában. A jelenlegi tanulmány először vizsgálta a három különböző dózisú laktát-intervenció (2000 mg/kg, 1000 mg/kg és 500 mg/kg) és a magas intenzitású intervallum edzés (HIIT) hosszú távú hatásait az öregedő egerekre (20-22 hónap) egy kísérleti vizsgálatként. Ezt követően megvizsgáltuk a hosszú távú, 500 mg/kg laktát-intervenció és a HIIT hatásait az agy neuroplaszticitására idős egerekben (25-27 hónap).

A kísérleti vizsgálat eredményei azt mutatták, hogy mind a HIIT, mind a különböző dózisú laktát-intervenció (500 mg/kg és 2000 mg/kg) pozitív hatást gyakorolt a neuroplaszticitás biomarker VEGF-re az öregedő egerek hippocampusában. Ezt követően a nyomonkövetési vizsgálat kimutatta, hogy a hosszú távú HIIT jelentősen javította az egerek teljesítményét a nyílt tér, a tárgyfelismerési és a passzív elkerülési tesztekben. Azonban a laktát-intervenció nem befolyásolta jelentősen ezeket a viselkedési teszteket. Ezenkívül, a kontrollcsoporthoz képest mind a HIIT, mind a laktát-intervenció pozitívan befolyásolta az angiogenezis jelátviteli útvonalat (p/t-AKT/eNOS/VEGF), a mitokondriális biomarkert (SDHA), és az anyagcserefehérjéket (p/t-CREB, p/t-HSL és LDH) az öregedő egerek hippocampusában. Figyelemre méltó, hogy csak a laktát-intervenció jelentősen növelte a BDNF (PGC-1α, SIRT1 és BDNF) jelátviteli útvonalat és az anyagcseretartalmat (laktát és piruvát). Végül, a hosszú távú HIIT és a laktát-intervenció nem változtatta meg a p/t-mTOR, iNOS, nNOS, HIF-1α, SYNAPSIN, SIRT3, NAMPT, CS, FNDC5 és a Pan Lactic aid-Lysine fehérje expresszióját az öregedő egerek hippocampusában.

Összefoglalva, a jelen tanulmány bebizonyította, hogy a hosszú távú HIIT és a laktátkezelés pozitív hatással van az öregedő egerek agyi funkcióira, ami a laktát potenciális használatát sugallja a neurodegeneratív betegségek terápiás stratégiájaként az idősebb populációban.

Kulcsszavak: Laktát-intervenció; Testmozgás; Öregedés; Agyműködés; Laktiláció; Hippocampus

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10. List of Own Publications

Publications related to this study

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Publications independent of this study

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