

Original Article

Chronic Swimming Routine Promotes Gut Microbiota Remodeling and Improvements in Physical Resilience, Episodic-Like Memory, and Inflammatory Status in Late Middle-Aged Mice

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ABSTRACT: Population aging is associated with progressive declines in physical and cognitive function driven by chronic low-grade inflammation and dysregulation of interconnected biological systems. Within the geroscience framework, we tested whether chronic swimming initiated in late midlife attenuates age-related deficits by modulating body composition, systemic and hippocampal inflammation, episodic-like memory and gut microbiota composition. Male C57BL/6 mice were trained for 8 weeks starting at 14 months; age-matched controls remained sedentary. Swimming reduced weight gain and epididymal fat mass and progressively improved muscle strength and endurance. Exercised mice showed enhanced short-term spatial discrimination and long-term associative recognition in object location and object-in-place tasks without changes in locomotion or anxiety-like behavior. These cognitive benefits coincided with reduced circulating pro-inflammatory cytokines and a hippocampal shift toward a neuroprotective profile, with down-regulation of inflammatory and glial activation markers and up-regulation of brain-derived neurotrophic factor (BDNF). Muscle performance correlated positively with hippocampus-dependent memory, linking physical resilience to cognitive outcomes. In parallel, 16S rRNA gene sequencing revealed an exercise-induced remodeling of the gut microbiota, characterized by significant changes in community structure and coordinated shifts in the relative abundance of shared taxa. Exercise enriched genera associated with metabolic homeostasis and anti-inflammatory

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functions, including *Akkermansia*, *Odoribacter* and *Alistipes*, while reducing taxa more prevalent in sedentary animals, such as *Romboutsia*. Collectively, these findings demonstrate that moderate-intensity swimming initiated during late middle age concurrently improves physical performance, cognitive resilience, inflammatory status and gut microbiota composition, supporting exercise as a multi-system intervention associated with coordinated adaptations across the muscle–gut–brain axis during aging.

Keywords: Aging, Exercise training, Gut microbiota, Inflammaging, Cognitive resilience

INTRODUCTION

Population aging is accelerating worldwide, with the proportion of individuals over 60 years expected to double by 2050, creating major challenges for maintaining functional independence, metabolic health, and cognitive resilience [1]. This demographic shift is accompanied by a substantial rise in morbidity and multimorbidity [2], largely attributable to a progressive decline in physiological resilience that compromises metabolic, musculoskeletal, and cognitive systems, thereby increasing vulnerability to chronic disease [3, 4]. At the biological level, mammalian aging reflects the cumulative disruption of evolutionarily conserved genetic and biochemical pathways, manifesting as genomic instability, telomere attrition, epigenetic remodeling, loss of proteostasis, deregulated nutrient sensing, mitochondrial dysfunction, cellular senescence, stem cell exhaustion, and altered intercellular communication [3]. In the central nervous system, age-associated impairments in learning and memory are often accompanied by subtle, gradual, and persistent changes in synaptic plasticity, neurovascular function, and glial homeostasis, with the hippocampus and interconnected cortical circuits [5]. At the systemic level, aging is characterized by a chronic, low-grade inflammatory state, referred to as “inflammaging”, evidenced by increased circulating pro-inflammatory cytokines and immune dysregulation. These changes can impact brain function through humoral signaling, altered blood–brain barrier permeability, and immune-to-glia communication [6]. Collectively, these interconnected processes contribute to cognitive frailty and increase the risk of adverse functional outcomes even in the absence of overt neurodegenerative disease.

Accumulating evidence identifies physical exercise as one of the most effective non-pharmacological interventions to counteract age-related functional decline [7]. In the central nervous system, exercise activates molecular and cellular pathways that support synaptic plasticity, neurotrophic signaling, and cerebrovascular function, with particularly strong effects observed in the hippocampus [8]. Both preclinical and clinical studies indicate that aerobic exercise enhances hippocampus-dependent learning and memory and can partially reverse

age-associated hippocampal vulnerability [9, 10]. Mechanistically, exercise exerts well characterized anti-inflammatory actions, including reductions in visceral adiposity–derived inflammatory signaling and repeated bouts of myokine-mediated immune modulation that shift the cytokine milieu toward a less pro-inflammatory profile [11, 12]. Within geroscience framework, elucidating how exercise modulates core biological hallmarks of aging is critical for identifying mechanisms that promote healthspan extension and for guiding the development of targeted interventions aimed to preserve cognitive and functional capacity in older adults [13].

Otherwise, alterations in the gut microbiota are increasingly recognized as contributors to age-related cognitive and systemic decline. Aging is associated with pronounced shifts in microbial community structure, reduced ecosystem stability, and loss of microbiota resilience [14]; in older adults, gut microbiota composition closely correlates with diet, health status, and markers of frailty [14]. The microbiota can influence host metabolism and immunity through microbial metabolites and immune signaling, and accumulating evidence supports bidirectional communication between the gut and the brain via neural, endocrine, immune, and metabolic routes [14]. This “microbiota–gut–brain axis” has been implicated in neurodevelopmental, psychiatric, and neurodegenerative conditions, and is increasingly recognized as a key determinant of age-related cognitive trajectories [15]. Importantly, physical activity is a potent modulator of the gut ecosystem. Exercise has been shown to increase gut microbial diversity and enrich taxa associated with metabolic and anti-inflammatory benefits, including *Akkermansia*, *Bifidobacterium*, and short-chain fatty acid (SCFA)-producing bacteria [16, 17]. Consistent with these observations, we have previously demonstrated that transplantation of gut microbiota from young and trained donors into aged mice markedly improves cognitive performance and synaptic plasticity, while reducing neuroinflammation through mechanisms involving enhanced gut permeability, changes in SCFA levels, and remodeling of bacterial community composition [18]. These microbiota-driven changes contribute to reduce circulating endotoxins, and lower systemic inflammation, thereby mitigating

neuroinflammatory processes that impair hippocampal function [18].

Here, we hypothesized that chronic swimming exercise during midlife-to-early-old age would counteract key age-associated physiological and cognitive declines by improving body composition, enhancing muscular performance, reducing systemic and hippocampal inflammation, and promoting a gut microbial profile enriched in beneficial taxa associated with metabolic and neuroimmune resilience. Consistent with this hypothesis, our findings demonstrate that exercise training not only decreases adiposity and strengthens muscle function but also improves episodic-like memory and shifts both peripheral and central inflammatory profiles toward a less pro-inflammatory and more neuroprotective state in the hippocampus of late middle-aged mice. Importantly, these effects were accompanied by an increased abundance of health-promoting microbial populations, supporting the notion that exercise-driven remodeling of the gut microbiota accompanies the observed physiological and neurobehavioral benefits and may represent a contributing mechanism, as suggested by prior microbiota-transfer studies.

MATERIAL AND METHODS

Animals

Male C57BL/6J mice were obtained from the Animal Facility of the Faculty of Sciences, Universidad de Valparaíso. Animals were housed under controlled environmental conditions (23 ± 1 °C, 12:12 h light–dark cycle) with ad libitum access to food and water. Mice were maintained on a normal control diet providing 10% of total energy from fat, 20% from protein, and 70% from carbohydrates. For the swimming exercise study, mice entered the experimental protocol at 14 months of age and were followed for 8 weeks until late middle age (≈ 16 months). Animals were randomly assigned to two experimental groups: (i) Sedentary controls, which remained physically inactive throughout the study period, and (ii) Exercise-trained mice, which underwent a structured swimming training protocol. Each mouse was considered an independent biological replicate across experiments unless otherwise specified.

Body weight was recorded weekly throughout the intervention period. Animal health status and general behavior were monitored daily to ensure welfare and to detect any signs of distress or illness. No adverse events related to the exercise intervention were observed.

All experimental procedures were conducted in accordance with the guidelines of the National Commission for Scientific and Technological Research (CONICYT, Chile) and were approved by the Bioethics

Committee of the Faculty of Sciences, Universidad de Valparaíso (protocol BEA172-21).

Swimming Training Protocol

Mice assigned to the exercise group underwent a chronic swimming training protocol adapted from previously validated paradigms for aging rodents [19]. Training started when animals were 14 months old and lasted for 8 consecutive weeks. Exercise sessions were performed 5 days per week, with each session consisting of 60 minutes of continuous swimming.

Swimming was conducted in a cylindrical tank (50 cm height, 30 cm diameter) filled with water maintained at 30 ± 1 °C to minimize thermal stress. During each session, mice swam individually under continuous supervision to ensure safety and proper execution of the protocol.

To provide a standardized workload, a small lead weight corresponding to 5% of the animal's body weight was attached to the base of the tail using adhesive tape. Body weight was measured weekly, and the load was adjusted accordingly to maintain a constant relative intensity throughout the intervention period. Animals were gently placed in the water at the beginning of each session and continuously monitored. If a mouse showed signs of excessive fatigue (e.g., floating without coordinated movement), it was briefly assisted and allowed to resume swimming once normal movement was restored. No animals required removal from the protocol due to exhaustion or adverse effects.

Sedentary control mice were handled daily but did not perform any physical exercise. They were placed in an empty tank for a similar duration to control for handling and environmental stress without exposure to swimming. At the end of the intervention, animals were euthanized at approximately 17 months of age. Immediately after euthanasia, gonadal adipose tissue pads associated with the epididymis were carefully dissected and weighed to assess adiposity. Fat mass was normalized to body weight for subsequent analyses.

Neuromuscular performance tests

Neuromuscular function was assessed weekly throughout the 8-week intervention using two complementary assays: the Kondziela's inverted screen test (whole-body strength/endurance) and a chain link weight-lifting test (forelimb strength).

Inverted screen test (Kondziela's test)

Muscle endurance/strength was evaluated using the inverted screen test originally described by Kondziela

(1964) and later standardized for mouse strength phenotyping [20]. In brief, each mouse was placed on a wire mesh mounted on a wooden frame; once the animal achieved a stable grip, the grid was smoothly inverted at a fixed height above the cage so the mouse hung upside down. Latency to fall was recorded, with a maximum cut-off of 4 minutes (240 s). To account for body size, performance was expressed as holding impulse, calculated as *latency to fall (s) × body weight (g) (g·s)*.

Chain link weight-lifting test

Forelimb strength was assessed using a graded chain link weight-lifting assay based on the weights test described by Deacon and others [20, 21]. A series of metal chain segments with increasing link number (here, 2 up to 11 links) was prepared, each attached to a small wire/mesh handle that the animal could grasp. Mice were gently held by the tail and allowed to grasp the handle with the forepaws; once a firm grip was established, the mouse was lifted so that the chain was fully suspended. The animal was required to hold the weight for up to 3 seconds; if successful, the next heavier chain was presented.

Performance was quantified as a lifting score defined as *link number × holding time (s)*, consistent with the scoring approach commonly used for this assay.

Behavioral assessments

All behavioral tests were conducted during the light phase of the cycle in a dedicated testing room under controlled illumination and noise conditions. Animals were habituated to the testing room for at least 30 min before each session to minimize stress-induced variability. The apparatus was cleaned with 5% ethanol between animals and with 70% ethanol at the end of each testing day to eliminate olfactory cues, as we previously described [18]. Behavior was recorded using a digital camera (Logitech C920, 30 fps, 640 × 480 px) and analyzed offline with ANY-maze software (v7.09, Stoelting Co., USA).

$$DI(\%) = \frac{Time_{\text{novel location}}}{Total\ exploration\ time} \times 100$$

Open Field (OF)

Locomotor activity and anxiety-like behavior were assessed using the open field test, a well-established paradigm for evaluating exploratory behavior and emotionality in rodents [22]. Each mouse was individually placed in the center of a circular arena (40 cm diameter) surrounded by 30 cm-high opaque walls under bright illumination (300 lux) and allowed to freely explore for 5 minutes. The arena was virtually divided into central

and peripheral zones using ANY-maze software. The central zone corresponded to the inner 30% of the arena area, whereas the peripheral zone comprised the remaining outer region adjacent to the walls. Parameters analyzed included: Total distance traveled, Mean and maximum speed and Time spent in center vs. periphery. Total distance and speed were used as indicators of general locomotor activity, whereas time spent in the center was used as an index of anxiety-like behavior.

Object Location Memory (OLM)

Spatial memory was assessed using the OLM test, which relies on rodents' natural preference for spatial novelty and is known to depend on hippocampal function [23].

Habituation (Day 1): Mice were allowed to freely explore the empty arena for 5 minutes. A fixed visual cue (vertical black tape) was placed at the center of one wall and remained constant throughout all phases.

Training (Day 2): Two identical objects were placed symmetrically in the arena. Mice were allowed to explore freely for 5 minutes.

Test (Day 3): One object was displaced to a new spatial location, while the other remained fixed. Memory performance was evaluated at two retention intervals: 1 hour and 24 hours after the training session. During each test, animals were allowed to explore for 5 minutes.

Exploration was defined as nose-directed investigation within a ~1 cm radius of the object. Sitting on, leaning against, or climbing the object was not considered exploration.

To control for potential object or spatial biases, object identity and spatial location were counterbalanced across animals. The object moved during the test phase and its left-right position was randomized between animals. The arena orientation and visual cue remained constant throughout the experiment.

The discrimination index (DI) was calculated as:

Object-in-Place (OiP)

Associative recognition memory was evaluated using the OiP test, which requires integration of object identity and spatial context and is dependent on coordinated activity between the hippocampus and medial prefrontal cortex [24].

Habituation (Day 1): Animals freely explored the empty arena for 5 minutes.

Training (Day 2): Four different objects were placed in fixed positions and mice explored for 5 minutes.

Test (Day 3): Two of the four objects were swapped to new positions, while the remaining two stayed in their original locations. Memory was assessed at 1 hour and 24 hours after the training session. During each test, animals explored freely for 5 minutes.

Object identity and spatial configuration were counterbalanced across animals to avoid systematic preferences for specific objects or positions. The pairs of objects that were swapped during the test phase varied across animals.

Exploration criteria were identical to OLM. The discrimination index was calculated as:

$$DI(\%) = \frac{Time_{displaced\ objects}}{Total\ exploration\ time} \times 100$$

Serum cytokine quantification (Multiplex ELISA)

Serum cytokine levels were quantified using a Q-Plex™ Chemiluminescent ELISA multiplex assay (Quansys Bioscience, Logan, UT, USA), following the manufacturer's instructions. Blood samples were collected at the end of the experimental protocol and serum was obtained by centrifugation. Thirteen inflammation-related cytokines were simultaneously measured, including granulocyte-macrophage colony-stimulating factor (GM-CSF), IL-1 α , IL-1 β , IL-2, IL-3, IL-4, IL-6, IL-10, IL-12p70, IL-17A, macrophage inflammatory protein-1 alpha (MIP-1 α), RANTES (CCL5), and TNF- α . Chemiluminescent signals were acquired using a Bio-Rad ChemiDoc imaging system (Bio-Rad, Hercules, CA, USA) and quantified with Q-View™ software (Quansys Bioscience). Cytokine concentrations were calculated from standard curves and expressed as pg/mL.

Quantitative real time PCR

Total RNA was extracted from mouse hippocampus (HPC) and medial prefrontal cortex (mPFC) using TRIzol reagent (Invitrogen), following the manufacturer's instructions. RNA samples were treated with DNase I to eliminate potential genomic DNA contamination using the DNA-free™ DNA Removal Kit (Invitrogen). RNA concentration and purity were assessed spectrophotometrically, and 1 μ g of total RNA was reverse-transcribed into cDNA using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems), according to the manufacturer's protocol.

Quantitative real-time PCR (qPCR) was performed using the AriaMx Real-Time PCR System (Agilent Technologies) with Brilliant II SYBR Green QPCR Master Mix (Agilent). All primer pairs exhibited optimal amplification efficiencies, ranging between 90% and 110%. Relative gene expression levels were calculated using the $2^{-\Delta\Delta C_t}$ method and are expressed as mean $\pm$ SEM. β -actin was used as the housekeeping gene for normalization. Primer sequences and thermocycling conditions are provided in the Supplementary Table 1.

Gut microbiota profiling by 16S rRNA gene sequencing

Microbial DNA was extracted from fecal samples using the QIAamp Fast DNA Stool Mini Kit (Qiagen, USA), following the manufacturer's instructions. Briefly, 180–220 mg of fecal material was homogenized in InhibitEX buffer to ensure efficient lysis and inhibitor removal. DNA concentration and purity were assessed spectrophotometrically before downstream processing. Fecal samples were collected individually from each mouse and processed independently.

The V3–V4 hypervariable regions of the bacterial 16S rRNA gene were amplified using the primer pair 357F (5'-CCTACGGGAGGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'). PCR reactions were performed in a final volume containing 1 $\times$ Taq buffer, 2 mM MgCl₂, 0.3 mM dNTPs, 0.3 μ M of each primer, 2.5 U GoTaq Flexi DNA polymerase (Promega), and 4 ng of template DNA. Thermal cycling conditions consisted of an initial denaturation at 94 °C for 3 min, followed by 28 cycles of 94 °C for 30 s, 57 °C for 1 min, and 72 °C for 90 s, with a final extension at 72 °C for 10 min.

Amplicons were purified, quantified using a KAPA Library Quantification Kit (Roche), and quality-checked on an Agilent 2100 Bioanalyzer using a DNA 1000 chip. Libraries were pooled at equimolar concentrations and sequenced on an Illumina MiSeq platform using paired-end chemistry (2 $\times$ 300 bp).

Sample size and outlier detection

A total of 14 mice were included in the study and randomized into sedentary and exercise groups (n = 7/group). All animals completed the intervention and were included in behavioral, body weight, and neuromuscular analyses. Outlier detection was performed using a predefined criterion of ± 2 standard deviations (SD) from the group mean. This rule was applied only to qPCR-derived measurements when extreme values were observed that likely reflected technical variability. In total, three values were excluded across all qPCR endpoints (one TNF- α value in the sedentary group, one IL-6 value in the sedentary group, and one GFAP value in the exercised group). No data points were excluded from the behavioral analyses, cytokine multiplex measurements, or correlation analyses. Sensitivity analyses including the excluded values confirmed that the overall conclusions of the study were unchanged. For molecular endpoints, sample sizes varied depending on sample availability and assay requirements. Serum cytokine measurements were performed on serum samples from 5 sedentary and 6 exercised mice. For gut

microbiota profiling, fecal samples from 6 animals per group were submitted for 16S rRNA gene sequencing. One sample from the exercised group was excluded prior to downstream analysis because it did not meet sequencing quality-control criteria, resulting in a final microbiota dataset of 11 samples (sedentary $n = 6$; exercise $n = 5$) long term. A flow diagram summarizing animal and sample inclusion across analyses is provided in Supplementary Figure 1.

Bioinformatic and statistical analysis of gut microbiota data

16S rRNA gene sequence processing and taxonomic assignment. Raw paired-end 16S rRNA gene sequencing reads (2×300 bp) targeting the V3–V4 region were processed using QIIME 2 (Quantitative Insights Into Microbial Ecology, version 2024.10) executed within a Docker containerized environment, ensuring reproducibility across computational analyses. Demultiplexed sequences were quality-checked and denoised using the DADA2 plugin, which performs quality filtering, trimming, error modeling, chimera removal, and inference of amplicon sequence variants (ASVs). Default parameters were used except for trimming and truncation lengths, which were optimized based on per-base quality score profiles to maximize overlap between forward and reverse reads while minimizing read loss. After quality filtering and denoising, sequencing depth ranged from 68,015 to 99,087 sequences per sample, with a median depth of 79,347 sequences (mean: 79,610). A total of 11 samples were retained for downstream analyses, comprising 875,712 total sequences and 2,383 inferred ASVs.

High-quality ASVs were taxonomically classified using a naïve Bayes classifier trained on the SILVA reference database (release 138), trimmed to the V3–V4 region amplified by the 357F/806R primer pair. ASVs assigned to non-bacterial sequences (e.g., mitochondria or chloroplasts) were removed prior to downstream analyses.

Feature table filtering and taxonomic aggregation. The ASV feature table was filtered to remove extremely low-abundance features and samples with insufficient sequencing depth, when applicable, using QIIME 2 filtering utilities. For community-level analyses and visualization, ASVs were collapsed to the genus level using the qiime taxa collapse function. Taxa annotated as *Unassigned* or *uncultured* at the genus level were retained for global diversity analyses but excluded from highlighted differential abundance results and figure annotations, to ensure biological interpretability.

Relative abundance tables were generated by normalizing genus-level counts to total sample reads.

These tables were used for stacked bar plots and downstream exploratory analyses.

Alpha- and beta-diversity analyses. Alpha-diversity metrics (Observed features, Shannon index, and Simpson index) and beta-diversity analyses were calculated on rarefied feature tables subsampled to 65,000 sequences per sample, a depth selected based on rarefaction curves to retain all samples while ensuring comparable sequencing effort across samples. Group comparisons were performed using non-parametric tests.

Beta-diversity was assessed using both abundance-weighted and presence–absence–based distance metrics. Bray–Curtis dissimilarity was used to evaluate differences in relative abundance structure, whereas Jaccard distance was used to assess differences in taxon presence–absence. Principal coordinates analysis (PCoA) was performed for visualization. Statistical significance of group separation was evaluated using PERMANOVA with 999 permutations. To ensure that observed differences were not driven by unequal dispersion between groups, PERMDISP tests were conducted.

Differential abundance analysis. Differential abundance analysis at the genus level was performed using DESeq2 (version ≥ 1.38) implemented in R (version ≥ 4.3). Raw (non-rarefied) genus-level count tables were imported into R and analyzed using a negative binomial generalized linear model, with experimental group (sedentary vs. exercise) specified as the main explanatory factor.

To control for multiple testing, p values were adjusted using the Benjamini–Hochberg false discovery rate (FDR) correction. Adjusted p values (p_{adj}) were used to assess statistical significance. Genera were considered statistically significant when FDR-adjusted p values were < 0.05 .

Prior to analysis and visualization, genera lacking reliable taxonomic annotation (e.g., labeled as *Unassigned* or *uncultured*) were excluded from highlighted results and figure annotations. In parallel, effect sizes ($\log_2$ fold change) were used to rank taxa and guide biological interpretation.

For figure generation and interpretative summaries, a subset of top exercise-associated genera (both increased and decreased with exercise) was selected based on a combination of FDR-adjusted significance, magnitude of fold change, and biological relevance, while maintaining transparency regarding the full statistical output.

Data visualization and figure generation. All microbiota-related figures were generated using R, primarily with the ggplot2 package, alongside dplyr and tidyr for data manipulation. Stacked bar plots of relative abundance were constructed from genus-level normalized tables. Beta-diversity PCoA plots were generated using

distance matrices exported from QIIME 2 and visualized in R.

Differential abundance results were visualized using custom plots highlighting selected genera associated with exercise. Color schemes were applied consistently across figures to distinguish exercise-associated increases and decreases, and axes were scaled independently when necessary to avoid compression of effect sizes.

Statistical analysis

The animals exhibited comparable baseline characteristics, including body weight and frailty scores, indicating a similar health status across the cohort. On this basis, animals were randomly assigned to experimental groups. All data are presented as mean $\pm$ SEM unless otherwise indicated. Sample sizes for each experiment are reported in the corresponding figure legends. Statistical analyses were performed using GraphPad Prism (version 10). Given the relatively small sample sizes and frequent deviation from normality, non-parametric statistical tests were applied unless otherwise specified. Comparisons between two independent groups (sedentary vs. exercise) were performed using the Mann–Whitney U test.

Longitudinal performance in the inverted screen test and the chain link lifting test was analyzed using mixed-effects models fitted by restricted maximum likelihood (REML). In each model, time (week) and group (sedentary vs. exercised) were included as fixed effects, and mouse was included as a random effect to account for repeated measurements within subjects. When appropriate, the Geisser–Greenhouse correction was applied to account for potential violations of sphericity. Post hoc comparisons were performed using Šidák's multiple comparisons test. Data are presented as mean $\pm$ SEM. Correlation analyses between muscle performance parameters (holding impulse and chain link lifting score) and cognitive outcomes (discrimination indices in the OLM and OiP tasks) were assessed using Spearman's rank correlation coefficient (ρ).

For serum cytokines, between-group comparisons (sedentary vs exercised) were performed using the Mann–Whitney U test. To account for multiple testing across the cytokine panel, p values were adjusted using the Benjamini–Hochberg false discovery rate (FDR) procedure. Adjusted q values < 0.05 were considered statistically significant.

Gene expression analyses were performed using qPCR on a limited set of candidate genes selected a priori based on their known involvement in inflammatory and neuroprotective pathways relevant to the study hypothesis. Because these targets were hypothesis-driven rather than part of an exploratory screen, each gene was

analyzed as an independent endpoint and no global multiple-testing correction was applied.

Importantly, all analyses of cognitive performance, serum cytokine levels, hippocampal and mPFC mRNA expression, and gut microbiota data were performed in a blinded manner, with group identity concealed until completion of statistical analyses. Statistical significance was set at $p < 0.05$.

RESULTS

Swimming training reduces body weight and epididymal fat mass in late middle-aged mice

To evaluate the impact of chronic swimming exercise on body composition during late middle age male C57BL/6 mice initiated an eight-week swimming training protocol at 14 months of age, consisting of 1 h/day, five days per week, with an external workload equivalent to 5% of their body weight attached to the tail. Age-matched sedentary controls did not undergo any exercise intervention. Throughout the training period, exercised and sedentary mice were monitored for changes in body weight. No differences were observed during the initial weeks of training; however, exercised animals exhibited a significant reduction in body weight during weeks 7 and 8, reaching a final body weight of 42 ± 1.1 g, in contrast to the 47.2 ± 1.5 g observed in sedentary mice (Supplementary Fig. 2A). Consistently, a decrease of 10.6 ± 1.4 g in body weight variation between weeks 0 and 8 was detected in the exercised group (Supplementary Fig. 2B). Moreover, exercised mice displayed lower epididymal fat mass normalized to body weight compared with sedentary controls (Supplementary Fig. 2C; 0.028 ± 0.002 g and 0.04 ± 0.003 g, respectively).

Progressive improvement in muscle strength induced by exercise in late middle-aged mice

To determine whether chronic swimming exercise enhances muscle strength during late middle age, motor performance was assessed longitudinally using complementary strength-based behavioral tests.

In the inverted screen test, exercised mice displayed higher holding impulse values than sedentary animals by the end of the training period (1575 ± 277 g·s vs 794 ± 216 g·s at week 8, respectively; Fig. 1A). Longitudinal analysis using a mixed-effects model (REML), accounting for repeated measurements within individual mice, revealed a significant main effect of time ($F(2.239, 26.86) = 4.11$, $p = 0.0241$) and a significant time $\times$ group interaction ($F(2.239, 26.86) = 7.57$, $p = 0.0018$). These results indicate that the temporal trajectory of strength performance differed between sedentary and exercised

animals across the intervention period. No overall main effect of group was detected when values were averaged across all time points ($F(1,12) = 0.36$, $p = 0.5584$), suggesting that group differences emerged progressively during the training period rather than representing a constant effect. Post hoc Šídák-adjusted comparisons indicated that exercised mice exhibited a significant increase in holding impulse at week 8 compared with baseline ($p = 0.029$), whereas sedentary mice did not show significant changes in performance across the intervention period.

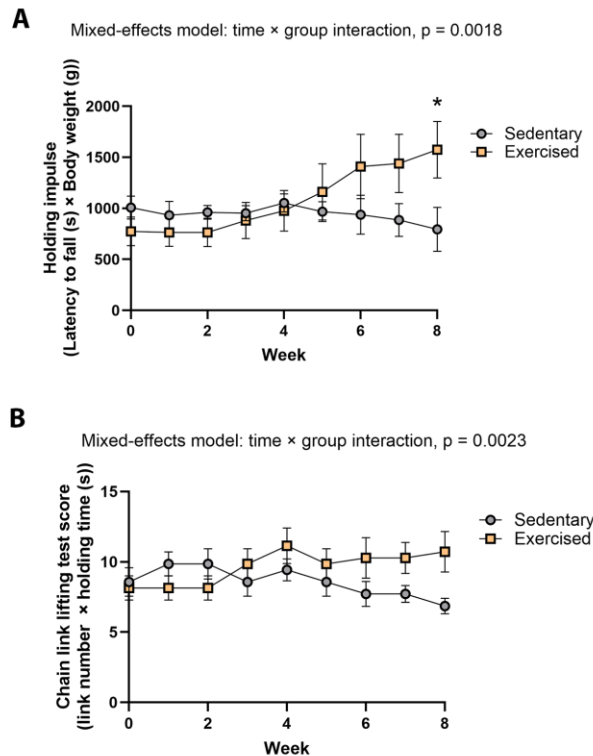


Figure 1. Chronic swimming exercise improves longitudinal strength performance in middle-aged mice. Muscle strength was assessed longitudinally during the 8-week swimming training protocol using complementary behavioral tests. (A) Inverted screen test. Holding impulse (latency to fall × body weight) was measured weekly to evaluate whole-body strength. Exercised mice progressively increased holding impulse over the training period, whereas sedentary mice showed a gradual decline. Longitudinal analysis using a mixed-effects model revealed a significant time × group interaction ($p = 0.0018$), indicating that the trajectory of strength performance differed between groups. (B) Chain link lifting test. Maximal lifting performance (link number × holding time) was assessed weekly across the training period. Exercised mice exhibited a progressive improvement in lifting capacity compared with sedentary controls. Mixed-effects model analysis revealed a significant time × group interaction ($p = 0.0023$). Data are presented as mean ± SEM ($n = 7$ mice per group). The asterisk indicates a significant difference between groups at the corresponding time point, $*p < 0.05$.

A similar longitudinal pattern was observed in the chain link weight-lifting test. Exercised mice displayed higher lifting performance by week 8 compared with sedentary animals (10.7 ± 1.4 vs 6.9 ± 1.5 ; Fig. 1B). Mixed-effects analysis revealed a significant time × group interaction ($F(3.330, 39.96) = 5.688$, $p = 0.0018$), indicating that the evolution of lifting performance across the intervention period differed between groups. However, no significant main effects of time ($F(3.330, 39.96) = 1.582$, $p = 0.2052$) or group ($F(1,12) = 0.746$, $p = 0.4048$) were detected. Post hoc Šídák-adjusted comparisons did not identify statistically significant differences between individual time points after correction for multiple testing.

Together, these findings indicate that chronic swimming exercise modifies the trajectory of neuromuscular performance across the intervention period and promotes improved strength outcomes in late middle-aged mice.

Chronic exercise enhances spatial discrimination and associative recognition memory in late middle-aged mice

To assess how chronic exercise influences spatial discrimination and associative recognition, we employed object-based memory tasks—the object location memory (OLM) and object-in-place (OiP) tests—designed to introduce a mismatch between learning and consolidation phases, promoting novelty-driven exploration. In the OLM test, mice were familiarized with two identical objects, and memory was evaluated after 1 h or 24 h by relocating one object (Fig. 2A). Exercised mice exhibited no differences in total exploration time at the 1 h retention interval (63 ± 8.6 s) relative to sedentary controls (47 ± 9 s; Mann–Whitney $U = 15$, $p = 0.2593$), and similarly no significant differences were detected at the 24 h interval (54 ± 8 s vs. 51.5 ± 9 s; Mann–Whitney $U = 21$, $p = 0.6807$) (Fig. 2C and E). These results suggest that chronic exercise did not modify general exploratory behavior. In contrast, object discrimination performance was significantly higher in the exercised group at the 1 h retention interval, reaching $60 \pm 5.4\%$ compared with $38.7 \pm 4.6\%$ in sedentary mice (Mann–Whitney $U = 6$, $p = 0.0157$) (Fig. 2B and C). This improvement was not maintained at the 24 h interval, where exercised mice showed only a non-significant trend toward higher discrimination performance compared with sedentary animals (Mann–Whitney $U = 9.5$, $p = 0.0571$) (Fig. 2D and E). In the OiP test, where two of four familiar objects were repositioned (Fig. 3a), both groups displayed comparable total exploration times at the 1 h retention interval (Mann–Whitney $U = 22.5$, $p = 0.8298$) and at the 24 h retention interval (Mann–Whitney $U = 20$, $p =$

0.5962), indicating no differences in general exploratory behavior between sedentary and exercised mice (Fig. 3C and E). Likewise, no significant differences were observed in the discrimination index at the 1 h retention interval (Mann–Whitney $U = 13.5$, $p = 0.1754$) (Fig. 3B and C). However, exercised mice exhibited significantly higher object discrimination at the 24 h retention interval compared with sedentary controls ($55.7 \pm 4\%$ vs. $38.7 \pm 3\%$; Mann–Whitney $U = 5$, $p = 0.0111$) (Fig. 3D and E).

Collectively, these findings indicate that chronic exercise mitigates age-related impairments in episodic-like memory, with significant effects on short-term spatial memory and long-term associative recognition. In addition, assessment of locomotor activity (Supplementary Fig. 3A–D) and anxiety-like behavior (Supplementary Fig. 3E–F) in the open field test revealed no differences between exercised and sedentary groups.

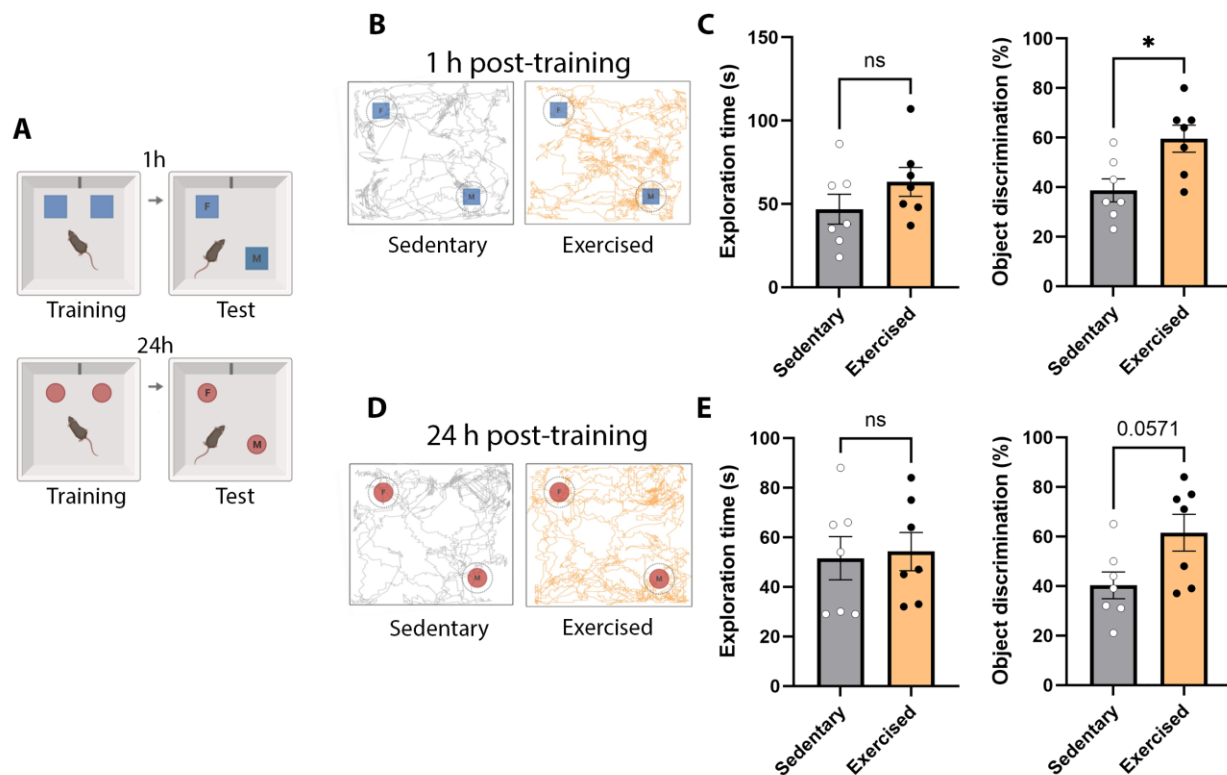


Figure 2. Chronic swimming exercise enhances performance in the object location memory test in middle-aged mice. Mice were subjected to the object location memory (OLM) test. Animals were first familiarized with two identical objects, and memory was assessed after a 1 h or 24 h retention interval by relocating one of the objects. Chronic swimming exercise significantly improved task performance at the 1 h retention interval and showed a trend toward improved performance at the 24 h retention interval. (A) Schematic representation of the test. (B) Representative trajectory maps showing exploratory behavior at the 1 h retention interval. (C) Total exploration time and discrimination performance at the 1 h retention interval. (D) Representative trajectory maps showing the exploratory behavior at the 24 h retention interval. (E) Total exploration time and discrimination performance at the 24 h retention interval. Grey columns represent sedentary controls; orange columns represent swimming-exercised mice. Data are presented as mean $\pm$ SEM ($n = 7$ mice per group). Statistical significance was determined using Mann–Whitney U test. * $p < 0.05$, ns: not significant versus sedentary mice.

Exercise reduces circulating pro-inflammatory cytokines in late middle-aged mice

To determine whether chronic swimming exercise modulates systemic inflammatory mediators during late middle age, we quantified serum cytokines using a multiplex panel comprising 13 analytes. Group differences were initially assessed using Mann–Whitney tests and p -values were subsequently adjusted for multiple

comparisons using the Benjamini–Hochberg false discovery rate (FDR).

After FDR correction ($q < 0.05$), exercised mice exhibited significantly lower circulating levels of the pro-inflammatory cytokines IL-1 β , IL-3, TNF- α , and IL-6 compared with sedentary controls (Fig. 4A–D). In addition, IL-17A and IL-10 displayed trends toward differential levels after FDR correction (IL-17A $q = 0.0788$; IL-10 $q = 0.0964$) (Fig. 4E and F), suggesting potential exercise-associated modulation that did not

reach the corrected significance threshold. No differences were observed for GM-CSF, IL-1 α , IL-2, IL-4, RANTES, MIP-1 α , or IL-12p70 after correction for multiple testing (Supplementary Fig. 4).

Together, these results indicate that chronic swimming exercise reduces a subset of circulating inflammatory mediators and may partially shift the systemic immune profile toward a less pro-inflammatory state in late middle-aged mice.

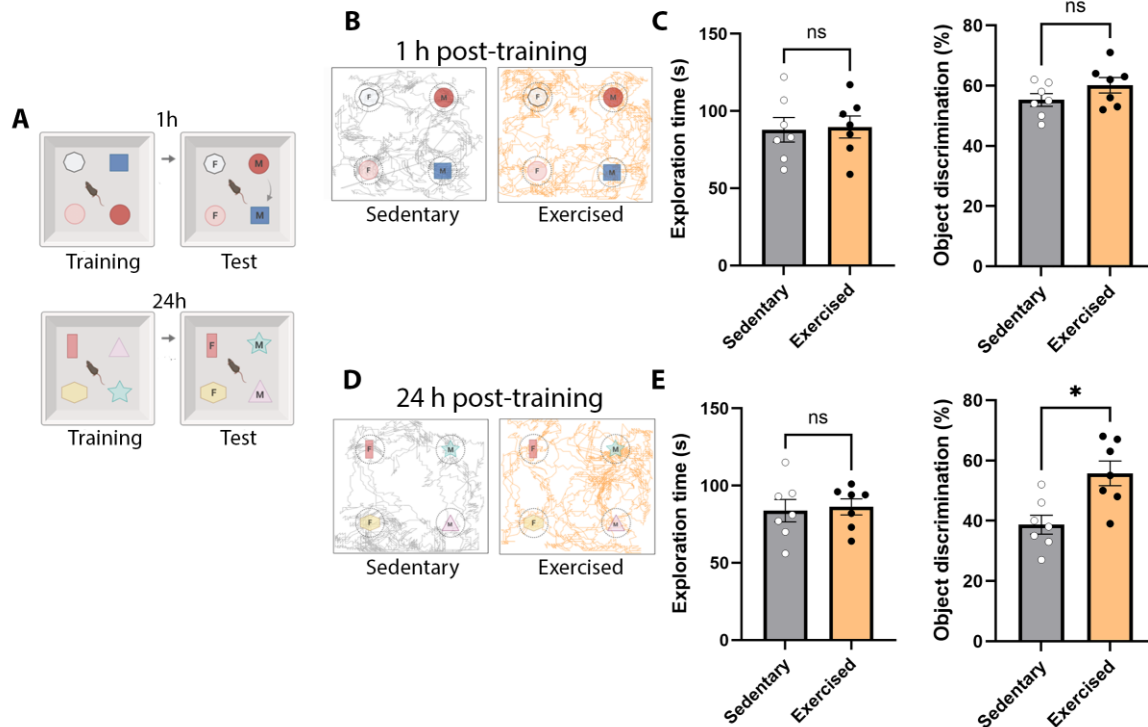


Figure 3. Chronic swimming exercise improves associative recognition memory assessed by the object-in-place test in middle-aged mice. Mice were subjected to the object-in-place (OiP) memory test. Animals were first familiarized with four distinct objects, and memory was assessed after a 1 h or 24 h retention interval by changing the spatial configuration of two objects. Chronic swimming exercise significantly improved task performance at the 24 h retention interval. (A) Schematic representation of the acquisition and retrieval phases of the OiP task. (B) Representative trajectory maps illustrating exploratory behavior at the 1 h retention interval. (C) Total exploration time and discrimination performance at the 1 h retention interval. (D) Representative trajectory maps showing exploratory behavior at the 24 h retention interval. (E) Total exploration time and discrimination performance at the 24 h retention interval. Grey columns represent sedentary controls; orange columns represent swimming-exercised mice. Data are presented as mean $\pm$ SEM ($n = 7$ mice per group). Statistical significance was determined using the Mann–Whitney U test. * $p < 0.05$, ns: not significant versus sedentary mice.

Exercise is associated with reduced inflammatory and glial-related transcripts and increased BDNF expression in the hippocampus of late middle-aged mice

To investigate whether chronic swimming exercise modulates hippocampal inflammatory, glial-related, and neurotrophic transcripts in aged animals, we analyzed the expression of key markers in hippocampal tissue. RT-qPCR analysis revealed that exercised mice exhibited reduced expression of IL-1 β (0.32 ± 0.1 -fold vs. 1.0 ± 0.17 -fold in sedentary mice; Mann–Whitney $U = 2$, $p = 0.0023$), GFAP (0.48 ± 0.15 -fold vs. 1.0 ± 0.13 -fold; $U = 6.5$, $p = 0.0379$), and Emr1 (0.45 ± 0.1 -fold vs. 1.0 ± 0.1 -fold; $U = 2$, $p = 0.0023$) compared with sedentary controls

(Fig. 5A, E, F). In contrast, TNF- α (0.98 ± 0.18 -fold vs. 1.0 ± 0.09 -fold in sedentary mice; $U = 17.5$, $p = 0.6550$) and IL-6 mRNA levels (1.03 ± 0.38 -fold vs. 1.0 ± 0.17 -fold; $U = 18$, $p = 0.7063$) were not significantly different between groups (Fig. 5B and C).

Importantly, hippocampal BDNF expression was significantly increased in exercised mice (1.83 ± 0.26 -fold vs. 1.0 ± 0.19 -fold in sedentary mice; $U = 7.5$, $p = 0.0286$) (Fig. 5D), consistent with a more neuroprotective transcriptional profile in the hippocampus. mRNA levels of the same biomarkers were evaluated in the medial prefrontal cortex (mPFC); however, no differences were observed between groups (Supplementary Fig. 5A–F).

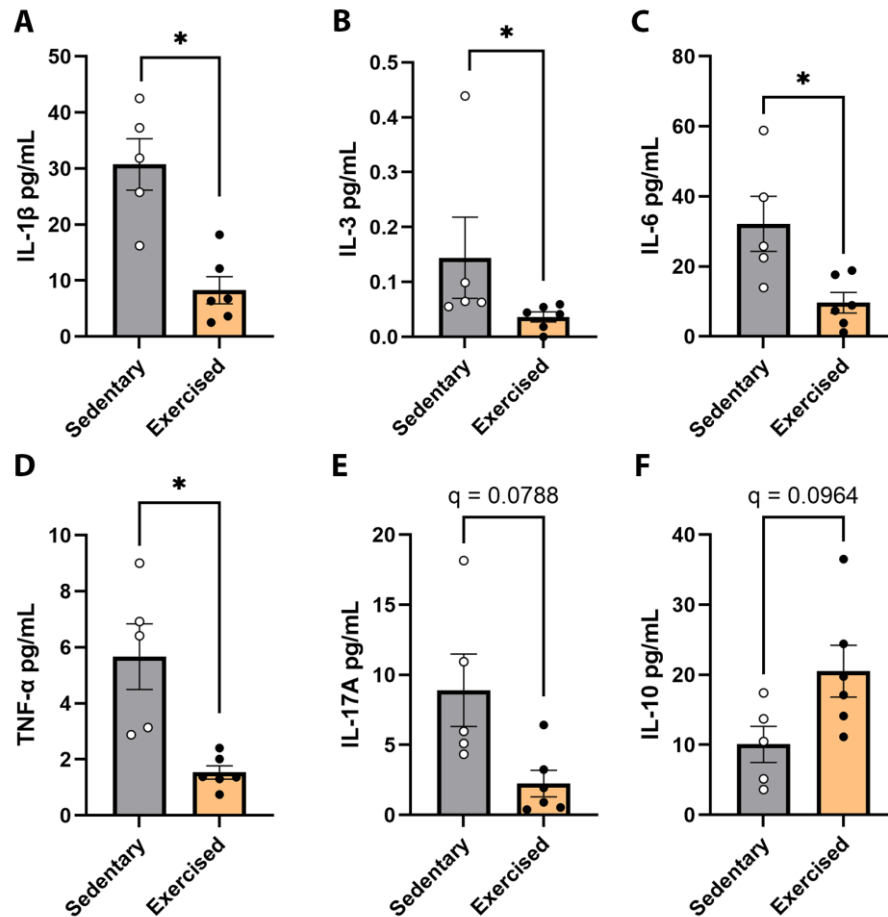


Figure 4. Swimming exercise attenuates circulating pro-inflammatory cytokine levels in middle-aged mice. Circulating cytokines were quantified in serum from swimming-exercised and sedentary middle-aged mice using antibody-based multiplex assays. Group comparisons were performed using the Mann–Whitney U test and p values were adjusted for multiple comparisons across the cytokine panel using the Benjamini–Hochberg false discovery rate (FDR). Chronic swimming exercise significantly reduced the circulating levels of the pro-inflammatory cytokines IL-1 β , IL-3, TNF- α , and IL-6 after FDR correction ($q < 0.05$). IL-17A displayed a trend toward reduced levels, whereas the anti-inflammatory cytokine IL-10 showed a trend toward increased levels in exercised mice following correction for multiple testing. (A) Serum levels of interleukin-1 β (IL-1 β). (B) Serum levels of interleukin-3 (IL-3). (C) Serum levels of interleukin-6 (IL-6). (D) Serum levels of tumor necrosis factor- α (TNF- α). (E) Serum levels of interleukin-17A (IL-17A). (F) Serum levels of interleukin-10 (IL-10). Grey columns represent mean sedentary controls; orange columns represent swimming-exercised mice. Data are presented as mean $\pm$ SEM ($n = 5$ –6 mice per group). Each dot represents an individual mouse. * $q < 0.05$ versus sedentary mice (Benjamini–Hochberg FDR correction).

Correlations between muscle performance and object memory in late middle-aged mice

To examine the relationship between muscular performance and cognitive function in late middle-aged mice, correlation analyses were performed between physical performance metrics measured at week 8 (holding impulse and chain link lifting score) and the discrimination index in the OLM test at 1 and 24 hours. Holding impulse performance correlated positively with spatial memory, with a Spearman coefficient $\rho = 0.54$ for

short-term memory (1 h; Fig. 6A) and $\rho = 0.55$ for long-term memory (24 h; Fig. 6B), suggesting that greater muscle endurance is associated with improved spatial memory. Similarly, the chain link lifting score showed a strong positive correlation with the OLM discrimination index both at 1 h ($\rho = 0.84$; Fig. 6C) and at 24 h ($\rho = 0.73$; Fig. 6D), suggesting that animals with greater muscle strength exhibit superior spatial discrimination across both time scales. Comparable analyses were conducted for the OiP test. The holding impulse was not associated with short-term OiP memory at 1 h ($\rho = 0.08$; Supplementary Fig. 6A), but displayed a significant

positive correlation at 24 h ($\rho = 0.72$; Supplementary Fig. 6B), indicating a potential link between muscle endurance and long-term object recognition memory consolidation. No significant correlations were observed between the chain link lifting score and OiP performance at 1 h (Supplementary Fig. 6C). At 24 h, only a positive trend was detected between lifting strength and OiP

performance ($\rho \approx 0.50$; Supplementary Fig. 6D). Taken together, these results indicate that better muscle performance in late middle age is robustly associated with improvements in hippocampus-dependent spatial memory, whereas associations with object-recognition memory are weaker and emerge primarily during long-term recall.

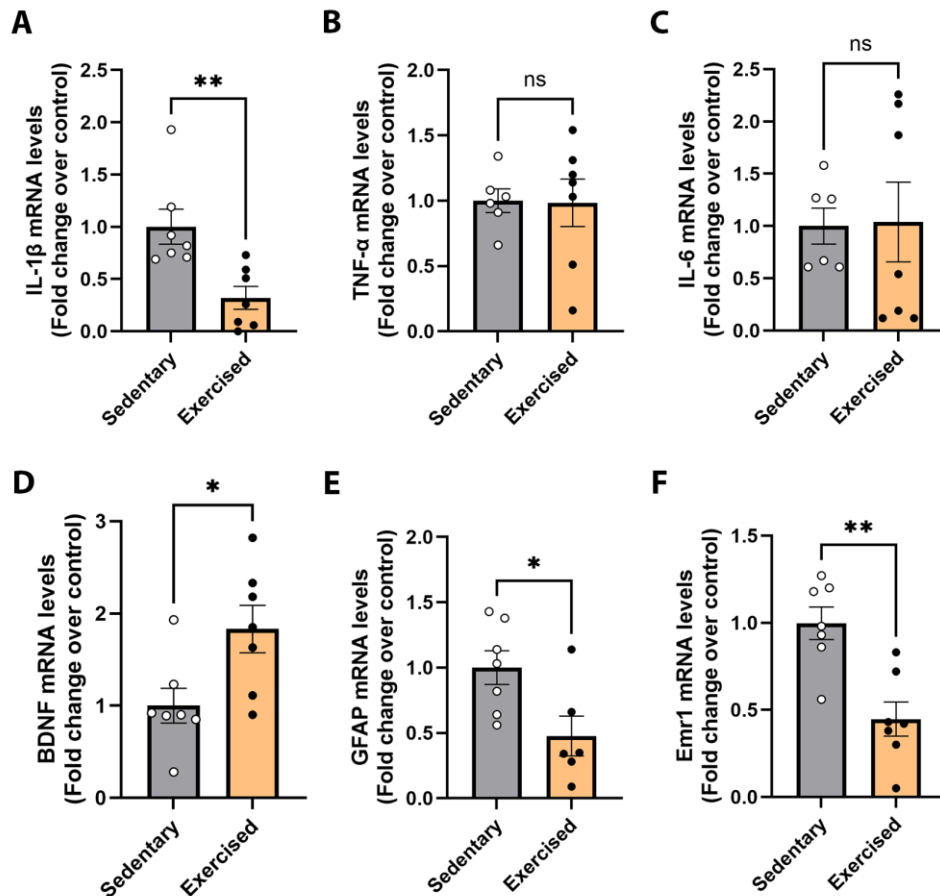


Figure 5. Exercise reduces neuroinflammatory markers and enhances neurotrophic factor expression in the hippocampus of middle-aged mice. mRNA expression of inflammatory, glial, and neurotrophic markers was analyzed in hippocampal tissue by RT-qPCR. Chronic swimming exercise significantly reduced interleukin-1 β (IL-1 β) mRNA levels but had no effect on tumor necrosis factor- α (TNF- α) or interleukin-6 (IL-6) transcript levels. In contrast, exercise increased brain-derived neurotrophic factor (BDNF) mRNA expression and reduced the expression of the gliosis markers glial fibrillary acidic protein (GFAP) and adhesion G protein-coupled receptor E1 (Emr1-F4/80). (A) Relative expression of IL-1 β . (B) Relative expression of TNF- α . (C) Relative expression of IL-6. (D) Relative expression of BDNF. (E) Relative expression of GFAP. (F) Relative expression of Emr1. Grey columns represent sedentary controls; orange columns represent swimming-exercised mice. Data are presented as mean $\pm$ SEM ($n = 6-7$ mice per group) and expressed as fold change relative to sedentary controls. Statistical significance was determined using the Mann-Whitney U test. * $p < 0.05$, ** $p < 0.01$, ns: not significant versus sedentary mice.

Gut microbiota adaptations accompany exercise-induced functional improvements in late middle-aged mice

To determine whether chronic swimming exercise is accompanied by adaptation in gut microbiota during late middle age, microbial community composition was

analyzed and compared between exercised and sedentary animals using complementary diversity and differential abundance approaches. Chronic swimming exercise induced a pronounced remodeling of gut microbiota composition in late middle-aged mice. At the genus level, relative abundance analysis of the most represented taxa revealed clear differences between sedentary and

exercised animals, indicating a redistribution of dominant bacterial populations rather than the appearance of rare taxa (Fig. 7A). Consistent with this observation, Bray–Curtis-based beta-diversity analysis showed a marked separation between groups, with minimal overlap in PCoA space (Fig. 7B). PERMANOVA confirmed a strong effect of exercise on overall community structure ($R^2 = 0.393$, $p = 0.005$), which was not driven by differences in within-group dispersion, as assessed by PERMDISP ($p > 0.05$). Differential abundance analysis further identified specific genera associated with exercise-induced microbiota remodeling (Fig. 7C). Exercised mice exhibited increased abundance of genera such as *Odoribacter*, *Alistipes*, *Clostridia_vadinBB60_group*, *Rikenellaceae_RC9_gut_group*, and *Akkermansia*, whereas sedentary mice showed higher levels of *Romboutsia*, *RF39*, *Prevotellaceae_UCG-001*, and *Alloprevotella*. These patterns were confirmed by

summarizing the top genus-level changes associated with exercise (Fig. 7D). Complementary analyses revealed that exercise-induced microbiota changes occurred predominantly at lower taxonomic levels. Relative abundance at the phylum level and alpha-diversity metrics (observed richness, Shannon diversity, and evenness) did not differ between groups (Supplementary Fig. 7A and B), indicating preserved global diversity. However, presence–absence–based beta-diversity analysis using Jaccard distance still demonstrated significant group separation ($R^2 = 0.172$, $p = 0.003$; Supplementary Fig. 7C), albeit with a smaller effect size compared with abundance-weighted metrics. Together, these findings indicate that chronic swimming exercise reshapes the relative abundance of shared bacterial taxa, resulting in a coordinated remodeling of gut microbiota structure that accompanies the physiological and cognitive adaptations observed in late middle-aged mice.

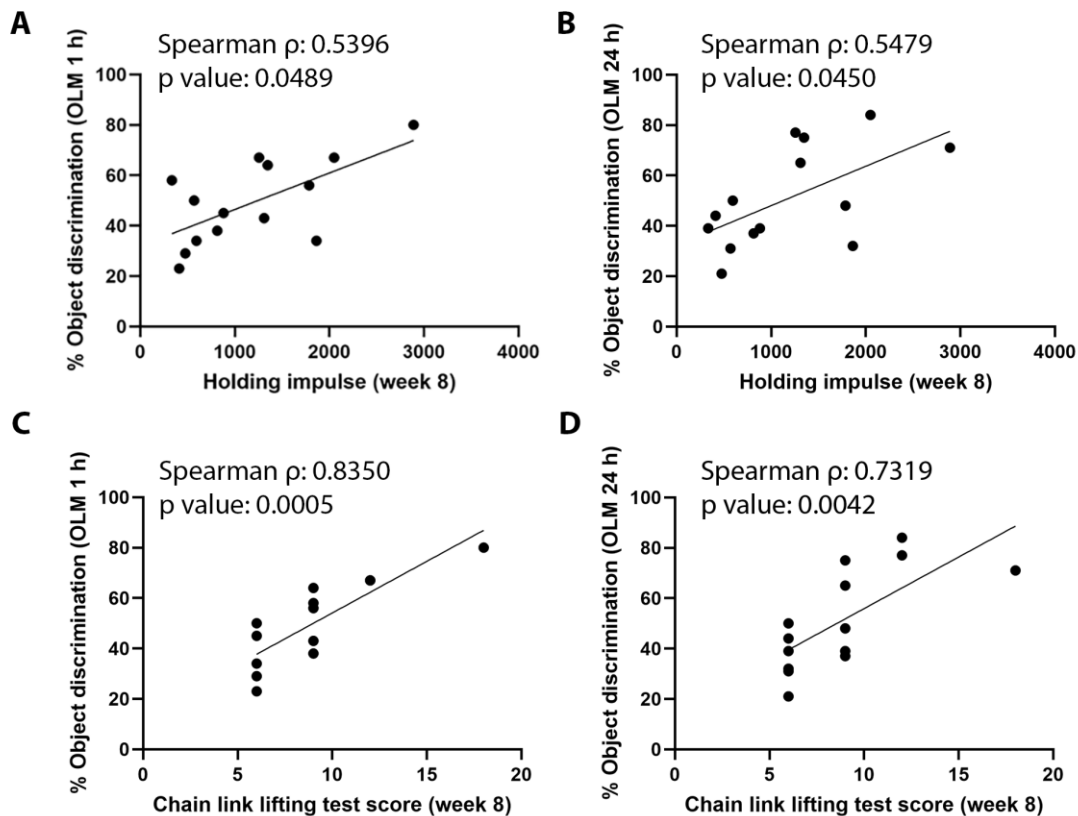


Figure 6. Muscle performance positively correlates with cognitive function assessed by the object location memory in middle-aged mice. Correlation analyses were performed between muscle function assessed at week 8 of the training protocol and discrimination performance in the OLM test. Overall, better muscle performance was associated with improved spatial memory performance in middle-aged mice. (A) Correlation between holding impulse and the OLM discrimination index at the 1 h retention interval. (B) Correlation between holding impulse and the OLM discrimination index at the 24 h retention interval. (C) Correlation between chain link lifting test score and the OLM discrimination index at the 1 h retention interval. (D) Correlation between chain link lifting test score and the OLM discrimination index at the 24 h retention interval. Correlations were assessed using Spearman's rank correlation coefficient (ρ), and the corresponding ρ and p values are indicated in each panel. Each dot represents an individual mouse.

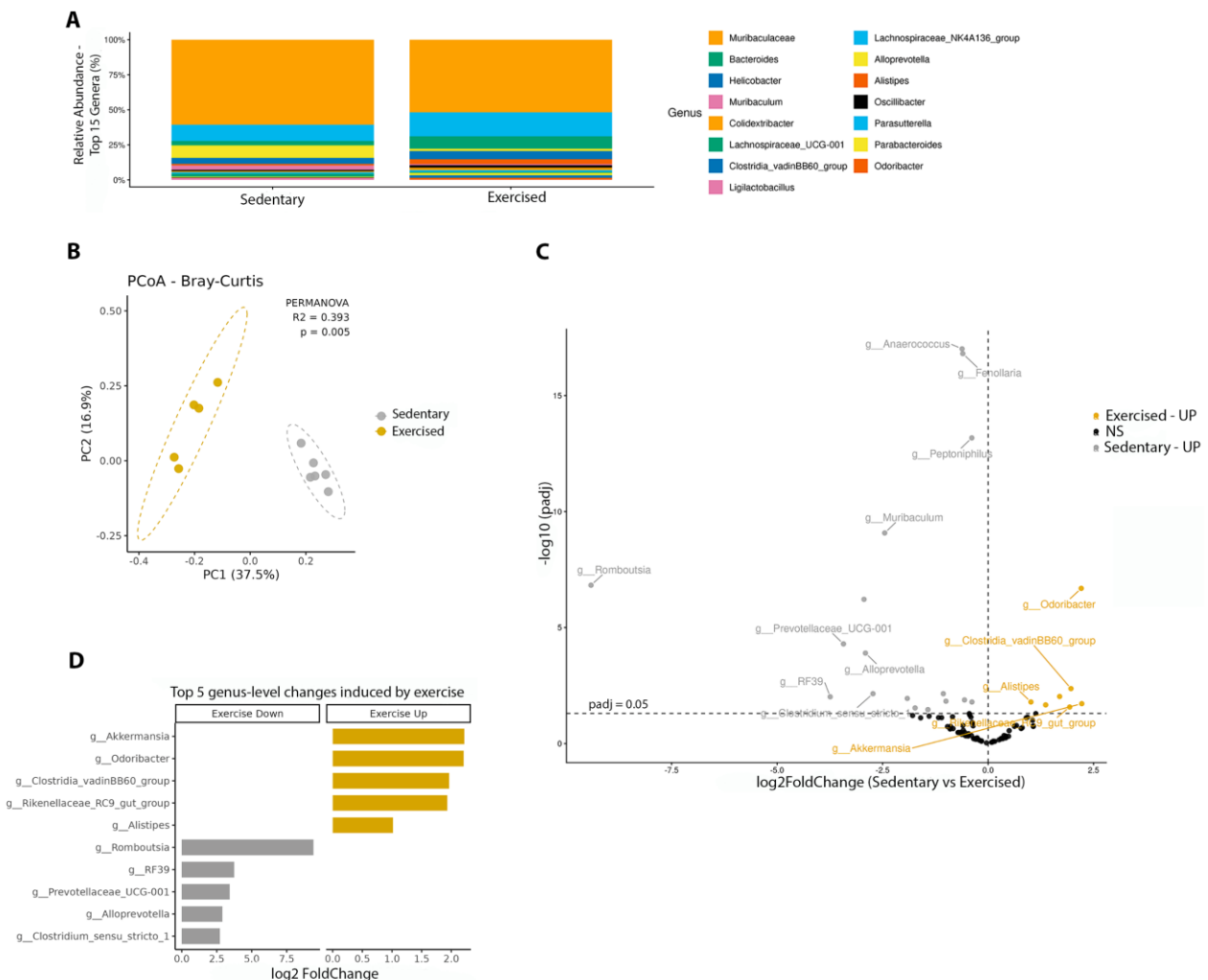


Figure 7. Chronic swimming exercise reshapes gut microbiota composition at the genus level in late middle-aged mice. Gut microbiota diversity and community structure were assessed by 16S rRNA gene sequencing following eight weeks of swimming exercise. Chronic swimming exercise induced a robust remodeling of gut microbiota composition in late middle-aged mice, predominantly affecting the relative abundance of shared bacterial taxa. (A) Stacked bar plots showing genus-level relative abundance of the most represented taxa in sedentary and exercised mice, highlighting a redistribution of dominant genera rather than the emergence of rare taxa. (B) Bray–Curtis dissimilarity–based principal coordinates analysis (PCoA) revealed a clear separation between sedentary and exercised animals. Community structure differences were statistically significant as determined by PERMANOVA ($R^2 = 0.393$, $p = 0.005$). (C) Differential abundance analysis at the genus level identified taxa significantly associated with exercise. Only genera with reliable taxonomic annotation were retained; unassigned or “uncultured” taxa were excluded from highlighted results. (D) Summary of the top exercise-associated genera showing increased (exercise-up) or decreased (exercise-down) relative abundance considered for visualization and interpretation. $n = 5$ –6 mice per group.

DISCUSSION

Summary and integration of findings.

This study demonstrates that eight weeks of moderate-intensity swimming initiated during late middle age elicits broad, coordinated benefits across multiple physiological systems in male C57BL/6J mice. Swimming significantly improved muscle strength and endurance while reducing epididymal fat mass compared with sedentary controls, without affecting overall locomotor activity or anxiety-

like behavior. At the cognitive level, exercised mice exhibit enhanced performance in both OLM and OiP tasks, with the most robust effect observed at the 24 h retention interval. This pattern suggests a preferential improvement in hippocampal-dependent spatial and associative memory processes, particularly those related to memory consolidation rather than short-term encoding. These behavioral improvements were accompanied by a marked attenuation of systemic inflammation, as reflected by reduced circulating levels of pro-inflammatory cytokines including IL-1 β , IL-3, IL-6, and TNF- α ,

together with a trend toward reduced IL-17A and increased anti-inflammatory IL-10. At the central level, exercise was associated with reduced hippocampal mRNA expression of inflammatory and glial-related markers, including IL-1 β , GFAP, and Emr1, together with increased BDNF mRNA levels. In contrast, TNF- α and IL-6 expression, as well as all assessed inflammatory and neurotrophic markers in the mPFC, remained unchanged, underscoring a region-specific sensitivity of the hippocampus to exercise-induced plasticity.

In parallel, 16S rRNA gene sequencing revealed that moderate-intensity swimming exercise reshaped the gut microbiota of late middle-aged mice. Exercise induced a pronounced reorganization of microbial community structure, as evidenced by significant changes in β -diversity, particularly when using abundance-weighted metrics, indicating that exercise primarily modulated the relative abundance of dominant taxa rather than causing extensive taxonomic turnover. At the genus level, exercised mice showed enrichment of bacterial taxa commonly associated with metabolic and anti-inflammatory functions, including *Odoribacter*, *Alistipes*, *Akkermansia*, and members of the *Rikenellaceae* and *Clostridia_yadinBB60* groups, while sedentary mice exhibited higher abundance of genera such as *Romboutsia* and *Prevotellaceae_UCG-001*. Importantly, correlation analyses revealed that individual differences in muscle performance (holding impulse and chain link lifting score) were strong predictors of OLM performance at both 1 h and 24 h, linking peripheral physical fitness with hippocampus-dependent cognitive outcomes. This relationship supports the concept that improvements in muscular function and endurance are functionally coupled with brain health during aging.

Collectively, these findings demonstrate that initiating moderate-intensity exercise during late middle age can concurrently enhance physical performance, hippocampal-dependent cognition, systemic and central inflammatory status, and gut microbiota composition. While many prior studies focus on single biological domain or have initiated interventions earlier in lifespan [25, 26], the present work provides integrative evidence that exercise remains a powerful, multi-system modulator of aging biology even when introduced later in life, reinforcing its value as a feasible strategy to promote resilience during aging.

Exercise as an anti-inflammatory and neurotrophic intervention in aging

Physical inactivity and sedentary behavior are highly prevalent among older adults and exacerbate age-related physiological decline [27, 28]. Prolonged sitting is associated with loss of aerobic fitness, musculoskeletal

and cognitive decline, and accounts for more than half of waking hours [27]. Sedentary lifestyles have been linked to impaired cognitive performance and increased risk of neurodegenerative disorders through alterations in synaptic plasticity and adult neurogenesis [29]. Moreover, physical inactivity elevates circulating pro-inflammatory IL-6, TNF- α and IL-1 β , reinforcing a chronic low-grade inflammatory state known as inflammaging [30, 31]. This pro-inflammatory milieu accelerates biological aging by promoting muscle catabolism, insulin resistance, endothelial dysfunction and neuroinflammation, thereby contributing to frailty and age-related morbidity [32]. By contrast, regular physical exercise improves executive function, memory and mood, reduces systemic inflammation and fosters a favorable neurotrophic environment [7]. Together, these findings underscore the importance of structured exercise strategies to counteract the detrimental effects of inactivity on cognitive, behavioral and immune function in older individuals.

Accumulating evidence indicates that physical activity induces a robust anti-inflammatory response both in peripheral tissues and within the central nervous system. Exercise reduces pro-inflammatory cytokines and increases anti-inflammatory mediators and neurotrophic factors, thereby mitigating cognitive impairments associated with aging and neurodegenerative diseases [33]. Microglia can shift from a classically activated M1 (pro inflammatory) phenotype toward an alternative an M2 (anti-inflammatory/resolution) state, characterized by the production of anti-inflammatory cytokines and trophic factors such as BDNF [34]. In aged mice, long-term wheel running reduced hippocampal IL 1 β and IL 10 and elevated BDNF and neuronal RE1-silencing transcription factor (REST) expression [35]. Similarly, in aged rats, treadmill exercise decreased hippocampal TNF α and IL 6 while increasing BDNF and TrkB signaling, improving memory performance and enhanced gut microbiota diversity [36]. Our findings are consistent with this body of literature, demonstrating that late life swimming reduces circulating pro inflammatory cytokines and suppresses hippocampal IL 1 β while increasing BDNF expression. The absence of changes in hippocampal TNF α and IL 6 expression in our study suggests that the anti-inflammatory effects of exercise may be selective or dependent on longer training durations. This interpretation aligns with reports showing duration-dependent effects of exercise in aged mice, where long term but not short-term interventions increased IL 10 and modulated IL 6 [37].

Importantly, our study provides evidence that initiating exercise during late middle age is sufficient to induce substantial neurobiological benefits. In aged female mice, cognitive deficits were reversed by a specific “sweet spot” of exercise intensity and duration, during

which growth hormone (GH) levels peaked; moreover, increasing GH in sedentary aged mice was sufficient to improve cognition [38]. These findings highlight the critical role of timing and duration in determining the efficacy of exercise interventions. Here, we show that initiating exercise at 14 months of age—corresponding to the onset of late middle age—enhance hippocampal BDNF expression and improved memory performance by 16 months, indicating that the aging brain retains a remarkable capacity for plasticity when appropriately challenged. Furthermore, observed correlation analyses between muscle performance and hippocampus-dependent memory support the existence of a functional muscle–brain axis, linking peripheral physical fitness to central cognitive outcomes.

Region-specific adaptations and possible mechanisms.

The absence of significant transcriptional changes in the mPFC despite robust hippocampal effects may reflect regional differences in aging and stress sensitivity. The hippocampus is particularly vulnerable to age-related inflammatory and neurotrophic dysregulation and responds strongly to exercise-induced modulation, whereas the mPFC may retain inflammatory homeostasis at this age [39]. In addition, the cognitive tasks employed in this study predominantly engaged hippocampal circuits, potentially reinforcing local plasticity while leaving the mPFC relatively unaffected. Our findings are consistent with reports that long-term exercise increases hippocampal neurogenesis and increases IL-10 without uniformly modulating all brain regions [37]. Finally, swimming-based exercise paradigms combine physical activity with a potential mild stress component depending on protocol parameters. However, several aspects of the present design were intended to maintain the intervention within a moderate physiological range, including controlled water temperature (30 ± 1 °C) and a limited workload (5% of body weight), which are commonly used in endurance swimming protocols that induce metabolic and neurobiological adaptations without exhaustion. Consistent with this interpretation, exercised mice did not display increased anxiety-like behavior in the open field test, suggesting that the intervention did not induce persistent stress-related behavioral alterations. Previous studies have similarly reported that moderate swimming exercise enhances hippocampal plasticity and cognitive performance through neurotrophic and anti-inflammatory mechanisms rather than chronic stress responses [19, 40]. Nevertheless, forced swimming may transiently activate physiological stress pathways, which could differentially affect specific brain regions. In particular, cortical regions such as the mPFC are highly sensitive to glucocorticoid signaling and stress-related modulation [40,41],

potentially dampening transcriptional responses in this region despite the clear adaptations observed in the hippocampus.

The mechanisms linking exercise to reduced inflammation and enhanced neurotrophic support likely involve multiple, converging pathways. Microglia and astrocytes respond to exercise-derived exerkines (e.g., IL-6, lactate, BDNF), which modulate their activation states [41]. IL-6 released from contracting skeletal muscles may cross the blood–brain barrier and initiate anti-inflammatory cascades, whereas lactate acts as a signaling molecule promoting BDNF expression [42]. Circulating growth hormone and insulin-like growth factor 1 (IGF-1) also increase during exercise and can enhance neurogenesis and synaptic plasticity [38, 43]. Moreover, epigenetic modifications in the hippocampus (e.g., histone acetylation at *Bdnf* and *cFos* promoters) are responsive to late-life exercise [44], and progressive weighted wheel running rejuvenates skeletal muscle epigenetic age by approximately eight weeks [45]. Together, these molecular adaptations may underlie the observed improvements in memory and physical fitness.

Exercise, gut microbiota and the muscle–gut–brain axis

Exercise induced a reproducible remodeling of the gut microbiota, including enrichment of *Akkermansia*, *Odoribacter*, *Alistipes*, and several anaerobic taxa within the class *Clostridia*, together with reductions in genera more frequently associated with sedentary or dysbiotic states. Among these, *Akkermansia muciniphila* is one of the most consistently exercise-responsive bacteria reported in both humans and rodents, and its increase has been linked to improved gut barrier integrity, reduced systemic inflammation, and healthier metabolic aging, all of which are relevant to brain health during aging [46]. Importantly, emerging preclinical and associative human evidence suggests a link between *Akkermansia* abundance and cognitive function: reduced levels of *Akkermansia* have been reported in individuals with hippocampal atrophy and cognitive decline, while restoration of *Akkermansia* in mouse models improves neuroinflammatory tone and cognitive performance, supporting a role for this genus in microbiota–gut–brain communication [47–49]. Notably, large multi-omics analyses in human populations have demonstrated that specific gut microbial genera are inversely associated with cognitive impairment and structural brain alterations. In particular, *Odoribacter* has been identified as a protective genus across independent cohorts, showing a robust positive association with hippocampal volume and white matter integrity, an effect partly mediated by circulating acetate levels [50]. In parallel, recent human data showing

that *Alistipes* has been repeatedly reported as a genus associated with age- and cognition-related phenotypes, including differential abundance in cohorts with cognitive impairment and neurodegenerative disease, meanwhile poorer cognitive performance is associated with lower abundance of *Clostridium* genera, which have been proposed as neuroprotective through their capacity to support gut homeostasis and short-chain fatty acid production [51, 52]. Importantly, age-related shifts in gut microbial communities, including changes in *Rikenellaceae*-associated taxa, have been linked to inflammaging and functional decline, supporting the concept that preservation of a more “youth-like” microbiota configuration may contribute to healthier aging trajectories [53, 54]. Chronic swimming exercise led to a marked reduction in the relative abundance of the genus *Romboutsia*. This finding is particularly relevant in light of previous human studies reporting an increased abundance of *Romboutsia* in patients with Alzheimer’s disease compared to cognitively healthy controls [55]. Notably, higher levels of *Romboutsia* have been shown to negatively correlate with right amygdala volume, a brain structure critically involved in memory processing and emotional regulation and known to undergo atrophy in Alzheimer’s disease [55]. Our data are consistent with the concept of a muscle–gut–brain axis, where improved muscle function co-occurs with favorable microbial shifts and enhanced cognitive performance.

Exercise-induced changes in gut microbiota can persist even after microbiota transfer. Germ-free mice colonized with microbiota from exercised donors exhibit altered β -diversity, reduced body mass and attenuated response to chemically induced colitis [56], highlighting the functional significance of exercise-driven microbiota adaptations. This microbiota may influence brain function via immune pathways and microbial metabolites such as short-chain fatty acids or eicosanoids. In a multi-omics study, exercise remodeled eicosanoid metabolism associated with *Akkermansia*, alleviating intestinal senescence in aged mice [57]. Such metabolites can cross the blood–brain barrier and modulate neuroinflammation and neuroplasticity [15]. Future studies should quantify microbial metabolites and determine whether transplanting microbiota from exercised mice recapitulates cognitive and physical benefits. Our previous work further demonstrated that transplanting microbiota from young-trained donors into aged mice enhances cognitive function and synaptic plasticity. In that study, aged mice receiving a fecal microbiota transplant (FMT) from young-trained donors exhibited improved recognition and spatial memory, preserved hippocampal long-term potentiation and upregulated synaptic markers compared with age-matched or sedentary donor FMT [18]. Young-exercised microbiota

transplant also reduced neuroinflammation, decreased gut permeability and increased cecal levels of butyrate and valerate, while enriching beneficial genera such as *Akkermansia* and *Prevotellaceae* [18]. These findings indicate that exercise-shaped microbial communities can contribute causally to brain health in experimental settings. However, the present study demonstrates associations between exercise, microbiota remodeling, and physiological outcomes, rather than direct causality within this dataset.

Despite its promise, microbiome manipulation through FMT carries inherent risks. The U.S. Food and Drug Administration (FDA) issued safety alert after following cases of pathogenic *Escherichia coli* transmission during investigational FMT, resulting in several infections and fatalities [58]. Consequently, FMT must be conducted under investigational new-drug protocols with rigorous donor screening and informed consent [59]. Clinical guidelines stress that potential donors undergo extensive questionnaires and serologic and stool testing to exclude asymptomatic pathogen carriers [60]. Even when precautions are followed, adverse events occur in roughly 19% of recipients, typically abdominal discomfort, bloating, nausea or diarrhea, and serious microbiota-related events such as bacteremia or sepsis occur in about 1% of patients, particularly those with compromised mucosal barrier [60, 61]. Moreover, FMT is considered investigational for indications other than recurrent *Clostridioides difficile* infection, and long-term safety data are limited [60]. In this context, exercise emerges as a safer, accessible and non-invasive alternative. We observed that moderate physical activity reshapes the gut microbiota, increases beneficial taxa such as those described above, reduces systemic inflammation and improves cognition without the need for invasive procedures or exposure to donor-derived pathogens. Our study demonstrates that initiating exercise at 14 months—an advanced stage of the mouse lifespan—elicits pronounced benefits across muscle strength, memory, inflammatory profiles and microbiota composition. Thus, although microbiota-targeted therapies hold promise, promoting regular, late-life exercise may provide a low-risk, non-pharmacological means to achieve similar neuroprotective and anti-inflammatory effects.

Novelty and significance

This study provides several novel and mechanistic contributions to the understanding of exercise-induced adaptations during aging. First, initiating exercise in late middle age improved muscle strength, reduced adiposity, and enhanced hippocampal-dependent memory, demonstrating that beneficial adaptations remain

attainable late in life. Second, the integration of behavioral, physiological, immunological, neurotrophic and microbiota measures revealed correlations between peripheral muscle performance and hippocampal memory, supporting a functional muscle–gut–brain axis. Third, the use of moderate-load swimming mirrors a widely practiced human exercise modality, enhancing translational relevance. Fourth, our 16S rRNA sequencing identified specific microbial taxa and diversity metrics altered by exercise, aligning with and extending previous findings in aged rodents. Together, these findings position chronic swimming as a potent geroprotective intervention targeting multiple hallmarks of aging.

Future directions

This study provides a conceptual framework for investigating how physical activity initiated in later life modulates interconnected mechanisms that govern aging and resilience. A central key future direction is to define the signaling logic through which skeletal muscle acts as a systemic communicator during exercise, coordinating adaptive responses across the brain and the gut. Understanding how transient exercise-induced signals are integrated over time to generate lasting effects on hippocampal plasticity and microbiota composition will be essential to conceptualize exercise as a resilience-promoting intervention rather than a transient stimulus. At the level of the aging brain, future studies should determine whether late-life exercise can reprogram neuroimmune states that typically drift toward chronic inflammation with age. Clarifying how microglial and astrocytic phenotypes are reshaped by physical activity, and whether these changes are associated with preserved synaptic function and memory, will help establish neuroimmune plasticity as a mediator of cognitive health span.

Another important avenue concerns the gut–brain axis as an integrative regulator of brain aging. Exercise-induced alterations in microbial metabolism may function as systemic modulators linking peripheral energy metabolism to central inflammatory tone. Establishing causal relationships between specific microbial metabolites and cognitive outcomes in aged organisms will be critical for identifying transferable and potentially targetable mediators of exercise benefits. These long-term adaptations are likely sustained by stable epigenetic and transcriptional regulation. Accordingly, future studies integrating epigenomic and transcriptomic analyses across skeletal muscle and hippocampus will provide insight into how late-life behavioral interventions induce durable molecular reprogramming, even in aged tissues traditionally considered less plastic. From a geroscience perspective, biological heterogeneity must be explicitly

addressed. Examining sex- and age-dependent responses to exercise will be necessary to determine whether resilience mechanisms differ across the lifespan, particularly given the influence of sex hormones on neurotrophic signaling and neuroimmune function. Finally, these conceptual advances highlight the importance of translation. Determining whether accessible, low-impact aquatic exercise programs in older adults engage similar anti-inflammatory, neurotrophic, and microbiota-associated pathways will be a critical step toward leveraging exercise as a scalable intervention to extend cognitive health span.

Limitations

Some limitations should be acknowledged. Sample size was modest, and only male mice were studied, limiting statistical power and generalizability to females. Forced swimming may introduce stress that could confound gene expression and cognitive outcomes, although no anxiety-like behavior was observed. Future studies incorporating physiological stress markers, such as circulating corticosterone levels or adrenal gland measurements, will help to further distinguish exercise-specific from stress-related effects in swimming-based exercise paradigms. We measured only mRNA levels of inflammatory and neurotrophic markers in the hippocampus; therefore, the observed changes in transcripts such as IL-1 β , GFAP, Emr1, and BDNF do not establish corresponding protein-level regulation or cell-type-specific localization. In particular, these data do not define astrocytic or microglial phenotypic shifts. Future studies incorporating protein analyses, immunohistochemistry, and cell-type-specific approaches will be necessary to better characterize the cellular mechanisms underlying these transcriptional changes. Gut microbiota profiling relied on 16S rRNA sequencing; metagenomic and metabolomic approaches would provide deeper functional insights. In addition, although exercise-induced microbiota shifts were observed, the present study does not establish direct causal links between specific microbial taxa and cognitive or inflammatory outcomes. Future studies integrating microbiota transfer or metabolite analyses will be required to address this question. Finally, our study focused on a single exercise intensity and duration, leaving dose–response relationships unexplored.

Conclusion

Chronic moderate swimming initiated in late middle age ameliorates multiple hallmarks of aging, including inflammation, neurotrophic decline, muscle weakness, cognitive impairment and gut dysbiosis. These findings underscore the capacity of the aged brain and body to

adapt to physical activity and highlight the coordinated interplay between muscle performance, gut microbiota and hippocampal function. Collectively, the data support the promotion of regular physical activity in mid-to-late life as a non-pharmacological geroprotective strategy and provide a foundation for future mechanistic studies and translational trials.

Data availability

Raw 16S rRNA gene sequencing reads have been deposited in the NCBI Sequence Read Archive (SRA) under BioProject accession number PRJNA1433506. Additional data supporting the findings of this study are available from the corresponding author upon request.

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Authors' Contributions

P.L.L., G.J., and R.P.-V. designed the experiments and contributed to the conceptualization of the study. P.L.L. and G.J. supervised the research. R.P.-V., L.AdS. and J.M. performed the behavioral experiments. C.I.-Q., F.F.-A., and G.J. developed and conducted the gut microbiota bioinformatic analyses. R.P.-V., G.J., J.R.-P., F.S.-O., and C.A.-G. developed the experiments presented in the main text and supplementary material and analyzed the corresponding experimental data. P.J.U., Y.A.N., and F.J.C. contributed to the conceptualization of the work and participated in manuscript review and editing. G.J. prepared the figures of the study. P.L.L., G.J., R.P.-V., P.J.U., Y.A.N., and F.J.C. contributed to data interpretation. P.L.L. and G.J. wrote, reviewed, and edited the manuscript. All authors read and approved the final manuscript.

Conflict of interest

The authors declare no competing interests.

Supplementary Materials

The Supplementary data can be found online at: www.aginganddisease.org/EN/10.14336/AD.2026.0192.

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