Cellular and Molecular Biology
E-ISSN : 1165-158X / P-ISSN : 0145-5680
www.cellmolbiol.org

Original Research

L-Carnitine supplementation increases expression of PPAR-γ and glucose transporters in skeletal muscle of chronically and acutely exercised rats

Ragip Pala[1], Eyup Genc[1], Mehmet Tuzcu[2], Cemal Orhan[3], Nurhan Sahin[3], Besir Er[2], Vedat Cinar[1], Kazim Sahin[3*]

[1] Department of Movement and Training Science, Faculty of Sports Sciences, Firat University, Elazig, Turkey
[2] Department of Biology, Faculty of Science, Firat University, Elazig, Turkey
[3] Department of Animal Nutrition, Faculty of Veterinary Medicine, Firat University, Elazig, Turkey

Correspondence to: nsahinkm@yahoo.com

Received May 11, 2017; **Accepted** January 25, 2018; **Published** January 31, 2018

Doi: http://dx.doi.org/10.14715/cmb/2018.64.1.1

Abstract: In this study, the effects of L-Carnitine supplementation on the lipid peroxidation and expression of PPAR-γ and glucose transporters in the liver and muscles of chronically and acutely exercised rats were investigated. A total of 42 male Wistar Albino rats (8-week-old) were divided into six groups as follows: Control, L-Carnitine, Chronic Exercise (CE), Chronic Exercise + L-Carnitine, Acute Exercise (AE) and L-Carnitine + Acute Exercise. Chronic exercise consists of 30 m/min, 30 min/day, and 5 days/week for 6 weeks. Rats in the acute exercise groups were run on the treadmill at 30 m/min until exhaustion. L-Carnitine was given at the level of 300 mg per kilogram of diet for 6 weeks. There was no significant difference in the levels of serum ALT, AST, urea, creatinine and glucose levels between the exercise and L-Carnitine groups ($P > 0.05$). Cholesterol and triglyceride levels decreased by L- carnitine supplementation and chronic exercise in control groups but increased in the AE groups compared to the control group without reinforcement ($P < 0.05$). Serum, muscle, heart, and liver malondialdehyde (MDA) concentrations were lower in CE and higher in the AE groups ($P < 0.001$). However, L-Carnitine supplementation reduced MDA levels ($P < 0.05$). Liver and muscle PPAR-γ, liver GLUT-2 and muscle GLUT-4 mRNA expressions were lower in AE group than in all other groups ($P < 0.001$). Both chronic exercise and supplemental L-Carnitine increased liver and muscle PPAR-γ, GLUT-2 and GLUT-4 mRNA expression ($P <0.05$). As a result, although acute exercise increased oxidative stress, chronic exercise reduced oxidative stress by lowering lipid peroxidation level. L-Carnitine supplementation decreased oxidative stress and improved glucose and lipid metabolism by regulation of PPAR-γ, GLUT-2 and GLUT-4 mRNA expression in rats.

Key words: L-Carnitine; Exercise; Oxidative stress; PPAR-γ; Glucose transporters.

Introduction

In the exhausting or excessive exercise, oxygen consumption increased systematically 10-20 times (1, 2) and in skeletal muscles 100-200 times increases (2). This increase, in the use of oxygen in the mitochondria of the cell known as the center of energy production, results by triggering the formation of free radicals and other reactive oxygen species (ROS) and thus in increased ROS release from mitochondria during exercise (3). Excessive ROS produced during exercise, resulting in lipid peroxidation and causes oxidative stress resulting DNA damage (4, 5). In those who run or doing excessive exercise, oxidative stress resulting by mitochondria, it manifested as muscle fatigue and muscle damage (2, 6). In order to mitigate or eliminate negativities which are exercise-induced, studies were made on athletes and experimental animals. These studies are based on the most basic elimination of oxidative stress and made in the shape of supplementation of materials which is considered to have antioxidant properties. L-Carnitine plays a very important role in fat and carbohydrate metabolism and it is needed to show the proper function of the heart and muscles in humans and animals (7). It has been reported that L-Carnitine has an antioxidant effect and it protects the damage of cell by membrane stabilization against free radicals, prevents mitochondrial damage and increase energy production, and reduces the passage of free radicals (8). L-Carnitine which helps to use fatty acids effectively is used to provide a healthy and balanced diet in humans and animals (9). PPAR-γ involved in adipocyte differentiation (10), lipid metabolism (11) and regulation of expression of the target gene in glucose homeostasis (12). PPAR-γ also has a suppressive immune function in favor of anti-atherosclerotic effects (13). PPAR-γ regulates genes that affect the release of free fatty acids from adipocytes and regulate genes encoding adipocyte hormone (14).

It is reported that physical exercise improves insulin sensitivity by increasing the expression of glucose transporters (GLUTs) (15, 16). Glucose connects GLUTs, becomes soluble in lipid and moves according to the gradient concentration. An isomer of glucose transport, GLUT-2, GLUT-4, plays an important role in glucose uptake of cells. GLUT-2 transporter molecule involves duplex in hepatocytes, in glucose transportation and the secretion of insulin from the pancreas by glucose stimulation (17). There is glucose isoform in tissues such as skeletal muscle, heart, and lipid tissue which are tissues that insulin-mediated glucose uptake. GLUT-4 is moved into intracellular vesicles. Insulin binds to insulin receptors in the cell membrane, allows the migration of GLUT-4, which is located in the cell, to the cell membrane by activating tyrosine kinase in the

receptor of the β subunit in the cell (18).

L-Carnitine plays an important role in β-oxidation in peripheral tissues and in mitochondrial membrane passage of long-chain fatty acids for ATP production (19). L-Carnitine helps to produce more energy from fat and the economic use of muscle glycogen stores by taking part in increasing the oxidation of fatty acids (20). Although the benefits of exercise training and L-Carnitine for several blood markers are well known, the effects of L-Carnitine supplementation on the muscle PPAR-γ and glucose transporters in healthy conditions (associated or not with physical activity) are unknown. Therefore, in this study, the effects of L-Carnitine supplementation were investigated on the changes of lipid peroxidation and PPAR-γ and glucose transporters in chronically and acutely exercised rats.

Materials and Methods

Animals and experimental design

A total of 42 male Wistar albino rats (8 weeks of age), 7 rats in each group, were kept in a controlled environment with a 12:12 light-dark cycle at 22 °C and were provided with rat chow and water *ad libitum.* This study was carried out by an appropriately respect the ethical rules for animal welfare and animal rights, after approval (2014/01/08) by Firat University Ethics Committee for Animal Experiments. The rats used in the experiments were obtained from Firat University Experimental Research Center. Rats were randomly divided into six groups: (1) Control: Rats fed a standard diet and not exercised, (2) L-Carnitine: Rats fed a standard diet containing L-Carnitine and not exercised, (3) Exercise (CE): Rats fed a standard diet and exercised (30 m / min, gradient 0%, 30 min; exercise was performed for 5 days per week for 6 weeks), (4) Exercise (CE)+L-Carnitine: Rats fed a standard diet containing L-Carnitine and exercised, (5) Acute Exercise (AE): Rats fed a standard diet containing L-Carnitine and exercised (30 m/ min, gradient 0%, 30 min) for 5 days per week during 6 weeks and exhaustion exercise was also performed. (6) Acute Exercise (AE)+L-Carnitine): Rats fed a standard diet containing L-Carnitine and exercised (30 m / min, gradient 0%, 30 min) for 5 days per week during 6 weeks, and exhaustion exercise was also performed. L-Carnitine was supplemented for six weeks at a level of 300 mg per kilogram of diet. The dose selection (300 mg per kilogram of diet) is based on previous studies in which this dosage has a significant effect on the rat (21). Experimental animals start to run initially 10 m / min. and at the end of a 2-week run-in period of 30 m / min, 0% grade, 30 minutes of jogging protocol has applied with controlled increments (Treadmill, MAYTME 0804, Commat Limited, Ankara). The treadmill is equipped with an electric shock grid on the rear obstacle to provide exercise motivation to the animals. Rats, introduced after dietary L-Carnitine was subjected to a running test for 5 days a week for 6 weeks and last day acute exercise (running on a treadmill until exhaustion) protocol was applied. Running test was made between the hours (8: 00-11: 00) in the morning to ignore the basal glucocorticoid activity.

Collection of samples

At the end of the experiment, blood, liver, heart and muscle specimens were taken from decapitated animals via cervical dislocation via anesthesia. Blood samples were collected by gel biochemical tubes and serum samples were taken and centrifuged at 4 °C at 5000 rpm for 10 minutes in a chilled centrifuge. In addition, the tissues obtained from the animals were stored in a freezer at -80 °C until analysis.

Laboratory analyses

Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), urea, creatinine, glucose, cholesterol, and triglyceride levels were analyzed using an automated analyzer (Samsung LABGEO PT10, Samsung Electronics Co., Suwon, Korea). Reproducibility and device/method sensitivity of LABGEOPT10 were established according to IVR-PT06 guidelines.

Serum and tissue MDA concentrations were measured according to the method described by Karatepe (22) with a Shimadzu UV-vis SPD-10 AVP detector, a CTO-10 AS VP column and 30 mM KH_2PO_4 and methanol (82.5: 17.5, v/v, pH 3.6) at a flow rate of 1.2 ml/ min. Column waste was monitored at 250 nm. Tissue homogenization (10%, w/v) in 10 mM phosphate buffer (pH 7.4) was prepared and centrifuged at 13,000 x g for 10 minutes at 4 °C. The resulting supernatant was collected and kept at -80 ° C for MDA estimation.

RNA isolation and quantitative reverse-transcriptase polymerase chain reaction

Liver, heart, and muscle samples were homogenized and total RNA was extracted using the RNeasy Mini kit (Valencia, CA, USA Qiagen) according to the manufacturer's animal cell extraction protocol instructions, including DN RNA therapy. Then, 500 ng of RNA was reverse transcribed with reverse transcriptase with random primers. RNA concentrations were determined using a NanoDrop (MaestroGen, Las Vegas, NV, USA). RNA samples were either stored at -80 ° C for long-term storage or immediately stored on ice for cDNA synthesis. For cDNA synthesis, 2 µg total RNA was reverse transcribed using TaqMan1 Reverse Transcription reagents (Qiagen, Valencia, CA, USA). qRT-PCR was performed on cDNA aliquots using the SYBR Green PCR Master Mix (catalog no. 330620, Qiagen) to quantitatively determine the gene expression of PAR-y, GLUT-2, GLUT-4 and glyceraldehyde 3-phosphate dehydrogenase (GAPDH, the internal standard) on a Rotor-Gen Q machine (Qiagen). Reactions were performed in triplicates with 2 µL primer pair, 5 µL SYBR green master mix, 1 µL RNA-free water and 2 µL cDNA templates. PCR was done with the following conditions: initial denaturation at 95 ° C for 10 minutes followed by 40 cycles denaturation at 95 °C for 10 seconds, annealing at 60 ° C and extension together at 60 °C at 45 °C. After the last cycle, the melting curve was determined at 60-95 ° C. Negative control samples were always included in the amplification reactions to check for contamination. The specificity of the amplification was confirmed by melting curve analysis. Each PCR reaction was made in triplicate and the mean Ct value was used for statistical analysis. Messenger RNA expression was standardized for GAPDH expression levels and then normalized to

Table 1. Effect of L-Carnitine on liver and kidney functions in exercised rats.

Parameters	Control	Carnitine	CE	CE+Carnitine	AE	AE+ Carnitine	*P*
AST U/L	234.17±18.55	243.67±15.34	245.50±26.68	247.00±24.42	241.85±19.33	238.42±21.92	> 0.05
ALT U/L	95.50±6.51	89.81±12.61	84.50±8.87	86.71±9.43	94.14±8.35	87.27±10.80	> 0.05
Urea mg/dL	28.48±2.44	26.13±2.76	28.40±3.21	27.76±3.65	27.59±2.52	25.99±2.43	> 0.05
Creatine mg/dL	0.39±0.02	0.38±0.03	0.38±0.03	0.37±0.04	0.40±0.03	0.39±0.04	> 0.05

Data were given as standard error mean ±. (a-d) Differences between groups with different letters are statistically significant (p<0.05). Control: sedentary and rats fed a standard diet and not exercised; Carnitine: rats fed a standard diet containing 300 mg/kg L-carnitine and exercised; CE: Rats fed a standard diet and exercised; CE+ Carnitine; Rats fed a standard diet containing L-carnitine and exercised; AE: Rats fed a standard diet containing L-carnitine and exhaustion exercise was performed; AE+Carnitine: Rats fed a standard diet containing L-carnitine and exhaustion exercise was also performed.

the control group.

Statistical analysis

Given this assumption, seven samples per treatment were included in the study. The sample size was calculated based on a power of 85% and P value was 0.05. The data were analyzed using a two-way ANOVA procedure of SPSS (IBM SPSS 2012 Version 21.0); P <0.05 was considered statistically significant. Intergroup comparisons were analyzed by the Tukey post hoc test. All data are shown as Mean ± Standard Deviation (SD).

Results

The liver (AST, ALT) and renal function (creatinine and blood urea nitrogen) parameters are reported in Table 1. There was no significant difference in the liver and renal function among all groups (Table 1, $P > 0.05$). As shown in Table 2, there was no significant difference in glucose levels between the experimental groups ($P > 0.05$). The changes in cholesterol and triglyceride levels in the animal group are also shown in Table 2. Both exercise training and L-Carnitine supplementation significantly reduced total cholesterol and triglyceride levels compared to the control group. Serum cholesterol and triglyceride levels were lower in all the L-Carnitine supplemented groups compared to the non-supplemented controls ($P < 0.001$). In addition, cholesterol and triglyceride levels did not differ according to the control group in the acute exercise groups and chronic exercise groups ($P < 0.05$). The lowest cholesterol and triglyceride levels were observed in the CE + L-Carnitine group and the highest cholesterol and triglyceride levels were observed in the control group ($P < 0.05$).

Effects of L-Carnitine supplementation and acute or chronic exercises on MDA levels are shown in Table 3. Serum, muscle, heart, and liver MDA concentrations were higher in the acutely exercised groups while significantly lower in the chronically exercised groups compared to the controls ($P < 0.001$). Serum, muscle, heart, and liver MDA levels were lower in all the L-Carnitine supplemented groups compared to the non-supplemented controls ($P < 0.05$). The highest MDA levels were observed in the AE group and the lowest MDA levels were observed in the CE+ L-Carnitine group (Table 3).

A significant difference was observed between groups in the liver and muscle PPAR-γ mRNA expression (Figure 1 and 2; Panel A). Accordingly, the lowest level of PPAR-γ gene expression was found in the acute exercise group. The highest levels expression of PPAR-γ gene has been found in the L-Carnitine group with chronic exercise ($P < 0.05$). Chronic exercise group and L-Carnitine supplemented group followed this group. However, there was no difference between

Table 2. Effect of L-Carnitine on glucose and lipid profile in exercised rats.

Parameters	Control	Carnitine	CE	CE+Carnitine	AE	AE+ Carnitine	*P*
Glucose, mg/dL	98.83±4.27	99.51±5.34	98.00±6.27	95.14±4.37	99.80±5.18	97.25±5.35	> 0.05
Cholesterol, mg/dL	74.83±2.36^{a}	66.00±2.96^{b}	65.83±2.88^{b}	57.29±2.71^{c}	67.25±2.48^{b}	66.57±2.96^{b}	< 0.001
Triglyceride, mg/dL	98.67±3.64^{a}	83.00±4.96^{b}	87.83±4.52^{b}	69.86±4.80^{d}	86.00±4.33^{b}	74.46±4.41^{c}	< 0.001

Data were given as standard error mean ±. (a-d) Differences between groups with different letters are statistically significant (p<0.05). Control: sedentary and rats fed a standard diet and not exercised; Carnitine: rats fed a standard diet containing 300 mg/kg L-carnitine and exercised; CE: Rats fed a standard diet and exercised; CE+ Carnitine; Rats fed a standard diet containing L-carnitine and exercised ; AE: Rats fed a standard diet containing L-carnitine and exhaustion exercise was performed; AE+Carnitine: Rats fed a standard diet containing L-carnitine and exhaustion exercise was also performed.

Table 2. Effect of L-Carnitine on glucose and lipid profile in exercised rats

Parameters	Control	Carnitine	CE	CE+ Carnitine	AE	AE+Carnitine	*P* <
Serum MDA	0.67±0.03^{c}	0.46±0.02^{d}	0.49±0.02^{d}	0.38±0.03^{e}	0.99±0.04^{a}	0.73±0.02^{b}	0.001
Muscle MDA	79.27±2.09^{c}	65.57±2.74^{d}	66.49±2.93^{d}	62.43±2.61^{de}	95.69±2.52^{a}	89.66±2.34^{b}	0.001
Heart MDA	47.23±2.73^{c}	41.31±2.92^{d}	39.79±3.15^{d}	34.50±3.21^{e}	60.01±3.04^{a}	53.88±2.94^{b}	0.001
Liver MDA	93.38±3.03^{c}	84.88±2.86^{d}	85.55±2.74^{d}	78.812.63^{e}	109.72±2.85^{a}	98.35±2.72^{b}	0.001

Data were given as standard error mean ±. (a-d) Differences between groups with different letters are statistically significant (p<0.05). Data were given as standard error mean ±. (a-d) Differences between groups with different letters are statistically significant (p<0.05). Control: sedentary and rats fed a standard diet and not exercised; Carnitine: rats fed a standard diet containing 300 mg/kg L-carnitine and exercised; CE: Rats fed a standard diet and exercised; CE+ Carnitine; Rats fed a standard diet containing L-carnitine and exercised; AE: Rats fed a standard diet containing L-carnitine and exhaustion exercise was performed; AE+Carnitine: Rats fed a standard diet containing L-carnitine and exhaustion exercise was also performed.

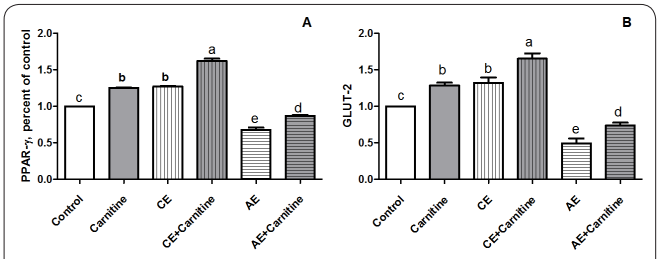

Figure 1. Effect of L-carnitine on the liver relative fold change of PPAR-γ (A) and GLUT-2 (B) expression in exercised rats**.** Control: sedentary and rats fed a standard diet and not exercised; Carnitine: rats fed a standard diet containing 300 mg/kg L-carnitine and exercised; CE: Rats fed a standard diet and exercised; CE+ Carnitine; Rats fed a standard diet containing L-carnitine and exercised; AE: Rats fed a standard diet containing L-carnitine and exhaustion exercise was performed; AE+Carnitine: Rats fed a standard diet containing L-carnitine and exhaustion exercise was also performed.

the groups of L-Carnitine supplemented and acute exercise group. PPAR-γ gene expression was found low in acute exercise and L-Carnitine supplemented group regarding the acute exercise group ($P < 0.05$).

As shown in Figure 1 (Panel B), it was determined that L-Carnitine and acute exercise causes a significant change in liver GLUT-2 gene expression levels ($P < 0.001$). Additionally, liver GLUT-2 expressions reduced in chronic exercise group when compared with the control group. However, this decrease in expression of GLUTs by L-Carnitine supplementation was statistically increased and closes to control group. A significant increase has been observed in GLUT-2 expressions when control group and the group with L-Carnitine compared ($P < 0.05$). Muscle GLUT-4 expression was significantly increased in chronic exercise group when compared with the control group ($P < 0.001$) after L-Carnitine was supplemented, expression levels increase more and more (Figure 2, Panel C). In addition, it was determined that GLUT-4 expression decreased significantly in the acutely exercised group when compared with control group. It was observed that GLUT-4 levels were increased in the acutely exercised group; the differences between these increases were statistically significant ($P < 0.05$).

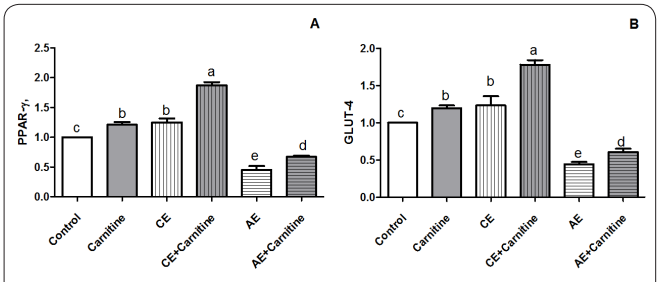

Figure 2. Effect of L-carnitine on the muscle relative fold change PPAR-γ (A) and GLUT-4 (B) expression in exercised rats. Control: sedentary and rats fed a standard diet and not exercised; Carnitine: rats fed a standard diet containing 300 mg/kg L-carnitine and exercised; CE: Rats fed a standard diet and exercised; CE+ Carnitine; Rats fed a standard diet containing L-carnitine and exercised; AE: Rats fed a standard diet containing L-carnitine and exhaustion exercise was performed; AE+Carnitine: Rats fed a standard diet containing L-carnitine and exhaustion exercise was also performed

Discussion

In the present study, we found that there was no significant difference in liver (ALT, AST) and kidney function (blood urea nitrogen and creatinine) and glucose levels in the exercise and carnitine groups (Table 1). However, carnitine and chronic exercise decreased serum cholesterol and triglyceride levels (Table 2). In addition, L-Carnitine supplementation and exercise training reduced the serum, muscle, heart, and liver MDA levels (Table 3). Moreover, L-Carnitine supplementation and exercise training increased the expression of PPAR-γ mRNA in liver and muscle. Especially, most increase determined in the groups which are chronic exercise and L-Carnitine applied together (Figure 1 (Panel A), Figure 2 (Panel A)). Statistically, a significant difference was found between L-Carnitine and exercise groups in liver and muscle GLUT-2 and GLUT-4 gene expression (Figure 1 (Panel B), Figure 2 (Panel B)).

L-Carnitine is available as endogenous in fat and carbohydrate metabolism and its biosynthesis can be performed in human skeletal muscle, liver, heart, kidney and brain tissue (23). It is reported that organism's resistance and energy capacity may increase by a combination of regular exercise and implementation of L-Carnitine supplement and also the increased generation of free radicals can be reduced by the effect of carnitine (24). In many studies has been reported to carnitine supplementation cause a decrease in total cholesterol and triglyceride levels in exercised rats (25-28). Similar to our results, Karanth and Jeevaratnam (29) have found a decrease in cholesterol and triglyceride levels in the exercised rats and fed with diet supplemented with carnitine. Also, Kim et al. (30) reported reductions in cholesterol and triglyceride levels were observed in endurance exercised rats fed with carnitine containing diet.

L-Carnitine also protects cellular respiration and enzyme activity against oxidative damage (31) and it prevents the accumulation of lipid oxidation products (32). In the present study, L-Carnitine supplementation advanced antioxidant capacity and decreased the level of MDA in chronic and acute exercises. Several animal studies reported that L-Carnitine supplementation increases the antioxidant defense metabolism and can prevent oxidative damage (33, 34). Parandak et al. (35) reported that L-Carnitine supplementation decreased MDA levels in running 21 healthy young men for 14 km. In another study done by Sıktar et al. (36) sixty days of swimming exercise increased oxidative stress in hypothermic conditions. However, L-Carnitine supplementation does not prevent the formation of free radicals in hypothermic conditions. In a study, it was reported that L-Carnitine helped to lipid peroxidation and can prevent oxidative damage by regulating the distribution of glutathione in the liver and muscle (37). Atalay et al. (38) also reported that L-Carnitine supplementation decreased the level of TBARS in 26 healthy young men soccer players aged between 17-19 years.

Peroxisome proliferator-activated receptors (PPARs) play a key role in regulating insulin resistance and adipogenesis, carbohydrate, and lipid metabolism (39). In previous studies, it was shown that exercise increased the level of PPAR-γ in liver (40, 41). GLUTs are a large group of membrane proteins and are downstream sub-

strates of the ACT and play a key role in the regulation of blood glucose levels (42). GLUT2, a transmembrane protein, is expressed by hepatic cells which are the main carrier for the transfer of glucose between the liver and blood (43). GLUT4 is an insulin receptor inducible glucose transporter and is specifically expressed in adipocytes and skeletal muscle (44). Previous studies have shown that elevation of GLUT4 protein expression and stimulation of GLUT4 vesicle translocation to the plasma membrane may improve glucose transports (45). On the other hand, glycogen is used for energy in skeletal muscles during exercise, but in long exercise, while glycogen depleted blood sugar and fatty acid rises, at the same time the glucose transporter GLUT-2 and GLUT-4 increases, this situation is called synthesis of glycogen. So in chronic exercise, GLUT-2 and GLUT-4 effects rise that helps regulate blood sugar (46, 47). GLUT4 is found in intracellular vesicles during normal situations and translocates to the plasma membrane in response to insulin and exercise. Previous studies have demonstrated that rats submitted to treadmill running had increased GLUT2 and GLUT4 gene and protein expressions in the muscle and liver (48-51). In the present study, we demonstrated for the first time that L-Carnitine significantly increased the expression of hepatic and muscle PPAR-γ, hepatic GLUT-2, and muscle GLUT-4. There are no earlier studies associated with examining the effects of L-Carnitine treatment on the expression of in rats acutely and chronically exercised rats to compare with this study. Nevertheless, it was reported that L-Carnitine prevented the hyperlipidemia and hyperglycemia in exercised rats and improved glucose tolerance (29).

In conclusion, while acute exercise increases oxidative stress, chronic exercise decreased oxidative stress by reducing lipid peroxidation. In addition, L-Carnitine consumption increased the expression of PPAR-γ, GLUT-2, and GLUT-4 in chronically and acutely exercised rats. Meanwhile, L-Carnitine reduced oxidative stress by showing a synergistic effect with chronic exercise.

Acknowledgements
This work was granted by Firat University Scientific Research Projects Unit (FUBAP-BSY.14.02) and the Turkish Academy of Sciences (KS).

Author's Contribution
KS participated in the study design and drafting the manuscript. RP, EG, MT and CO, participated in the data collection and assays and data analysis. NS, BE, VC participated in the data analysis and statistical analysis for the variables and drafting the manuscript. All authors read and approved the final manuscript.

Interest Conflict
The authors declare no conflicts of interest.

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