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ORIGINAL ARTICLE

Endurance training and MitoQ supplementation improve spatial memory, VEGF expression, and neurogenic factors in hippocampal tissue of rats

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ABSTRACT

Background and Aim: The hippocampus has a key role in memory and learning, which means that this brain structure has high-energy demand. Accordingly, mitochondrial dysfunction in the hippocampus has deleterious effects on brain function. MitoQ is an antioxidant that accumulates selectively in mitochondria at high concentration. In this study, the effect of MitoQ alone and in combination with endurance training (ET) was investigated on spatial memory (distance, time, and number of passes in the target quarter), antioxidant status (superoxide dismutase [SOD] and glutathione peroxidase [GPx]), and neurogenic factor levels (vascular endothelial growth factor [VEGF] and brain-derived neurotrophic factor [BDNF]) in male Wistar rats.

Methods: Rats were assigned to a control (CTL) group, ET group, ET+MitoQ group, and a MitoQ group. Rats were trained on a treadmill for 8 weeks, 5 days/week, and 50 min/day. MitoQ (250 µM daily) was administered through drinking water for 8 weeks. Spatial memory (Morris water maze test), gene expression (real-time PCR), protein expression (Western blotting), and antioxidants (ELISA method) were determined.

Results: Distance and number of passes in the target quarter in the ET, MitoQ, and ET+MitoQ groups were higher than in the CTL group (P=0.001). MitoQ+ET had more impact on the abovementioned indices than MitoQ or ET alone. Simultaneous use of MitoQ and ET significantly increased gene and protein expression of VEGF (P=0.0001) and gene expression of BDNF (P=0.004) and Sestrin 2 (*SESN2*) (P=0.0001) in hippocampal tissue. The expression of VEGF (P=0.007) and *SESN2* (P=0.001) was higher in the MitoQ group compared to the CTL group. Tissue GPx levels were increased following all three interventions ($P\leq$0.013) compared to the CTL group while SOD levels remained unchanged in all groups.

Conclusions: The combination of ET and MitoQ has additive effects on spatial memory in rats by modulating parameters that are involved in hippocampal neurogenesis. In addition, MitoQ may have positive effects on the antioxidant defense by improving GPx activity.

Relevance for Patients: Considering the positive effects of MitoQ on improving the memory and the antioxidant defense, it seems that it can play a positive role in improving the diseases associated with memory loss in the long term, and ET along with this supplement can increase the possible positive effects.

1. Introduction

The hippocampus plays a key role in memory formation by providing sensory, emotional, and cognitive components together [1]. The dentate gyrus of the hippocampus is a highly vascularized

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environment, and vascular endothelial growth factor (VEGF) can cross the blood–brain barrier. VEGF mediates hippocampal neurogenesis and angiogenesis and the role of myofiber-derived VEGF is stimulating hippocampal proliferation [2].

Among the neurotrophins, brain-derived neurotrophic factor (BDNF) has received more attention than others [3]. As a regulator of neuronal survival, growth, and differentiation during development, BDNF is highly expressed in the brain [4]. BDNF can mediate neuronal survival, neurogenesis, cell death, and plasticity, and exercise training can regulate BDNF synthesis [5]. BDNF also mediates neuronal plasticity that can promote learning and memory [3]. Thus, BDNF can transform physiological stimuli of neural activity into molecular and morphological changes in the nervous system [4].

Sestrin 2 (*SESN2*) can suppress reactive oxygen species (ROS) and protect against various stimuli, including oxidative stress and hypoxia [6,7]. *SESN2* also plays a key role in metabolic regulation through activation of the key energy sensor AMP-dependent protein kinase and inhibition of the mammalian target of rapamycin complex 1 [6].

MitoQ is an advanced mitochondria-targeted antioxidant that has been shown to protect against oxidative stress [8]. MitoQ consists of a ubiquinone moiety, which prevents lipid peroxidation by acting as a chain-breaking antioxidant and recycling the α-tocopheroxyl radical to its active form, conjugated to a triphenylphosphonium cation to facilitate accumulation within mitochondria [9-11].

Exercise training can lead to strengthening of the functional connection of the brain and hippocampus-cortex network, so the extent of hippocampal changes in this functional pattern is critical [12]. The positive effects induced by exercise training in the brain are carried out by the induction of BDNF expression in the hippocampus and the activation of its tropomyosin kinase receptor B receptor [4]. Indeed, blocking BDNF signaling in the hippocampus attenuates exercise-induced learning and memory formation [5,13]. In addition, VEGF released from exercising myofibers can enter the brain and directly stimulate hippocampal neurogenesis [3]. Two weeks of exercise training with a complex exercise pattern can increase BDNF expression [14]. Literature on the relationship between *SESN2* and exercise training is limited [7]. However, exercise training can stimulate *SESN2* accumulation and autophagic response in the skeletal muscle of old mice, which can improve insulin sensitivity in these animals [9,10].

Therefore, given the importance of neurogenesis parameters and mitochondrial ROS production in hippocampal performance, this study aimed to investigate the effects of MitoQ supplementation, alone and in combination with ET, on the spatial memory and neurogenesis factors (VEGF and BDNF) and *SESN2*, which regulates inflammation and ROS generation in mitochondria. We hypothesized that MitoQ supplementation to ET may improve hippocampal function by increasing the expression of neurogenesis factors and by positively regulating *SESN2* expression in rats more than ET alone.

2. Materials and Methods

Thirty-two male Wistar rats were randomly divided into four groups (n=8): Control (CTL), endurance training (ET), MitoQ, and ET+MitoQ. Animals were purchased from Kerman University of Medical Science. The animals were kept in conventional conditions (12 h of light and 12 h of darkness) with free water and normal food access. MitoQ was provided by MitoQ Company (MitoQ Ltd., New Zealand). RNA isolation kit (Bio Basic, Canada), cDNA synthesis kit (Parstous Biotechnology, Iran), Master Mix Green (Ampliqon, Denmark), and rat superoxide dismutase (SOD) and glutathione peroxidase (GPx) ELISA kits (Sunlong Biotech, China) were used in this study. The experimental protocol was approved by the Ethics Committee of Kerman University of Medical Sciences (Ethics No: IR.KMU.REC.1398.678).

2.1. Exercise protocol

During familiarity training, the rats were trained on the treadmill at a speed of 15 m/min for 15 min for 2 weeks. For calculating the maximal oxygen consumption (VO_{2max}), we used the maximal rate (V_{max}) and the incremental test was initiated with a 10 m/min warm-up that gradually increased (0.3 m/min) until exhaustion [15]. After the incremental test, the lactate levels were measured using a lactometer (Lactate Scout Company/Code: 37, Germany), and values above 6 mmol/L were considered high intensity [16]. Then, we calculated VO_{2max}. ET was performed based on Table 1 [17]. The CTL and MitoQ supplementation groups were not trained during the protocol and were placed on the treadmill each session to induce stress.

2.2. MitoQ supplementation

MitoQ was given to rats at a dose of 250 μM in drinking water for 8 weeks [18].

2.3. Morris water maze test

Morris water maze is a circular metal pool with a black wall 140 cm in diameter and 60 cm high, filled with water to a depth of 30–25 cm. Twenty-four hours after adapting to the device, rats in each group were trained for 6 consecutive days, each day in six separate 60 s attempts. The training method was similar in each effort; each rat was accidentally released into the water from one of the four main directions (north, south, east, and west) from the tail so that its face was toward the wall of the pool. The rats swam to find a hidden platform 10 cm in diameter and 3 cm below the water surface in the middle of one of the reservoir quarters (e.g., northeast) and rest on it for 30 s. In each training session, the time to find the platform, the total distance traveled, and the times that the rats passed the platform were measured using the

Table 1. Endurance training schedule

Week	1	2	3	4	5	6	7	8
Speed (m/min)	60% Vmax	65% Vmax	70% Vmax	70% Vmax	75% Vmax	75% Vmax	75% Vmax	75% Vmax
Time (min)	20	30	30	40	40	45	50	50

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camera above the pool. On the test day, the pool was divided into four equal quarters, and the indicators were measured (the target quarter was the place where the platform was already located). Kinovea software (Kinovea-08.15) was used in this study. Kinovea is a two-dimensional motion analysis software that can be used to measure kinematic parameters [19].

2.4. Determination of SOD activity

The ELISA kit was used according to the manufacturer's instructions to measure SOD levels in tissue lysis. SOD, which absorbs light at 560 nm, acts as a catalyst in the dismutation of O_2 radicals to hydrogen peroxide (H_2O_2) and in the conversion of NBT to NBT-diformazan [20].

2.5. Determination of GPx activity

An ELISA kit was used to measure GPx activity using the method described by Paglia and Valentine. The assay kit indirectly measures GPx activity through a reaction with glutathione reductase, the enzyme responsible for regenerating the reduced form of oxidized glutathione (GSSG). When NADPH is oxidized to NADP+, its absorbance at 340 nm decreases [21].

2.6. Real-time PCR

Total RNA extraction was conducted by EZ-10 spin column total RNA Mini-Preps Super Kit following manufacturer instructions, and for this purpose, 20 mg of brain tissue was excised from storage and used for RNA extraction. For cDNA synthesis, we used 100 ng of total RNA and Easy cDNA synthesis kit for real-time PCR. Real-time PCR was performed by Real Plus 2× Master Mix Green high ROX, and PCR reaction also contained forward and reverse primers, sterile water, and 100 ng cDNA. Real-time PCR was performed on an ABI StepOnePlus instrument. The thermal reaction was as follows: Stage 1: Denaturation, 95°C for 10 min, Stage 2: 40 cycles at 95°C for 20 s, and 60°C for 30 s, and finally, melt curve analysis was performed, which started from 60°C and increased by 0.3°C. Primers were purchased from Metabion (Metabion International, Germany) and are listed in Table 2. Finally, the expression level was determined by the $2^{-\Delta\Delta Ct}$ method and normalized to 18S rRNA as a housekeeping gene [22].

Table 2. Sequences of primers used for real-time PCR

Gene	Primer sequence
VEGF	F-CACTGGACCCTGGCTTTACT
	R-GACGTCCATGAACTTCACCA
BDNF	F-GTCCCTTCTACACTTACCTCTTG
	R-CTTTGTTTCACCCTTTCCACTCCT
SESN2	F-GCGGCTCGAGATAACTCGGCATCTGACCT
	R-AATGCGGCCGCGTTTCTCCCCTGTGACAAT
18S rRNA	F-GCAATTATTCCCCATGAACG
	R-GGCCTCACTAAACCATCCAA

VEGF: Vascular endothelial growth factor, BDNF: Brain-derived neurotrophic factor, *SESN2*: Sestrin 2

2.7. Western blotting

The hippocampal tissue was quickly removed and placed in liquid nitrogen and stored at −80°C for Western blotting. Protein levels of VEGF were measured by Western blotting. Initially, 30 mg of brain tissue was homogenized. The supernatant was collected and separated by 10% SDS-PAGE. After electrophoresis, the samples were transferred to the PVDF membrane and incubated with an anti-VEGF-A antibody. The blots were then incubated with conjugated peroxidase secondary antibody. After washing, the blots were imaged using chemiluminescence. The amount of protein was measured by quantitative density analysis and compared to β-actin as a CTL by Image J software [23].

2.8. Statistical analysis

Data are expressed as mean ± SD. The analysis of data was performed by SPSS software (SPSS, Chicago, IL, USA; Version 22). Data normality was analyzed by the Shapiro–Wilk test. Then, we confirmed that the distribution of the data was normal. For comparison between the groups, we conducted one-way ANOVA, and pairwise comparison was performed by Tukey's *post hoc* test. $P<0.05$ was considered statistically significant.

3. Results

3.1. High-performance liquid chromatography-tandem mass spectrometry (HPLC-MS)

To assess the targeting of MitoQ in hippocampal tissue, the concentration of MitoQ was measured in the whole lysate hippocampal tissue by HPLC-MS [24]. For HPLC-MS calibration, we used the MitoQ internal standard (MRC Mitochondrial Biology Unit and Department of Medicine, University of Cambridge), and the tissue levels of MitoQ in the CTL group and MitoQ group were 0 and 5.68 ± 0.81 pmoles/100 mg protein, respectively.

3.2. Gene and protein expression

ET significantly increased VEGF protein and gene expression, distance, and the number of passes in the target quarter compared to the CTL group ($P<0.001$) (Table 3, Figures 1 and 2). Furthermore, time in the target quarter increased in the MitoQ group compared to the CTL group. MitoQ supplementation increased protein and gene expression of VEGF and gene expression of *SESN2* compared to the CTL group (Figures 1-3). The increase in the expression of VEGF, BDNF, and *SESN2* caused by exercise training was enhanced by MitoQ supplementation in the groups with the combination of MitoQ and exercise training (Figures 1-4).

GPx levels were increased following all three interventions (MitoQ: $P=0.013$, ET: $P<0.0001$, and ET+MitoQ: $P=0.003$) (Figure 5). There was no change in SOD levels in any of the groups (MitoQ: $P=0.70$, ET: $P>0.99$, and ET+MitoQ: $P=0.98$) (Figure 6).

4. Discussion

Our study showed that ET might improve some spatial memory indicators (distance and number of passes in the target quarter)

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as well as protein and transcript levels of VEGF in hippocampal tissue. In this regard, ET and MitoQ supplementation may have positive effects on antioxidant defense and genes involved in neurogenesis. MitoQ supplementation alone positively modulated GPx levels, gene and protein expression of VEGF, and transcript levels of *SESN2*.

Our results showed that MitoQ has no effect on the gene expression of BDNF in the hippocampus, but exercise training and MitoQ supplementation combined can increase the expression of BDNF in the brain (Figure 4). In our experiment, ET alone did not significantly change the gene expression of BDNF in the brain. Other research has shown that during recovery, levels of BDNF can decrease below the baseline [25], and maybe the shortness of the training period was one of the reasons for the ineffectiveness of ET on the expression pattern of this gene.

The present study demonstrated that MitoQ supplementation alone and in combination with ET could increase the gene and

Table 3. Parameters of Morris water maze test

Groups/ scale	Distance in target quarter (Mean±SD)	Time in target quarter (Mean±SD)	Number of passes (Mean±SD)
CTL	328±21	15±3	2.0±0.5
ET	441±32*	18±2	4.0±0.5*
MitoQ	467±23*	20±2*	6.0±0.8*
ET+MitoQ	458±83*	19±1	5.0±0.4*

*Statistically significant compared to the control group. CTL: Control, ET: Endurance training

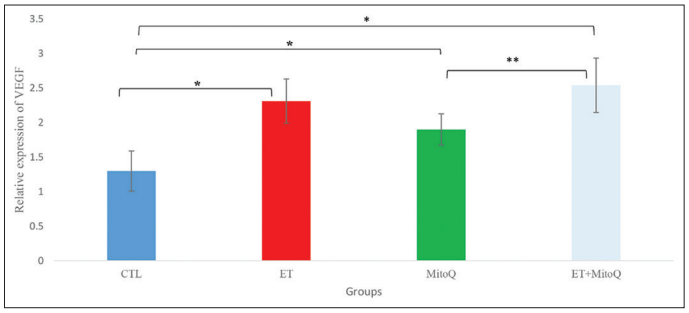

Figure 1. Relative vascular endothelial growth factor (VEGF) gene expression in the control (CTL), endurance training (ET), MitoQ supplementation (MitoQ), and ET+MitoQ supplementation (ET+MitoQ) group (n=8/group). Data are expressed as mean±SD; *P<0.05 compared to CTL group; **P<0.05 compared to MitoQ group.

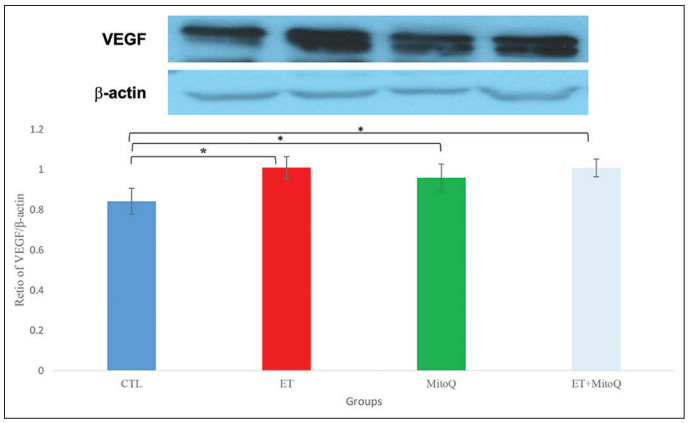

Figure 2. Protein expression of vascular endothelial growth factor (VEGF) in the control (CTL), endurance training (ET), MitoQ supplementation (MitoQ), and ET+MitoQ supplementation group (n=8/group). Data are expressed as mean±SD; *P<0.05 compared to CTL group.

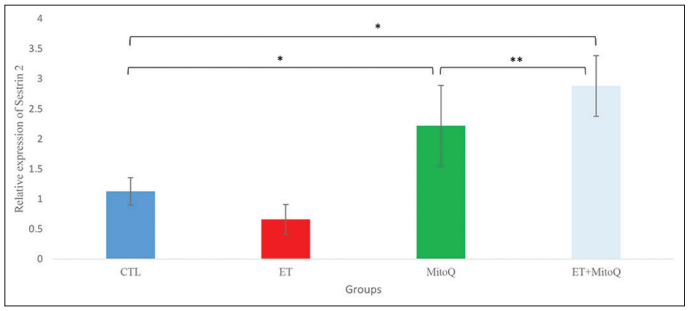

Figure 3. Sestrin 2 gene relative expression in the control (CTL), endurance training (ET), MitoQ supplementation (MitoQ), and ET+MitoQ supplementation group (n=8/group). Data are expressed as mean±SD; *P<0.05 compared to CTL group; **P<0.05 compared to MitoQ group.

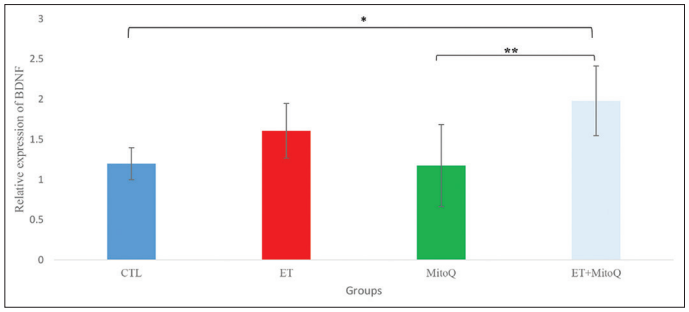

Figure 4. Brain-derived neurotrophic factor (BDNF) gene relative expression in the control (CTL), endurance training (ET), MitoQ supplementation (MitoQ), and ET+MitoQ supplementation group (n=8/group). Data are expressed as mean±SD; *P<0.05 compared to CTL group; **P<0.05 compared to MitoQ group.

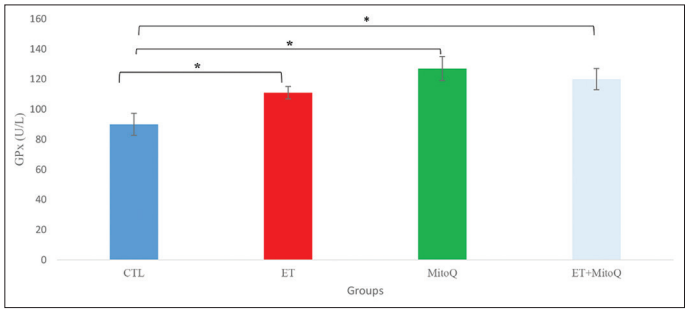

Figure 5. Tissue levels of glutathione peroxidase (GPx) in the control (CTL), endurance training (ET), MitoQ supplementation (MitoQ), and ET+MitoQ supplementation group (n=8/group). Data are expressed as mean±SD; *P<0.05 compared to CTL group.

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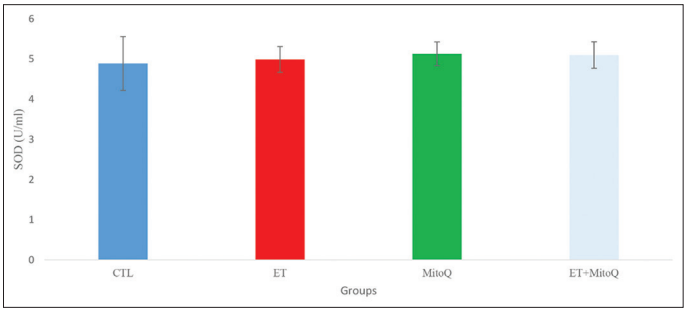

Figure 6. Tissue levels of superoxide dismutase (SOD) in the control (CTL), endurance training (ET), MitoQ supplementation (MitoQ), and ET+MitoQ supplementation group (n=8/group). Data are expressed as mean±SD.

protein expression of VEGF in hippocampal tissue (Figures 1 and 2). Broome *et al*. showed that MitoQ supplementation could increase the gene expression of VEGF in skeletal muscle [11], which is related to an improvement of the endothelial function [10] and VEGF-mediated angiogenesis through PGC-1α in untrained middle-aged humans [26]. Our study indicates that MitoQ supplementation increases gene and protein levels of VEGF in the brain, which, in turn, coincided with improved memory-related outcome parameters.

In our experiment, ET and ET+MitoQ did not change the time in the target quarter, but in both groups, the distance and number of passes in the target quarter improved. MitoQ supplementation could improve cognitive performance and inhibit memory loss [27]. Based on the data, we obtained from the Morris water maize test, there were improvements in some indicators of spatial memory (distance and number of passes in the target quarter) that is aligned with the changes in the expression of VEGF, which is involved in neurogenesis. Other studies show that ET can enhance memory and the expression of genes involved in neurogenesis [11,28,29].

The previous studies have presented similar results, that is, antioxidant supplementation increased plasma levels of GPx but had no significant effect on SOD [30,31]. GPx and SOD serum levels as oxidative stress markers generally increase following vigorous exercise [32,33] or long-term training [34], which suggests activated antioxidant defense. Similarly, this study found that GPx levels increased following MitoQ alone, and this increase was slightly higher than ET alone and ET supplemented with MitoQ. These results imply that MitoQ supplementation of ET might mitigate the exercise-induced increase in GPx due to the overall improvement of antioxidative function in mitochondria [35]. On the other hand, as there was no change in SOD levels following all interventions and no difference between interventions, the positive effect of MitoQ on the prevention of ROS overproduction might be relatively less dependent on SOD activity even though MitoQ is known as a mitochondria-targeted antioxidant.

The previous studies have shown the beneficial effects of MitoQ on inflammation and antioxidant enzymes in the brain [36-39]. In addition, in this study, the gene expression of *SESN2* showed a specific pattern compared to the other genes. In the groups that exercised, gene expression of *SESN2* did not change compared to the CTL group, and in the groups that exercised and received MitoQ, *SESN2* gene expression increased significantly compared to the CTL group (Figure 3). *SESN2* can protect neurons in the hippocampus against ischemia-induced apoptosis through phosphorylation of ribosomal protein in rats [40]. *SESN2* is a novel target for the prevention of hypoxia and metabolic disorders as it can reduce ROS accumulation and regulate autophagy [41]. A related study showed the protective effect of MitoQ against oxidative stress on mitochondrial DNA through activation of Nrf/ARE signaling [42], which regulates the induction of *SESN2* expression [43]. In our study, MitoQ was able to increase *SESN2* expression, and this effect can enhance the benefits of ET. Other research has shown the same increase in antioxidant defense in hypertensive patients [39].

5. Conclusions

MitoQ alone has positive effects on VEGF in the brain, and this improvement is accompanied by enhancement in some memory-related indices. MitoQ, in combination with ET, can have increased effect on antioxidant status and angiogenesis factors. There are few clinical studies in this area. Follow-up translational research and more comprehensive assessment of these findings in clinical studies are warranted.

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Conflicts of Interest

All the authors declare that they have no conflicts of interest.

Ethics Approval and Consent to Participate

The animal experiments were carried out in accordance with the National Institutes of Health (NIH) Guideline for the Care and Use of Laboratory Animals and were approved by the Ethics Committee on the use of animals at Kerman University of Medical Sciences, Kerman, Iran (Ethics No: IR.KMU.REC.1398.678).

Availability of Data

The datasets generated during and/or analyzed during the present study are available from the corresponding author on reasonable request.

References

[1] Knierim JJ. The Hippocampus. Curr Biol 2015;25:R1116-21.

[2] Ballard HJ. Exercise Makes your Brain Bigger: Skeletal Muscle VEGF and Hippocampal Neurogenesis. J Physiol 2017;595:5721-2.

[3] Gates M, Tai CC, Macklis JD. Neocortical Neurons Lacking the Protein-tyrosine Kinase B Receptor Display Abnormal Differentiation and Process Elongation *in vitro* and *in vivo*. Neuroscience 2000;98:437-47.

[4] El Hayek L, Khalifeh M, Zibara V, Assaad RA, Emmanuel N, Karnib N, *et al*. Lactate Mediates the Effects of Exercise on Learning and Memory Through SIRT1-Dependent Activation of Hippocampal Brain-derived Neurotrophic Factor (BDNF). J Neurosci 2019;39:2369-82.

[5] Sleiman SF, Henry J, Al-Haddad R, El Hayek L, Abou Haidar E, Stringer T, *et al*. Exercise Promotes the Expression of Brain Derived Neurotrophic Factor (BDNF) Through the Action of the Ketone Body β-hydroxybutyrate. Elife 2016;5:e15092.

[6] Pasha M, Eid AH, Eid AA, Gorin Y, Munusamy SJ. Sestrin2 as a Novel Biomarker and Therapeutic Target for Various Diseases. Oxid Med Cell Longev 2017;2017:3296294.

[7] Wang LX, Zhu XM, Yao YM. Sestrin2: Its Potential Role and Regulatory Mechanism in Host Immune Response in Diseases. Front Immunol 2019;10:2797.

[8] Sukjamnong S, Chan YL, Zakarya R, Nguyen LT, Anwer AG, Zaky AA, *et al*. MitoQ Supplementation Prevent Long-term Impact of Maternal Smoking on Renal Development, Oxidative Stress and Mitochondrial Density in Male Mice Offspring. Sci Rep 2018;8:6631.

[9] Pham T, MacRae CL, Broome SC, D'souza RF, Narang R, Wang HW, *et al*. MitoQ and CoQ10 Supplementation Mildly Suppresses Skeletal Muscle Mitochondrial Hydrogen Peroxide Levels without Impacting Mitochondrial Function in Middle-aged Men. Eur J Appl Physiol 2020;120:1657-69.

[10] Rossman MJ, Santos-Parker JR, Steward CA, Bispham NZ, Cuevas LM, Rosenberg HL, *et al*. Chronic Supplementation with a Mitochondrial Antioxidant (MitoQ) Improves Vascular Function in Healthy Older Adults. Hypertension 2018;71:1056-63.

[11] Broome SC, Pham T, Braakhuis AJ, Narang R, Wang HW, Hickey AJ, *et al*. MitoQ Supplementation Augments Acute Exercise-induced Increases in Muscle PGC1α MRNA and Improves Training-induced Increases in Peak Power Independent of Mitochondrial Content and Function in Untrained Middle-aged Men. Redox Biol 2022;53:102341.

[12] Erickson KI, Voss MW, Prakash RS, Basak C, Szabo A, Chaddock L, *et al*. Exercise Training Increases Size of Hippocampus and Improves Memory. Proc Natl Acad Sci U S A 2011;108:3017-22.

[13] Voss MW, Soto C, Yoo S, Sodoma M, Vivar C, van Praag H. Exercise and Hippocampal Memory Systems. Trends Cogn Sci 2019;23:318-33.

[14] Berchtold N, Chinn G, Chou M, Kesslak JP, Cotman CW. Exercise Primes a Molecular Memory for Brain-derived Neurotrophic Factor Protein Induction in the Rat Hippocampus. Neuroscience 2005;133:853-61.

[15] Parnow A, Gharakhanlou R, Gorginkaraji Z, Rajabi S, Eslami R, Hedayati M, *et al*. Effects of Endurance and Resistance Training on Calcitonin Gene-related Peptide and Acetylcholine Receptor at Slow and Fast Twitch Skeletal Muscles and Sciatic Nerve in Male Wistar Rats. Int J Pept 2012;2012:962651.

[16] Lonbro S, Wiggins JM, Wittenborn T, Elming PB, Rice L, Pampo C, *et al*. Reliability of Blood Lactate as a Measure of Exercise Intensity in Different Strains of Mice During Forced Treadmill Running. PLoS One 2019;14:e0215584.

[17] Mansouri M, Nikooie R, Keshtkar A, Larijani B, Omidfar K. Effect of Endurance Training on Retinol-binding Protein 4 Gene Expression and its Protein Level in Adipose Tissue and the Liver in Diabetic Rats Induced by a High-fat Diet and Streptozotocin. J Diabetes Investig 2014;5:484-91.

[18] Yang Y, Xu P, Zhu F, Liao J, Wu Y, Hu M, *et al*. The Potent Antioxidant MitoQ Protects against Preeclampsia during Late Gestation but Increases the Risk of Preeclampsia When Administered in Early Pregnancy. Antioxid Redox Signal 2021;34:118-36.

[19] Garthe A, Kempermann G. An Old Test for New Neurons: Refining the Morris Water Maze to Study the Functional Relevance of Adult Hippocampal Neurogenesis. Front Neurosci 2013;7:63.

[20] Weydert CJ, Cullen JJ. Measurement of Superoxide Dismutase, Catalase and Glutathione Peroxidase in Cultured Cells and Tissue. Nat Protoc 2010;5:51-66.

[21] Paglia DE, Valentine WN. Studies on the Quantitative and Qualitative Characterization of Erythrocyte Glutathione Peroxidase. J Lab Clin Med 1967;70:158-69.

[22] Mohammadi A, Fallah H, Shahouzehi B, Najafipour H. Mir-33 Inhibition Attenuates the Effect of Liver X Receptor Agonist T0901317 on Expression of Liver X Receptor Alpha in Mice Liver. ARYA Atheroscler 2017;13:257-63.

[23] Pour MB, Joukar S, Hovanloo F, Najafipour H. Long-term Low-intensity Endurance Exercise along with Blood-flow Restriction Improves Muscle Mass and Neuromuscular Junction Compartments in Old Rats. Iran J Med Sci 2017;42:569-76.

[24] Sun C, Liu X, Di C, Wang Z, Mi X, Liu Y, *et al*. MitoQ Regulates Autophagy by Inducing a Pseudo-mitochondrial Membrane Potential. Autophagy 2017;13:730-8.

[25] Nofuji Y, Suwa M, Moriyama Y, Nakano H, Ichimiya A, Nishichi R, *et al*. Decreased Serum Brain-derived Neurotrophic Factor in Trained Men. Neurosci Lett 2008;437:29-32.

[26] Arany Z, Foo SY, Ma Y, Ruas JL, Bommi-Reddy A, Girnun G, *et al*. HIF-independent Regulation of VEGF and Angiogenesis by the Transcriptional Coactivator PGC-1alpha. Nature 2008;451:1008-12.

[27] Young ML, Franklin JL. The Mitochondria-targeted antioxidant MitoQ Inhibits Memory Loss, Neuropathology, and Extends Lifespan in Aged 3XTG-AD Mice. Mol Cell Neurosci 2019;101:103409.

DOI: http://dx.doi.org/10.18053/jctres.09.202301.001

[28] Babaei P, Azari HB. Exercise Training Improves Memory Performance in Older Adults: A Narrative Review of Evidence and Possible Mechanisms. Front Hum Neurosci 2021;15:771553.

[29] De la Rosa A, Solana E, Corpas R, Bartres-Faz D, Pallas M, Vina J, *et al*. Long-term Exercise Training Improves Memory in Middle-aged Men and Modulates Peripheral Levels of BDNF and Cathepsin B. Sci Rep 2019;9:3337.

[30] Oliveras-López MJ, Berná G, Jurado-Ruiz E, de la Serrana HL, Martín F. Consumption of Extra-virgin Olive Oil Rich in Phenolic Compounds has Beneficial Antioxidant Effects in Healthy Human Adults. J Funct Foods 2014;10:475-84.

[31] De Moffarts B, Kirschvink N, Art T, Pincemail J, Lekeux P. Effect of Oral Antioxidant Supplementation on Blood Antioxidant Status in Trained Thoroughbred Horses. Vet J 2005;169:65-74.

[32] Fisher G, Schwartz DD, Quindry J, Barberio MD, Foster EB, Jones KW, *et al*. Lymphocyte Enzymatic Antioxidant Responses to Oxidative Stress Following High-intensity Interval Exercise. J Appl Physiol (1985) 2011;110:730-7.

[33] Lekhi C, Gupta PH, Singh B. Influence of Exercise on Oxidant Stress Products in Elite Indian Cyclists. Br J Sports Med 2007;41:691-3.

[34] Melikoglu MA, Kaldirimci M, Katkat D, Sen I, Kaplan I, Senel K. The Effect of Regular Long Term Training on Antioxidant Enzymatic Activities. J Sports Med Phys Fitness 2008;48:388-90.

[35] Powers SK, Bomkamp M, Ozdemir M, Hyatt H. Mechanisms of Exercise-Induced Preconditioning in Skeletal Muscles. Redox Biol 2020;35:101462.

[36] Jeong JH, Koo JH, Yook JS, Cho JY, Kang EB. Neuroprotective Benefits of Exercise and MitoQ on Memory Function, Mitochondrial Dynamics, Oxidative Stress, and Neuroinflammation in D-galactose-induced Aging Rats. Brain Sci 2021;11:164.

[37] Zhou J, Wang H, Shen R, Fang J, Yang Y, Dai W, *et al*. Mitochondrial-targeted Antioxidant MitoQ Provides Neuroprotection and Reduces Neuronal Apoptosis in Experimental Traumatic Brain Injury Possibly via the NRF2-ARE Pathway. Am J Transl Res 2018;10:1887-99.

[38] Piscianz E, Tesser A, Rimondi E, Melloni E, Celeghini C, Marcuzzi A. MitoQ is able to Modulate Apoptosis and Inflammation. Int J Mol Sci 2021;22:4753.

[39] Masoumi-Ardakani Y, Najafipour H, Nasri HR, Aminizadeh S, Jafari S, Moflehi D. Effect of Combined Endurance Training and MitoQ on Cardiac Function and Serum Level of Antioxidants, NO, miR-126, and miR-27a in Hypertensive Individuals. Biomed Res Int 2022;2022:8720661.

[40] Shi X, Doycheva DM, Xu L, Tang J, Yan M, Zhang JH. Sestrin2 Induced by Hypoxia Inducible Factor1 Alpha Protects the Blood-brain Barrier via Inhibiting VEGF after Severe Hypoxic-ischemic Injury in Neonatal Rats. Neurobiol Dis 2016;95:111-21.

[41] Pan C, Chen Z, Li C, Han T, Liu H, Wang X. Sestrin2 as a Gatekeeper of Cellular Homeostasis: Physiological Effects for the Regulation of Hypoxia-related Diseases. J Cell Mol Med 2021;25:5341-50.

[42] Hu Q, Ren J, Li G, Wu J, Wu X, Wang G, *et al*. The Mitochondrially Targeted Antioxidant MitoQ Protects the Intestinal Barrier by Ameliorating Mitochondrial DNA Damage via the NRF2/ARE Signaling Pathway. Cell Death Dis 2018;9:403.

[43] Shin BY, Jin SH, Cho IJ, Ki SH. Nrf2-are Pathway Regulates Induction of Sestrin-2 Expression. Free Radic Biol Med 2012;53:834-41.

DOI: http://dx.doi.org/10.18053/jctres.09.202301.001