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Exercise rescues cognitive deterioration in naturally aged rats via PGC1α/FNDC5/irisin/AMPK signaling pathway to restore redox, endothelial, and neuronal homeostasis

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Keywords

- Exercise
- Cognitive dysfunction
- Irisin/AMPK
- eNOS/NO/BDNF pathway

Abstract

Background: Aging-associated cognitive impairments become a global phenomenon, especially with the increase in life expectancy and sedentary lifestyle. Thus, the present study aimed to assess the cognitive functions in aged rats and explore the potential involvement of the endogenous exercise-induced myokine irisin in such an effect. Lastly, it was to identify the possible irisin downstream adenosine monophosphate-activated protein kinase (AMPK) signaling pathway to restore hippocampal redox and eNOS/NO/brain-derived neurotrophic factor (BDNF) homeostasis. **Materials and Method:** Three groups of rats were conducted; young (3-month-old), non-trained aged (20-month-old), and exercise (EX)-aged group performing swimming EX 1h/day/5 days /week for 8 weeks. **Results:** Our findings revealed aging was associated with impaired cognitive parameters, increased total oxidant status (TOS) with a reduction in total antioxidant capacity (TAC), eNOS/NOx, and BDNF in the aged group versus the young. Such changes were improved by EX-induced upraised PGC1α/ FNDC5/irisin/AMPK pathway. The increased irisin is positively correlated with the hippocampal TAC, eNOS, NOx, BDNF, and AMPK levels, while negatively correlated with TOS. **Conclusion:** Bolstering irisin/AMPK levels via training would be an approach to prevent or delay an aging-associated cognitive decline or its progression.

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Introduction

Aging is a physiological and inevitable part of life. It is characterized by a decline in the homeostasis of body systems and health span [1]. Such decline involves the hippocampus, the primary central area concerned with learning and memory, causing evident neurodegeneration and diminution of neurotransmitters with reduced cerebral blood flow [2]. Thus, senility becomes the most concrete identified risk factor for the worldwide cognitive deficit [3].

Free radical theory of aging is one of the hallmarks of aging. There is plentiful evidence that oxidative injury plays a crucial role in the development and progression of aging and its associated organ dysfunctions [4].

Nitric oxide (NO), a gaseous signaling molecule, has plenty of physiological effects; neurotransmission, vascular homeostasis, induction and preservation of the synaptic plasticity that essential for memory consolidation. In addition, it is a neuro-protective molecule in the dopaminergic pathways predominantly those impact motor dysfunctions [5]**.** It is synthesized by endothelial NO Synthase (eNOS) in addition to, neuronal NOS (nNOS), and inducible NOS (iNOS). eNOS is primarily expressed by cerebral endothelial cells and is found also, in the hippocampus. It is concerned with the regulation of cerebral blood flow and positively related to brain-derived neurotrophic factor (BDNF) synthesis [6]. BDNF is crucial for neuronal existence and development. It tackles as a modulator for neural transition and contributes to its plasticity which is vital for cognitive purposes [7]. Based on the above-mentioned, targeting redox balance and eNOS/NO/BDNF is an inevitable pathway to preserve brain health and function.

The beneficial effects of exercise are well documented; however, the underlying molecular mechanisms are still less well understood. Remarkably exerkines or myokines, muscular hormones secreted in response to exercise, gained attention. They certify crosstalk between the muscle and brain in addition to other organs, resulting in physiological benefits from exercise [8].Among these, irisin was just acknowledged as an important exerkine. It is cleaved from fibronectin type III domain-containing protein 5 (FNDC5), a transmembrane precursor protein expressed in exercised muscle, brain, and among others. The peroxisome proliferator-activated receptor gamma coactivator-1α (PGC1α) is an important transcriptional coactivator, required for irisin production [9]. It is also, a master regulator of mitochondrial biogenesis and can antagonize aging-associated frailty and improve physical endurance [1]. Thus, the involvement of PGC1α /FNDC5/irisin in the pathogenesis and protection against aging-associated cognitive dysfunction would be predictable to have somewhat valuable effects.

Adenosine monophosphate-activated protein kinase (AMPK) is a metabolic kinase that regulates glucose uptake and secures cellular survival under stress conditions. It becomes attractive as a promoter of healthy aging owing to the capability to integrate several signaling and transcription pathways that favor longevity [10]. Till now, to the best of our knowledge, no study has investigated the irisin/AMPK pathway in combating aging-associated decline in cognitive functions.

Based on the above mentioned, the present study aimed to assess the cognitive functions in aged rats and explore the effect of EX, using the open field (OF) and novel object recognition (NOR) tests to assess the locomotor activity, animal exploratory behavior, and memory states. Also, it aimed to illustrate the potential involvement of the endogenous EX-induced myokine irisin in such effect by measuring its circulating level and its upstream pathway PGC-1α and FNDC5 in the hippocampus. Lastly, it was to identify the possible irisin downstream AMPK signaling pathway to restore hippocampal redox and eNOS/NO/BDNF homeostasis.

Material and methods

Animals used

Male Wistar rats, young (3-month-old) and aged (20-month-old) were conducted in this study. They were purchased from Animal House, Faculty of Veterinary Medicine, Benha University (Egypt). They were housed in metallic cages (three per cage) and maintained on prevailing atmospheric conditions and room temperature about 25°C. They were fed standard pellet diets and drinking water ad libitum. Rats were acclimatized to the environmental conditions for one week before the experiment. The experimental procedures, animal handling, sampling, and scarification were done in accordance with the Guide for the Care and Use of Laboratory Animals, Eighth Edition 2011 [11].The protocol of this study was revised and approved by the Ethical Committee of the Care and Use of Experimental animals, Faculty of Medicine, Benha University, Egypt (REC-FOMBU, MoHP, No. 19-11-2022).

Experimental groups and procedure (Fig. 1)

The rats were divided into three groups (n = 6), Young (3-month-old); Aged (20-month-old), and exercise trained-aged (20-month-old) group (EX-Aged) that swam for 1h/day and 5 days /week for 8 weeks [12]

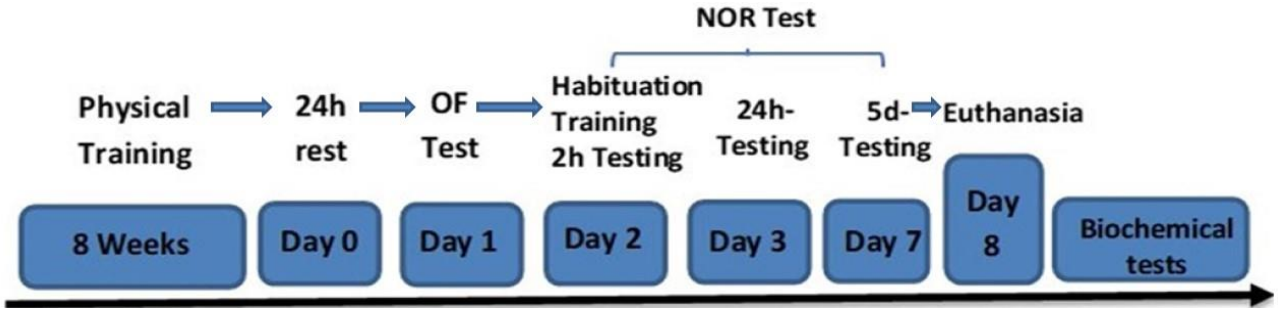

Figure 1 Illustrating diagram for the general experimental procedure.
OF, open field test; NOR, novel object recognition test. It was created using the software: PowerPoint.

The **Swimming exercise training protocol was as follow:** The rats were adapted to swimming in a cylindrical tank of diameter 80 cm and depth 90 cm. It was filled with 60 cm water at temperature of 33 - 36°C. The swimming exercise protocol (equivalent to moderate intensity) was composed of training once daily for 15 min for 2 days then; the duration was gradually increased until rats swam for 60 min, on the 5th day of first week of training then continued till the end of the 8th weeks at a rate of 5 days/week. The sedentary groups, young and aged, were immersed in the tank with water at a depth of 5cm daily, 5 days/week for 8 weeks to serve as controls for the effects of handling and exposure to water. A rest

for 24 hours was allowed before rats were subjected to the behavioral tests [13]

Behavioral Testing:

Open field (OF) test

The OF was performed to assess the locomotor and exploratory activities of rats. It is composed of a black-painted box measuring 100 × 100 × 40-cm. The rats were individually placed in the center of the box and allowed to explore the arena for 5 min. Number of crossing lines as a measure of locomotor activity, number of times a rat rears (standing on hind limbs) as a measure of exploratory behavior, and number of times a rat defecates, or grooms (licking the body and paws) as a measure of anxiety-like behavior, were recorded [14]

Memory assessments by novel object recognition (NOR) test (Fig. 2)

One day following OF, NOR has been executed in the same box. NOR can examine short, intermediate, and long-term memory using 2 objects; novel and familiar. It is based on innate preference for novelty of rats. This makes them take longer time at the novel object (N) than the familiar one (F) which reflects intact memory concerning the familiar object [15].The test is composed of 3 phases:

Habituation: Each rat was allowed to move freely in an empty box for 3-min. **Training session:** 24hs later, each rat was allowed to recognize (touching or sniffing) two identical objects placed in the boxfor 3-min. **Testing sessions:** 2 hours, 24 hours, and 5 days, following the training session were done for testing short-term, intermediate-term, and long-term memory respectively. The animals were positioned in the box where one of the previously introduced objects has been replaced by a novel one, for 3-min. The time spent exploring each object (sec), was recorded. All behavioral data were collected via a camera positioned above the test area. To avoid confuses by odor cues, 70% ethanol was used to clean the arena and objects after each trial [15].

Later, the following indices were estimated.

Discrimination index (DI) which calculated by dividing the difference between the time exploring the novel (N) and the familiar object (F), by the total time exploring both objects; (N – F / N + F). A positive result reveals more time spent with the novel object (preserved memory) and a negative result reveals more time spent with the familiar object (poor memory). Recognition index (RI) % = (N / N + F**),** it is the % of time exploring the N to the total time of both objects exploration [16].

Samples Collection and hippocampal tissue preparation

24hs after the end of the experiment all rats were anesthetized by pentobarbital (40 mg/kg, i.p.). Blood samples were obtained from the retro-orbital plexus, centrifuged, and serum was collected for analysis of irisin. Hippocampi were immediately excised and stored at -80^{o}C till examination. One part was used for the measurement of phosphorylated-AMPK (p-AMPK) and BDNF proteins in addition to the biochemical analysis of oxidative stress-related markers and nitrite/nitrate (NOx). RNA*later*™ stabilization solution (Thermo Fisher Scientific, Madison, WI, USA) was used to store the other part at -80 ^{o}C for assessment of PGC-1α, FNDC5, and eNOS mRNA expressions.

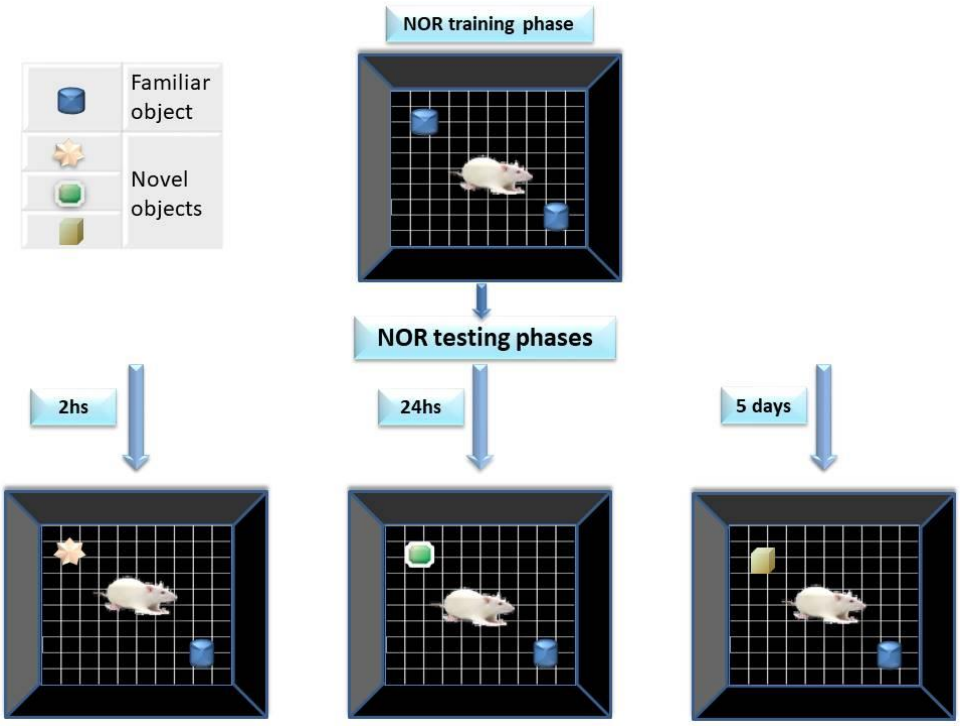

Figure 2 Illustrating diagram for the training and testing phases of NOR memory test
NOR, novel object recognition test. It was created using the software: PowerPoint.

Enzyme-Linked Immuno-Sorbent Assay (ELISA) for hippocampal irisin, p-AMPK, and BDNF proteins

Irisin ELISA kits (Catalog # EK-067-29, Phoenix Pharmaceuticals, Burlingame, CA, USA), p-AMPK ELISA Kits (Catalog # MBS2501430, MyBiosource, Inc. San Diego, CA, USA), and BDNF ELISA kits (Catalog # ab213899, Abcam, Cambridge, USA) were used in this study according to the manual instructions. The method depends on competitive enzyme immunoassay technique. Incubating the polyclonal anti-parameter antibody and a parameter- horseradish peroxidase (HRP) conjugates with the assay sample and buffer was done in a pre-coated plate. Wells were decanted and washed five times after incubation. An HRP enzyme substrate was added to the wells. A blue complex formed due to the enzyme-substrate reaction. Color intensity was measured at 450 nm spectrophotometrically (spectrophotometer, Bio-Rad Laboratories, Hercules, CA, USA).

Measurement of hippocampal oxidative stress-related markers

Total oxidant status (TOS) (No., IKJU-002CL, Creative Diagnostics Co., NY, USA) and total antioxidant capacity (TAC) (No.,TA 25 13, Biodiagnostic Co., Gizza, Egypt) were measured using colorimetric assay kits in the hippocampus supernatant. For reading the absorbance UV-160 1PC UV-visible spectrophotometer was used [17].

Assessment of hippocampal nitrite/nitrate (NOx)

Measurement of NO metabolites nitrite and nitrate (NOx) was documented to assess NO synthesis due to NO short half-life. The most frequently used method of NOx analysis is the Griess assay (R&D Systems, Minneapolis, MN). The optical density was qualified at 540 nm using a microplate

reader (xMarkmicroplate spectrophotometer, Bio-Rad Laboratories) [18].

Assessment of hippocampal PGC-1α, FNDC5, andeNOS genes by reverse transcription-quantitative PCR (RT-qPCR):

Primer sequences specific to each gene are shown in **Table 1.**

The quantity and quality of total RNA (Jena Bioscience, Germany), isolated from 25mg hippocampal tissue, was analyzed usingNano-Drop™ One UV-Vis Spectrophotometer. A quantity of 500 ng of mRNA was used for cDNA synthesis, then amplified with SYBR Green One-Step PCR Master Kit (Applied Biosystems, Foster City, CA USA). Sigmoid-shaped amplification plots were obtained using RQ manager program. A critical threshold (CT) value was used to analyse mRNA levels for target genes and Glyceraldehyde phosphate dehydrogenase (GAPDH), as a housekeeping gene. The mRNA expression of each gene was normalized relative to the GAPDH mean CT values according to the $2^{-\Delta\Delta CT}$ formula [19]. RT-qPCR reactions were conducted in duplicate for each sample, and data analysis was performed using average values. Melting curve analyses were performed after qPCR to prove amplification of the specific products of interest. To ensure the absence of non-specific amplification for all used primers, non-template controls were included within each run.

Statistical analysis

Statistical Package for Social Sciences (SPSS) program version 26 (SPSS Inc., Chicago, IL, USA), was used to analyze data. These data were introduced as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) test and post hoc multiple comparisons (LSD test) were used to compare between all groups. The statistical significance was defined when (*P* value) < 0.05. Pearson correlation coefficients were used to determine the association between the serum irisin and TOS, TAC, eNOS, NOx, BDNF, and p-AMPK.

Table 1: Oligonucleotide sequences of the assessed genes

Genes	Forward primer (sense)	Reverse primer (antisense)
PGC-1α	GAG AAC AAG ACT ATT GAGCGAAC	GTG GAG TGG CTG CCT TGGGT
FNDC5	AGCTGGGATGTCCTGGAGGA	GCACATGGACGATATATTCT
eNOS	TACGGAGCAGCA AAT CCA C	GAT CAA AGG ACT GCA GCC TG
GAPDH	ATGTGTCCGTCGTGGATCTGAC	AGACAACCTGGTCCTCAGTGTAG

eNOS, endothelial nitric oxide synthase; FNDC5, fibronectin type III domain-containing protein 5; GAPDH, glyceraldehyde phosphate dehydrogenase;PGC-1α, peroxisome proliferator-activated receptor gamma coactivator-1α.

Results

Effect of aging and exercise training on rats' memory performance in the experimental groups (Table 2)

Our results revealed that in the testing sessions 2 hours, 24 hours, and 5 days of NOR test, aged rats took significantly longer time to discover familiar objects than the novel ones compared to the young group. Subsequently, there were significant

decreases in the DI and RI % in the aged group compared to the young group ($P < 0.05$). These findings indicated disturbed short-term, intermediate-term, and long-term memory for the familiar object with an inability to identify it.

Conversely, exercise training by aged rats in the EX-Aged group resulted in an improvement in memory performance; the exercised aged rats spent more time exploring the novel object compared to the familiar one. This indicates retained information concerning the familiar object. There were also, significant increases in DI and RI % 2hs, 24hs, and 5days testing sessions in the EX-Aged group when compared to the Aged group ($P < 0.05$).

Table 2: Time-spent at the novel and familiar objects, Discrimination index, and recognition index % of NOR test in the experimental groups

NOR parameters		Young	Aged	Ex-Aged
Time spent at the Novel object (sec)	2hs	62.5±3.2	33.3±5.3 [a]	59.3±5.3 [b]
	24hs	68.3±2.1	42.0±3.8 [a]	64.3±2.9 [a, b]
	5days	70.1±3.7	52.3±4.7 [a]	69.6±3.1 [b]
Time spent at the Familiar object (sec)	2hs	21.2±2.6	45.6±3.5 [a]	24.6±3.2 [b]
	24hs	16.3±2.7	54.3±3.5 [a]	16.6±4.4 [a, b]
	5days	12.3±3.1	45.6±4 [a]	14.1±3.4 [b]
Discrimination index (DI)	2hs	0.49 ±.04	- 0.16±.06 [a]	0.41±.07 [a, b]
	24hs	0.61 ±.05	- 0.12±.05 [a]	0.59±.1 [a, b]
	5days	0.70 ±.06	0.06 ±.07 [a]	0.66±.06 [a, b]
Recognition Index (RI %)	2hs	74.73 ±2.38	41.99 ±3.08 [a]	70.58 ±3.93 [a, b]
	24hs	80.77 ±2.62	43.57 ±2.81 [a]	79.51 ±5.09 [a, b]
	5days	85.12 ±3.23	53.38 ±3.66 [a]	83.22 ±3.29 [a, b]

Data are expressed as mean ± standard deviation SD; n = 6, [a] $P < 0.05$ significant difference compared with the young group; [b] $P < 0.05$ significant difference compared with the aged group.EX, exercise; NOR, novel object recognition.

Effect of aging and exercise training on rats' locomotor activity and exploratory behavior in the experimental groups (Fig. 3)

During the OF, the aged rats showed a significant ($P< 0.05$) decline in the number of crossing lines (Fig. 3A) and rearing (Fig. 3D), but significant increase in the defecation droppings (Fig. 3B) and grooming (Fig. 3C) when compared to the young group. These findings indicated aging-associated reduction in locomotor activity and exploratory behavior. On the other hand, the exercise training in the Ex-Aged group significantly ($P< 0.05$) reversed the aforementioned parameters when compared to the aged group.

Effect of aging and exercise training on the hippocampal TOS and TAC levels in the experimental groups (Table 3)

Importantly, there was a significant decrease in the hippocampal TAC levels, but significant increase in TOS in the aged rat group versus the young group ($P < 0.05$). On the contrary, The EX-Aged group exhibited a significant ($P< 0.05$) uprising in the TAC levels but a significant reduction in TOS when compared to the aged group.

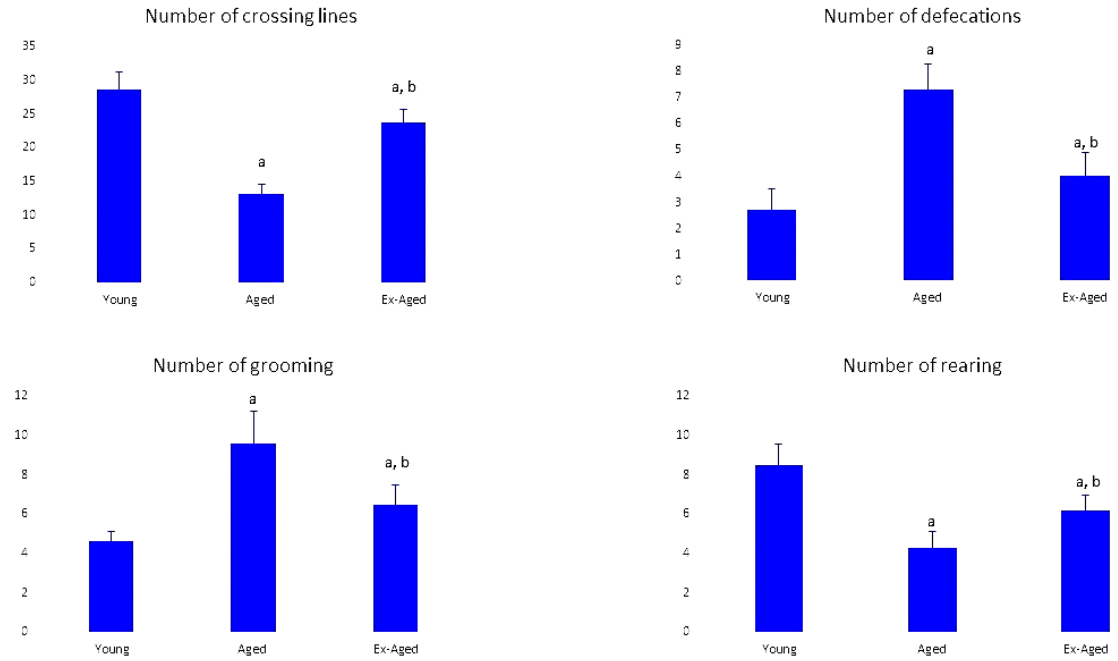

Figure 3 Changes in animal behavior in the experimental groups.

Data are expressed as mean ± standard deviation SD; $n = 6$, [a] $P < 0.05$ significant difference compared with the young group; [b] $P < 0.05$ significant difference compared with the aged group. EX, exercise.

Table 3: Hippocampal TOS and TAC levels in the experimental groups

	Young	Aged	Ex-Aged
TOS (nmol/mg protein)	8.1 ± 0.73	13.7 ± 1.03 [a]	10.4 ± 1.0 [a, b]
TAC (nmol/mg protein)	3.2 ± 0.6	1.46 ± 0.42 [a]	2.6 ± 0.35 [a, b]

Data are expressed as mean ± standard deviation SD; $n = 6$, [a] $P < 0.05$ significant difference compared with the young group; [b] $P < 0.05$ significant difference compared with the aged group. EX, exercise; TAC, total antioxidant capacity; TOS, total oxidant status.

Effect of aging and exercise training on the hippocampal PGC-1α, FNDC5, andeNOS genes expression (Fig. 4)

The hippocampal PGC-1α, FNDC5, and eNOS mRNA expressions were significantly ($P < 0.05$) down- regulated in the aged group when compared to their corresponding in the young group. Noteworthy, the exercise training in the EX-Aged group caused significant up regulation in these genes versus aged group ($P < 0.05$).

Effect of aging and exercise training on the serum irisin and hippocampal p-AMPK, NOx

and BDNF protein levels in the experimental groups (Table 4)

The results of the present study revealed significant decline in serum irisin and hippocampal p-AMPK level in addition to NOx and BDNF in the aged rats when compared to their corresponding in the young ($P < 0.05$). On the other hand, exercise training caused significant increases in their levels in the EX-Aged group in comparison to the aged group ($P < 0.05$).

Correlation between serum irisin level and other parameters (Fig. 5)

There was significant correlation between serum irisin and other parameters ($P < 0.05$); it was positively correlated with the hippocampal levels of p-AMPK ($r = 0.877$), TAC ($r = 0.813$), eNOS ($r = 0.812$), NOx ($r = 0.822$), and BDNF ($r = 0.901$) while, negatively correlated with the TOS ($r = -0.846$).

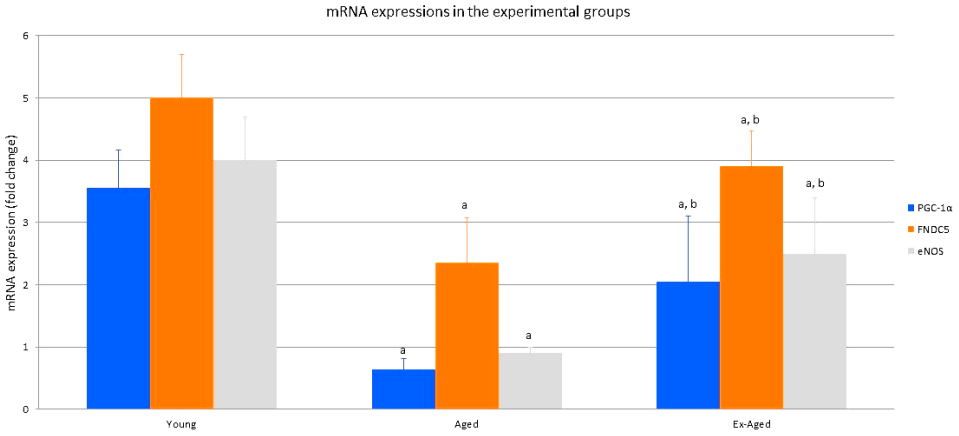

Figure 4 Changes in hippocampal relative mRNA expressions of PGC-1α, FNDC5, and eNOS in the experimental groups

Data are expressed as mean ± standard deviation SD; *n* = 6, [a] $P < 0.05$ significant difference compared with the young group; [b] $P < 0.05$ significant difference compared with the aged group. EX, exercise; eNOS, endothelial nitric oxide synthase; FNDC5, fibronectin type III domain-containing protein 5; GAPDH, glyceraldehyde phosphate dehydrogenase; PGC-1α, peroxisome proliferator-activated receptor gamma coactivator-1α.

Table 4: Serum irisin and hippocampal p-AMPK, NOx, and BDNF levels in the experimental groups

	Young	Aged	Ex-Aged
Irisin (ng/mL)	133.5 ± 6.7	89 ± 9.34 [a]	121 ± 7.69 [a, b]
p-AMPK (ng/ml)	8.6 ± 0.98	3.1 ± 0.6 [a]	5.2 ± 0.81 [a, b]
NOx (µmol/g tissue)	37.8 ± 3.9	23 ± 4.5 [a]	31.5 ± 2.6 [a, b]
BDNF (pg/mg protein)	17.1 ± 1.5	5.1 ± 0.96 [a]	14.3 ± 1.3 [a, b]

Data are expressed as mean ± standard deviation SD; *n* = 6, [a] $P < 0.05$ significant difference compared with the young group; [b] $P < 0.05$ significant difference compared with the aged group. EX, exercise; BDNF, brain-derived neurotrophic factor; NOx, nitrite/nitrate; p-AMPK, phosphorylated adenosine monophosphate-activated protein kinase.

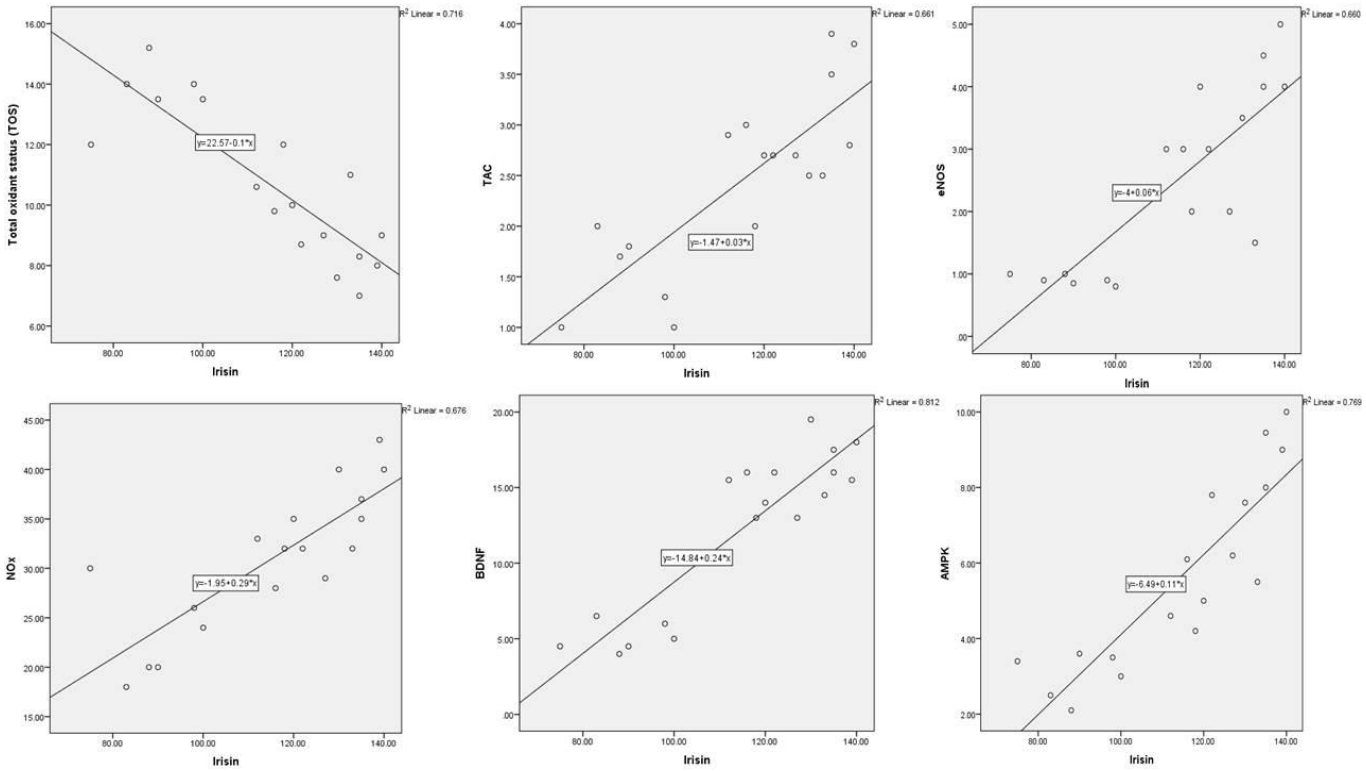

Figure 5 Correlation between serum irisin and TOS, TAC, eNOS, NOx, BDNF, and AMPK levels.
BDNF, brain-derived neurotrophic factor; eNOS, endothelial nitric oxide synthase NOx, nitrite/nitrate; p-AMPK, phosphorylated adenosine monophosphate-activated protein kinase; TOS, total oxidant status; TAC, total antioxidant capacity.

Discussion

The main finding in the current study is the observation of PGC-1α/FNDC5/ irisin/AMPK positive feedback relation on mediating the nootropic, cognitive preservation, and anti-aging effect of exercise in naturally aged rats. It was achieved by preserving the redox, eNOS/NOx, and BDNF homeostasis throughout the aging process.

The aged rats in this study exhibited cognitive impairments compared to young rats. There were defective short-term, intermediate-term, and long-term memory as concluded from the NOR test. The aged rats spent less time exploring the novel object rather than the familiar during the testing phases 2hs, 24hs, and 5 days after training. In line with this, a significant decrease in DI and RI was reported. Moreover, during the open field test, the aged rat group exhibited a significant decline in locomotor activity, physical performance, exploratory behavior, and learning. A lower number of crossing lines and rearing, but a higher number of grooming and defecation were reported in comparison to the young group. Our behavioral findings are consistent with those made by Belviranlı and Okudan [14].

The present work next explored the mechanism involved in such aging-associated cognitive deficit. There was an evident decline in eNOS/NO/BDNF levels that were triggered by oxidative stress. These eventually cause hippocampal vascular-neural dyshomeostasis.

The aged rats group exhibited a statistically substantial increase in TOS while, a decline in TAC when compared to the young group. These findings are in line with the oxidative theory of aging; due to the diminution of the antioxidant

capacity to buffer oxidants and it becomes overwhelmed by the generated free radicals leading to oxidative injury to lipids, proteins, and DNA [4].

It is noteworthy that the brain, especially the cerebral endothelial cells, is highly susceptible to oxidative injury, due to its plentiful lipid content and great consumption of O2. In addition, a solid linkage exists between oxidative stress-induced cell senescence and endothelial dysfunction, which was in a NO-dependent pathway [20]. These data eventually explain our finding regarding the significant down-regulation of eNOS gene expression and NOx concentration in aged rats versus the young. Our findings agreed with those of Yang's research group [21].

Most importantly, the present results revealed a significant decrease in BDNF in the aged group versus the young. It was agreed with those of Monnier [22] and Hariharan [6]. They verified that the production of BDNF is dependent on the eNOS/NO pathway and is produced by cerebral endothelial cells 50 times faster than cortical nerve cells. Also there was a positive feedback loop exists between BDNF and eNOS/NO. Furthermore, partial eNOS/NO deficiency roots cognitive impairment and amyloid angiopathy [20] with the loss of neurotropic effects of BDNF. These ultimately provide the mechanism supposed for the hippocampal vascular-neural dyshomeostasis and explain the cognitive impairments that commonly associate with the aging process.

Irisin, as an EX-induced myokine and a mediator for muscle-brain cross talk, it became the focus of increased concern. It is interesting that aged rats exhibited a reduction in the hippocampal PGC-1α and FNDC5 mRNA levels in addition to the serum irisin when compared to the young group. This ensures age-specific irisin decline, both central and peripheral, with diminution of the mitochondrial biogenesis. In addition, there was a statistically substantial positive correlation between irisin and eNOS, NO, and BDNF levels reported in the current work. This suggested that increasing PGC-1α/FNDC5/irisin may causally protect against aging-associated cognitive dysfunction.

The crucial determining factors of circulating irisin are age and muscle performance [1]. Thus, the increased age and the associated decrease in locomotor activity and physical endurance could explain age-associated irisin decline and also, provide a strategy to preserve it by training.

In consistent with this concept, our results revealed that EX training by aged rats for 8 weeks significantly prevented such cognitive decline with a significant reversal of the assessed parameters in NOR and OF tests compared to the non-trained aged rats. This pinpoints the valuable positive impacts of EX against cognitive deterioration. Our results go hands with those of Amirazodi et al. [23].

In this regard, EX training prevented central PGC-1α/FNDC5 and peripheral irisin decrease in aged rats and caused a significant rise compared to the non-trained aged group. Thus, the EX-induced PGC-1α/FNDC5/irisin could prevent the aging-associated cognitive deficit. In consistent with this, Islam's research group has recently, shown that irisin is sufficient to confer the benefits of exercise on cognitive function and that genetic deletion of FNDC5/irisin impairs cognitive function in exercise and aging [24].

A mounting group of evidence proposes that enhancing AMPK activity can prevent and even reverse the neurological deficits of aging. Lacking AMPK activity may be linked to almost all pathological aging processes [10]. In accordance, lower levels of p-AMPK were documented in aged rats versus the young group. Moreover, exercise training at older age prevented such aging-associated decline. In addition, AMPK activates PGC-1α and subsequently irisin production through direct or indirect pathways [25] indicating a positive feedback loop exists between AMPK and irisin.

To the best of our knowledge no study, to date, has evaluated the role of irisin/AMPK on the basis of eNOS/NO-BDNF downstream signaling pathway in the neuroprotective and anti-aging nootropic effects.

In support of our findings, some studies have revealed an important role of irisin in the activation of eNOS, via AMPK signaling both in vivo and in vitro; cultured vascular endothelial cells [26], spontaneously hypertensive rats [17], and in obese human [27]. Furthermore, our data go hand with Azimi and his colleagues who revealed that the hippocampal FNDC5/BDNF axis is amplified via activating the AMPK signaling pathway by training [28]. In addition, when exogenous Irisin, used to imitate exercise, was administrated at a dose of (0.5 μg/g i.p), it increased BDNF expression in the brain [29].

Significantly, TAC was increased and positively correlated, while TOS was decreased and negatively correlated, with serum irisin levels in EX-aged rats versus the non-trained Aged group. These results identify irisin as an antioxidant myokine. This was in accordance with the findings of both teams of Bi [30] and Jiang [31] in the setting of aged liver and spinal cord injury, respectively. They reported that irisin reduced malondialdehyde and increased antioxidant enzymes both in vivo and in vitro. This antioxidant property could be attributed to direct redox regulation by AMPK being a positive regulator of nuclear factor erythroid 2-related factor 2 (Nrf-2) [32]. Collectively, these outcomes pinpoint a downstream mechanistic pathway on the appreciated actions of the EX-induced irisin in the hippocampus that impacts neurogenesis, neurotransmitters viability, and redox balance by which it preserves memory and locomotor activity in aged rats through AMPK.

Conclusion

This study demonstrated that PGC1α, FNDC5, and irisin levels are reduced in aged rats and that exercise boosts their levels and attenuates cognitive impairment. We further show that through the AMPK pathway, irisin restores redox, eNOS/NOx, and BDNF homeostasis in the aged brain. Our results recommend that irisin could embrace an attractive therapy pointed to prevent dementia or delay its progression as well, in risky people or in late stages patients. Forthcoming, an exogenous irisin may be used to imitate exercise's beneficial effects, especially for those who cannot be engaged in regular physical exercise.

Declarations:

Compliance with ethical standards: The study was executed by following the rules and guidelines of the Institutional Animal Ethics Committee, Benha University, Egypt. Approval Protocol Number (REC-FOMBU, MoHP, No. 19-11-2022).

Author contributions: All authors contribute equally in the study design, collection of samples, data analysis, and manuscript writing.

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