

Interaction Between Acute Exercise and Carnitine Supplementation on the Expression of Genes Involved in Liver Metabolism in Male Rats

Beydolah Shahouzehi^{1,2} , Soheil Aminizadeh³ , Yaser Masoumi-Ardakani^{3,*} 

¹ Student Research Committee, Kerman University of Medical Science, Kerman, Iran; bshahouzehi@gmail.com, b.shahouzehi@kmu.ac.ir (B.S.);

² Cardiovascular Research Center, Institute of Basic and Clinical Physiology Sciences, Kerman University of Medical Sciences, Kerman, Iran

³ Physiology Research Center, Institute of Neuropharmacology, Kerman University of Medical Sciences, Kerman, Iran; soheilaminizadeh@gmail.com (S.A.); ymasoumi@kmu.ac.ir (Y.M.A.);

* Correspondence: ymasoumi@kmu.ac.ir (Y.M.A.);

Scopus Author ID 36617532100

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Abstract: Acute exercise induces rapid and dramatic induction of transcription in the liver. The beneficial effects of carnitine on serum factors and gene expression have been proven. This study examined the interaction between acute exercise and carnitine supplementation on the expression of genes involved in liver metabolism. Thirty-two male Wistar rats were randomly assigned into 4 groups (n = 8): Group 1 control, Group 2 received 200 mg/kg/day LCAR, Group 3 performed acute exercise, and Group 4 received LCAR and performed acute exercise. Gene expression in the liver was evaluated by Real-time PCR. Acute exercise significantly increased PDK4 expression compared to other groups. Also, carnitine administration, performing an acute exercise, and combination of LCAR-Acute significantly increased AMPK and PGC-1 α expression compared with the control group. The expression of SREBP-1c and SCD1 was not significantly changed between studies. The combination of acute exercise and carnitine administration increased PGC-1 α expression, indicating the importance of carnitine with exercise as a beneficial supplement.

Keywords: carnitine; exercise; real-time PCR; AMPK; PGC-1 α ; PDK4.

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1. Introduction

The capacity of a system to regulate the oxidation of energy sources based on the availability of these fuels is called metabolic flexibility. The ability to change the substrate consumption in response to the metabolic requirements depends on the balance between the oxidation of the fuel and the tissue's capacity to store it. In the meantime, competition between fatty acids and glucose for entry into metabolic oxidation pathways occurs at the level of pyruvate dehydrogenase complex (PDC) [1]. Sources of cellular fuel (carbohydrate and lipid) can compete and interact with oxidation [1, 2].

The PDC complex performs oxidative decarboxylation of pyruvate, linking the metabolism of fatty acids and carbohydrates [3]. PDC inactivation is catalyzed by the pyruvate dehydrogenase kinases (PDKs) [3]. PDK activity can be regulated by levels of metabolic intermediates such as pyruvate and transcription factors such as PGC-1 α under different conditions in different tissues [1, 4-6]. AMPK plays a vital role in maintaining energy balance

in the cell [7-9]. Overall, AMPK activation increases PGC1 expression, and AMPK requires activity to modulate the expression of many key genes involved in mitochondrial and glucose metabolism [10-12]. Other genes involved in energy metabolism include SREBPs, PPARs, SCD1, and MCAD [13]. The enzyme MCAD plays an important role in the oxidation of lipids.

The main role of carnitine (beta-hydroxy-gamma trimethyl ammonium butyrate) in the body is to facilitate the oxidation of long-chain fatty acids by transferring them into the mitochondrial [14, 15]. Therefore, without carnitine, most dietary lipids cannot be used and accumulate in the body, eventually leading to obesity. Carnitine is absorbed in the intestine through sodium-dependent active transport and inactive transport [14, 15]. Carnitine is considered a worldwide supplement, and its most important effects are weight loss and fat mass reduction (so-called fat burning) [14-16].

It was shown that people who took carnitine supplements and exercised improved lipid profile, oxidative muscle capacity, and higher athletic performance [2, 16-18]. Additionally, it was reported that carnitine supplementation ameliorated muscle damage [19]. Exercise leads to an increase in PGC-1 α expression that affects a wide range of cellular functions [4, 5, 20]. It was described that exercise training affects PDK4 levels [1]. Exercise reduced hepatic PPAR γ expression and downstream targets such as SREBP-1c, ACC1, SCD-1, and fatty acid synthase (FAS). Pala *et al.* (2018) showed that acute exercise could affect MDA levels and reduce the expression of glucotransporters and PPAR-gamma in the liver and muscle [21].

Broderick *et al.* also investigated the effect of acute exercise on the expression of genes involved in fat metabolism and showed that the expression of PGC-1a and CPT1 genes was increased after exercise [22]. L-carnitine supplementation with regular aerobic exercise improves liver tissue apoptosis in type 2 diabetic subjects [23]. The beneficial effects of an exercise session have been demonstrated in some studies. For example, Taybi *et al.* showed that an exercise session increased hepatic ABCA1 expression [24].

Acute exercise induces rapid and dramatic induction of transcription in the liver. Huen *et al.* showed that acute exercise increased the expression of PDK4, PGC-1a, AMPK, and IRS-2 genes in the liver, while FAS expression decreased and CPT1 α expression remained unchanged [25]. Also, another study showed that an acute exercise session increased serum FGF21 levels in mice and healthy males. In addition, they found that FGF21 expression was increased in the liver but not in the white adipose tissue and skeletal muscle of mice with acute exercise. Hepatic enhancement was associated with increased hepatic PPAR α expression [26].

The beneficial effects of carnitine on serum factors and gene expression have been proven, and even in some pathological conditions in animal models have had significant and beneficial effects. The body's metabolic system as a coordinated and integrated system is actually a function of changes caused by exercise and the use of carnitine supplements. Given these cases, the association between metabolic integration, carnitine supplementation, and exercise needs further investigation.

One of the reasons is the limited number of studies on the effects of carnitine supplementation. Most previous studies have investigated anthropometric alterations, insulin sensitivity, and decreased fat mass. On the other hand, there was a lack of a more detailed study on the effects of carnitine administration as well as the simultaneous effect of carnitine supplementation and acute exercise on the expression of genes involved in metabolism. We designed this study to determine the interaction between performing acute exercise and L-carnitine intraperitoneal administration on the expression of PDK4, AMPK, MCAD, CS, SREBP1, SCD1, and PGC-1 α genes in the liver of male Wistar rats.

2. Materials and Methods

2.1. Materials.

The materials that used in this study were including; L-carnitine (Sigma, L-Carnitine hydrochloride, Cat number: C0283), Total RNA extraction kit (BioBasic, EZ-10 Spin Column Total RNA Miniprep Kit, Cat number: BS82312), cDNA synthesis kit (TAKARA, Prime Script RT Reagent Kit, Cat number: RR037A), 2X SYBR Green (Ampliqon, Cat number: A325402), and primers (purchased from Metabion, Germany).

2.2. Methods.

All animal care and procedures were conducted according to the European Convention for the protection of animals used for experimental and other scientific purposes. This study was approved by the ethics committee of Kerman University of Medical Sciences with number, IR.KMU.REC.1399.536.

2.3. Animals.

Thirty-two male Wistar rats in the age range of 8 weeks were purchased from the pet center of Kerman Physiology Research Center and kept in the laboratory with a temperature of 22 ± 2 ° C and a light-dark cycle of 12/12 hours. Animals will have free access to water and food. Rats will first be tested for two weeks to familiarize themselves with the laboratory environment. After acclimatization, the animals were randomly assigned into 4 groups (n = 8) as follow; Group 1 control which did not receive carnitine, Group 2 received 200 mg/kg/day LCAR by i.p. injections (4 weeks), Group 3 acute exercise group, and Group 4 that received 200 mg/kg/day LCAR and performed acute exercise.

2.4. Exercise protocol.

The acute exercise was performed in one session in the relevant groups 12 hours before killing the animals. Thus, the animals on the treadmill start at a speed of 10 meters per second and slowly increase to a speed of 30 meters per second. The duration of the exercise was 45 minutes [24-26].

2.5. Total RNA extraction, cDNA synthesis, and real-time PCR.

The liver tissue was used for total RNA extraction. Twenty milligrams of liver tissue were dissected and homogenized using lysis buffer supplied in the kit by Hielscher Sonicator (Hielscher H200, Germany). The obtained homogenate was loaded into the EZ-10 spin column, and RNAs were isolated according to the kit instructions. Then, isolated RNA quality and quantity were determined by the Nano-drop instrument. Finally, we used 500 ng of total extracted RNA in order to synthesize cDNA that was performed by Parstous cDNA synthesis kit according to the kit instructions [24-26].

We used ABI Step one plus instrument to perform Real-time PCR reactions. Each reaction mixture was contained 10 µl SYBR green, 100 ng of synthesized cDNA, primers (forward & reverse primers; 1µl from each primer), and the reaction volume was reached to 20 µl by distilled water. We used primer's T_m to determine the annealing temperature and then performed gradient PCR using Bio-Rad thermocycler. The thermal protocol in real-time PCR

was as follows: 95 (10 min), 95 (20 sec), annealing temperature (45 sec), 40 cycles, and after the thermal cycles were finished, the melt curve analysis was performed. In the current study, 18S rRNA was used as the housekeeping gene. The relative expression of genes was determined by $2^{-\Delta\Delta C_t}$ method [24-26]. Gene-specific primers were used to perform Real-time PCR measurements (Table 1).

Table 1. The primers sequences that used in this study in order to perform real-time PCR.

Gene	Forward	Reverse
AMPK	TTAAACCCACAGAAATCCAAACAC	CTTCGCACACGCAAATAATAG
CS	CGGTTCTTGATCCTGATGAGGG	ACTGTTGAGGGCTGTGATGGC
MCAD	CGCCCCAGACTACGATAAAA	CAAGACCACCACAACCTCTCC
PDK4	AAGCCCTGATGGACACCTC	GAAGCCTGGGATGCTCTTG
Srebp-1c	GACGACGGAGCCATGGATT	GGGAAGTCACTGTCTTGGTTGTT
SCD1	AAAGTTTCTAAGGCCGCTG	GTCTGAGCCAGCAATCTCAA
PGC-1 α	ACCCACAGGATCAGAACAAACC	GACAAATGCTCTTTGCTTTATTGC
18S	GCAATTATCCCCATGAACG	GGCCTCACTAAACCATCCAA

2.6. Statistical analysis.

Data analysis was performed using SPSS software (Version 20). One-way analysis of variance (ANOVA) was used to determine the differences in variables between groups along with Tukey's HSD post-hoc test. In all statistical comparisons, a significance level of $P < 0.05$ was considered significant.

3. Results and discussion

Acute exercise significantly increased PDK4 expression compared to other groups (p values; vs control $p = 0.011$, vs LCAR and combination of LCAR-Acute $p < 0.001$) (Figure 1).

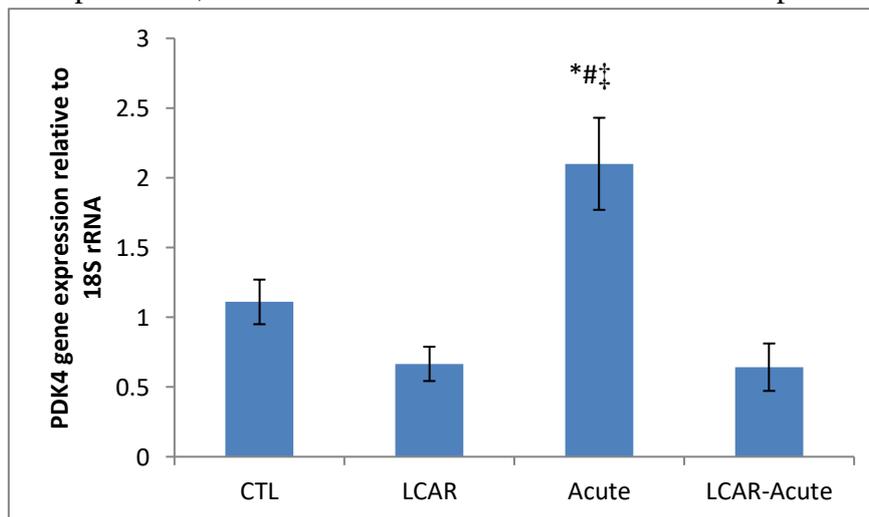


Figure 1. PDK4 gene relative expression in 4 studied groups, including control (CTL), received L-carnitine (LCAR), performed acute exercise, and LCAR-Acute. Data are expressed as Mean SEM, $p < 0.05$ was considered as a significant difference. * statistically significant compared to control group, # statistically significant compared to LCAR group, ‡ statistically significant compared to LCAR-Acute group.

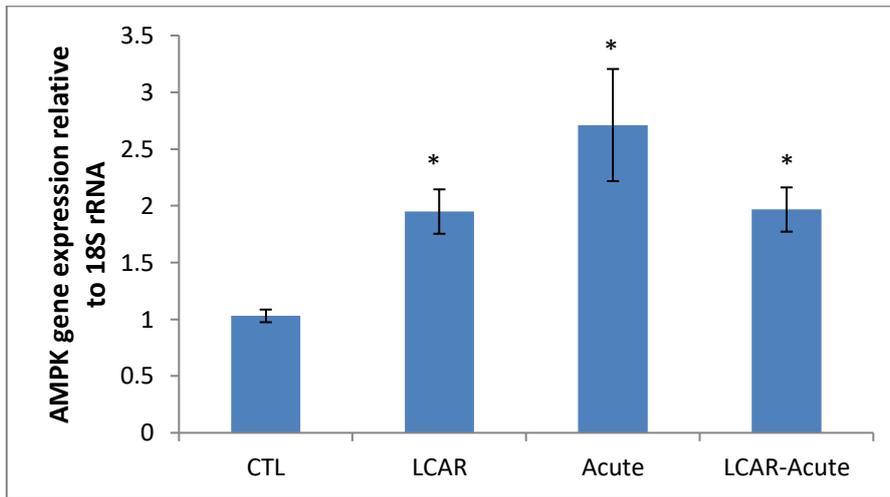


Figure 2. AMPK gene relative expression in 4 studied groups, including control (CTL), received L-carnitine (LCAR), performed acute exercise, and LCAR-Acute. Data are expressed as Mean SEM, $p < 0.05$ was considered as a significant difference. * statistically significant compared to the control group.

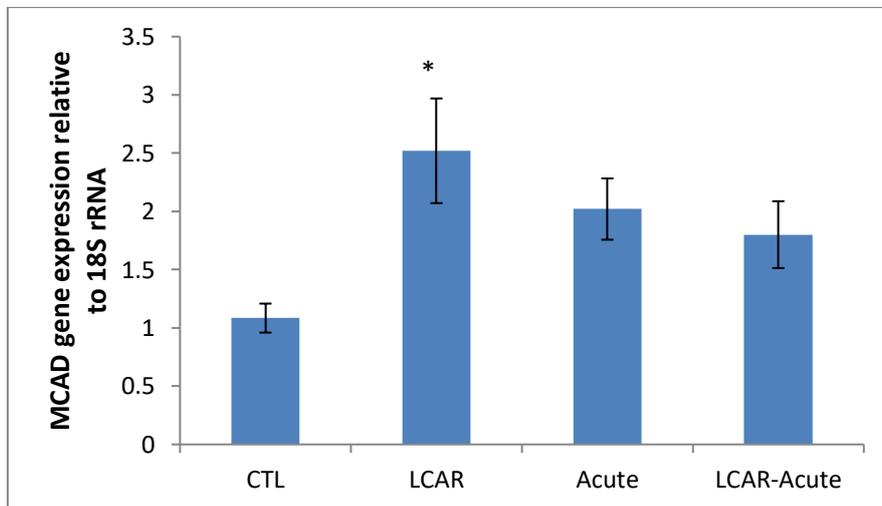


Figure 3. MCAD gene relative expression in 4 studied groups, including control (CTL), received L-carnitine (LCAR), performed acute exercise, and LCAR-Acute. Data are expressed as Mean SEM, $p < 0.05$ was considered as a significant difference. * statistically significant compared to the control group.

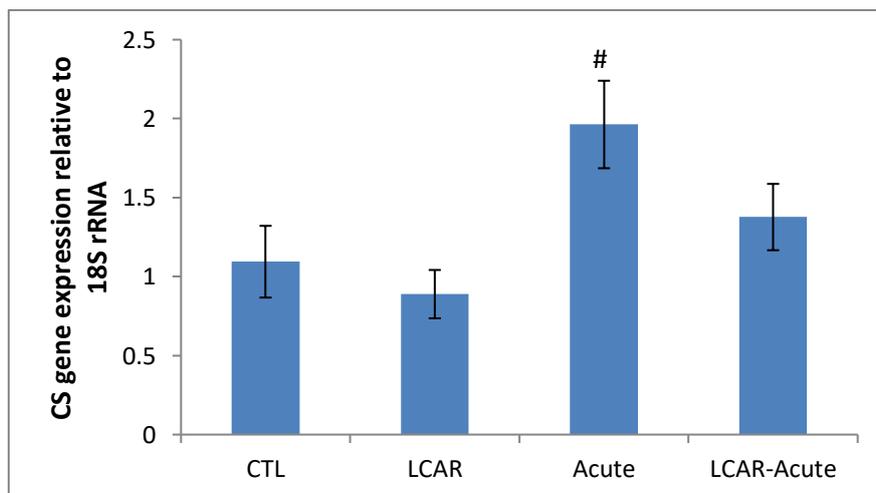


Figure 4. CS gene relative expression in 4 studied groups, including control (CTL), received L-carnitine (LCAR), performed acute exercise, and LCAR-Acute. Data are expressed as Mean SEM, $p < 0.05$ was considered as a significant difference. # statistically significant compared to LCAR group.

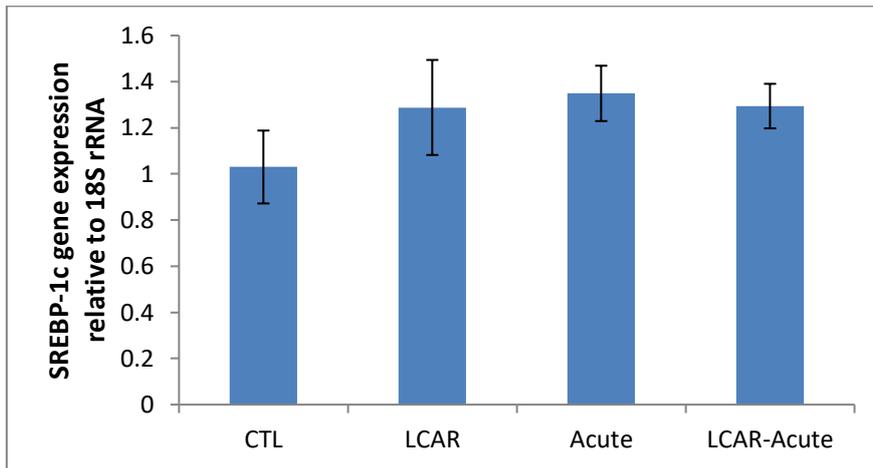


Figure 5. SREBP-1c gene relative expression in 4 studied groups, including control (CTL), received L-carnitine (LCAR), performed acute exercise, and LCAR-Acute. Data are expressed as Mean SEM, $p < 0.05$ was considered as a significant difference.

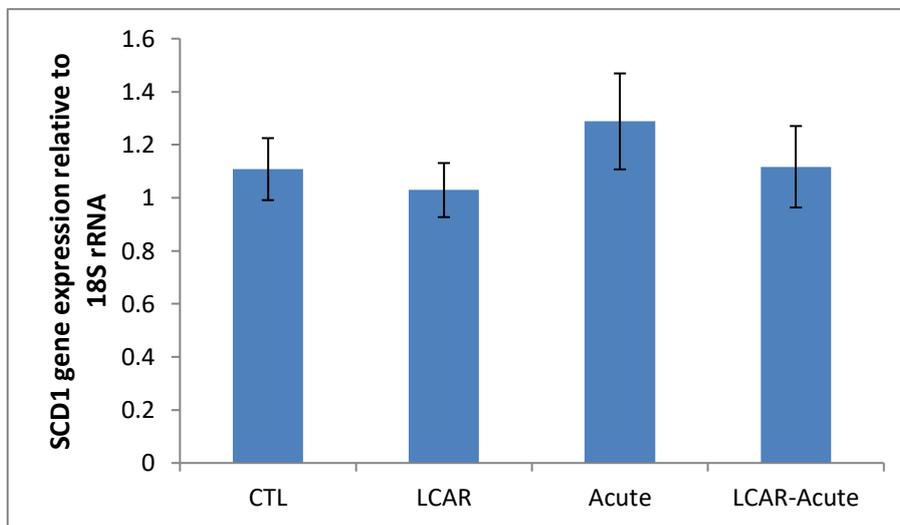


Figure 6. SCD-1 gene relative expression in 4 studied groups, including control (CTL), received L-carnitine (LCAR), performed acute exercise, and LCAR-Acute. Data are expressed as Mean SEM, $p < 0.05$ was considered as a significant difference.

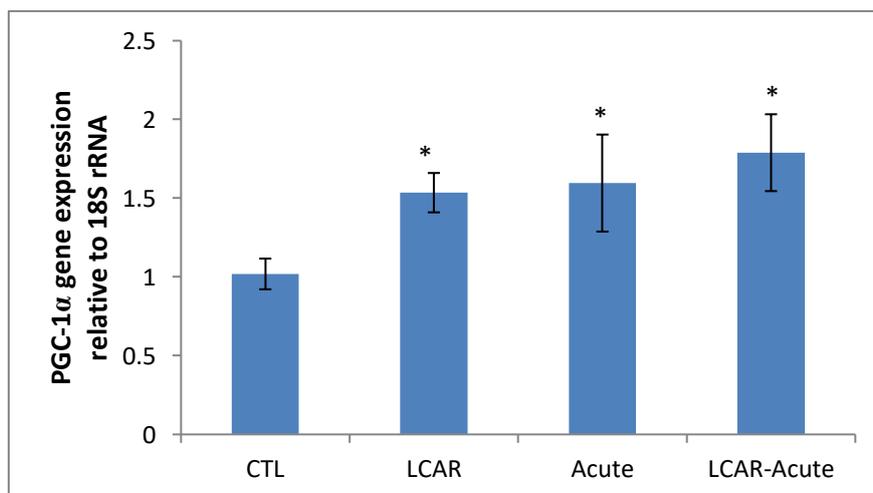


Figure 7. PGC-1 α gene relative expression in 4 studied groups, including control (CTL), received L-carnitine (LCAR), performed acute exercise, and LCAR-Acute. Data are expressed as Mean SEM, $p < 0.05$ was considered as a significant difference.* statistically significant compared to the control group.

Compared with the control group, carnitine administration, performing the acute exercise, and combination of LCAR-Acute significantly increased AMPK and PGC-1 α expression (Figures 2 and 7). MCAD level was significantly higher in the group that received carnitine ($p = 0.026$) (Figure 3), and CS expression was upregulated by exercise ($p = 0.012$) (Figure 4). The expression of SREBP-1c and SCD1 was not significantly changed between the studied groups (Figures 5 and 6).

Acute exercise induces rapid and dramatic induction of transcription in the liver. Carnitine supplementation's beneficial effects were reported previously [2-6, 16-19, 25, 26]. In the current study, we have evaluated the interaction between carnitine supplementation (200 mg/kg/d, i.p) and acute exercise on the expression of genes involved in liver metabolism. Acute exercise increased PDK4 expression. PDK4 inhibits pyruvate oxidation in mitochondria by inhibiting PDC activity and increasing the conversion of pyruvate to lactate. The study by Huen *et al.* showed that acute exercise increased the expression of PDK4 in the liver [25], which is consistent with the results of our study. However, in the group that combined acute exercise with carnitine intake (group 4), carnitine administration neutralized the effect of acute exercise on PDK4 expression. By decreasing PDK4, carnitine appears to cause the pyruvate pathway to flow through the PDC, increase acetyl CoA, and increase the Krebs cycle [2].

AMPK activates catabolic pathways and inhibits anabolic pathways. It was documented that exercise training increases AMPK expression and activity [7, 8]. Pataky *et al.* (2020) have shown that AMPK-gamma was upregulated 3 hours after exercise training [10]. It was described previously that carnitine supplementation increases AMPK expression in the liver, which is consistent with our present results [27]. Acute exercise has also increased AMPK expression, which is in line with the study of Huen *et al.* [25]. During exercise, the AMP / ATP ratio increases and therefore increases and activates AMPK, which ultimately leads to increased glycolysis and glucose oxidation pathway activity [7, 8]. In the present study, the animals performed acute exercise, and due to acute exercise, there was an urgent need for energy, and therefore the body prefers pyruvate to produce lactate and obtain ATP faster.

MCAD is essential for the oxidation of fatty acids by the beta-oxidation pathway. Additionally, carnitine has been shown to increase MCAD expression due to its fat-burning properties through transferring fatty acids into the mitochondria matrix [28]. However, acute exercise and the combination of acute exercise and carnitine did not significantly alter MCAD expression. Given the need to oxidize carbohydrates relative to lipids, this is not unexpected during acute exercise. But the important point here is that acute exercise has been able to eliminate the effect of carnitine on MCAD expression. The lipogenic genes expression was not changed significantly in the current study. Neither carnitine supplementation nor acute exercise was able to later SREBP-1c and SCD1 expression in the liver.

PGC-1 α , which plays an important role in mitochondrial biogenesis, was significantly increased in all three studied groups compared to controls. In previous studies, such as the study of Huen *et al.* and the study of Broderick *et al.*, acute exercise has been shown to increase PGC-1 α expression, which is similar to the finding in our study [22, 25]. Also, Park and colleagues (2020) reported that acute exercise increased PGC-1 α expression [5]. PGC-1 α plays an important role in mitochondrial respiration, antioxidant defense, and lipid and carbohydrate metabolism, and therefore, acute exercise can exert some beneficial effects through the upregulation of PGC-1 α .

4. Conclusions

The combination of acute exercise and carnitine administration increased PGC-1 α and AMPK expression, indicating the importance of carnitine with exercise as a beneficial supplement. But, it is necessary to evaluate the protein level and activity of these factors in this model to find out more precisely about the adaptation which results from acute exercise in animal models.

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Conflict of interest

The authors declare no conflict of interest.

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