Exercise hormone irisin prevents physical inactivity-induced cognitive decline in mice

Jonghyuk Park a, Jimmy Kim a, Toshio Mikami b,∗

a Department of Pharmacology, Graduate School of Medicine, Nippon Medical School, 1-1-5 Sendagi, Bunkyo-ku, Tokyo 113-8602, Japan
b Department of Health and Sports Science, Nippon Medical School, 1-7-1 Kyoan-cho, Musashino, Tokyo 180-0023, Japan

1. Introduction

Several studies have reported the benefits of regular exercise in maintaining and improving cognitive function. For example, human studies have found that regular exercise prevents age-induced cognitive decline [1,2] and reduces the progression of dementia in Alzheimer’s disease (AD) [3,4]. In animal experiments, regular exercise has been demonstrated to prevent cognitive impairment due to aging [5], chronic stress [6], and drug-induced cognitive impairment [7].

Brain-derived neurotrophic factor (BDNF) present in the hippocampus is among the vital substances that prevents and improves cognitive decline via regular exercise. Hippocampal BDNF affects synaptic plasticity and neurogenesis in the hippocampal dentate gyrus (DG) [5,8]. Furthermore, hippocampal neurogenesis greatly influences the maintenance of cognitive function and prevention of neurodegenerative disorders [9,10]. Moreover, some substances released by the peripheral organs during exercise are known to increase hippocampal BDNF levels and prevent cognitive decline. One such substance is insulin-like growth factor-1 (IGF-1). IGF-1, which is secreted by the liver into the circulating blood, is transported to the brain, and contributes to the increase in adult hippocampal neurogenesis, thereby improving cognition [11]. Vascular endothelial growth factor (VEGF) secreted by the liver also contributes toward the maintenance of cognitive function [6,12]. Adiponectin released by fatty tissue also affects cognitive function [13]. In addition to these substances, myokines released by the skeletal muscle, especially irisin, have also garnered considerable attention as modulators of cognitive function.

Irisin is cleaved from fibronectin type III domain-containing 5 (FNDC5) present in the cell membrane and released into the blood by skeletal muscles, and the latter is enhanced during exercise [14–16].

Abbreviations: PI, physical inactivity.
∗ Corresponding author.
E-mail addresses: jpark@nms.ac.jp (J. Park), jimi-kim@nms.ac.jp (J. Kim), mikami@nms.ac.jp (T. Mikami).
Irisin directly provokes an increase in hippocampal BDNF levels through the peroxisome proliferator-activated receptor gamma coactivator 1α (PGC-1α)/FDNCS pathway [17], contributing toward improvement in cognition in AD mice [15]. Further, peripheral administration of recombinant FDNCS/irisin prevented memory impairment [15], and intra-hippocampal administration of irisin attenuated synaptic impairment in AD mice [18]. Furthermore, exercise intervention before brain ischemia decreased brain infarct volume, but the therapeutic effect of exercise was vitiated by pretreatment with an irisin-neutralizing antibody [19]. These results suggest that elevated levels of irisin in circulating blood, as induced by physical activity, play a vital role in improving cognitive function, which led to the designation of irisin as an exercise hormone.

Decreased physical activity in daily life, i.e., physical inactivity (PI), markedly affects the onset of metabolic diseases, such as diabetes and hyperlipidemia [20–22], and cognitive decline [23,24]. Numerous human studies have reported the influence of PI on cognitive function; however, only a few animal studies have examined the effect of PI on cognition. Therefore, we developed a new experimental animal model to investigate the effect of PI on cognition. We housed the mice in the PI cage, which restricted the amount of physical activity at night by approximately 50% compared to that of the mice housed in the standard cage (as described later). PI induced cognitive decline and a depressive state without increasing plasma corticosterone levels. Simultaneously, regular exercise after PI prevents cognitive decline and mental deterioration in mice [25]. These results showed that cognitive and mental alterations mediated by PI and regular exercise could be attributed to alterations in irisin levels in circulating blood. It has been hypothesized that PI may decrease muscle contraction, followed by the decline in irisin release from skeletal muscles, resulting in cognitive decline and mental deterioration. Consequently, it could be assumed that regular exercise restores the irisin levels, preventing cognitive decline. However, this hypothesis has not been proven by previous studies.

The present study investigated whether changes in cognitive function and mental states via PI or regular exercise would be related to irisin levels in the blood, skeletal muscle, and hippocampus using intravenous administration of irisin-neutralizing antibodies in mice.

2. Material and methods

2.1. Animals and groups

A total of thirty 7-week-old male C57BL/6 J mice (21–23 g; Sanyko Laboratories, Tokyo, Japan) were allowed to adapt to standard transparent mouse cages (29 × 18 × 13 cm) for one week before being assigned to the experimental conditions. The animals were provided ad libitum access to standard chow (MF; Oriental Yeast Co., Ltd., Tokyo, Japan) and water. After one week of acclimatization, the animals were randomly divided into four experimental groups: control mice (Cont, n = 8), physically inactive mice (PI, n = 7), PI-exercise mice (PI+Ex, n = 7), and PI+Ex–irisin-neutralizing antibody mice (PI+Ex+Ab, n = 8). All mice were housed at a stable room temperature of 22–24 °C with 50% humidity under a 12-h/12-h light/dark cycle (lights on 08:00–20:00 h). All mice were administered various treatments and behavioral tests and were used for molecular/biochemical and immunohistochemical analysis. The animal handling protocols and study procedures conformed to the National Institute of Health guidelines. The study was approved by the Animal Care and Use Committee of Nippon Medical School (approval no. 30–028). Furthermore, all possible efforts were made to minimize animal pain and discomfort.

2.2. Housing conditioning

The Cont mice were housed with four mice in each standard transparent mouse cage, while the PI, PI+Ex, and PI+Ex+Ab mice were housed in a cage made by dividing the standard mouse cage into six sections to restrict the living space (PI cage). The living space per mouse in this cage was 11 cm wide, 11 cm long, and 10.5 cm high. Additionally, the PI cage was designed with a transparent acrylic plate with a view of all sides and a 12-mm hole in the wall to allow mouse-to-mouse contact, as described previously [25] (Fig. 1A). Each mouse’s body weight was measured weekly on Monday. At this time, we also measured each mouse’s chow weight in the bait box, remeasured it at the same time on the following day, and calculated each mouse’s food consumption per day by measuring the consumed chow.

2.3. Physical exercise protocol

All mice were allowed to adapt to treadmill running for 15 min at a speed of 10–15 m/min for three consecutive days. After acclimatization, the PI+Ex and PI+Ex+Ab mice were subjected to treadmill running at a speed of 15 m/min for 30 min daily, preceded by a 5-min warm-up at a speed of 5–7 m/min for 7 min, three days per week for 8 weeks (10–11:00 am) (Fig. 1B). According to a previous study, this exercise intensity attains 60% maximal oxygen consumption in mice [26]. The Cont and PI mice were allowed to rest on the treadmill (0 m/min, 15 min).

2.4. Administration of irisin-neutralizing antibody

Irisn-neutralizing antibody (G-067–17, Phoenix Pharmaceuticals, USA) was administered by intravenous injection to the PI+Ex+Ab mice (10 μg/mouse) at the 6th, 7th, and 8th weeks of exercise. Control IgG was administered to the other three groups via intravenous injection (Fig. 1B).

2.5. Behavioral testing

All mice (n = 30) underwent behavioral tests after the last exercise training session in the following order. The Y-maze test and open-field test (OFT) were performed during the 9th week, while the sucrose preference and exercise capacity tests (muscle strength and endurance performance) were performed during the 10th week (Fig. 1B).

2.5.1. Y-maze test (Y-maze)

The Y-maze test was used to measure spatial working memory by observing the spontaneous alternation behavior of the mice. The Y-maze consisted of three equally spaced arms (12 cm, height; 3 cm, width; and 40 cm, length). First, a mouse was placed at the end of one of the maze’s arms and allowed to move freely for 10 min. Thereafter, the Y-maze was cleaned before and after each test using spray bottles containing 70% ethanol and paper towels to prevent olfactory cues.

Actual alternation was defined as the entry of a mouse into all three arms in consecutive choices. Spontaneous alternation (%) was derived from the total number of actual alternations divided by the total number of arm entries minus 2, which was then multiplied by 100, as shown in the following equation: % alternation = [((actual alternations)/(total number of arm entries - 2)) × 100.

2.5.2. OFT

The OFT was performed to assess anxiety-like behavior in mice. The black box used in the test (length × width × height: 40 cm × 40 cm × 50 cm) was divided into 16 square grids. At the start of each trial, the mice were placed in the right-hand corner of the field and allowed to explore the arena freely, and their locomotor activity was recorded videographically for 10 min. The equipment was cleaned with 70% ethanol after each test. The animals’ behavior was scored using SMART® ver. 3.0 software (Panlab Inc., Spain), and the parameters scored included the center-staying duration, rearing, and distance traveled.
2.5.3. Sucrose preference test (SPT)

The SPT was used to assess anhedonia depression-like behavior in mice. On the first day, two bottles containing tap water were placed on either side of the cage to acclimatize the mice for two days. The next day, one of the bottles was replaced with 1% sucrose solution, and the mice were allowed to choose freely during the night (19:00–09:00 h). The position of the two bottles was reversed on the next day to avoid the mice’s preference for any one position, which was conducted four times. The sucrose preference ratio (%) was derived by dividing the volume of sucrose intake by the total volume of sucrose and water intake, which was multiplied by 100.

2.5.4. Grip strength test

A grip strength meter (GPM-100; Melquest, Toyama, Japan) was used to test muscle force in mice, according to the methods described by Takeshita et al. [27]. To measure the grip strength of the forelimbs (two paws) or four limbs (four paws), each mouse was made to grasp the device’s grip with its forelimbs (two paws) or four limbs (four paws) and its tail was pulled from behind. The tension recorded by the gauge when the mouse released its limbs from the bar was measured and expressed as the grip strength (g).

2.5.5. Endurance exercise capacity

The endurance exercise capacity of all mice was measured using a treadmill. All mice, including the control and PI groups, were acclimated to the exercise by running on the treadmill for 10 min at a speed of 7–12 m/min for three consecutive days before the endurance test. The initial treadmill speed for the endurance test was 10 m/min. After 5 min, the speed was increased by 3 m/min at intervals of 5 min, until a point of exhaustion was reached. Exhaustion was defined as the point at which the mice stayed on the grid at the back of the treadmill for a period of 30 s despite mild touching.

2.6. Tissue preparation

Two days after completing behavioral and endurance exercise tests, all mice were anesthetized with the mixed anesthesia containing medetomidine (0.75 mg/kg), midazolam (4 mg/kg), and butorphanol (5 mg/kg) and transcardially perfused with saline through the left ventricle. The mice brains were then carefully removed and hemisected on ice. The right hemisphere was fixed in 4% paraformaldehyde solution for immunohistochemical analysis described below. Left hippocampus was quickly isolated from left hemisphere, divided into two equal pieces, frozen in liquid nitrogen, and stored at −80°C until further analyses. One piece of hippocampus was used for RNA isolation and the other piece for Western blot analysis, as described below. Subsequently, the weights of the muscles (soleus, EDL, gastrocnemius) and epididymal adipose tissue were measured, after which the muscles and tissue were frozen in liquid nitrogen and stored at −80°C until analysis.

2.7. Enzyme-linked immunosorbent assay (ELISA)

Blood was collected in tubes pretreated with heparin sodium and centrifuged at 3000 g for 15 min at 4°C to obtain plasma. Plasma
2.8. mRNA extraction and quantitative real-time polymerase chain reaction analysis

mRNA extraction and quantitative real-time polymerase chain reaction (PCR) were performed as described previously [28]. Briefly, the hippocampal and skeletal muscle samples of mice were homogenized in TRIzol Reagent (Invitrogen, CA, United States) on ice, and the total RNA was extracted, according to the manufacturer’s instructions. Complementary DNA was synthesized using 1 μg of total RNA in 20 μl of reaction mixture, i.e., the ReverTra Ace™ qPCR RT Master Mix with gDNA Remover (FSQ-301; Toyobo, Osaka, Japan), as per the manufacturer’s instructions. Quantitative real-time PCR was performed using the SsoAdvanced Universal SYBR Green Supermix (Bio-Rad) and CFX Connect Real-time PCR system (Bio-Rad) to quantify the mRNA levels. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as the endogenous control. The following mouse-specific primers were used: PGC1α: forward 5′-ACCTCCTGACCTTGAAGC-3′, reverse 5′-CTGGGCTGTCTTCTGGTTC-3′; FNDC5: forward 5′-GAGGCACCAGTAACAAACTAG-3′, reverse 5′-GAGGATAATTAGCCCGATG-3′; BDNF: forward 5′-TGACGGGCGTATAGCAAAAAGG-3′; reverse 5′-CTTATGAACTGCCAGCCTTAATC-3′; and GAPDH forward 5′-CATACTGCGCACCA GAAGA-3′, reverse 5′-ATGCTTCTGGGCCAGCCG-3′. The 2−ΔΔCt method was used to analyze relative mRNA expression values. Sample analysis for each gene was performed in duplicate.

2.9. Western blot

Total protein was extracted from the hippocampus and skeletal muscles [extensor digitorum longus (EDL), soleus, and gastrocnemius], and Western blotting was performed as previously described [25]. Briefly, the sample was homogenized in radioimmunoprecipitation lysis buffer (50 mM Tris-HCL [Ph: 7.4]; 150 mM NaCl; 1% Triton X-100; 0.5% sodium deoxycholate; 0.1% sodium dodecyl sulfate) containing 14,000 g of total RNA in 20 μl of reaction mixture, i.e., the ReverTra Ace™ qPCR RT Master Mix with gDNA Remover (FSQ-301; Toyobo, Osaka, Japan), as per the manufacturer’s instructions. Quantitative real-time PCR was performed using the SsoAdvanced Universal SYBR Green Supermix (Bio-Rad) and CFX Connect Real-time PCR system (Bio-Rad) to quantify the mRNA levels. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as the endogenous control. The following mouse-specific primers were used: PGC1α: forward 5′-ACCTCCTGACCTTGAAGC-3′, reverse 5′-CTGGGCTGTCTTCTGGTTC-3′; FNDC5: forward 5′-GAGGCACCAGTAACAAACTAG-3′, reverse 5′-GAGGATAATTAGCCCGATG-3′; BDNF: forward 5′-TGACGGGCGTATAGCAAAAAGG-3′; reverse 5′-CTTATGAACTGCCAGCCTTAATC-3′; and GAPDH forward 5′-CATACTGCGCACCA GAAGA-3′, reverse 5′-ATGCTTCTGGGCCAGCCG-3′. The 2−ΔΔCt method was used to analyze relative mRNA expression values. Sample analysis for each gene was performed in duplicate.

2.10. Immunohistochemical analysis

Mice were intraperitoneally injected with bromodeoxyuridine and 5-bromo-2’-deoxyuridine (BrDU; Sigma) at 50 mg/kg per day for three consecutive days during the 10th week and sacrificed after all behavioral tests (Fig. 1B). For histochemical analysis, right brain hemispheres were post-fixed in 4% parafomaldehyde at 4 °C for 24 h. After the fixation step, the brains were equilibrated in 30% sucrose containing 0.1 M phosphate buffer for 48 h. Coronal sections measuring 40 μm were sequentially cut through the entire hippocampus using a vibratome (Leica, Germany). The sections were incubated in 2 M HCl at 37 °C for 30 min to denature the DNA, followed by immunostaining with mouse monoclonal anti-BrDU (BD Pharmingen, 1:100) using the M.O.M kit (Vector Laboratories), while gently shaking, for two nights at 4 °C. After washing with PBS, the sections were incubated with biotinylated anti-mouse IgG in M.O.M. diluent (1:250) for 2 h at room temperature. Then, the sections were incubated with avidin-biotin-peroxidase complex for 2 h. Lastly, the sections were washed in PBS and visualized using 0.67 mg/ml 3’-3’-diaminobenzidine. The sections that reacted with the antibodies were mounted, dehydrated, and coverslipped using CC/Mount™.

Six coronal sections per mouse were randomly selected (approximately 1.86–2.36 mm posterior to the bregma). To estimate the total number of BrDU-positive cells in the hippocampus, the labeled cells in the subgranular zone (SGZ) and the granule cell layer (GCL) of the DG were counted manually under a Leica DM3000 microscope (Leica, Germany) with a 20 × objective. The areas of hippocampal DG for each section were measured using NIH ImageJ software (NIH Image Engineering, Bethesda, MD, United States); the cell density per mm² was calculated as described previously [29].

3. Results

3.1. Effect of PI and exercise training on body weight, muscle weight, and plasma irisin concentration

We established a PI model in mice by limiting their living space compared to that of a standard cage (Fig. 1A). Simultaneously, the mice were subjected to regular exercise in the presence or absence of an irisin-neutralizing antibody (Fig. 1B). The repeated-measures ANOVA revealed that the interaction between group and time for body weight was not significant (F(24,234) = 0.329, P = 0.999; Fig. 2A). Post hoc analysis showed that body weight was lower in the PI+Ex mice than in the Cont mice between the 3rd and 5th weeks (p < 0.05). The body weight of the PI+Ex+Ab mice was lower than that of the Cont mice at the 6th week (p < 0.05), while there was no significant difference in body weight between the Cont and PI+Ex mice throughout the study period (Fig. 2A). The interaction of group and time for food intake was not significant and was similar for all groups during the eight-week period (F(24,234) = 1.207, P = 0.2367; Fig. 2B). Moreover, the body weight did not differ significantly among the four groups at sacrifice (F(3,26) = 1.103, P = 0.366; Fig. 2C). The weight of the EDL was higher in the PI+Ex mice than in the PI mice (F(3,26) = 2.794, P = 0.060), while the

FDNC5/irisin and corticosterone concentrations were measured using a commercially available mouse FDNC5/irisin ELISA kit (NBP3–08118, Novus Biologicals, USA) and a corticosterone ELISA kit (AB108821, Abcam, UK), according to the manufacturer’s specifications. The absorbance of the samples was read at 450 nm using a 96-well microplate reader (Varioskan MultiSkan FC, Thermo Scientific, USA).
weights of the soleus ($F_{(3,26)} = 0.5241, P = 0.670$) and gastrocnemius muscles did not differ significantly among the four groups ($F_{(3,26)} = 2.651, P = 0.070$; Fig. 2D). In contrast, the weight of the epididymal fat was significantly higher in the PI mice than in the other three groups ($F_{(3,26)} = 4.893, P < 0.01$; Fig. 2E). Moreover, the plasma corticosterone concentration did not differ among the four groups ($F_{(3,26)} = 0.039, P = 0.989$; Fig. 2F). The plasma irisin concentration of the PI+Ex mice only was elevated compared to that of the PI group ($F_{(3,26)} = 3.847, P < 0.05$; Fig. 2G).

3.2. Effect of PI and exercise training on working memory, anxiety, and depression-like behaviors

The total number of arms entered did not differ significantly among the four groups ($F_{(3,26)} = 0.249, P = 0.861$; Fig. 3A). The results of the Y-maze test showed a decrease in spontaneous alternation for the PI mice compared to that for the Cont mice. While regular exercise restored spontaneous alternation, irisin-neutralizing antibody administration suppressed the effect of exercise training ($F_{(3,26)} = 7.612, P < 0.001$; Fig. 3B). Additionally, plasma irisin levels were significantly correlated with the percentage of alternations ($r = 0.402, P < 0.05$; Fig. 3C). Although the distances traveled in the OFT did not differ among the four groups ($F_{(3,26)} = 1.755, P = 0.181$; Fig. 3D), the vertical activity quantified by rearing was significantly higher for the PI+Ex mice than for the Cont and PI mice ($F_{(3,26)} = 1.798, P < 0.05$; Fig. 3E). Furthermore, the time spent in the center zone was significantly lower for the PI mice than for the Cont mice ($F_{(3,26)} = 4.415, P < 0.05$). The time in the center in the OFT tended to be higher for the PI+Ex mice than for the PI mice (Fig. 3F), and the plasma irisin levels tended to correlate with the percentage of time in the center ($r = 0.342, P = 0.064$; Fig. 3G), albeit without statistical significance. The results of the SPT showed a decrease in sucrose preference in the PI mice than that in the Cont mice, while exercise training prevented these depression-like states ($F_{(3,26)} = 4.421, P < 0.05$; Fig. 3H); however, there was no correlation between the plasma irisin levels and sucrose preference ratio ($r = 0.129, P = 0.497$; Fig. 3I).

3.3. Effect of PI and exercise training on grip strength and endurance exercise capacity

The grip strength of the forelimbs (two paws) was significantly lower in the PI group than that in the other three groups ($F_{(3,26)} = 8.245, P < 0.001$, Fig. 4A). The grip strength of all four limbs (four paws) was significantly higher in the PI+Ex mice than that in the PI mice ($F_{(3,25)} = 8.423, P < 0.001$; Fig. 4B). Regarding the endurance exercise capacity, the running distance ($F_{(3,26)} = 7.669, P < 0.001$) and time to exhaustion ($F_{(3,26)} = 8.423, P < 0.001$) were significantly longer for the PI+Ex group than for the Cont and PI groups. The running distance and time to exhaustion of the PI+Ex-Ab mice were also significantly longer than those of the PI mice (Fig. 4C,D).

3.4. Effect of PI and exercise training on PGC-1α and FNDC5 expression in skeletal muscle

The expression of PGC-1α ($F_{(3,26)} = 2.208, P = 0.111$) and FNDC5 mRNA in the EDL muscle did not differ significantly among the four
groups \((F(3,26) = 1.431, P = 0.256; \text{Fig. 5A})\). Similar results were obtained for PGC-1α \((F(3,26) = 2.086, P = 0.127)\) and FNDC5 protein expression levels \((F(3,26) = 0.184, P = 0.906; \text{Fig. 5B})\). Although PGC-1α mRNA expression in the soleus tended to be lower in the PI group than in the Cont group, its expression in the PI+Ex group was higher than that in the PI group \((F(3,26) = 4.828, P < 0.01)\). FNDC5 mRNA expression was higher in PI+Ex mice than in PI mice, but exercise-induced FNDC5 mRNA expression was vitiated in the PI+Ex+Ab mice \((F(3,26) = 5.815, P < 0.01; \text{Fig. 5C})\). PGC-1α protein levels were higher in the PI+Ex mice than in the PI mice \((F(3,26) = 3.603, P < 0.01)\), whereas FNDC5 protein levels in the soleus did not differ significantly among the groups \((F(3,26) = 2.419, P = 0.089; \text{Fig. 5D})\).

Furthermore, PGC-1α \((F(3,26) = 3.488, P < 0.05)\) and FNDC5 mRNA expressions in the gastrocnemius was elevated only in the PI+Ex mice.
Fig. 4. Effects of physical inactivity and exercise on muscle strength and endurance exercise capacity, (A) Grip strength of the forelimbs (2 paws) and (B) four limbs (4 paws) (C) Total distance (m) and (D) time (min) to exhaustion during the endurance exercise capacity test Cont; control mice, PI; physical inactivity mice, PI+Ex; PI+exercise mice, PI+Ex+Ab; PI+Ex+irisin-neutralizing-antibody administered mice The values represent the means ± SEM. *P < 0.05; **P < 0.01; ***P < 0.001 (n = 7–8 per group).

compared to the Cont and PI mice (F(3,26) = 5.425, P < 0.01; Fig. 5E). The protein levels of PGC-1α in the gastrocnemius muscle were lower in the PI mice than in the Cont mice. Exercise training restored PGC-1α (F(3,24) = 8.680, P < 0.001) protein levels in the gastrocnemius, but these effects were inhibited by irisin-neutralizing antibody administration. Only the PI+Ex mice showed significantly elevated FNDC5 protein levels in the gastrocnemius compared to the PI mice (F(3,25) = 5.856, P < 0.01; Fig. 5F).

3.5. Effect of PI and exercise training on PGC-1α, FNDC5, BDNF expression, and cell proliferation in the hippocampus

The expression of PGC-1α (F(3,24) = 7.245, P < 0.01) mRNA in the hippocampus was higher in the PI+Ex mice than in the Cont and PI mice. Regular exercise also increased FNDC5 (F(3,25) = 4.841, P < 0.01) and BDNF mRNA expression in the hippocampus compared to that in the PI mice (F(3,24) = 5.118, P < 0.01; Fig. 6A). The protein levels of PGC-1α in the hippocampus were lower in the PI mice than in the Cont mice. Exercise training significantly increased PGC-1α (F(3,26) = 6.996, P < 0.01) and BDNF (F(3,26) = 4.862, P < 0.01) protein levels in the hippocampus compared to those in the PI mice; however, the increase in these genes by exercise was vitiated by irisin-neutralizing antibody administration. The FNDC5 protein level in the hippocampus tended to be higher in the PI+Ex mice than in the PI mice (F(3,26) = 2.612, P = 0.073; Fig. 6B). We counted the number of BrdU-positive cells in the DG, indicating cell proliferation, to identify the factors underlying exercise-induced improvement in cognitive function and irisin levels. The number of BrdU-positive cells in the DG was higher in the PI+Ex mice, and this effect was vitiated by the administration of irisin-neutralizing antibody in the PI+Ex+Ab mice (F(3,26) = 5.138, P < 0.01; Fig. 6C). Additionally, plasma irisin levels were significantly correlated with hippocampal BDNF protein levels (r = 0.424, P < 0.05; Fig. 6D) and the number of hippocampal BrdU-positive cells (r = 0.606, P < 0.001; Fig. 6E). The hippocampal BDNF protein levels were also significantly correlated with the percentage of alternation in the Y-maze test (r = 0.404, P < 0.05; Fig. 6F), while no correlation was observed between the hippocampal BDNF protein levels and percentage of time in the center in OFT (r = 0.329, P = 0.076; Fig. 6G) and sucrose preference ratio (r = 0.262, P = 0.163; Fig. 6H).

4. Discussion

Several studies have reported that regular exercise can prevent and improve cognitive decline in both humans and animals. The beneficial effects of regular exercise have been attributed to circulating myokines in blood, especially irisin. In contrast, PI results in cognitive decline, while regular exercise prevents it. However, the mechanism underlying the preventive effect of regular exercise remains unelucidated. Therefore, this study investigated the relationship between cognitive decline and irisin in an animal model of PI. The results demonstrated that regular exercise contributed toward the prevention of cognitive decline due to PI via the increase in plasma irisin, which subsequently enhances BDNF expression and cell proliferation in the hippocampus.

We found no significant differences in body weight and muscle weight among the study groups. However, epididymal fat mass increased by approximately 20% in the PI group compared to that in the other three groups of mice. Our previous study indicated that even mice housed in the PI cage for 20 weeks showed a significant increase in epididymal fat weight without an increase in body weight compared to the mice housed in standard cages [25]. However, mice fed a 20% high-fat diet when housed in the PI cage showed a two-fold increase in epididymal fat mass with a remarkable increase in body weight compared to mice housed in a standard cage and fed a standard diet [30]. Although we could only demonstrate the weight of the epididymal fat and some hindlimb muscles, we assume that an increase in fat mass without body weight gain reflects a decrease in lean body mass due to long-standing PI. Skeletal muscles are the primary components of lean body tissue. Therefore, the decrease in skeletal muscle mass in the whole body could have inhibited irisin release from skeletal muscles, consistent with the decrease in plasma irisin in physically inactive mice. Future studies that measure the fat and lean mass using computed tomography while the mice are housed in standard and PI cages are necessary to prove this hypothesis.

Regular treadmill exercise decreased epididymal fat and elevated circulating irisin levels, consistent with previous studies, which demonstrated that acute and chronic exercise increase circulating irisin levels [26, 31–34]. The elevation in irisin promotes thermogenesis in adipose tissue by the process of “browning” white adipose tissue into brown adipose tissue [14]. A significant correlation was observed between plasma irisin levels and 24-h energy expenditure in healthy postmenopausal women [35]. Moreover, the exercise-induced reduction in the visceral adipose tissue area was negatively correlated with the
change in serum irisin levels in middle-aged individuals who underwent exercise training [34]. Thus, it can be inferred that reducing body fat mass via exercise would depend on the function of plasma irisin. However, in the present study, an exercise-induced reduction in fat mass occurred, irrespective of the absence or presence of an irisin-neutralizing antibody. Similarly, one study reported that there was no relationship between the browning of subcutaneous white adipose tissue and irisin in exercise-trained human participants [36]. Therefore, we speculated that fat mass reduction via regular exercise observed in this study was due to increased energy consumption during exercise, independent of irisin action. Future studies are needed to examine the indicators of energy metabolism, such as oxygen consumption and respiratory quotient, in exercise-trained mice in the presence and absence of irisin-neutralizing antibodies to clarify the relationship between exercise-induced fat loss and irisin.

The present study showed that PI resulted in cognitive decline, depression, and anxiety in mice, consistent with the findings of our previous study [25]. Simultaneously, regular exercise prevented cognitive and mental deterioration, although this effect was hindered by the administration of irisin-neutralizing antibodies. Moreover, exercise-induced elevation in BDNF and cell proliferation in the hippocampus was also altered depending on the presence or absence of irisin-neutralizing antibodies. Both BDNF protein and hippocampal cell proliferation significantly affect cognitive function by mediating improvement and deterioration [29,37]. Intraperitoneal administration of recombinant irisin increases PGC-1α, FNDC5, and BDNF mRNA levels in the hippocampus [38]. Moreover, four weeks of treadmill running was sufficient to increase cell proliferation and neurogenesis in the
Fig. 6. Effects of physical inactivity and exercise on the PGC-1α/FNDC5/BDNF pathway and neuronal cell proliferation in the hippocampus, (A) PGC-1α, FNDC5, and BDNF mRNA and (B) protein expression levels in the hippocampus (C) Number of BrdU-positive cells in the dentate gyrus (DG). (D) Correlation between plasma irisin levels and BDNF protein expression and (E) BrdU-positive cells in the hippocampus (F) Correlation between hippocampal BDNF protein expression and percentage of alternations in the Y-maze test, (G) time spent in the open-field test (OFT), and (H) sucrose preference ratio. BrdU, 5-bromo-2'-deoxyuridine; BDNF, brain-derived neurotrophic factor; EDL, extensor digitorum longus; PGC-1α, peroxisome proliferator-activated receptor gamma coactivator 1α; FNDC5, fibronectin type III domain-containing 5. The values are the means ± SEM. *P < 0.05; **P < 0.01 (n = 7–8 per group).
hippocampal DG. Interestingly, at that time, an increase in the number of FNDC5-terminal fragment-positive cells was observed in the SGZ of the hippocampal DG of exercised mice [39]. These results suggest that exercise-induced FNDC5/irisin may contribute to the effects of exercise on hippocampal cell proliferation, neuroprotective genes, and hippocampal-dependent memory. The present study showed a positive correlation between plasma irisin levels and performance on the Y-maze test, hippocampal BDNF protein levels, and hippocampal cell proliferation. Furthermore, a positive correlation was observed between hippocampal BDNF protein levels and Y-maze performance. This result indicates that circulating irisin could affect cognitive function by altering BDNF expression and cell proliferation in the hippocampus, which is consistent with the results of the following (previous) studies. Four-week voluntary wheel running stimulates BDNF expression through the PGC-1α/FNDC5 pathway in the hippocampus [17]. The preventive effect of regular exercise on memory defects in Alzheimer’s model mice is inhibited by the administration of irisin-neutralizing antibodies [15]. The prevention of cognitive decline via regular exercise was not observed in genetically modified FNDC5 knockout mice [18].

Although circulating irisin is recognized as a myokine released from skeletal muscle, other organs besides skeletal muscle, such as fatty tissue, the kidneys, and liver, also reportedly release irisin [32,40]. In particular, the following intriguing results have been reported for fatty tissue. Serum irisin levels are elevated in mice fed a high-fat diet compared to those fed a standard diet [41]. Plasma irisin levels are associated with body mass index [42], and PGC-1α and FNDC5 mRNA expression in muscles was higher in OLETF rats (a model of type 2 diabetes) compared to genetically controlled LETO rats [43]. These results suggest that irisin is a myokine released by skeletal muscles and, at the same time, an adipokine released from adipose tissues, indicating that plasma irisin could be a plasma indicator of obesity. Furthermore, a recent study reported that plasma irisin levels undergo alteration depending on the hepatic irisin levels, and liver-derived circulating irisin improved cognitive deficits via exercise in an AD mouse model [18]. Therefore, the primary organ of origin for plasma irisin is controversial [14,44,45]. Thus, the organ that primarily releases irisin in response to exercise requires elucidation in future studies. It is thought that exercise promotes the release of irisin from peripheral organs, possibly skeletal muscle, which is transported to the brain, and plays a role in improving or maintaining brain functions. However, the present study showed that exercise increases hippocampal FNDC5 mRNA and protein levels, and that intravenous administration of irisin-neutralizing antibodies suppresses its expression. As the increase in hippocampal FNDC5 was in line with PGC-1α, we postulated that factors causing elevation in PGC-1α, possibly IGF-1 [46] or lactate [28], were increased by exercise, which subsequently enhanced FNDC5 expression. Moreover, FNDC5 expression in skeletal muscles was suppressed by the intravenous administration of irisin-neutralizing antibodies. These results cannot explain why FNDC5 expression was suppressed by irisin-neutralizing antibodies injected into circulating blood. However, neutralizing antibodies against several proteins have been reported to suppress protein expression. For example, IGF-1 receptor-neutralizing antibody administration suppresses IGF-1 receptor expression [47]. Moreover, bevacizumab, a neutralizing antibody for VEGF, suppresses VEGF expression in cancer cells [48]. Therefore, our finding that irisin-neutralizing antibodies suppressed FNDC5 expression in both the hippocampus and skeletal muscle does not seem to be erroneous, although the underlying mechanism remains unknown.

5. Conclusion

This study demonstrated that PI resulted in the deterioration of working memory and onset of depressive and anxiety states in a mouse model of PI. In contrast, regular exercise prevented these deteriorative changes wrought by PI. Simultaneously, plasma irisin was associated with working memory, hippocampal cell proliferation, and hippocampal BDNF protein expression, suggesting that plasma irisin released by peripheral organs, perhaps skeletal muscles, is critical for maintaining cognitive function even in a state of PI.

Funding

This study was supported by the Meiji Yasuda Life Foundation of Health and Welfare, Japan, and in part by a Grant-in-Aid for Early Career Scientists (21K17605) from the Japan Society for the Promotion of Science (JSPS).

CRediT authorship contribution statement

J.P. and T.M. conceived and designed the experiments, interpreted the results, and drafted the manuscript. J.P., J.K., and T.M. performed the experiments and analyzed the data. J.P. prepared figures. All the authors approved the final version of the manuscript.

Acknowledgments

We would like to thank Dr. R. Arakawa (Nippon Medical School) for critical review and discussion of the manuscript and Editage (www.editage.com) for English language editing.

References

[1] K.I. Erickson, M.W. Voss, R.S. Prakash, C. Basak, A. Szabo, L. Chaddock, J.S. Kim, S. Heo, H. Alves, S.M. White, T.R. Wojcicki, E. Mailey, V.J. Vieira, S.A. Martin, B.D. Pence, J.A. Woods, E. McAuley, A.F. Kramer, Exercise training increases size of hippocampus and improves memory, Proc. Natl. Acad. Sci. USA, 108, 2011, pp. 3017–3022. (https://doi.org/10.1073/pnas.1019505108).
[2] A. Maass, S. Duzel, M. Goerke, A. Becke, U. Sobieray, K. Neumann, M. Lovden, U. Lindenberger, L. Backman, R. Braun-Dullea, D. Ahrens, H.J. Heinze, N. G. Muller, E. Duzel, Vascular hippocampal plasticity after aerobic exercise in older adults, Mol. Psychiatry 20 (2015) 585–593, https://doi.org/10.1038/mp.2014.114.
[3] E.B. Larson, L. Wang, J.D. Bowen, W.C. McCormick, L. Teri, P. Crane, W. Kukull, Exercise is associated with reduced risk for incident dementia among persons 65 years of age and older, Ann. Intern. Med. 144 (2006) 73–81, https://doi.org/10.1001/anninternmed.144.1.73.
[4] N. Scarmeas, J.A. Luchsinger, N. Schupf, A. Minic UK, M.A. Tang, Y. Stern, Physical activity, diet, and risk of Alzheimer disease, JAMA 302 (2009) 627–637, https://doi.org/10.1001/jama.2009.1144.
[5] H. van Praag, T. Shubert, C. Zhao, F.H. Gage, Exercise enhances learning and hippocampal neurogenesis in aged mice, J. Neurosci. 25 (2005) 8660–8665, https://doi.org/10.1523/JNEUROSCI.1751-05.2005.
[6] T. Kiuchi, H. Lee, T. Misawa, T. Mikawa, Regular exercise exercises depression-like behavior via VEGF-Flk-1 signaling in chronically stressed mice, Neuroscience 207 (2012) 208–217, https://doi.org/10.1016/j.neuroscience.2012.01.023.
[7] A.M. Sohroforouzani, S. Shakarian, M. Ghanbarzadeh, H. Alaei, Effect of forced treadmill exercise on stimulation of BDNF expression, depression symptoms, tactile memory and working memory in LPS-treated rats, Behav. Brain Res. 418 (2022), 113645, https://doi.org/10.1016/j.bbr.2021.113645.
[8] S.H. Choi, E. Bylykbash, Z.K. Chatila, S.W. Lee, B. Palli, G.D. Clemenson, E. Kim, A. Rompala, M.K. Oram, C. Asselin, J. Aronson, C. Zhang, S.J. Miller, A. Lesinski, J. Chen, D.Y. Kim, D. van Praag, B.M. Spiegelman, F.H. Gage, R.E. Tanzi, Combined adult neurogenesis and BDNF mimic exercise effects on cognition in an Alzheimer’s mouse model, Science 361 (2018), https://doi.org/10.1126/science.aau8581.
[9] P. Bekinschtein, M. Cammarota, K. Katze, L. Slipczuk, J.I. Rossato, A. Goldin, I. Izquierdo, J.H. Medina, BDNF is essential to promote persistence of long-term memory storage, Proc. Natl. Acad. Sci. USA, 105, 2008, pp. 2711–2716, (https://doi.org/10.1073/pnas.0711863105).
[10] C. Hock, K. Heese, C. Hulette, C. Rosenberg, U. Otten, Region-specific neurotrophin imbalances in Alzheimer disease: decreased levels of brain-derived neurotrophic factor and increased levels of nerve growth factor in hippocampus and cortical areas, Arch. Neurol. 57 (2000) 845–851, https://doi.org/10.1001/archneur.57.6.846.
[11] J.L. Trejo, M.V. Llorens-Martín, I. Torres-Aleman, The effects of exercise on spatial learning and anxiety-like behavior are mediated by an IGF-I-dependent mechanism related to hippocampal neurogenesis, Mol. Cell Neurosci. 37 (2008) 402–411, https://doi.org/10.1016/j.mcn.2007.10.016.
[12] J.L. Trejo, E. Carro, I. Torres-Aleman, Circulating insulin-like growth factor I mediates exercise-induced increases in the number of new neurons in the adult hippocampus, J. Neurosci. 21 (2001) 1628–1634, https://doi.org/10.1523/JNEUROSCI.21-05-01628.2001.
[13] J. Bloemer, P.D. Pinky, W.D. Smith, D. Bhattacharya, A. Chauhan, M. Govindarajulu, H. Hong, M. Dhanasekaran, R. Judd, R.H. Amin, M.N. Reed,
Behavioural Brain Research 433 (2022) 114008

T. Mikami, J. Kim, J. Park, H. Lee, P. Yaicharoen, S. Suidasari, M. Yokozawa, M.K. Edwards, P.D. Loprinzi, Experimentally increasing sedentary behavior results in increased levels of plasma irisin and muscle PGC-1α and browning of subcutaneous adipose tissue in mice, Sci. Rep. 4 (2014) 4199, https://doi.org/10.1038/srep04199.

J. Park et al. Physiol. 12 (2021), 736905, https://doi.org/10.3389/fphys.2021.736905.

J. Huh, V. Mougos, A. Kabasakalis, I. Faturouz, A. Siopi, A. Douroudos II, treatment of obesity prevents aging-related cognitive impairment and enhances spatial memory in mice, Aging 13 (2021), 1087–1093, https://doi.org/10.1007/s40520-021-01134-6.

J. Gil-Mohapel, M.P. Cunha, A.L.S. Rodrigues, Antidepressant-like and pro-neurogenic effects of physical exercise: the putative role of FNDC5/irisin pathway, J. Neural Transm. 127 (2020) 355, https://doi.org/10.1007/s00702-020-112516.

R. Ronnestad, S. Ellefsen, Irisin in blood increases transiently after single sessions of intense endurance exercise and heavy strength training, PLoS One 10 (2015), e0121367, https://doi.org/10.1371/journal.pone.0121367.

A. Siteneski, G. Olescowicz, F.L. Pazini, A. Camargo, D.B. Fraga, P.S. Brocardo, A. Menard, H. Matsuo, E. Tanzi, B.M. Spiegelman, C.D. Wrann, Exercise hormone irisin is a critical regulator of cognitive function, Nat. Metab. 3 (2021) 1058–1070, https://doi.org/10.1038/s42255-021-00476-7.

F. Prado, M.A.M. Prado, J.F. Abisambra, F. Tovar-Moll, P. Mattos, O. Arancio, S. Cinti, K. Hojlund, S.P. Gygi, B.M. Spiegelman, Adiponectin knockout mice display cognitive and synaptic plasticity deficits, Front. Neurosci. 16 (2022), 866405, https://doi.org/10.3389/fnbeh.2022.866405.

J. Soberman, A. Besnard, M.P. Jedrychowski, H. Kim, H. Tu, E. Kim, S.H. Choi, R. E. Tanzi, B.M. Spiegelman, C.D. Wrann, Exercise hormone irisin is a critical regulator of cognitive function, Nat. Metab. 3 (2021) 1058–1070, https://doi.org/10.1038/s42255-021-00476-7.

M.V. Lourenco, R.L. Frozza, G.B. de Freitas, H. Zhang, G.C. Kincheski, F.C. Ribeiro, J.Y. Huh, V. Mougios, A. Kabasakalis, I. Faturouz, A. Siopi, A. Douroudos II, exercise-induced hormone irisin effects in mice, Sci. Rep. 4 (2014) 4199, https://doi.org/10.1038/srep04199.

V. Suppiramaniam, Adiponectin knockout mice display cognitive and synaptic plasticity deficits, Front. Endocrinol. 10 (2019) 819, https://doi.org/10.3389/fendo.2019.00819.

X. Ge, A. Cho, M.A. Ciol, C. Pettan-Brewer, J. Snyder, P. Rabinovitch, W. Ladiges, D.J. Li, Y.H. Li, H.B. Yuan, L.F. Qu, P. Wang, The novel exercise-induced hormone irisin protects against neuronal injury via activation of the Akt and ERK1/2 signaling pathways and contributes to the neuroprotection of physical exercise in cerebral ischemia, Metabolism 68 (2017) 31–42, https://doi.org/10.1016/j.metabol.2016.12.002.

C. Yang, G. Sui, D. Li, L. Wang, S. Zhang, P. Lei, Z. Chen, F. Wang, Exogenous IGF-1 injection alleviates depression-like behavior and hippocampal mitochondrial dysfunction in mice, Sci. Rep. 6 (2016) 3548, https://doi.org/10.1038/srep3548.

J.Y. Huh, V. Mougos, A. Kabasakalis, I. Faturouz, A. Siopi, A. Douroudos II, G. Filipaopoulos, K.H. Panagiotou, C.S. Park, Mantzoros, Exercise-induced irisin secretion is independent of age or fitness level and increased irisin may directly modulate muscle metabolism through AMPK activation, J. Clin. Endocrinol. Metab. 99 (2014) E2154–E2161, https://doi.org/10.1210/jc.2014-1437.

J.Y. Huh, G. Panagiotou, V. Mougos, M. Brinkoetter, M.T. Vamvri, B. E. Schneider, C.S. Mantzoros, FNDC5 and irisin in humans: I. Predictors of circulating concentrations in serum and plasma and II. mRNA expression and circulating concentrations in response to weight loss and exercise, Metabolism 61 (2012) 1725–1736, https://doi.org/10.1016/j.metabol.2012.09.002.

R.R. Kramer, P. Shockett, N.D. Webb, U. Shah, V.D. Castracane, A transient elevated irisin blood concentration in response to prolonged, moderate aerobic exercise in young men and women, Horm. Metab. Res. 46 (2014) 150–154, https://doi.org/10.1055/s-0033-1355391.

S. Cinti, K. Hojlund, S.P. Gygi, B.M. Spiegelman, A. PGC1-alpha-dependent, muscle function in male mice, Sci. Rep. 7 (2017) 4232, https://doi.org/10.1038/s41598-017-0589-6.

C. Yang, G. Sui, D. Li, L. Wang, S. Zhang, P. Lei, Z. Chen, F. Wang, Exogenous IGF-1 injection alleviates depression-like behavior and hippocampal mitochondrial dysfunction in high-fat diet mice, Physiol. Behav. 229 (2021), 112356, https://doi.org/10.1016/j.physbeh.2021.112356.

J. Hailey, E. Maxwell, K. Koukouras, W.R. Bishop, J.A. Pachter, Y. Wang, Neutralizing anti-insulin-like growth factor receptor 1 antibodies inhibit receptor function and induce receptor degradation in tumor cells, Mol. Cancer Ther. 1 (2002) 1349–1355.

Y. Hoshino, T. Hayashida, A. Hirata, H. Takahashi, N. Chiba, M. Ohmura, M. Wakis, H. Jinno, H. Hasegawa, S. Maheswaran, S. Suematsu, Y. Kitagawa, Bevacizumab terminates homeobox B9-induced tumor proliferation by silencing microenvironmental communication, Mol. Cancer 13 (2014) 102, https://doi.org/10.1186/1476-4598-13-102.