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The effect of treadmill exercise on memory function and gut microbiota composition in old rats

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Aging is associated with declines in memory function and significant change in gut microbiota. In this study, we investigated how exercise affects age-related memory decline and inflammation, and gut microbiota diversity. Bl6 mice were divided into control, control and exercise, old, and old and exercise groups. Treadmill exercise was performed once a day, 5 days a week for 8 consecutive weeks. Short-term memory was assessed using step-through test and spatial learning memory was assessed using Morris water maze task. Enzyme-linked immunosorbent assay was performed for the proinflammatory cytokines, tumor necrosis factor (TNF)-α and interleukin (IL)-6, in the hippocampus. Western blot analysis was conducted for the neurotrophic factors, brain-derived neurotrophic factor (BDNF) and tyrosine kinase B (TrkB), in the hippocampus. In addition, fecal samples were collected for sequencing and metagenomic analysis. Old rats showed decline in short-term memory and spatial learning memory. Increment of TNF-α and IL-6 concentration with decrement of BDNF and TrkB expression were observed in the old rats. Decreased diversity of gut microbiota composition and decreased beneficial gut microbiota were found in the old rats. However, treadmill exercise improved short-term memory, decreased TNF-α and IL-6 concentration, and increased BDNF and TrkB expression in the old rats. Treadmill exercise also increased the diversity of gut microbiota composition and affected the increase of beneficial gut microbiota in the old rats. In conclusion, treadmill exercise reduced age-related inflammatory markers and effectively improved memory decline while enhancing the diversity and abundance of beneficial gut microbiota.

Keywords: Aging, Exercise, Microbiome, Memory, Proinflammatory cytokines

INTRODUCTION

Aging is a universal and natural process in which physical and cognitive functions decline progressively over time. This process also affects the gut microbiota, resulting in decreased microbial diversity, impaired immune function, and impaired inflammatory regulation (Ticinesi et al., 2019). Age-related changes in the gut microbiota contribute to increased inflammation through various pathophysiological mechanisms (Biagi et al., 2016). Compared to younger populations, older people have an increased Bacteroidetes population, a decrease in beneficial microbes such as *Bifidobacteria* and *Firmicutes*, and a decrease in overall gut microbial diversity (Ticinesi et al., 2017). These changes in microbial composition and community diversity during aging are attributed to increased inflammatory responses, contributing to the development of metabolic, cancer, and degenerative diseases (Ticinesi et al., 2019; Vaiserman et al., 2017). These findings highlight the important role of changes in the gut environment in disease pathogenesis and emphasize the need for improved lifestyle and dietary interventions to support gut microbial diversity, particularly during aging.

Exercise is well known as a means of improving cardiorespirato-

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ry fitness, increasing muscle strength and power, and improving overall health when performed regularly (Russell et al., 2014). Sustained physical activity has also been shown to positively impact cognitive functions such as memory and learning (Hopkins et al., 2012). Furthermore, exercise is recognized as an effective strategy to enhance neuroplasticity and mitigate neuronal cell death, particularly in the context of aging and Alzheimer's disease (Kramer et al., 2006; Öhman et al., 2014). In addition to its systemic benefits, exercise has a profound effect on the gut microbiome. There is evidence that regular exercise may help maintain a healthy gut environment by increasing beneficial bacteria populations and decreasing harmful bacteria populations (Petersen et al., 2017).

The aging immune system is characterized by immunosenescence and a paradoxical state of increased inflammation, which can lead to immune dysfunction. Chronic systemic inflammation has been implicated in the pathogenesis of neurodegenerative and metabolic disorders (De Luca d'Alessandro et al., 2011). Managing inflammation associated with aging is an important strategy for maintaining health and preventing disease.

Disruption of the gut microbiome has been shown to be associated with neurological function and brain metabolite regulation (Erny et al., 2021; Hao et al., 2019). Dysbiosis can be caused by factors such as poor diet, overgrowth of pathogenic microbes, and stress-induced mental health problems, all of which can alter the composition of the gut microbiome (Collins and Bercik, 2009). The gut-brain axis refers to the two-way communication between the gut and the brain, either directly or indirectly. The composition of the gut microbiome changes significantly with age. *Akkermansia muciniphila* supports gut health and alleviates conditions such as metabolic diseases, diabetes, and inflammation. Aging decreases gut stem cell regeneration, leading to symptoms such as epithelial cell imbalance and digestive dysfunction. Additionally, the abundance of *A. muciniphila* decreases with age (Collado et al., 2007).

Brain-derived neurotrophic factor (BDNF) plays a critical role in brain neuroplasticity and development of immature neurons through its interaction with tyrosine kinase B (TrkB). It is an important factor in neurogenesis and neuronal growth within the central nervous system, including cell growth and synaptogenesis. BDNF levels are affected by age, and circulating levels typically decline as individuals age (Webster et al., 2006). However, increased physical activity and exercise have been shown to improve decreased BDNF levels. In particular, exercise has been shown to positively affect both BDNF and TrkB levels in older adults (Northey et al., 2018). Exercise-induced changes in BDNF levels are closely related to cognitive function and memory, with aerobic exercise having a particularly significant effect (Northey et al., 2018). This suggests that cognitive decline in older adults may be alleviated by aerobic exercise.

Exercise has been shown to improve overall gut health by increasing the diversity and abundance of beneficial gut microbes, including commensal bacteria (Monda et al., 2017). In addition, gut microbes have been shown to modulate antioxidant enzyme activity in response to exercise (Hsu et al., 2015). These microbes also play an important role in regulating oxidative stress and inflammatory responses, and influence metabolism and energy expenditure during physical activity (Mach and Fuster-Botella, 2017). Aging not only leads to declines in physical and cognitive functions, but also causes significant changes in the composition of the gut microbiome. Building on previous research, this study aimed to investigate the effects of exercise on gut microbiome diversity and the abundance of beneficial microbes, and explore its association with improving age-related cognitive decline and inflammation.

MATERIALS AND METHODS

Experiment animals and groups

All animal experiments were conducted in compliance with the guidelines of the National Institutes of Health and the Korean Academy of Medical Sciences. The study protocol was approved by the Animal Experiment Ethics Committee of Kyung Hee University (approval No. KHUASP [SE]-20-573). All BL6 mice were housed in a controlled environment with a temperature of 25°C± 1°C and a 12-hour light/dark cycle (lights on from 7:00 a.m. to 7:00 p.m.), and food and water were supplied ad libitum. All animals were randomly divided into four groups (n=10 per group): control group (12 weeks old), control and exercise group (12 weeks old), old group (20 months old), and old and exercise group (20 months old).

Exercise protocol

According to previously described methods (Park et al., 2020), after an initial 1-week acclimation period, mice in the treadmill exercise groups ran on the treadmill for 30 min/day, 5 days/wk for 8 weeks. For the exercise protocol, mice warmed up for 5 min at 3 m/min at a 0° incline, performed the main exercise for 20 min at 10 m/min, and cooled down for 5 min at 3 m/min.

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Step-through test

Short-term memory was assessed using the step-through test, as described previously (Park et al., 2020). In the training phase, the mice were placed in the entrance identified by the halogen bulb and the box door was opened. When the mice entered the dark compartment, the door was closed and the mice were allowed to remain there for 20 sec. This training was repeated twice. In the third training session, when the mice entered the dark compartment, the door was simultaneously closed and the mice received a 1 mA scrambled foot shock for 2 sec. After 24 hr, the mice were returned to the entrance identified by the halogen bulb. When the door was opened, the latency time for the mice to enter the dark compartment was measured. If the latency exceeded 300 sec, it was recorded as 300 sec.

Morris water maze task

Spatial learning memory was assessed using the Morris water maze task as described by Park et al. (2020). Mice were habituated to swimming in a pool without a platform for 60 sec the day before training. Training sessions were performed 3 times a day for 5 consecutive days. Probe trials were performed 24 hr after the last training session. Once the platform was found, the mouse was allowed to remain there for 30 sec. If the mouse did not find the platform within 60 sec, it was gently guided to the platform by hand. The mouse then underwent a 60-sec retention probe test, during which the platform was removed from the pool. Data collection was automated using the Smart Video Tracking System (Smart version 2.5, Panlab, Barcelona, Spain).

Enzyme-linked immunosorbent assay for proinflammatory cytokines

The concentration of proinflammatory cytokines, such as tumor necrosis factor (TNF)-α and interleukin (IL)-6, were measured using an enzyme-linked immunosorbent assay kit (Abcam, Cambridge, UK) according to the previously described method (Park et al., 2023).

Western blot analysis for BDNF and TrkB

As described in a previous method (Park et al., 2023), hippocampal tissues were homogenized on ice using a homogenizer and lysed in a lysis buffer. Forty micrograms of protein were separated into SDS-polyacrylamide gels and transferred onto a nitrocellulose membrane. This membrane was then incubated with primary antibodies: mouse β-actin antibody (1:3,000; Santa Cruz Biotech, Inc., Dallas, TX, USA), rabbit BDNF (1:1,000; Bioss) and TrkB (1:1,000; Cell Signaling Technology, Inc., Danvers, MA, USA). Horseradish peroxidase-conjugated anti-mouse and anti-rabbit antibodies were used as secondary antibodies for β-actin and BDNF and TrkB respectively.

Collection of fecal samples and sequencing/metagenomic analysis

Fecal samples, collected as previously described method (Park et al., 2022), were placed in sterile test tubes. Total DNA was extracted from 200 mg of feces per sample using QIAamp Fast DNA Stool Mini Kit (QIAGEN, Hilden, Germany) as the manufacturer's instructions. For sequencing library preparation targeting the V3 and V4 regions, 2 ng of input gDNA was prepared using a 5× reaction buffer, 1 mM dNTP mixture, 500 nM of universal F/R polymerase chain reaction (PCR) primers, and Herculase II Fusion DNA Polymerase (Agilent Technologies, Santa Clara, CA, USA). The primary PCR conditions included 25 cycles of 3 min at 95°C, followed by 30 sec at 95°C, 30 sec at 55°C, and 30 sec at 72°C, with a final extension for 5 min at 72°C.

The primary PCR product was purified using AMPure beads (Agencourt Bioscience, Beverly, MA, USA). After purification, 2 μL of the primary PCR product was further amplified using NexteraXT Indexed Primers to construct the final library containing the index. The second PCR cycle conditions were identical to those of the primary PCR, except that the amplification was limited to 10 cycles. The PCR product was purified again with AMPure beads. The final purified product was quantified using qPCR following the KAPA Library Quantification Kit protocol for Illumina Sequencing Platforms and qualified using a TapeStation D1000 ScreenTape (Agilent Technologies, Waldbronn, Germany). Sequencing of barcoded 16S rRNA gene amplicons was performed using the Illumina MiSeq platform (Illumina, San Diego, CA, USA) by Macrogen Inc. (Seoul, Korea). Whole metagenomic DNA extracted from fecal samples was subjected to paired-end shotgun sequencing using the Illumina Hi-Seq 2000 platform, also conducted by Macrogen Inc.

Data analysis

IBM SPSS Statistics version 26.0 (IBM Corp., Armonk, NY, USA) was used for data analysis. One-way analysis of variance followed by Tukey *post hoc* test was performed to compare differences between groups. Results are presented as the mean ± standard error of the mean, and a P-value < 0.05 was considered statistically significant.

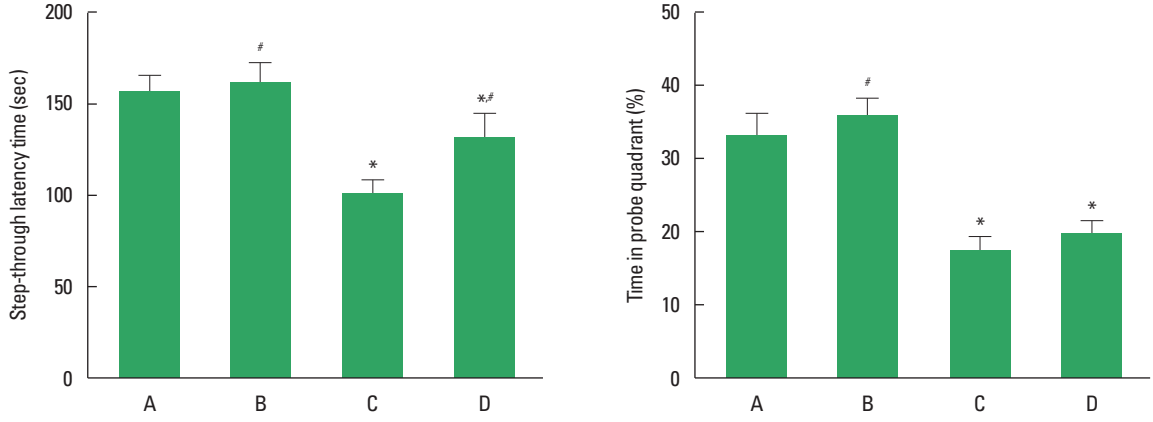

Fig. 1. The effects of treadmill exercise on short-term memory and spatial learning memory. Left panel: step-through test. Right panel: Morris water maze task. Data are expressed as the mean ± standard error of the mean. A, control group; B, control and exercise group; C, old group; D, old and exercise group. *P< 0.05 compared to the control group. #P< 0.05 compared to the old group.

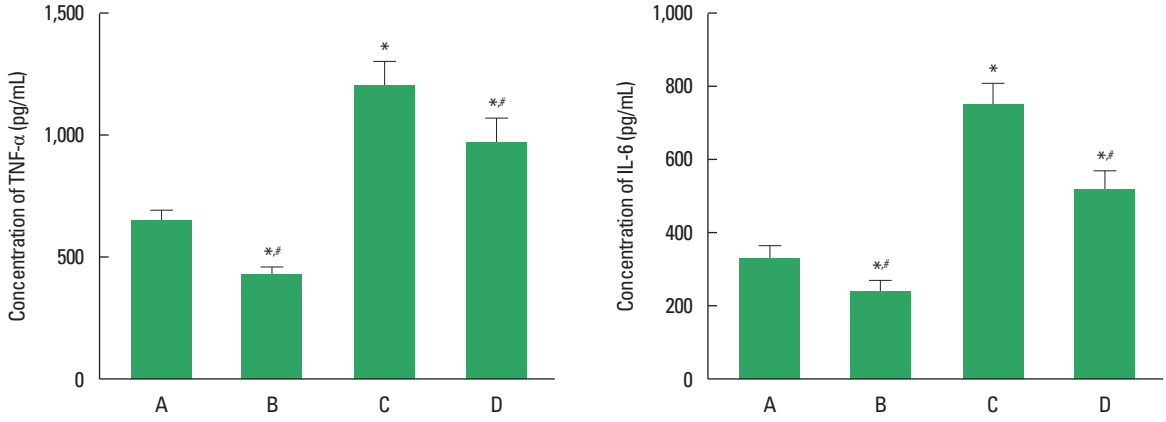

Fig. 2. The effects of treadmill exercise on hippocampal concentrations of tumor necrosis factor (TNF)-α (left panel) and interleukin (IL)-6 (right panel). Data are expressed as the mean ± standard error of the mean. A, control group; B, control and exercise group; C, old group; D, old and exercise group. *P< 0.05 compared to the control group. #P< 0.05 compared to the old group.

RESULTS

Short-term memory and spatial learning memory

Fig. 1 show the results of short-term memory and spatial learning memory. Short-term memory was assessed using step-through test. Short-term memory was lower in the old group (P < 0.05), but treadmill exercise improved short-term memory in the young group and in the old group (P < 0.05) (Fig. 1, left panel).

Spatial learning memory was assessed using Morris water maze task. The probe quadrant time in the Morris water maze task was reduced in the old group (P < 0.05), but treadmill exercise improved spatial learning memory in the young group (P < 0.05) not in the old group. (Fig. 1, right panel).

Concentration of TNF-α and IL-6

Concentration of proinflammatory cytokines, TNF-α and IL-6, are presented in Fig. 2. Concentration of TNF-α and IL-6 was significantly elevated in the old group (P < 0.05), but treadmill exercise significantly reduced the concentration of TNF-α and IL-6 in the young group and in the old group (P < 0.05).

Expression of BDNF and TrkB

To investigate expression of the neurotrophic factors, BDNF and TrkB, in the hippocampus, Western blot of BDNF and TrkB was analyzed (Fig. 3). The expression level in the control group were normalized to 1.00. Expression of BDNF and TrkB was significantly decreased in the old group (P < 0.05), but treadmill exercise significantly increased the expression of BDNF and TrkB in

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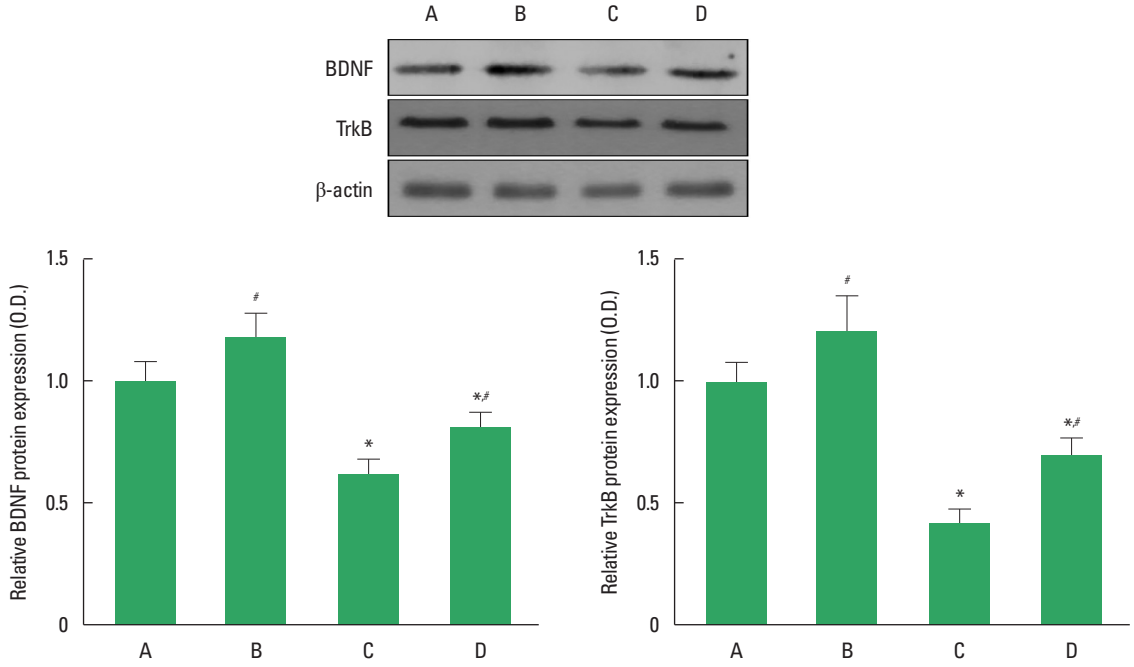

Fig. 3. The effects of treadmill exercise on brain-derived neurotrophic factor (BDNF) and tropomyosin receptor kinase B (TrkB) expression in the hippocampus. Upper panel: representative expression of BDNF and TrkB. Left lower panel: relative BDNF expression. Right lower panel: relative TrkB expression. Data are expressed as the mean ± standard error of the mean. A, control group; B, control and exercise group; C, old group; D, old and exercise group. *$P < 0.05$ compared to the control group. #$P < 0.05$ compared to the old group.

the old group ($P < 0.05$).

Microbiome in the gut microbiota

The diversity of gut microbiota is presented in Fig. 4. Aging shows the reduced diversity of intestinal microflora composition. *Lactobacillus*, *Bifidobacterium*, and *A. muciniphila* were lower in the old group ($P < 0.05$), but treadmill exercise significantly increased *Lactobacillus* and *Bifidobacterium* ($P < 0.05$). Treadmill exercise did not exert significant effect on the abundance of *A. muciniphila* in the old group.

DISCUSSION

Aging is a complex process characterized by a decline in physiological function due to continuous changes at the cellular and tissue levels. Aging leads to various changes, including neurodegeneration due to impaired neuroplasticity and dysregulation of homeostatic signaling systems (Bektas et al., 2018). Aging also affects the composition of beneficial gut microbiota, resulting in a decrease in the richness and diversity of the microbiota (Finamore et al., 2019). In this study, the old group that participated in exercise had increased gut microbiome diversity compared to the control group.

In particular, the populations of *Lactobacillus* and *Bifidobacterium*, well-known beneficial gut bacteria commonly used in probiotics for gut health, increased (Maftei et al., 2024; Sanders et al., 2019). These microbes have been reported to have positive effects on various processes, including neurogenesis and neurodevelopment, and also contribute to immune regulation (Kong et al., 2022). Our results confirmed that exercise increased *Lactobacillus* and *Bifidobacterium*, suggesting that physical activity promotes the growth of beneficial gut microbiome and induces positive changes in the gut microbiome. *A. muciniphila* is another major gut microbe known to decline with aging. *A. muciniphila*, present in the intestinal mucosa, has been shown to help promote gut health and improve conditions such as metabolic diseases, diabetes, inflammatory diseases, and even cancer. The abundance of *A. muciniphila* in the human gut decreases with age (Collado et al., 2007). In the present study, *A. muciniphila* levels were lower in the old group compared to the young group, but no significant difference was observed with exercise. This suggests that the decline in *A. muciniphila* is mainly caused by aging rather than exercise.

Aging disrupts the peripheral immune system, promoting an inflammatory state characterized by the release of proinflammato-

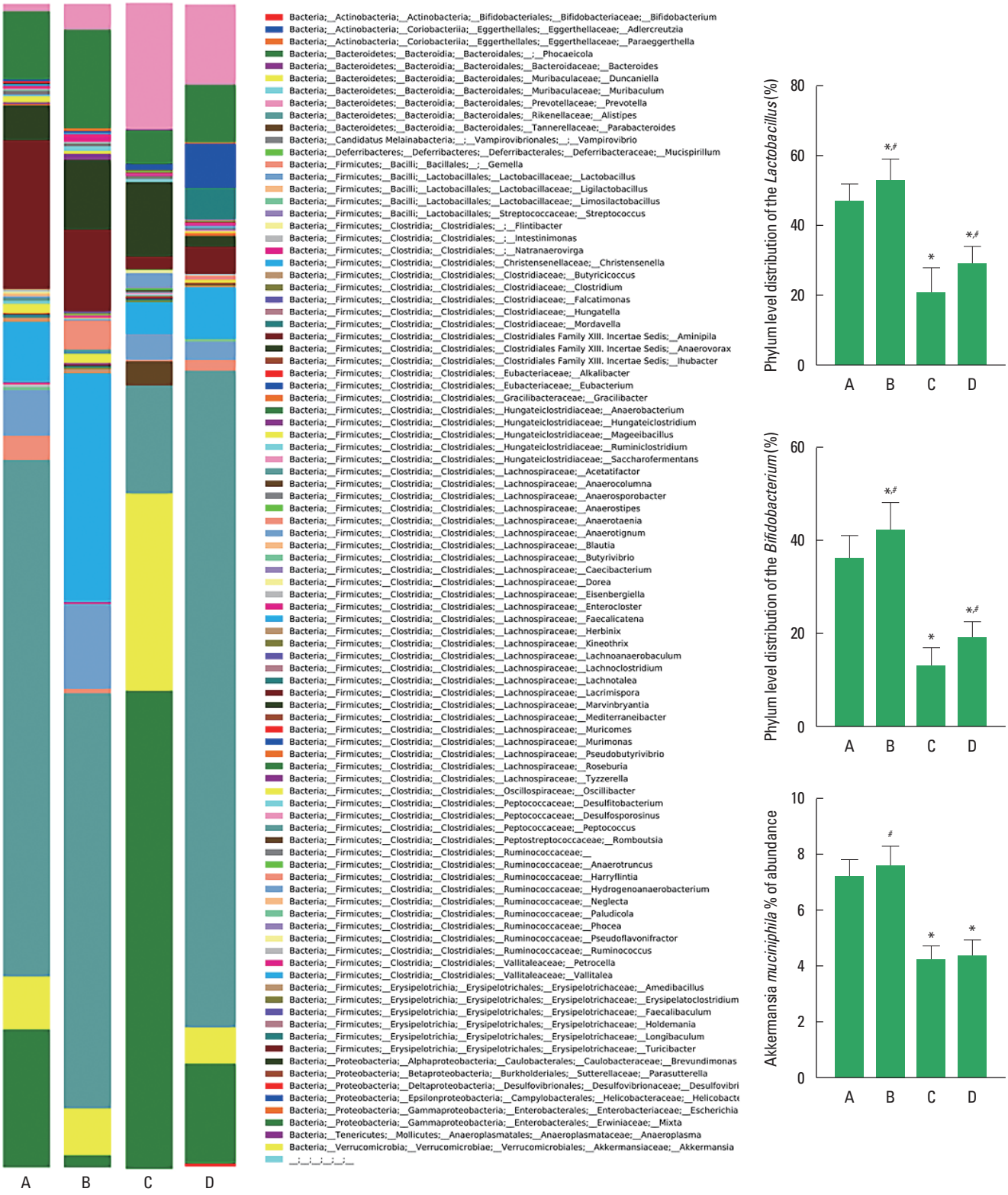

Fig. 4. Effects of aging and exercise on the gut microbiome. Left panel: gut microbiome diversity. Right panel: Phylum-level distribution of the gut microbiota determined by cloning fecal 16S rRNA genes and sequencing the resulting clones. Data are expressed as the mean ± standard error of the mean. A, control group; B, control and exercise group; C, old group; D, old and exercise group. *$P<0.05$ compared to the control group. $^{\#}P<0.05$ compared to the old group.

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ry cytokines and a decrease in anti-inflammatory cytokines, resulting in excessive innate immune activity (Gruver et al., 2007). Major proinflammatory cytokines, such as IL-1β, IL-6, IL-12, IL-18, interferon-γ, and TNF-α, secreted by immune cells, and anti-inflammatory cytokines, such as IL-4, IL-10, IL-13, and IL-19, mediate immune responses through interactions with body cells (Wong et al., 2001). Elevated levels of IL-6 and TNF-α are associated with increased morbidity, disability, and mortality in older adults. In this study, the exercise group showed positive outcomes, including decreased levels of IL-6 and TNF-α.

BDNF signaling pathway plays an important role in learning and memory (Huang et al., 2006). BDNF binds to a specific receptor, TrkB, and promotes learning and memory performance, while also influencing neuronal growth, differentiation, and repair (Tyler et al., 2002). Exercise has been shown to positively affect learning and memory retention by increasing the expression of BDNF and TrkB (Vaynman et al., 2004). In this study, the exercise group showed increased expression of BDNF and TrkB, and these benefits were observed regardless of age. In the current results, short-term memory and spatial learning memory were assessed using the step-through test and the Morris water maze task, and the old group showed significant improvement in reduced short-term memory through exercise.

This study showed that treadmill exercise effectively improves age-related memory decline and immune marker levels. In addition, treadmill exercise was shown to reduce inflammation by increasing beneficial gut microbes and the diversity of microbial communities.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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