ORGINAL ARTICLE

The Effect of Aerobic Training and L- Carnitine on BCL2 and Mitochondrial Enzymes of Rat's Kidney

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Abstract

Objective: Diabetes causes free radical production, oxidative stress and alterations in mitochondrial enzymes and apoptosis. The purpose of this study was to investigate the effect of aerobic exercise and consumption of L-carnitine on BCL2 and some mitochondrial enzymes of the kidney in diabetic rats.

Materials and Methods: In this experimental study, 45 male wistar rats (200-300 gr) were simple randomly divided into six groups: 1) placebo group, 2) healthy control group, 3) diabetic control group, 4) diabetic group receiving L- carnitine, 5) diabetic group of aerobic training and 6) diabetic group of aerobic training and recipient of L-carnitine. Rats with a serum glucose level higher than 300 mg/ dL were considered diabetic. L-carnitine recipients received 100 mg of L-carnitine daily orally. The dependent variables of the study were measured 24 hours after the last training program session by ELISA in kidney tissue. Data were analyzed by Shapiro-Wilk and two way ANOVA and Tukey post hoc test at *P*-value< 0.05.

Results: The results showed that the combined effect of aerobic training and supplementation of L-carnitine on Bcl2 factor of kidney tissue of rats with diabetes has a significant effect (*P*-value: 0.019). But aerobic exercise (*P*-value: 0.969) and supplementation of L-carnitine (*P*-value: 0.584) were not significant.

Conclusion: The results of this study showed that the combined effect of aerobic exercise and supplementation of L-carnitine on CPT2 and Malonyl COa and Bcl2 have a significant effect on the kidney of diabetic rats. Also, exercise alone and consumption of L-carnitine alone do not have a significant effect.

Keywords: Aerobic training, L-carnitine, Diabetes, CPT2, Malonyl CO A, Bcl2, Kidney.

Introduction

Diabetes is a serious medical problem. Its prevalence in worldwide is rising dramatically. Increased blood glucose is the main attribute of this disease and in the long term causes damage to various tissues of the body, although its mechanism varies in different tissues (1). Diabetes can cause tissue damage, cell death, or apoptosis. Apoptosis is

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a planetary death and fully protected cell that plays an important role in the growth and development of organs, homoeostasis and destruction of worn cells (2). Past studies indicate that defect in this pathway causes the accumulation of mutated cells and ultimately death of the patient (3). One of the organs that may be damaged by diabetes and lead to death is the kidney. Recently, it has been shown that an increase in the number of patients with renal failure and chronic kidney disease is associated with an increase in diseases with systemic effects associated with CKD and specifically type 2 diabetes (4). However, the association between the death of apoptosis and the kidney tissue with hyperglycemia is still not well known. Oxidative stress is produced by an imbalance between the production of reactive oxygen species (ROS) and antioxidant agents that eliminate ROS (5).

The exercise improves insulin resistance; it increases insulin sensitivity, decreases oxidative stress by improving antioxidant system and protects beta cells (6). In addition, exhausting exercise is associated with oxidative stress and the production of free oxygen radicals (7). Oxidative stress is one of several mechanisms that induce apoptosis (8). Some studies have reported that severe exercise induces apoptosis in the intestinal lymphocyte of rat mice, but the optional running on the treadmill decreases apoptosis, while the mandatory exercise training increases the levels of antioxidants to give (9). On the other hand, mitochondria, including the effective organs in apoptosis, are an integral part of the internal pathway of apoptosis and the location of many of the proteins involved in the early stages of this process, including the members of the Bcl-2 family. It is also involved in the external pathway of apoptosis (4). The mitochondria provide the common energy of the cell and plays a vital role in the metabolism of all types of foods (10). Antioxidants, by various mechanisms, reduce the severity of oxidative stress reactions and their molecular effects on macromolecules such as lipids, proteins, and DNA, and reduce cellular effects and, ultimately, their clinical problems (11). L-carnitine is a non-proteinaceous amino acid produced from lysine and methionine amino acids that have antioxidant properties (12). L-carnitine facilitates the β-oxidation of long-chain fatty acids, participates in the metabolism of branched-chain amino acids, and proves cellular membranes (13). Research has reported that l-carnitine protects antioxidant enzymes against oxidative damage (14). Various studies have also been conducted on the effects of L-carnitine on antioxidant enzymes and apoptotic factors, and contradictory results have been obtained (15). The purpose of this study was to investigate the effect of aerobic exercise and consumption of L-carnitine on BCL2 and some mitochondrial enzymes of the kidney in diabetic rats.

Materials and Methods

In the empirical research, the ethics of working with laboratory animals were considered and how mice were slaughtered. In the present study, 45 rats weighing 250 to 300 grams from the breeding laboratory of Razi serum were collected and transferred to the research center. After entering the research environment and one week's acquaintance with a new environment the animals were simple randomly divided into six groups : 1) placebo group (5 rats), 2) healthy control group (8 rats) 3) diabetic control group (8 rats), 4) diabetic group receiving L- carnitine (8 rats), 5) diabetic group of aerobic training (8 rats), 6) diabetic group of aerobic training and recipient of L-carnitine (8 rats). During the research period, animals were kept in polycarbonate transparent cages of 15× 15× 30 cm in size at a temperature of 20 to 22° c, a cycle of light to darkness of 12:12 and humidity of 55 to 65 percent. They were fed in the form of pellet from livestock feed production centers. Rats were diabetic by STZ injected. Rats of control groups received the same buffer. About 48 hours after STZ injection, rats with a serum glucose level above 300 mg/ dL were

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considered diabetic. Rats receiving L-carnitine daily cached 100 mg of L-carnitine (16,17).
Aerobic training groups performed an aerobic training program on a treadmill, 5 days a week, from 9 am to 11am for six weeks (18) as follows: In resources, this intensity for diabetic rats is equivalent to the intensity of the lactate threshold (18) and equivalent to about 75% of the maximum oxygen consumed (19) which is relatively high for diabetic rats (20). In order to stimulate the mice to run an acoustic stimuli (hit to the treadmill wall) was used, during this 6 weeks, control rats walked on treadmill one session per week, for 5 minutes, at a speed of 10 m / min and a zero gradient to know more about it. After 6 weeks, all rats were anesthetized with subcutaneous injection of 10% alcohol hydrate at 5 ml per kg body weight and then biopsy was done. Finally, for the concentration of BCL-2, CPT2, and Malonyl CoA, the German ASSAY kit was used. To describe the data, central tendency indicators were used and the data distribution was normalized by Shapiro Wilk test. Inferential analysis of data was performed by two way ANOVA and Tukey post hoc test using SPSS 21 software. Also, the significance level of a ≤0/05 was considered for testing the research hypotheses. Finally, Excel software was used to draw charts.

Ethical considerations

This study was approved by committee of Ethics in Research of Institute of Physical Education and Sport Science, Ministry of Science, Research and Technology, Tehran, Iran, with number of .IR.SSRI.REC.1397.334

Results

The results showed that the combined effect of aerobic training and supplementation of L-carnitine on Bcl2 factor of kidney tissue of rats with diabetes has a significant effect (*P*-value: 0.019). But aerobic exercise (*P*-value: 0.969) and supplementation of L-carnitine (*P*-value: 0.584) were not significant.
It was also found that the combined intervention of aerobic exercise and supplementation of L-carnitine had a significant effect on CPT2 in kidney tissues of diabetic rats (*P*-value: 0.021). But supplementation consumption (*P*-value: 0.31) alone and aerobic exercise (*P*-value: 0.226) alone do not affect.
Finally, it was found that six weeks of aerobic training (*P*-value: 0.08), supplementation of L-carnitine (*P*-value: 0.547), and combined aerobic exercise and supplementation of L-carnitine (*P*-value: 0.961) did not have a significant effect on the malonyl CoA factor of kidney of rats with diabetes mellitus.

Discussion

The study findings showed that aerobic exercise and supplementation of L-carnitine separately did not have a significant effect on Bcl2 in kidney tissue of diabetic rats, but the combination of aerobic training and supplementation of L-carnitine increased Bcl2 in kidney tissues of diabetic rats. There was no significant difference in liver expression level of Bcl-2. Diabetes is clinically one of the most important causes of abnormalities such as nephropathy, retinopathy, neuropathy, and cardiovascular disease (21). Bcl-2 family proteins are the most important type of protein in the regulation of apoptosis. Although the precise mechanism of apoptosis is still unclear, it may differ depending on the cell type and type of stimuli (22).
The exercise induces apoptosis, which is a natural process for destroying damaged cells in which significant inflammatory reactions do not occur. This process ensures the normal functioning of the body. Protective mechanisms against apoptosis due to prevention may be affected by NF-kB, which prevents sensitivity to apoptosis and can reinforce the incremental regulation of the anti-apoptotic cells of Bcl-2 (23). Caspase 9 can also positively regulate apoptosis by activating Caspase 3 (24). Although the present study did not determine the levels of caspases 9 and 3, which could be considered as limiting the present study, but in other studies, exercise activity was observed with

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decreasing activity of initiator caspase 9 and executive caspase 3 from both internal and external pathways prevent apoptosis and fragmentation of DNA (19). Considering the use of the six-week protocol in the present study, it is likely that adaptations resulting from the training will activate the anti-apoptotic pathways. Contradiction in results may be related to factors such as the low duration of exercise in each session or training period, abnormal levels of apoptotic regulator factors in the subjects, the type of subject, and the type of exercise protocol. In most of the above studies, the resistance protocol and also the heart tissue have been used.

The results showed that supplementation of L-carnitine alone did not have a significant effect on Bcl2 in kidney tissue of diabetic rats. L-carnitine has been reported to reduce obesity caused by a high-fat diet and additional carnitine inhibits the increase of total triglycerides and lipids (25). The main function of L-carnitine is to facilitate fat oxidation by transporting long-chain fatty acids to mitochondria, whereby beta-oxidation is performed. Hence, most dietary lipids using carnitine in the body can be used as a source of energy (25). However, the combination of aerobic exercise and supplementation of L-carnitine increased Bcl2 in kidney tissues of diabetic rats. It has been shown that the factor of tumor necrosis alpha (TNF-α) increases apoptosis by expressing nitric oxide synthase (iNOS) and nitric oxide (26). Exercise with L-carnitine supplementation can help reduce the apoptosis induced tumor necrosis factor signaling in this study. However, in the present study, the levels of the alpha necrosis factor were not measured, which includes the limitations of the present study. However, increasing the anti-apoptotic agent Bcl-2 in the kidney tissue with exercise with the L-carnitine supplementation may be due to the interactions of antioxidant and lipid-lowering agents of L-carnitine and exercise. The results of this study showed that six weeks of combined aerobic training and supplementation of L-carnitine significantly increased the CPT2 factor of kidney tissue of diabetic rats. But the use of supplement alone and aerobic exercise alone did not have a significant effect.

The CPT I enzyme is located in the outer membrane and CPT II in the mitochondrial membrane (5). investigated the expression of estrogen-related receptor alpha (ERRα) as an important factor in regulating cellular energy hemostasis in skeletal muscle of male rats following four weeks of endurance training and determining its role with fat metabolism indices. Their results indicated that expression of carnitine palmitoyl transferase 1 beta gene in the endurance training group was significantly higher than the control group. The precise mechanisms of the effect of exercise on the regulation of CPT II in kidney tissues are not well defined. However, due to the high sensitivity of CPT II to malonyl Coa, it can be stated that reduced malonyl Coa levels during fasting are enough to inhibit CPT II activity in patients with CPT II deficiency and can cause symptoms in fasting and prolonged exercise (27). The plasma concentration of fatty acids is directly related to the entry of fatty acids into the liver (28). Therefore, the liver is stacked with fatty acids and lipids. The increase in deep receptors of insulin and carrier protein GLUT4 is essential for glucose uptake by the muscle (29). Enough intensity and duration of aerobic exercise activity has beneficial effects on the improvement of insulin sensitivity and changes in deep surfaces and skeletal muscle receptors, and also increases the content of GLUT4 mRNA and skeletal muscle protein, so, better glucose utilization, decreased resistance to insulin and consequently reduce the entry of lipids to the liver (29). Brad et al. showed that daily intake of 3 g of L-carnitine supplementation for 4 weeks did not effect on taking the substrate, endurance function and carbohydrate and fat oxidation during the performance of sub-maximal activities. It seems that these differences are depended to the amount and duration of supplements usage and the type of exercise. The results showed

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that six weeks of aerobic training, supplementation of L-carnitine, and combined intervention of aerobic exercise and L-carnitine complementary did not have a significant effect on malonyl Coa of fatty acids by inhibiting the fatty acid synthase in satiety. During fasting, reducing the concentration of malonyl Coa may facilitate the use of mitochondrial fatty acids. Exercise activity can stimulate lipid oxidation and inhibit lipid synthesis, which is done through the activation of the AMPK pathway (30). This enzyme is stimulated and activated by increasing the ratio of AMP to ATP in the tissues, which is a result of the physiological stimulant of exercise activity (28). During exercise, AMPK is activated and its activity remains in the muscle, liver and adipose tissue after exercise activity (28). Activating AMPK by deactivating the Acetyl-CoA carboxylase enzyme, activating the Malonyl CoA decarboxylase enzyme and inhibiting the expression of the gene of Lipogenic enzymes, Acetyl-CoA carboxylase and synthetic fatty acid, inhibits the synthesis of leptides. Basically stimulates the oxidation of lipids in the liver, through the reduction in the amount of malonyl Coa, which itself is an allosteric inhibitor of the carnitine-palmitoyl transferase enzyme, which controls the transmission of high chain cytosolic fatty acids to mitochondria (31). A role of carnitine is buffering additional short chain Acyl groups by means of Acyl Carnitine, a short and long chain of Acyl Carnitine (mainly Acetyl carnitine) formed through the mitochondrial enzyme activity called Acyl coa transferase (32).This reaction results in the release of coenzyme A, an important substrate for the various stages of energy metabolism in mitochondria (33). Most research on L-carnitine and exercise mainly focuses on the role of L-carnitine in the transfer of fats from cytosol to the mitochondrial membrane, while other roles such as membrane composition protection, the sustainability of the physiological ratio of coa to acetylcoa, reduced lactate production and detoxification also referred to it (32). Altogether, although there are several mechanisms for promoting kidney support for exercise activity in diabetes, overall, it has been observed that exercise can improve kidney function by improving metabolic factors such as plasma lipid levels, blood glucose and blood pressure (34). Sports activity may be protected by the reduction of oxidative stress and associated inflammation, as well as antioxidant defense of the kidney tissue against diabetes mellitus (35).

Conclusions

The results of this study showed that supplementation with L-carnitine combined with regular aerobic exercise improves the oxidative stress and mitochondrial enzymes of kidney tissue in diabetic subjects. One of the limitations of the present study was the lack of control over the mortality of a number of rats throughout the study .It is recommended to determine the levels of caspases 3 and 9 in future research ,but given the recent research ,there are still many question in future research.

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