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

Abstract of PhD Thesis

Zhou Lei

Doctoral School of Sport Sciences

Hungarian University of Sports Science

Supervisor: Dr. Zsolt Radák, professor, DSc

Official reviewers: Dr. Csaba Csonka, associate professor, PhD
Dr. Karolina Pircs, senior researcher, PhD

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1. Introduction

The prevalence of age-related cognitive decline and neurodegenerative diseases such as Alzheimer's and Parkinson's disease is expected to rise dramatically with global population aging. Aging is accompanied by mitochondrial dysfunction, oxidative stress, impaired autophagy, chronic neuroinflammation, and diminished neurogenesis, all contributing to memory deficits and reduced brain plasticity. Thus, developing interventions to mitigate brain aging is a major biomedical priority.

Exercise has consistently been demonstrated to protect the brain, improving cognitive performance and neuroplasticity through induction of neurotrophic (e.g., brain-derived neurotrophic factor, BDNF), angiogenic (e.g., vascular endothelial growth factor, VEGF), and metabolic (e.g., sirtuins, PGC-1α) signaling pathways. Endurance and resistance exercise are known to slow down age-associated decline, but high-intensity interval training (HIIT) has recently emerged as a time-efficient and potent modality to enhance maximal oxygen uptake, mitochondrial biogenesis, and brain-derived signaling.

Beyond oxygen delivery and mechanical stimulation, exercise substantially alters systemic metabolism. Lactate, once regarded merely as a metabolic byproduct, is now recognized as an essential exerkine—a metabolite with signaling functions. Lactate crosses the blood-brain barrier via monocarboxylate transporters (MCT1/2), activates hydroxycarboxylic acid receptor 1 (HCAR1/GPR81), and has been implicated in neurogenesis, angiogenesis, and memory regulation. Evidence shows that lactate administration can reproduce some exercise-induced benefits, partly via the SIRT1–PGC-1α–FNDC5–BDNF axis.

However, critical gaps remain: (i) most lactate studies have been performed in adult rather than aged animals, (ii) dose-dependent effects of chronic lactate exposure are poorly defined, and (iii) direct comparisons between lactate and exercise in aged brain are lacking. Moreover, while acute lactate can induce protein lactylation, the role of long-term lactate exposure in histone or non-histone lactylation within aged hippocampus remains uncertain.

This thesis addresses these gaps by systematically testing dose-dependent lactate effects in aged mice, and by performing a head-to-head comparison of long-term lactate versus

HIIT on behavior, angiogenesis, neurotrophic pathways, mitochondrial markers, and hippocampal protein lactylation.

2. Objectives

2.1 Overall aim

To determine how long-term lactate administration compares with HIIT in modulating neuroplasticity and behavior in aged mice.

2.2 Specific Objectives

Dose-finding (20–22 months): Establish a tolerated lactate dose that elevates hippocampal pro-angiogenic signaling (AKT/eNOS/VEGF) without adverse effects.

Efficacy comparison (25–27 months): Compare low-dose lactate with HIIT across:
(i) Behavior: open-field exploration, novel object recognition (recognition index), and passive-avoidance memory (latency).
(ii) Angiogenesis signaling: p/t-AKT (Ser473), eNOS, and VEGF abundance in hippocampus.
(iii) Neurotrophic and metabolic pathways: PGC-1α, SIRT1, BDNF, mitochondrial/energetic markers, and tissue lactate/pyruvate content.

2.3 Hypotheses

HIIT would improve cognition and activate angiogenic pathways in aged mice.

Chronic lactate administration would stimulate angiogenic and neurotrophic signaling but may not fully replicate exercise-induced behavioral improvements.

3. Methods

3.1 Animals, Ethics, and Housing

Wild-type male mice were studied in two age windows: aging (20–22 months; dose-finding) and aged (25–27 months; efficacy). Animals were housed under standard conditions (12-h light/dark, temperature-controlled rooms), with ad libitum access to chow and water.

3.2 Experimental Design

Experiment 1 (dose-finding, aging mice): Groups (n=4/group): Control (PBS), HIIT, high-dose lactate, medium-dose lactate, low-dose lactate. Endpoints: behavior, hippocampal angiogenic markers.

Experiment 2 (efficacy, aged mice): Groups (n=7/group): Control (PBS), HIIT, low-dose lactate (dose selected from Experiment 1). Endpoints: behavior, hippocampal angiogenesis and neurotrophic signaling, metabolites.

3.3 Interventions

Lactate administration: Sodium lactate was injected intraperitoneally 5×/week for 6–7 weeks (depending on experiment). In Experiment 1, three doses (500/1000/2000 mg/kg) were used; in Experiment 2, the 500 mg/kg was used.

HIIT protocol: Treadmill running at 10% incline, following an individualized familiarization and speed calibration. Each HIIT session comprised 3-min intervals (~85% of the calibrated maximum speed) alternating with 2-min recovery (~45%), repeated for 10 cycles after warm-up, 5×/week for 6–7 weeks.

3.4 Behavioral Testing

Open Field Test: Exploration and anxiety-like behavior assessed via center-zone time and locomotion.

Novel Object Recognition: Memory performance measured by recognition index after 24-h retention.

Passive Avoidance: Retention latency used as a marker of aversive memory.

3.5 Tissue Collection and Molecular Assays

Mice were euthanized 48 h after the final session to avoid acute exercise effects. Hippocampus were dissected, snap-frozen, and processed for Western blot. Targets included angiogenic (p/tAKT, eNOS, VEGF), neurotrophic/metabolic (PGC-1α, SIRT1, BDNF, FNDC5), mitochondrial (SDHA, CS, LDH, SIRT3, NAMPT), and signaling proteins (p/tCREB, p/tHSL). Band intensities were normalized to appropriate loading controls and expressed relative to Control.

3.6 Metabolite Assays

Tissue lactate and pyruvate were quantified using enzymatic colorimetric/fluorometric assays according to manufacturer instructions. The lactate/pyruvate (L/P) ratio was calculated as an index of cytosolic redox state.

3.7 Statistics

Data are presented as mean ± SD. Group differences were analyzed using one-way ANOVA followed by Dunnett's post-hoc test against Control. Significance was set at $p = 0.05$.

4. Results

4.1 Pilot Study

Blood lactate kinetics: Lactate rose dose-dependently with peaks at ~10 min post-injection (Lac-L ~4.7 mmol/L; Lac-M ~7.2; Lac-H ~13.9). Recovery time ranged from ~20 min (low) to ~180 min (high). Transient lethargy was observed at the highest dose.

Behavior: HIIT significantly improved NOR performance; no significant behavioral changes were seen with lactate.

Signaling: VEGF and AKT increased at Lac-L and Lac-H. Based on efficacy and safety, 500 mg/kg was chosen for the main study.

4.2 Efficacy Study

Behavioral outcomes: HIIT significantly improved Open Field Test center time, Novel Object Recognition recognition index, and Passive Avoidance retention latency. Lactate did not significantly alter behavioral performance.

Angiogenesis signaling: Both HIIT and lactate increased p/t AKT, eNOS, and VEGF, while mTOR remained unchanged.

Neurotrophic pathways: Lactate significantly increased BDNF, PGC-1α, and SIRT1, with FNDC5 trending upward. HIIT increased SIRT1 but had modest effects on BDNF and PGC-1α.

Mitochondrial markers: Both interventions upregulated SDHA and LDH; CS, SIRT3, and NAMPT remained unchanged.

Metabolites: Lactate increased hippocampal lactate and pyruvate concentrations but did not alter the lactate/pyruvate ratio.

Pan-lactylation: No significant changes were detected in hippocampal pan-lysine lactylation across groups.

5. Conclusion

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.

6. List of own publications

6.1 Publications related to this study

Zhou L, Mozaffaritabar S, Kawamura T, Koike A, Kolonics A, Kéringer J, Pinho RA, Sun J, Shangguan R, Radák Z. The effects of long-term lactate and high-intensity interval training (HIIT) on brain neuroplasticity of aged mice. Heliyon. 2024 Jan 10;10(2):e24421. doi: 10.1016/j.heliyon.2024.e24421. PMID: 38293399; PMCID: PMC10826720.

Zhou L, Mozaffaritabar S, Koltai E, Giannopoulou S, Kolonics A, Gu Y, Pinho RA, Miklossy I, Boldogh I, Radák Z. Consecutive skeletal muscle PGC-1α overexpression: A double-edged sword for mitochondrial health in the aging brain. Biochim Biophys Acta Mol Basis Dis. 2025 Aug;1871(6):167851. doi: 10.1016/j.bbadis.2025.167851. Epub 2025 Apr 12. PMID: 40228677.

Zhou L, Mozaffaritabar S, Kolonics A, Kawamura T, Koike A, Kéringer J, Gu Y, Karabanov R, Radák Z. Long-term iron supplementation combined with vitamin B6 enhances maximal oxygen uptake and promotes skeletal muscle-specific mitochondrial biogenesis in rats. Front Nutr. 2024 Jan 15;10:1335187. doi: 10.3389/fnut.2023.1335187. PMID: 38288063; PMCID: PMC10823527.

6.2 Publications independent of this study

Zhou L, Pinho R, Gu Y, Radak Z. The Role of SIRT3 in Exercise and Aging. Cells. 2022 Aug 20;11(16):2596. doi: 10.3390/cells11162596. PMID: 36010672; PMCID: PMC9406297.

Radak Z, Pan L, Zhou L, Mozaffaritabar S, Gu Y, A Pinho R, Zheng X, Ba X, Boldogh I. Epigenetic and "redoxogenetic" adaptation to physical exercise. Free Radic Biol Med. 2024 Jan;210:65-74. doi: 10.1016/j.freeradbiomed.2023.11.005. Epub 2023 Nov 17. PMID: 37977212.

Mozaffaritabar S, Koltai E, Zhou L, Bori Z, Kolonics A, Kujach S, Gu Y, Koike A, Boros A, Radák Z. PGC-1α activation boosts exercise-dependent cellular response in the skeletal muscle. J Physiol Biochem. 2024 Jan 23. doi: 10.1007/s13105-024-01006-1. Epub ahead of print. PMID: 38261146.

Bakonyi P, Kolonics A, Aczel D, Zhou L, Mozaffaritabar S, Molnár K, László L, Kutasi B, Tanisawa K, Park J, Gu Y, Pinho RA, Radak Z. Voluntary exercise does not increase gastrointestinal motility but increases spatial memory, intestinal eNOS, Akt levels, and *Bifidobacteria* abundance in the microbiome. Front Physiol. 2023 Aug 16;14:1173636. doi: 10.3389/fphys.2023.1173636. PMID: 37664431; PMCID: PMC10468588.

Kokas M, Budai A, Kádár A, Mozaffaritabar S, Zhou L, Téglás T, Orova RS, Gáspár D, Németh K, Toth DM, Sayour NV, Kovácsházi C, Xue A, Szatmári RZ, Törőcsik B, Máthé D, Kovács N, Szigeti K, Nagy P, Szatmári I, Fekete C, Arányi T, Varga ZV, Ferdinandy P, Radák Z, Kozlov AV, Tretter L, Komlódi T, Ambrus A. Microgliosis, neuronal death, minor behavioral abnormalities and reduced endurance performance in alpha-ketoglutarate dehydrogenase complex deficient mice. Redox Biol. 2025 Jun 27;85:103743. doi: 10.1016/j.redox.2025.103743. Epub ahead of print. PMID: 40609475; PMCID: PMC12271819.

Kawamura T, Kerepesi C, Sarkar JP, Torma F, Bori Z, Zhou L, Bakonyi P, Kolonics A, Balogh L, Higuchi M, Pillár V, Pircs K, Koch LG, Britton SL, Koltai E, Radak Z. Organ Specificity and Commonality of Epigenetic Aging in Low- and High-Running Capacity Rats. Aging Cell. 2025 Jun 8:e70110. doi: 10.1111/acel.70110. Epub ahead of print. PMID: 40485193.

Koltai E, Mozaffaritabar S, Zhou L, Kolonics A, Koike A, Tanisawa K, Park J, Torma F, Radak Z. PGC-1 alpha overexpression in the skeletal muscle results in a metabolically active microbiome which is independent of redox signaling. Sci Rep. 2025 Jul 1;15(1):20527. doi: 10.1038/s41598-025-05594-w. PMID: 40595952; PMCID: PMC12215982.