Effect of Aerobic Training and L-Carnitine Supplementation on Hepatic Oxidative Stress Factors in Diabetic Rat

Seyed Esmaeil Jabbari[1], Mandana Gholami*[2], Hojatollah Nikbakht[2], Nader Shakeri[2], Farshad Ghazalian[2]

1. Ph.D Student, Department of Physical Education and Sport Sciences, Science and Research Branch, Islamic Azad University, Tehran, Iran.
2.Ph.D Department of Physical Education and Sport Sciences, Science and Research Branch, Islamic Azad University, Tehran, Iran.

***Correspondence:**
Mandana Gholami, Department of Physical Education and Sport Sciences, Science and Research Branch, Islamic Azad University, Tehran, Iran.
Tel: (98) 912 149 1868
Email: m.gholami@srbiau.ac.ir

Received: 19 January 2020

Accepted: 12 May 2020

Published in June 2020

Abstract

Objective: The use of non-enzymatic antioxidants in food supplements and proper exercise can have a positive effect on decreasing oxidative stress by free radical hunting. The purpose of this study was to investigate the effect of aerobic training and L-carnitine supplementation on some of the oxidative stress factors in the liver of diabetic rats.

Materials and Methods: In this experimental study, 45 male wistar rats (200-300 gr) were randomly divided into six groups: 1) sham group, 2) healthy control group 3) diabetic control group, 4) diabetic group receiving L-carnitine, 5) diabetic group of aerobic training, 6) diabetic group of aerobic training and receiving L-carnitine. The aerobic exercise protocol included six weeks, five sessions per week on the treadmill. After intervention, malondialdehyde (MDA), superoxide dismutase (SOD), and glutathione peroxidase (GPX) levels were determined in liver tissue.

Results: Six weeks of aerobic exercise had a significant effect on MDA factor in hepatic tissue in diabetic rats (*P*-value: 0.024). However, supplementation (*P*-value: 0.868) and combined intervention of aerobic exercise and supplementation of L-carnitine (*P*-value: 0.465) did not have the significant effect on MDA factor. Also, 6 weeks of aerobic training, supplementation of L-carnitine, and combined intervention had no significant effect on SOD and GPX factors of hepatic tissue in diabetic rats (*P*-value> 0.05).

Conclusion: L-carnitine supplementation with regular exercise can have beneficial effects on hepatic antioxidant defense system in rats with type 2 diabetes.

Keywords: Aerobic training, L-carnitine, Antioxidant enzymes, Oxidative stress

Introduction

Diabetes is the most common metabolic disorder characterized by hyperglycemia due to absolute or partial insulin deficiency (1). The liver is one of the major organs in the body that plays an important role in metabolism, preservation and

[DOI: 10.18502/ijdo.v12i1.3635]

maintenance of blood glucose levels in the normal range, so that increasing blood glucose leads to an imbalance in oxidation-reduction reactions within hepatocytes. In this way, hyperglycemia, by increasing the production of advanced glycolysis end products, facilitates the production of free radicals by interfering with the production of intrinsic scavengers of free radicals such as superoxide dismutase and catalase, leading to cell damage (2).

In general, it seems that two processes play a role in the development of liver lesions. A state is a metabolic disorder that occurs in all patients and is probably related to advance glycosylated end products that are responsible for thickening and increasing the fibrosis, and the other is increasing oxidative stress (1). Oxidative stress, which is an imbalance between the production of free oxygen radicals and the body's antioxidant defense capacity, is strongly associated with diabetes and its complications. Hepatic cells in patients with diabetes may develop degenerative changes due to excessive glycogen accumulation. Sometimes severe hyperglycemia also increases the osmolality of hepatic cells, and cells develop degenerative changes. The aforementioned set indicates the importance of diabetes in developing liver lesions (1,3).

Regarding the production of free radicals by diabetes and exercise, the ways to reduce the negative consequences of diabetes and the production of free radicals is questionable. Using anti-oxidant agents can be useful in this regard. The antioxidants with different mechanisms decrease the intensity of oxidative stress reactions and their molecular effects on macromolecules such as lipids, proteins and DNA. (4)

L-carnitine has antioxidant properties. L-carnitine is a non-protein amino acid produced from lysine and methionine amino acids (5). L-carnitine facilitates the β-oxidation of long chain fatty acids, affects the metabolism of branched-chain amino acids, and fixed cell membranes (6). Many studies have reported that L-carnitine protects antioxidant enzymes against oxidative damage. Various studies have also been conducted on the effects of L-carnitine on antioxidant enzymes and contradictory results have been obtained (7,8). The purpose of this study was to investigate the effect of aerobic training and L-carnitine supplementation on some of the oxidative stress factors in the liver of diabetic rats.

Materials and Methods

In the present study, 45 rats weighing 200 to 300 grams were raised at the Razi Vaccine and Serum Research Institute. The male rats were randomly divided into 6 groups. The untrained animals set on the treadmill for one week's to maintain familiarity with the new environment without training them. These six groups include: sham group (5 rats), healthy control group (8 rats), diabetic control group (8 rats), diabetic group plus L-carnitine (8 rats), diabetic group of aerobic training (8 rats) and diabetic group of aerobic training plus L-carnitine (8 rats).

During the research period, animals were kept in transparent polycarbonate cages with dimensions of 15 × 15 × 30 cm manufactured by Razi Rad Company at ambient temperature from 20 to 22º C with a light cycle of 12.12 hours of darkness and air humidity of 55 to 65 Percentages. They were also maintained with proper ventilation and were fed to pellets using feeds from livestock feed production centers. Diabetes was induced to 32 rats with peritoneal injection of 95 mg/kg nicotinic amide and a single dose of STZ (55 mg / kg). The rats in control group received the same buffer value. Rats with a serum glucose level between 300-360 mg / dL were considered diabetic. L- Carnitine dose was 100 mg orally (9,10).

The aerobic training groups also performed an exercise program, including aerobic training on a treadmill, five days a week, from 9:00 to 11:00, for six weeks (11), as follows: The aerobic exercise protocol included five sessions per week, 20 minutes, speed 10 meter/minute and a zero-degree gradient in the first week, gradually reaching 40 minutes at 6

[DOI: 10.18502/ijdo.v12i1.3635]

weeks, speed 20 meter/minute and 5 degrees' gradient. It should be noted that in the literature, this training intensity for diabetic rats is equivalent to the intensity of the lactate threshold (11, 12), and equivalent to approximately 75% of the maximum oxygen consumed (13), which is considered to be relatively high for diabetic rats (14). In order to stimulate the rats to run, the audio stimuli (hit the treadmill wall) was used. During 6 weeks, control group rats walked on a treadmill for one session per week for 5 minutes, speed 10 meters per minute and with a zero gradient to know treadmill. After 6 weeks, all rats were anesthetized with chloroform solution through breathing and texture was done. Finally, for the measurement of malondialdehyde (MDA), the Satho method evaluated the GPX activity of glutathione peroxidase using the Biorex kit and the SOD superoxide dismutase activity by Winterbourne method. To describe the data, central tendency indicators were used. Shapiro Wilk test was also used to check the normality of the data distribution. For analyzing the data, two-way ANOVA and Tukey's post hoc test were used with SPSS - 21 and the significance level was *P*-value≤ 0.05. Finally, Excel software was used to draw charts.

Ethical considerations

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

Results

The results showed that six weeks of aerobic exercise had a significant effect on MDA factor of hepatic tissue in diabetic rats(*P*-value:0.024). However, L-carnitine supplementation (*P*-value: 0.868) and combined intervention of aerobic exercise and L-carnitine supplementation (*P*-value: 0.465) did not had a significant effect on MDA factor (figure 1). Regarding to SOD, six-week of aerobic training (*P*-value: 0.626), L-carnitine supplementation (*P*-value: 0.671), and combined aerobic training and L-carnitine supplementation (*P*-value: 0.173) had no significant effect on SOD factor (figure 2). The results also showed that six weeks of aerobic training (*P*-value: 0.102), L-carnitine supplementation (*P*-value: 0.172) and combination of aerobic training and L-carnitine supplementation (*P*-value: 0.140) rats did not had a significant effect on the GPX (figure 3). Table 1 shows the effects of six weeks of aerobic exercise on MDA factor in hepatic tissue in diabetic rats.

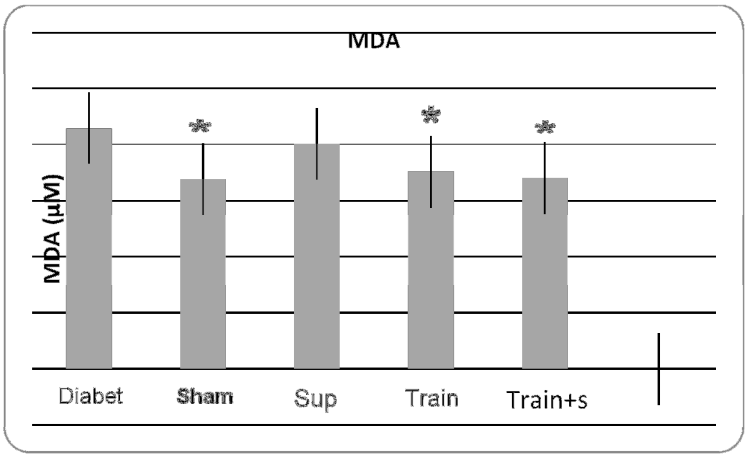

Figure 1. Difference between MDA Levels of hepatic tissue after interventions

[DOI: 10.18502/ijdo.v12i1.3635]

Discussion

In the present study, SOD did not change significantly in experimental groups. Regarding the indicators of oxidative stress and physical activity, studies have been conducted that their results vary according to the type, intensity and duration of training, also in different sexes and on humans and animals (15).

It has also been shown that L-carnitine improves liver dysfunctions and lipid disorders caused by a high-fat diet in through regulating fat metabolism and antioxidant capacity (16).

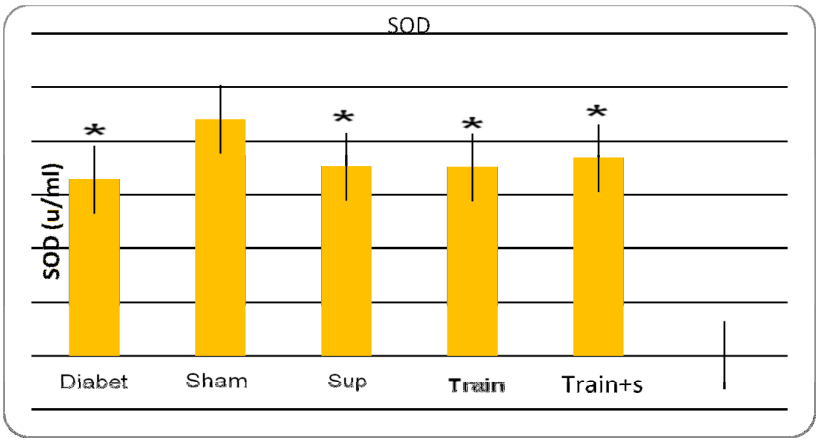

Figure2. Difference between SOD Levels of hepatic tissue after interventions

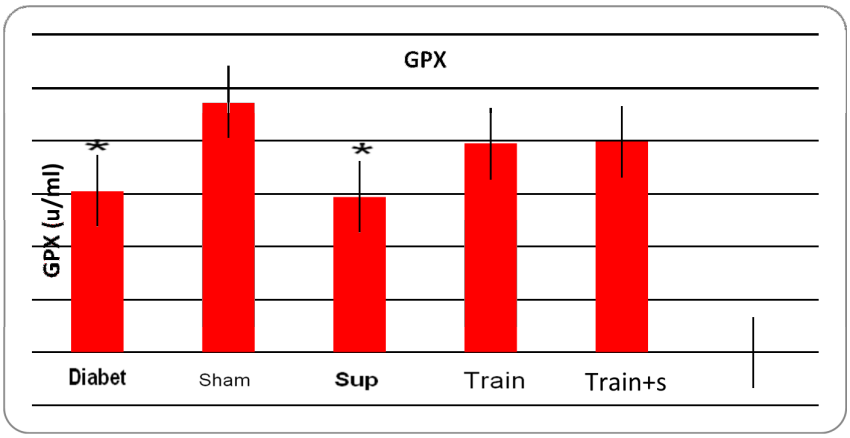

Figure 3. Difference between GPX Levels of hepatic tissue after interventions

Table 1. The effects of six weeks of aerobic exercise on MDA factor in hepatic tissue

Groups	Mean difference	*P*-value*
Sham group - diabetic control group	4.533	0.012 *
Aerobic training – Diabetic control	3.848	0.044 *
L-carnitine - Diabetic control	1.382	0.82
L-carnitine and aerobic training - Diabetic control	4.586	0.011
Aerobic training - sham group	0.684	0.985
L-carnitine - sham group	3.151	0.139
L-carnitine and aerobic training – sham group	0.052	1
L-carnitine - Aerobic training	2.466	0.347
L-carnitine and Aerobic training- Aerobic training	0.737	0.980
L-carnitine and Aerobic training - L-carnitine	3.204	0.129

*-ANOVA and Tukey's post hoc test

[DOI: 10.18502/ijdo.v12i1.3635]

The mechanism of the effect of L-carnitine on the oxidative indices is not specified accurately. The carbonyl L-carnitine group can stabilize free radicals from the carbon-alpha side by pair and protect the components of the plasma against the toxic effects of the active oxygen species and nitrogen. Cao et al in 2013 (17) showed that intravenous injection of a dose of 1000 milligrams of L-carnitine in 12 healthy individuals increased the activity of the enzyme superoxide dismutase. In Samadi et al study in 2014 (18), the mean serum glutathione level in healthy subjects was significant in the L-carnitine group. According to the type of patient and the dose of L-carnitine, the contradiction with the above research can be justified. It has been argued that L-carnitine can potentiate its antioxidant effects by increasing glutathione levels by inducing transcription of genes involved in the glutathin biosynthesis, increasing bioavailability, producing and maintaining it. Shokrzadeh et al. (7) showed that L-carnitine increases the glutathione level of the liver in rats, according to the dose. Therefore, in the current study, brighter results can be obtained in hepatic levels of SOD in diabetic subjects with changing the dose of L-carnitine. Several mechanisms have been proposed to justify the response of antioxidant enzymes to exercise. In some studies, increases in glutathione peroxidase levels have been observed after exercises (19), which may indicate an increase in glutathione stores as glutathione oxidase coenzyme. During the action of glutathione peroxidase, Glutathione resuscitation is converted to glutathione oxide, which itself is also converted into Glutathione resuscitation by glutathione reductase enzyme using NADPH (19).

Physical activity plays a key role in regulating the balance between the formation of reactive species and the antioxidant mechanisms, thereby reducing oxidative stress, reducing the risk of chronic diseases. The first mechanism that affects the oxidative stress indices after exercise is the training status (type, intensity, and duration of training). Interestingly, acute exercise not only causes a temporary increase in oxygen production of reactive oxygen species and nitrogen species, but also provides stimuli for the defense of endogenous antioxidants (20). This issue has been confirmed in Animals without activity whose levels of oxidants have increased in response to acute exercise. Long-term exercise with this effect confronts the increase of antioxidant enzymes and, consequently, the reduction of the production of free radicals. Studies on mice also show that endurance exercises increase levels of antioxidants and antioxidant enzymes in the skeletal and cardiac muscles. Long-term exercise with this change is countered by the increase in antioxidant enzymes, resulting in reduced free radical production. Studies on mice also show that endurance exercises increase levels of antioxidants and antioxidant enzymes in skeletal and cardiovascular muscles, thereby protecting against oxidative stress (19).

In many studies on the effects of training on oxidative stress, acute and endurance exercises reduce the risk of exercise-induced oxidative stress (21). The findings of the present study are not compatible with the results of Shreelaxmi et al., in 2011 (22) and Thirumalai et al. in 2011 (15) which showed that acute and endurance sports activities reduce the oxidative stress of exercise. L-carnitine has antioxidant properties and its deficiency can lead to increased oxidative stress (23). Also, the plasma concentration of L-carnitine has a positive correlation with its nutritional intake. Therefore, supplementation with L-carnitine may be helpful in reducing oxidative stress and improving the inflammatory status of people with diabetes (24).

The baseline level of hepatic L-carnitine in the present study may have a direct impact on its level after intervention. It seems that there is a contradiction between the findings of various studies on the effects of L-carnitine supplementation during sport activities due to differences in exercise methods and exercise tests, the amount or length of use of L-carnitine, Intensity of work or volume and

[DOI: 10.18502/ijdo.v12i1.3635]

time of activity that requires more studies with all aspects and simultaneous measurement of the antioxidant variables of the liver. Previous studies have shown that increasing the activity of antioxidant enzymes and decreasing the level of lipid peroxidation that occurs after exercise, has important implications for the prevention of complications of diabetic apoptosis and oxidative stress induced tissue damage (25). Regular exercise activity has been shown to increase the activity of antioxidant enzymes, increase the resistance to oxidative stress and, consequently, reduce oxidative damage (26). In addition, regular exercise has been effective in preventing and delaying diabetes, increasing insulin sensitivity, and improving glucose metabolism (27).

The mechanism that reduces the lipid peroxidation by reducing the concentration of MDA in the exercise group is glycemic control and reducing the parameters of the fat profile in the exercises, which have important effects on the reduction of oxidative stress parameters and provide more support for evidence of the potential protective effect of exercise against stress Oxidative in diabetic patients. Significantly, the results of the studies show the effects of exercise on glycemic control and oxidative stress, which can also be useful in treating MDA concentration in diabetic and non-diabetic patients, which decreases after six hours of exercise per week (22). One of the other important mechanisms for the ability to protect cells from exercise can be the ability to block the formation of free radicals. The reactive oxygen species in the mitochondrial electron transfer chain are produced as a natural product, but when their levels exceed the antioxidant capacity of the cell, they can lead to cell death. Oxidative stress caused by active oxygen species is highly associated with diabetes and its complications, and can cause cell death through various pathways (28).

Increasing the transmission of fatty acids to mitochondria and reducing fatty acids appears to be responsible for the main reason for reducing peroxidation of the hepatic tissue of diabetic rats after exercise. Further studies are needed to explore this mechanism. One of the reasons for the increase of malondialdehyde index may be due to increased production of free radicals in the electron transfer chain, which is proportional to the increase in oxygen consumption. Also, the reduction of malondialdehyde in the liver tissue may be due to the antioxidant effect or the effects of reducing sugar and reducing exercise lipid. Some studies have reported that there is a direct relationship between increased serum malondialdehyde and increased malondialdehyde in red blood cells. That is, severe physical activity leads to oxidative damage in blood cells such as red blood cells and lymphocytes (29). However, some other studies have reported that serum malondialdehyde does not change or decrease with antioxidant supplements after moderate or severe aerobic activity (29), which is in line with the results of this research. The results of this study are not consistent with Mahfouz, et al in 2009 (30) which indicate that the change in malondialdehyde does not change with use of L-carnitine supplementation in diabetic rats. Hajian et al. in 2017 (31) showed that the malondialdehyde of the liver tissue was decreased in the group treated with L-carnitine (300 mg / kg) compared to the diabetic control group and they showed that in the diabetic group treated with L-carnitine (300 mg / kg) in the form of gavage for 30 days compared to the non-treated diabetic group, the level of malondialdehyde in the liver tissue of diabetic rats was significantly reduced. Considering that high oxygen saturation is one of the most important reasons for increasing oxidative stress factors, and the response of oxidative stress to exercise and supplements of L-carnitine is influenced by factors such as health status, age, gender, race, genetics, physical fitness, individual differences, different tissue responses, muscle fibers and its types, severity and duration of exercise, and reduction in the intake of antioxidant food in the daily diet (32). Finally, the variety of lipid peroxidation indices, different measurement

[DOI: 10.18502/ijdo.v12i1.3635]

methods and sensitivity in different researches can lead to different results.
The limitations of the present study are lack of measurement of L-carnitine at the baseline and after intervention, and differences in nutrition, rest and daily activity of rats during the study.

Conclusions

L-carnitine supplementation intake with regular exercise can have beneficial effects on body's antioxidant defense system and decrease oxidative stress in rats with type 2 diabetes. So, in diabetic patients L-carnitine supplementation with regular exercise are recommended.

Acknowledgements

The authors want to thank all people who are sincerely cooperating in doing this research.

Funding

There were no financial support.

Conflict of Interest

The authors declare that there are no conflicts of interest.

References

1. Doustar YMD, Rezaei A, Hashemi M. The effect of endurance swimming exercise on the occurrence of apoptosis in experimental diabetic myopathic rats. Vet J of Islamic Azad Uni Tabriz Branch. 2009;3(4):629-36. (in Persian)
2. Mohajeri D, Mousavi Gh, Kaffashi Elahi R, Neshat Gharamaleki M. Study on protective effect of Naringenin (Citrus flavonone) on incipient diabetic hepatopathy in alloxan-induced diabetic rats. Veterinary Clinic Pathology. 2016;10 (1):39-52.
3. Cheng M, Gao HQ, Xu L, Li BY, Zhang H, Li XH. Cardioprotective effects of grape seed proanthocyanidins extracts in streptozocin induced diabetic rats. Journal of cardiovascular pharmacology. 2007;50(5):503-9.
4. Shirpoor A, Ilkhanizadeh B, Saadatian R, Darvari BS, Behtaj F, Karimipour M, et al. Effect of vitamin E on diabetes-induced changes in small intestine and plasma antioxidant capacity in rat. Journal of physiology and biochemistry. 2006;62(3):171-7.
5. Leeuwenburgh CH, Hollander J, Leichtweis S, Griffiths M, Gore M, Ji LL. Adaptations of glutathione antioxidant system to endurance training are tissue and muscle fiber specific. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology. 1997;272(1):363-9.
6. Flanagan JL, Simmons PA, Vehige J, Willcox MD, Garrett Q. Role of carnitine in disease. Nutrition & metabolism. 2010;7(1):1-4.
7. Shokrzadeh M, Ahangar N, Zargari M, Gilani Z, Shadboorestan A, Omidi M. Protective effect of L-carnitine on level of malondialdehyde in diazinon-induced lipid peroxidation in rats. Journal of Mazandaran University of Medical Sciences. 2013;22(97):198-206.(in Persian)
8. Tousson E, Hafez E, Zaki S, Gad A. The cardioprotective effects of L-carnitine on rat cardiac injury, apoptosis, and oxidative stress caused by amethopterin. Environmental Science and Pollution Research. 2016;23(20):20600-8.
9. Mansour HH. Protective role of carnitine ester against radiation-induced oxidative stress in rats. Pharmacological research. 2006;54(3):165-71.
10. Bodea F, Bocea A, Decea N. L-carnitine decreases oxidative stress induced by experimental hypobaric hypoxia. Pediatric Endocrinology, Diabetes and Metabolism. 2010;16(2):78-81.
11. Kim HJ, Park JY, Oh SL, Kim YA, So B, Seong JK, et al. Effect of treadmill exercise on interleukin-15 expression and glucose tolerance in zucker diabetic fatty rats. Diabetes & metabolism journal. 2013;37(5):358-64.
12. E Nico ET, de Oliveira PR, de Souza LP, Pereira FD, Delbin MA, Zanesco A, et al. The action of aminoguanidine on the liver of trained diabetic rats. Journal of Diabetes & Metabolic Disorders. 2013;12(1):40.
13. Rodrigues B, Figueroa DM, Mostarda CT, Heeren MV, Irigoyen MC, De Angelis K. Maximal exercise test is a useful method for physical capacity and oxygen consumption determination in streptozotocin-diabetic rats. Cardiovascular diabetology. 2007;6(1):38.
14. Falah S, Kordi MR, Ahmadizad S, Ravasi AA, Hedayati M. The effect of eight weeks of endurance training on resting levels and visfatin response and insulin resistance index to acute endurance activity in diabetic rats. Physiology and Management Research in Sport, 2011; No. 8, 83-93. (in persian)
15. Thirumalai T, David E, Viviyan TS, Elumalai EK. Effect of Solanum surattense seed on the oxidative potential of cauda epididymal spermatozoa. Asian Pacific Journal of Tropical Biomedicine. 2012;2(1):21-3.

[DOI: 10.18502/ijdo.v12i1.3635]

16. Seo H, Park CH, Choi S, Kim W, Jeon BD, Ryu S. Effects of voluntary exercise on apoptosis and cortisol after chronic restraint stress in mice. Journal of exercise nutrition & biochemistry. 2016;20(3):16.
17. Cao Y, Hao CJ, Wang CJ, Li PL, Wang LX, Guan HS, et al. Urinary excretion of L-carnitine, acetyl-L-carnitine, propionyl-L-carnitine and their antioxidant activities after single dose administration of L-carnitine in healthy subjects. Brazilian Journal of Pharmaceutical Sciences. 2013;49(1):185-91.
18. Mohammad S, Alinejad HA, Jafari M, Khalagi K, Asjodi F, Falah E. Effect of L-carnitine Supplementation on Health Indicators of Untrained Men Over a Period of Resistance Training: A Randomized, Placebo-Controlled Trial. Iranian Journal of Health Education and Health Promotion. 2014;2(3):232-41. (in Persian)
19. Powers SK, Criswell D, Lawler JO, Martin D, Lieu FK, Ji LL, et al. Rigorous exercise training increases superoxide dismutase activity in ventricular myocardium. American Journal of Physiology-Heart and Circulatory Physiology. 1993;265(6):H2094-8.
20. Radak Z, Chung HY, Goto S. Systemic adaptation to oxidative challenge induced by regular exercise. Free Radical Biology and Medicine. 2008;44(2):153-9.
21. Shreelaxmi VH, Prabha A, Shashidhar K, Rajeshwari S, Kalpana A. Effect of yoga on oxidative stress in elderly type 2 diabetes subjects. Journal of Yoga & Physical Therapy. 2016;6:258.
22. Kolodziejczyk J, Saluk-Juszczak J, Wachowicz B. L-Carnitine protects plasma components against oxidative alterations. Nutrition. 2011;27(6):693-9.
23. Marcovina SM, Sirtori C, Peracino A, Gheorghiade M, Borum P, Remuzzi G, et al. Translating the basic knowledge of mitochondrial functions to metabolic therapy: role of L-carnitine. Translational research. 2013;161(2):73-84.
24. Mohammadi M, Salehi I, Farajnia SA. Effect of swimming exercise on oxidative stress in hippocampus of diabetic male rats. 2008. (in Persian)
25. Mazzola PN, Terra M, Rosa AP, Mescka CP, Moraes TB, Piccoli B, et al. Regular exercise prevents oxidative stress in the brain of hyperphenylalaninemic rats. Metabolic brain disease. 2011;26(4):291.
26. Derouich M, Boutayeb A. The effect of physical exercise on the dynamics of glucose and insulin. Journal of biomechanics. 2002;35(7):911-7.
27. Hong JH, Kim MJ, Park MR, Kwag OG, Lee IS, Byun BH, et al. Effects of vitamin E on oxidative stress and membrane fluidity in brain of streptozotocin-induced diabetic rats. Clinica chimica acta. 2004;340(1-2):107-15.
28. Afzalpour ME, Gharakhanlou R, Gaeini AA, Mohebbi H, Hedayati M, Khazaei M. The effects of aerobic exercises on the serum oxidized LDL and total antioxidant capacity in non-active men. CVD prevention and control. 2008;3(2):77-82.
29. Mahfouz MH, Ghanem HM, Mohamed MA. Therapeutic effect of L-carnitine on sialic acid, soluble Fas (sFas) and other biochemical variables in hyperinsulinemic rats. 1 The artificial microRNA mediates GUS-GFP gene silencing using ath-miR169d precursor as backbone. 2009.
30. Hajian-Shahri S, Hajinezhad MR, Jahantigh M, Miri HR. The antidiabetic effect of L-carnitine in rats: the role of nitric oxide system. Feyz Journal of Kashan University of Medical Sciences. 2017;21(5):413-20.
31. Chevion S, Moran DS, Heled Y, Shani Y, Regev G, Abbou B, et al. Plasma antioxidant status and cell injury after severe physical exercise. Proceedings of the National Academy of Sciences. 2003;100(9):5119-23.

[DOI: 10.18502/ijdo.v12i1.3635]