Effect of Aerobic Training and L-carnitine on MDA, GPX and Hippocampal Neurogenesis in Diabetic Rats

Alireza Shahsavar[1]*, Hamid Rajabi[2], Reza Gharakhanlou[3], Mohammad Reza Dehkhoda[4]

1. PhD Student of Exercise Physiology, Kharazmi University, Tehran, Iran.
2. Professor of Exercise Physiology, Kharazmi University, Tehran, Iran.
3. Professor of Exercise Physiology, Tarbiat Modares University, Tehran, Iran.
4. Associate Professor of Exercise Physiology, Kharazmi University, Tehran, Iran.

***Correspondence:**
Alireza Shahsavar, PhD Student of Exercise Physiology, Kharazmi University, Tehran, Iran.
Tel: (98) 912 475 4530
Email: shahsavar6@gmail.com

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Abstract

Objective: L-carnitine is associated with an increase in antioxidant enzymes activities and reduction in oxidative damage. The aim of this study was to evaluate the effect of aerobic training and L-carnitine cconsumption on hippocampal oxidative stress and neurogenesis factors in diabetic rats.

Materials and Methods: In this experimental study, 45 male wistar rats were divided into six groups including sham (5 rats), healthy control (8 rats), diabetic control (8 rats), diabetic receiving L-carnitine (8 rats), diabetic receiving aerobic exercise (8 rats), diabetic receiving L-carnitine and aerobic exercise (8 rats). Rats with serum glucose level higher than 300 mg/dL were considered diabetic. L-carnitine supplemented dose was 100 mg per day. The aerobic exercise protocol including five sessions per week started at a speed of 10 for 20 min at a zero-degree grade in the first week and gradually reached a speed of 20 for 40 min at a grade of 5 in the sixth week. The Hippocampal tissues of the dependent variables (BDNF, MDA and GPX) were measured by ELISA 24 hours after the last session of the exercise program. One-way ANOVA and Tukey's post hoc test at *P*-value< 0.05 were used to analyze the data.

Results: The results of the study revealed that aerobic exercise and L-carnitine consumption have a significant effect on BDNF (*P*-value= 001), MDA (*P*-value= 001) and GPX (*P*-value= 001) of the diabetic rats.

Conclusion: According to the results, it is suggested that diabetic patients use aerobic exercise and supplementation with carnitine with caution and physician advice.

Keywords: Aerobic exercise, L-carnitine, Diabetes, BDNF, MDA, GPX.

Introduction

One of the most common complications of diabetes is diabetic neuropathy which causes autonomic nervous system disorders. Hippocampus, in the brain, seems to be sensitive to increased blood glucose levels in diabetes state (1). Anyway, the mechanisms of damage to nerve cells in the hippocampus caused by diabetes are not clear (1). The role of free radicals or ROS in the outbreak and progression of diseases

including neurological and cardiovascular disorders has been considerably appreciated (2). Free radicals cause damage to the most macromolecules. Metal ions such as iron have an important catalytic role in the function of ROS (3). Oxidative stress is also tightly related to the onset of diabetes and its complications. Moreover, it has been proved that oxidative stress increases in blood in diabetes (4).

So, the therapeutic goals of diabetes mainly include reducing insulin resistance and stimulating insulin secretion through nutritional reform, exercise and drug therapy (5). In this regard, Dabidi Roshan et al (2010) proved that inducing homocysteine into the dorsal hippocampus significantly increases MDA in this area and causes memory disorder in rats. Moreover, 4-week aerobic exercise led to a significant decrease in MDA in comparison to the control group (6). Islami (2015) confirmed that diabetic neuropathy lead to a significant decrease in BDNF expression in sciatic nerve motor roots in rats. Also endurance training can partly compensate for the reduction of BDNF expression caused by diabetic neuropathy (7).

It has been revealed that physical exercise reduces neuronal disorders and improves cognitive function. It is likely that various growth factors such as BDNF which induces neurogenesis, protects neural cells against neural analysis, and positively affects neural formation, serve as the mediator of exercise-induced nerve protection (8). Also BDNF can probably contribute to increasing brain resistance against impairment and aging-induced neuronal degeneration (9). In this regard, the evidences show that physical activity has beneficial effects on brain health and reduces the risk of developing diseases. Some evidences that have recently been found indicate that exercise promotes neuronal formation of the brain, which is associated with the increase in neurotrophic factors such as exercise-induced BDNF. However, its mechanisms have not been appreciated yet (10).

One of the methods of free radical production is the use of dietary supplements such as L-carnitine (11). L-carnitine facilitates the β-oxidation of long-chain fatty acids and participates in the metabolism of branched-chain amino acids (12). Studies have reported that L-carnitine protects antioxidant enzymes against oxidative damage (13). L-carnitine also can be effective in improving the function of nerve differentiated fat –derived mesenchymal stem cells and leads to an increase in the expression of BDNF and NGF genes (14).

Regarding the diabetes has negative effects on life. Finding a way to minimize the negative effects of diabetes is especially important. On the other hand, no research has been done on the effect of L-carnitine and endurance training on diabetic subjects. Therefore, the aim of this study was to determine the effect of aerobic exercise and L-carnitine consumption on research variables.

Materials and Methods

This is an experimental study in which the ethics of working with laboratory animals including the availability of water and food and the appropriate maintenance conditions were considered. The protocol that rats were killed was also observed. In the present study, 45 rats weighting 250-300 g based on the Cochrane formula were provided from Razi vaccine & serum research institute and moved to the research center. After one- week familiarity with the new environment, the rats were randomly divided into six groups including sham group (5 rats) ((To control the effect of injection, subjects in this group received only saline solution)., healthy control group (8 rats), diabetic control group (8 rats), diabetic group receiving L-carnitine (8 rats), diabetic group receiving aerobic exercise (8 rats), diabetic group receiving L-carnitine and aerobic exercise (8 rats). During the research period, the animals were kept in transparent 15 × 15 × 30 cm polycarbonate cages manufactured by Razi Rad company at 20-22°C and 55-65% moisture and 12:12 light

cycles; the animals were also fed with pellet-form food provided from livestock feed production centers. 32 rats attained a diabetic state with a single dose of nicotine amide and STZ injection. First, nicotine amide (95 mg / kg of body weight) was intraperitoneally injected into the rats. After 15 minutes, STZ (55 mg/ kg of body weight) prepared in sodium citrate buffer with pH=7.4 was intraperitoneally injected. Rats of control groups received the same amount of buffer. Five days after the injection, blood sample was drawn from the tail of the rats and then placed on glucometer strips. The rats with serum glucose level higher than 300 mg/dL were considered diabetic (15). The rats receiving L-carnitine received 100 mg of oral L-carnitine per day (16-17). The aerobic exercise group performed aerobic exercise on treadmill for six weeks, five days a week from 9 AM to 11 AM (18); the protocol started with the speed of 10 m/min for 10 min at a grade of zero percent in the first week and gradually reached the speed of 20 m/min for 40 min at the grade of 5 in the sixth week taking into account the principle of overload.

Based on previous research, the exercise intensity of the diabetic rats is at the same intensity of the threshold of lactate (18-19); it is also equivalent of about 75% of maximum oxygen consumption (20) which is a relatively high intensity for the diabetic rats (21). An acoustic stimulant (hitting the treadmill) was used to stimulate the rats to run. In order to familiarize the rats of the control group with the treadmill, they walked on it once a week for 5 min, with the speed of 10 m/min and at a grade of zero percent. After 6 weeks, all the rats were anesthetized with chlorophyram through the respiratory tract and a surgical biopsy was performed.

Finally, BDNF, MDA and GPX concentration in Hippocampal tissues was measured using Assay with a sensitivity of 0.1. Indicators of central tendency and Shapiro-Wilk Test were respectively used to describe the data and analyze the normality of data distribution. Regarding the inferential analysis of data, one-way ANOVA and Tukey's post hoc test were used via SPSS/21. In order to verify the research hypotheses, the significance level $\alpha \leq 0.05$ was used.

Ethical considerations

This study was approved by the ethics committee of the Physical Education Research Institute of the Ministry of Science, Research and Technology, and the code of (IR.SSRI.REC.1397.337) ethics was issued.

Results

The results of study indicated that aerobic exercise and L-carnitine consumption have a significant effect on BDNF (*P*-value= 0.001), MDA (*P*-value= 0.001) and GPX (*P*-value= 0.001) in rats**.** To find out the difference see the Tukey follow up test in Table 1.

Table 1. Tukey follow-up test results for BDNF, MDA and GPX

Compare Groups	BDNF *P*-value	MDA *P*-value	GPX *P*-value
Healthy control-Diabetic Control	0.001*	0.001*	0.001*
Healthy control-Sham	0.300	0.785	0.001*
Healthy control-aerobic training	0.001*	0.009*	0.709
Healthy control-L carnitine supplement	0.001*	0.001*	0.197
Healthy control-aerobic training and L carnitine supplement	0.262	0.991	0.001*
Diabetic Control-Sham	0.001*	0.001*	1
Diabetic Control- aerobic training	0.001*	0.001*	0.001*
Diabetic Control- L carnitine supplement	0.001*	0.001*	0.009*
Diabetic control-aerobic training and L carnitine supplement	0.001*	0.001*	0.001*
Sham-aerobic training	0.022*	0.001*	0.001*
Sham- L carnitine supplement	0.001*	0.001*	0.008*
Sham-aerobic training and L carnitine supplement	1	0.426	0.001*
Aerobic training- L carnitine supplement	0.545	0.001*	0.006*
Aerobic training-aerobic training and L carnitine supplement	0.028*	0.043*	0.048*
L carnitine supplement -aerobic training and L carnitine supplement	0.001*	0.001*	0.001*

*=significant in level of (*P*-value≤ 0/05)

Discussion

The results of the present study in relation to GPX are consistent with the results of research by Kanter et al (2017), Alipour et al (2012), Gaeini and Ghardashi (2017); they funded that interval aerobic exercises with 80% HRmax significantly increases GPX in type 2 diabetics (15,23-24). The studies have revealed that inhibition of oxidative processes in diabetics can reduce the incidence and development of delayed complications in these patients. Therefore, using antioxidant supplements can be an appropriate way to reduce the oxidative stress and its complications (24).

On the other hand, L-carnitine has antioxidant properties and its deficiency can lead to increased oxidative stress (13). Moreover, plasma concentration of L-carnitine has a positive correlation with its nutritional value. Hence, L-carnitine supplement is likely to help diabetics by reducing oxidative stress and improving inflammatory status (25).

The results showed a significant reduction in MDA levels. In this regard, similar studies have revealed that the production of oxygen-reactive compounds increases and antioxidant defense decreases in diabetics. Malondialdehyde, as an indicator of lipid peroxidation, is the final product of lipid decomposition by oxidative active species. Therefore, an increased level of Malondialdehyde indicates cell membrane damage and impairment in the defense mechanism of enzymatic and non-enzymatic antioxidants. It is well worth mentioning that increased levels of malondialdehyde were observerved in diabetics (26). Yarmohamadi and Mahjoub (2017) confirmed that six weeks of aerobic exercise significantly reduced malondialdehyde levels in postmenopausal diabetic women (27).

Moreover, the results of the studies conducted by Ebrahimpour et al (2013) are not consistent with the findings of the present study (28). It seems that the difference in the research findings of various studies can be attributed to the type of the participants, supplement dosage, different combinations of supplements, duration of consumption, and the type of exercise.

In the present study, MDA levels decreased in the groups consuming L-carnitine supplement in comparison to the groups which did not consume it.

High tissue oxygenation is one of the most important reasons of increased oxidative stress factors, and oxidative stress response to exercise and L-carnitine supplements are influenced by health status, age, gender, genetics, physical fitness, individual differences, different tissue responses, muscle fibers and its types, intensity and duration of exercise, and reduction in the intake of antioxidant food in the daily diet of individuals (29).

The result of this study showed that increased levels of BDNF after six weeks of aerobic exercise that was consistent with the findings of the studies carried out by Salimi Avansar et al (2017), Osali et al (2017), Fuhr et al (2018) (30-32). Various mechanisms lead to an increase in BDNF in the hippocampus, including Production of IGF1, increased levels of BDNF mRNA, other neurochemical materials such as corticosteroids, exercise intensity, age, neurotrophin 3, increased neurological activity or changes in activity patterns during exercises (33).

In the study carried out by Osali et al (2017), the BDNF levels significantly increased after 6 months of aerobic exercise in 50 to 65-year-old women with metabolic syndrome (31). On the other hand, the previous studies have revealed that the production and adjustment of the hippocampus BDNF via exercise is mediated by the neurotransmitter systems, the neuroendocrine system and insulin-like growth factor-1 (IGF for people with schizophrenia-1) (34). The result of the present study show that BDNF levels increases in diabetic participants does not expand upon the results of the studies carried out by Lee et al (2014) and Kim et al (2015) (11,18). The inconsistency in the results of the studies may be due to the difference in the types of the participants, the

hippocampus area where BDNF level is measured, and research methodology.

The results of the present study demonstrated that L-carnitine supplement significantly increased hippocampus BDNF in the diabetic rats. in this regard, Farahzadi et al (2015) revealed that L-carnitine can be effective in improving the function of nerve differentiated fat –derived mesenchymal stem cells and leads to an increase in the expression of BDNF and NGFgenes (14). As it has been mentioned, the main function of L-carnitine is to facilitate fat oxidation by transporting long chain fatty acids to mitochondria, whereby beta-oxidation is performed (35). Therefore, one of the beneficial mechanisms of L-carnitine is fat oxidation and subsequently increased levels of BDNF. As a protective agent and neurological treatment for reducing the inflammatory damage in CNS associated with BDNF, carnitine acts in a dose-dependent way. Lack of control of rat mortality during the study was the limitation of the present study.The protocol implemented in this study was approved by the Ethics Committee of Physical Education Research Institute from the Institute of Physical Education in 2018. (Trace Code: 51707).

Conclusions

In summary, the results of the present study showed that six weeks of aerobic training with concomitant administration of L-carnitine had an effect on MDA, GPX and Hippocampal Neurogenesis in Diabetic Rats. It was also found that aerobic exercise and consumption of L-carnitine had more influence on research variables than exercise and L-carnitine consumption. Therefore, aerobic exercise can be safely recommended to reduce the adverse effects of diabetes, but supplementation should be consulted with a physician.

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Conflict of Interest

There are no conflicts of interest.

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