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The Effect of High-intensity Interval Training and L-carnitine on the Expression of Genes Involved in Lipid and Glucose Metabolism in the Liver of Wistar Rats

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HIGHLIGHTS

- L-carnitine supplementation and performing High-intensity interval training (HIIT) showed beneficial effects in rats.
- L-carnitine + HIIT reduced lipogenic genes expression.
- The combination of L-carnitine and HIIT caused a significant weight control in rats.

Abstract: Metabolic flexibility is the capacity of a system to adjust fuel (primarily glucose and fatty acids) oxidation based on nutrient availability. Pyruvate Dehydrogenase Kinase 4 (PDK4) is one of the main enzymes that plays a critical role in metabolic flexibility. In the current study, we examined the expression of genes involved in metabolism in the liver of male rats. Thirty-two male Wistar rats (8 week-old) were randomly divided into 4 groups (n = 8); 1. Control, 2. Training (do HIIT training for 4 weeks), 3. LCAR (received 200 mg / kg of L-carnitine, daily), 4. Training + L-carnitine (LCAR-HIIT). The gene expression was measured by Real-time PCR and quantified by $2^{-\Delta\Delta Ct}$ method. Sterol regulatory element-binding protein-1c (SREBP-1c) expression was significantly higher in the LCAR ($p = 0.018$) and HIIT ($p = 0.001$) group than in the LCAR- HIIT group. Stearoyl-Coenzyme A desaturase-1 (SCD1) expression in HIIT ($p = 0.007$, $p < 0.001$) and LCAR-HIIT ($p < 0.001$, $p = 0.003$) groups was significantly increased and decreased compared to the control group and LCAR group, respectively. PDK4 expression was significantly reduced by combination of LCAR-HIIT compared to the control group. In sum, the administration of carnitine and HIIT is very useful and can conduct this by reducing the expression of lipogenic genes such as SREBP-1c and SCD1 as well as increasing the expression of Carnitine palmitoyltransferase 1 (CPT1).

Keywords: High-intensity interval training; L-carnitine; AMPK; SREBP-1c; CPT1.

INTRODUCTION

Metabolic flexibility is the capacity of a system to regulate the oxidation of either glucose or fatty acids (FAs) based on the availability of these energy sources. It is the result of the activity of various enzymes, among which pyruvate dehydrogenase complex (PDC) is of particular importance. The PDC complex performs oxidative decarboxylation of pyruvate, linking the metabolism of FAs and carbohydrates [1]. PDC inactivation is catalyzed by 4 isoenzymes of pyruvate dehydrogenase kinase (PDK). PDK4 is abundant in the liver, heart, and skeletal muscle [1-3]. PDK activity can be regulated by levels of metabolic intermediates such as pyruvate and Peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) under different conditions [1]. PGC-1α has been shown to be a key regulator of cellular energy metabolism along with AMP-activated protein kinase (AMPK). PGC-1α has roles in the mitochondrial biogenesis, FAs oxidation, and hepatic gluconeogenesis [4-6]. Expression of PGC-1α rapidly increases in the liver and heart following short-term starvation, and in skeletal muscle following exercise. Previous studies showed that PGC-1α is used to increase mitochondrial content, and aerobic capacity in response to stimulation of the skeletal muscle and the heart tissues [4-7]. AMPK plays a vital role in maintaining energy balance. AMPK affects PGC-1α activity, but its consequences are not yet well understood, but overall, AMPK activation increases PGC1α expression [8].

Carnitine palmitoyl transferase I (CPT1) mediates the entry of FAs into the mitochondria, and helps initiate the oxidation of those energy sources. Short-chain acyl-CoA dehydrogenase (SCAD) and Medium-chain acyl-CoA dehydrogenase (MCAD) play an important role in the oxidation of FAs, and SCAD catalyzes the first reaction in this pathway. Other genes involved in lipid metabolism are Liver X receptor alpha (LXR-α) and Sterol regulatory element-binding protein-1c (SREBP-1c). LXR nuclear receptor is also an important and upstream regulator of SREBP-1, thereby increasing hepatic lipogenesis [9, 10].

L-carnitine (beta-hydroxy-gamma trimethylammonium butyrate) is well known as a vitamin and amino acid-like substance, and the L-isoform of carnitine has physiological activity. The main role of carnitine in the body is to facilitate the oxidation of lipids by transferring long-chain fatty acids (LCFA) into the mitochondrial matrix (the site of β-oxidation). Therefore, without carnitine, most dietary lipids cannot be used as a food source, and FAs accumulate in the body [11]. Carnitine administration has shown beneficial effects in metabolic diseases such as diabetes and has also been reported to reduce triglyceride and cholesterol levels [12, 13]. Carnitine administration has also been reported to be useful in the prevention and treatment of metabolic disorders [14].

Exercise training increases PGC-1α expression [15], and also affects PDK4 levels [1]. It was reported that exercise decreased SREBP-1C, ACC1, and SCD-1 expression, and up regulated the expression of SCAD and CPT1-α [9]. High-intensity interval training (HIIT) has been shown to increase HDL-c and reduced TG and VLDL levels [16]. A study by Carnevali and coauthors showed that HIIT increases mitochondrial lipid transfer capacity (by increasing the activity of the enzyme CPT-1), which facilitates the oxidative process [17]. Overall, HIIT increases energy efficiency and physical function [18, 19].

People who took carnitine supplements and exercised had improved lipid profile. Carnitine has also been reported to improve athletic performance [11]. Previous data over the combination effects of carnitine and high intensity-interval training are limited and there is the lack of comprehensive study to evaluate its effects on the expression of genes involved in metabolism [11, 12], therefore we presented this study to evaluate the combination effects of HIIT and carnitine on the expression of PGC1, PDK4, AMPK, MCAD, CPT1, CS, LXR-α, SREBP1, SCD1, SCAD genes in the liver of male rats. The results of the present study can provide the interactive effect of HIIT and carnitine administration as a new proposed alternative to improve metabolic status during exercise.

MATERIAL AND METHODS

Material

L-carnitine hydrochloride (C0283-100g, SIGMA, USA), Total RNA Mini-Prep extraction kit (Bs1361, BioBasic, Canada), Prime Script RT Reagent cDNA synthesis kit (RR037A, TAKARA, Japan), RealQ Plus Master Mix Green High ROX (A325406, Ampliqon, Denmark), Primers (Metabion, Germany).

Animals

All the animal procedures were in accordance with the requirements of the Animal Research: Reporting in Vivo Experiments used for experimental and other scientific purposes. This study was approved by the ethic committee of Kerman Medical University Research Council (No. IR.KMU.REC1398.015). Thirty-two male Wistar rats in the age range of 8 weeks were purchased from the animal center of Physiology Research Center of Kerman and kept in the laboratory with a temperature of 22 ± 2^{O}C and a light-dark cycle of 12:12 h. Animals have free access to water and food. Rats will first be examined for two weeks to familiarize themselves with the laboratory environment and determine their health. After obtaining the license of the University Ethics Committee and matching based on body weight, rats will be randomly divided into 4 groups (n = 8) as follows: 1. Control 2. Training (do HIIT training for 4 weeks) 3. LCAR (received 200 mg / kg of L-carnitine, daily) 4. Training + LCAR [20].

Exercise protocol

Group 2 and 4 consisted in 10 bouts of high-intensity treadmill running (20 m/min, 30° inclination), separated by 1 min of active rest, 5 days/week (HIIT, N = 8). Sample size was calculated by a Power analysis based on previous results. The intensity of exercise training was assessed by measuring the maximum speed of rats on the treadmill and blood lactate levels directly after exercise with a lactometer (Lactate Scout Company/Code: 37), and Levels > 4 mmol / l lactate were considered HIIT. In addition, in each session, the lactate was the criteria for evaluating the exercise training intensity. After each session of exercise training, the levels of lactate was measured and based on the lactate levels the intensity of exercise training was optimized [21, 22].

RNA extraction, cDNA synthesis and Real-time PCR

Liver tissue (30 mg) was dissected and homogenized at lysis buffer by Sonicator (Heilscher-H200, Germany). Total RNA was extracted according to kit protocol. Then, complementary DNA (cDNA) was synthesized from extracted RNA (500 ng) by cDNA synthesis kit according to kit's procedure. Real-time PCR was performed by specific primer and the primers' sequences are listed below (Table 1).

Table 1. Primers sequence which used for gene expression measurements by Real-Time PCR

Gene	Forward Sequence	Reverse Sequence
AMPK	TTAAACCCACAGAAATCCAAACAC	CTTCGCACACGCAAATAATAG
CPT1	GTGCTGGAGGTGGCTTTGGT	TGCTTGACGGATGTGGTTCC
CS	CGGTTCTTGATCCTGATGAGGG	ACTGTTGAGGGCTGTGATGGC
LXR-α	CCTGATGTTTCTCCTGACTC	TGACTCCAACCCTATCCTTA
MCAD	CGCCCCAGACTACGATAAAA	CAAGACCACCACAACTCTCC
PDK4	AAGCCCTGATGGACACCTC	GAAGCCTGGGATGCTCTTG
PGC-1α	ACCCACAGGATCAGAACAAACC	GACAAATGCTCTTTGCTTTATTGC
SCAD	TGCCCTATGTTTCGCACCTC	TTCAATGCCCATCATCCCTT
SCD1	AAAGTTTCTAAGGCCGCTG	GTCTGAGCCAGCAATCTCAA
SREBP-1c	GACGACGGAGCCATGGATT	GGGAAGTCACTGTCTTGGTTGTT
18S	GCAATTATTCCCCATGAACG	GGCCTCACTAAACCATCCAA

The real-time PCR reaction was contained 10 µL SYBR green, forward and reverse primers (1µL of each primer), cDNA (100 ng), and reach the reaction volume to 20 µL by distilled DNase free water. The annealing temperatures were according to each primers Tm. The thermal protocol was as follow; 95 °C (5min), 95 °C (15 s), annealing temperature (45 s), 40 cycles and after the thermal cycles were finished the melt curve analysis was performed automatically by ABI Step One Plus instrument. We used 18S rRNA as housekeeping gene. The relative expression of genes were determined by $2^{-\Delta\Delta Ct}$ method [23].

Statistical analysis

Statistical analysis performed by SPSS software (SPSS version 20, SPSS Inc., Chicago, USA) and the data expressed as mean ± SEM. For comparison between groups, we used one-way analysis of variance (ANOVA) followed by the Tukey's post hoc test. The p values < 0.05 were considered significant.

RESULTS

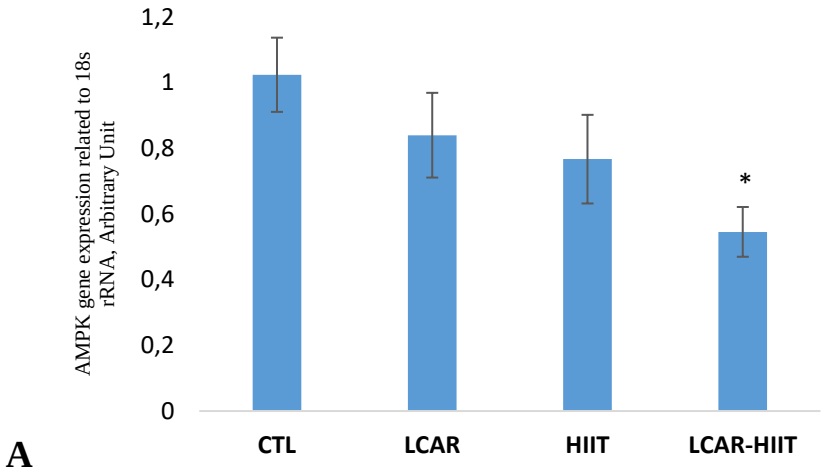
Rat weight values on the first day of the study were determined. We analyzed the data in different groups and no significant difference was observed. Therefore, the weight values of first day of all animals included in the study were considered as "baseline" (167.75 ± 1.0 g). The weight values also measured in the final day of the study and for examined the means between different groups we used baseline value. The results are given in Table 2. The combination of carnitine and HIIT caused significant weight loss compared to the control and the HIIT groups (p = 0.018; p = 0.015, respectively). In the control (p = 0.015) and HIIT (p = 0.013) groups, the amount of weight compared to baseline showed a significant increase. Also, we calculate the body weight gain (BWG) (as Weight Day 30-Weight Day 1). The data showed that LCAR (p = 0.009) and combination of LCAR-HIIT (p < 0.0001) significantly reduced BWG compared to control group. The combination of LCAR-HIIT showed that there was almost no BWG during the study and the BWG was increased in control (p < 0.0001), LCAR (p = 0.02), and HIIT (p < 0.0001) groups compared to combination of LCAR-HIIT (Table 2).

Table 2. Weight gain after 30 days of treatment in 4 studies groups.

	Control (CTL)	L-carnitine (LCAR)	HIIT	LCAR-HIIT
Weight Day 1	165.9±6.5	169.0±12.1	169.4±5.7	168.7±4.3
Weight Day 30	194.6±6.5	181.8±9.2	191.0±4.7	168.9±3.9*‡
Weight Gain	29.0±1.4	12.8±3.5*	21.6±2.1	0.22±3.1*†‡

Data are expressed as Mean±SEM, p<0.05 was considered as significant, n=8. * Statistically significant compared to CTL group, † statistically significant compared to LCAR group, ‡ Statistically significant compared to HIIT group.

The results showed that AMPK expression was significantly decreased in the LCAR-HIIT group compared to the control group (p = 0.047) (Figure 1a). The expression of PGC-1α in the LCAR-HIIT group was significantly reduced compared to the LCAR group as well as the group that underwent HIIT training (p = 0.042 [LCAR], p = 0.027 [HIIT]) (Figure 1b). PDK4 expression was significantly decreased by combination of LCAR-HIIT (p = 0.004) compared to the control group (Figure 2c). Changes in LXR-α gene expression were not significant among the study groups (Figure 2d).



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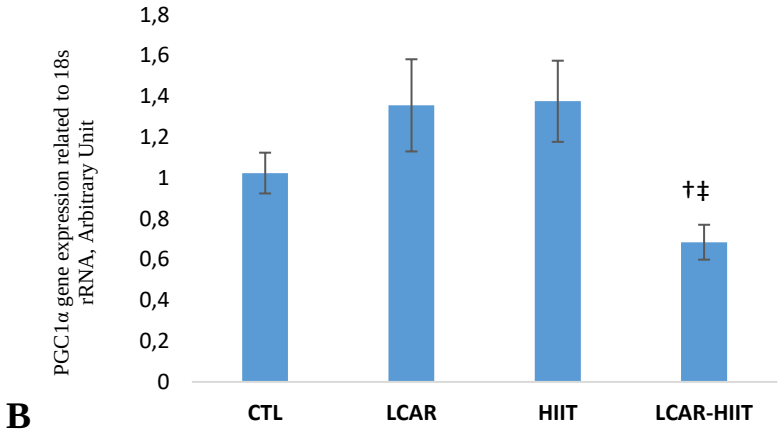


Figure 1. Relative AMPK (A) and PGC-1α (B) gene expression quantified by Real-Time PCR method in liver of studied groups. The groups were as follow; Untreated Control (CTL); received L-carnitine (LCAR), performed High intensity interval training (HIIT) and L-carnitine + HIIT (LCAR-HIIT), Data are expressed as Mean±SEM, p<0.05 was considered as significant, n=8. * Statistically significant compared to CTL group, †statistically significant compared to LCAR group, ‡ statistically significant compared to HIIT group.

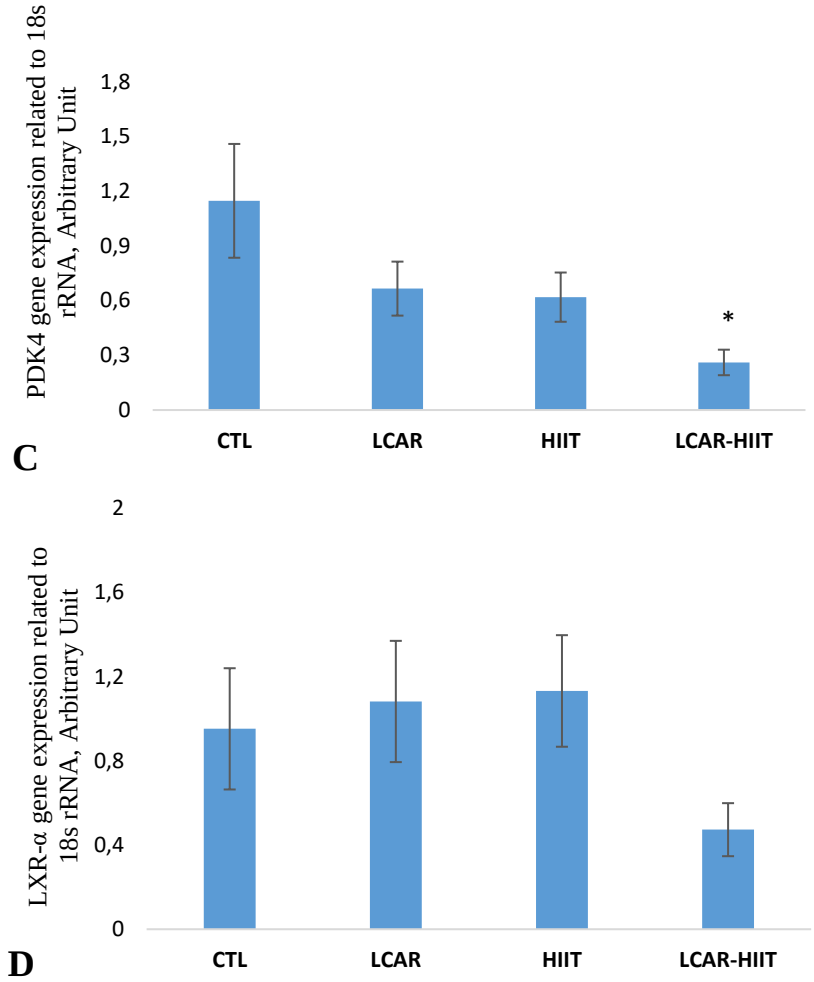


Figure 2. Relative PDK4 (C) and LXR-α (D) gene expression quantified by Real-Time PCR method in liver of studied groups. The groups were as follow; Untreated Control (CTL); received L-carnitine (LCAR), performed High intensity interval training (HIIT) and L-carnitine + HIIT (LCAR-HIIT), Data are expressed as Mean±SEM, p<0.05 was considered as significant, n=8. * Statistically significant compared to CTL group.

SREBP-1c expression was significantly higher in the carnitine (p = 0.018) and HIIT (p = 0.001) group than in the LCAR-HIIT group (Figure 3e). SCD1 expression in HIIT (p = 0.007) and LCAR-HIIT (p < 0.001) groups was significantly increased and decreased compared to the control group, respectively. SCD1 expression in HIIT (p < 0.001) and LCAR-HIIT (p = 0.003) groups was significantly increased and decreased compared to LCAR group (Figure 3f). CS expression in the HIIT group was not increased to the control (p = 0.015), LCAR (p = 0.001) and LCAR-HIIT (p < 0.001) groups (Figure 4g). SCAD expression was significantly decreased in the LCAR-HIIT group compared to the LCAR group (p = 0.003) (Figure 4h).

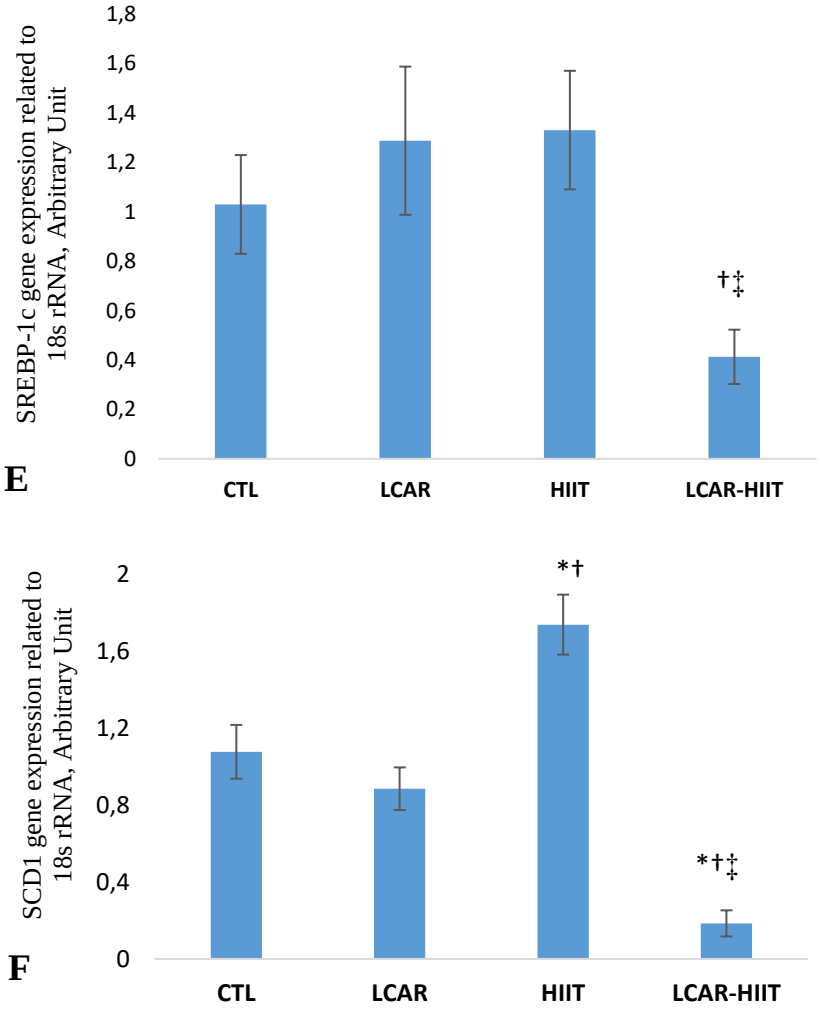


Figure 3. Relative SREBP-1c (E) and SCD1 (F) gene expression quantified by Real-Time PCR method in liver of studied groups. The groups were as follow; Untreated Control (CTL); received L-carnitine (LCAR), performed High intensity interval training (HIIT) and L-carnitine + HIIT (LCAR-HIIT), Data are expressed as Mean±SEM, p<0.05 was considered as significant, n=8. * Statistically significant compared to LCAR group, †statistically significant compared to LCAR group, ‡ statistically significant compared to HIIT group.

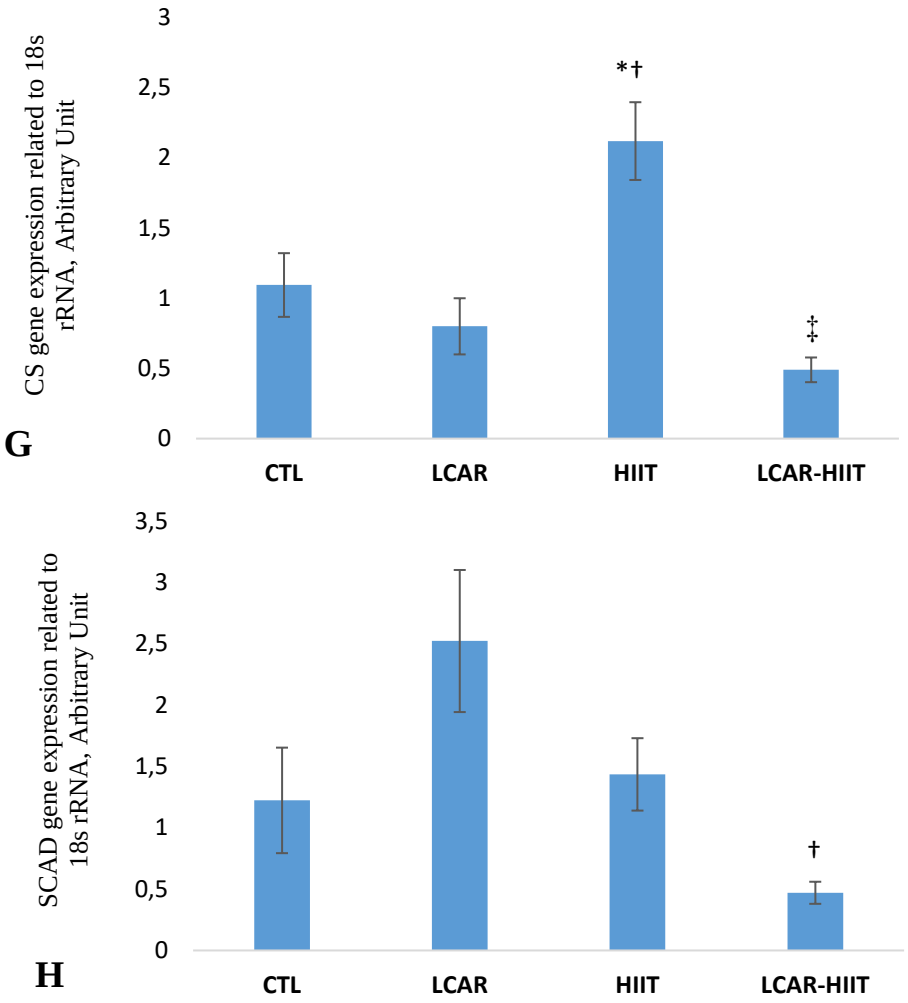
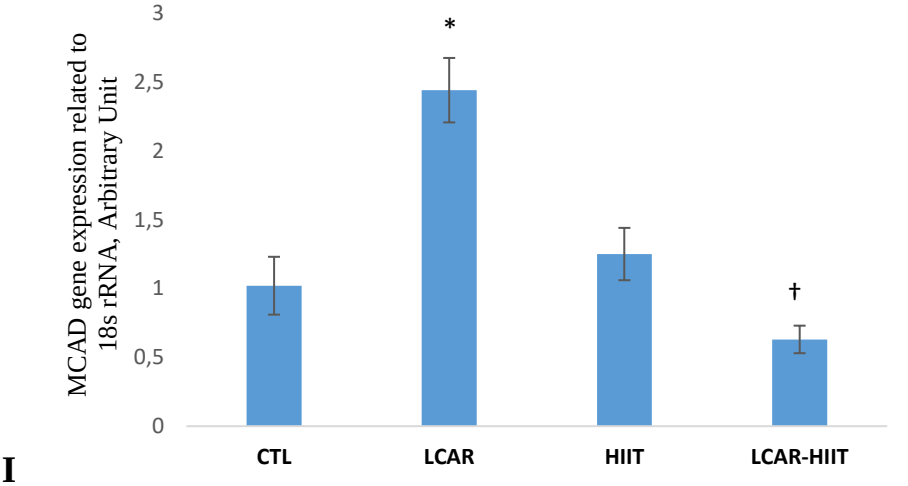


Figure 4. Relative CS (G) and SCAD (H) gene expression quantified by Real-Time PCR method in liver of studied groups. The groups were as follow; Untreated Control (CTL); received L-carnitine (LCAR), performed High intensity interval training (HIIT) and L-carnitine + HIIT (LCAR-HIIT), Data are expressed as Mean±SEM, $p<0.05$ was considered as significant, n=8. * Statistically significant compared to LCAR group, †statistically significant compared to LCAR group, ‡ statistically significant compared to HIIT group.

Carnitine administration increased MCAD expression and LCAR-HIIT decreased MCAD expression ($p < 0.001$) (Figure 5i). CPT1 expression was increased by carnitine administration and HIIT, and the LCAR-HIIT combination also increased CPT1 expression ($p < 0.05$) (Figure 5j).



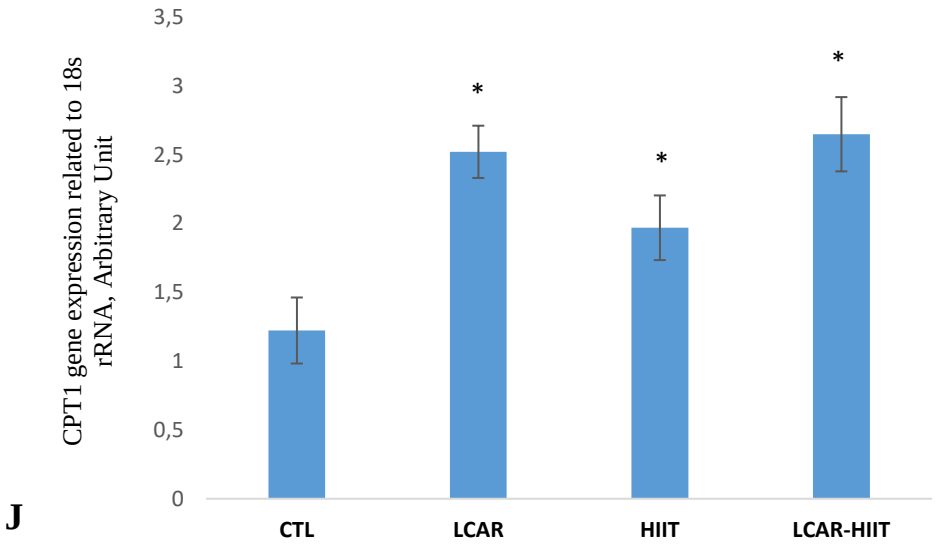


Figure 5. Relative MCAD (I) and CPT1 (J) gene expression quantified by Real-Time PCR method in liver of studied groups. The groups were as follow; Untreated Control (CTL); received L-carnitine (LCAR), performed High intensity interval training (HIIT) and L-carnitine + HIIT (LCAR-HIIT), Data are expressed as Mean±SEM, $p<0.05$ was considered as significant, n=8. * Statistically significant compared to CTL group, †statistically significant compared to LCAR group.

DISCUSSION

HIIT improves the physical function, cardiovascular health and energy metabolism [24]. But, it is not yet clear how HIIT affects the transcription factors involved in lipid metabolism and which pathways are most influenced by performing HIIT [25]. In current study, we have evaluated carnitine, HIIT, and a combination of both on the expression of genes involved in metabolism. We found that the combination of LCAR-HIIT result in significant alterations in genes expression that we discussed here.

The results of previous studies show that there is no agreement on the expression of PGC-1α after HIIT. Twelve weeks of HIIT in obese / overweight individuals increased PGC-1α expression [26]. Eight weeks of HIIT in mice increased hepatic expression of PGC-1α, PPARα and CPT1 [10]. Little and coworkers have reported that PGC-1α expression was increased in the liver of female rats after 8 weeks of HIIT [27]. On the other hand, 10 weeks of HIIT training in mice did not cause a significant change in PGC-1α expression [28]. Another study showed that 10 weeks of HIIT increased PGC-1α expression but failed to reverse the decrease in HFD diet-induced PGC-1α expression in skeletal muscle [29]. Interestingly, the combination of carnitine-HIIT led to a significant reduction in PGC-1α gene expression in this study. However, carnitine supplementation and HIIT alone slightly increased its expression but it was not significant.

AMPK expression was similar to PGC-1α expression, combination of carnitine-HIIT significantly reduced AMPK expression, while no significant change was observed between other 3 groups. Gibala and coauthors showed that HIIT increased the expression of PGC-1α and AMPK in skeletal muscle [15]. In young men, 4 weeks of HIIT resulted in significant elevation of AMPK expression, but PGC-1α expression was not changed significantly [30]. AMPK in the liver increases the expression of PGC-1α [31]. Therefore, the decrease in PGC-1α expression in the present study can be attributed to the decrease in hepatic AMPK expression. The combination of carnitine-HIIT leads to metabolic adaptations that reduce AMPK expression. It is possible that carnitine increases ATP levels by increasing lipid catabolism [32] during HIIT (it in turn increases the need for an energy source), which can lead to down-regulation of AMPK expression.

PDK4 expression decreased under the influence of the combination of carnitine-HIIT. It has previously been reported that HIIT increases PDC activity and possibly one of the mechanisms involved may be a decrease in PDK4 expression [33, 34]. In the study of Aguiar and coauthors, although the expression of PDK4 decreased after 4 weeks of HIIT training but this decrease was not significant, which is similar to the separate effect of either carnitine or HIIT on the expression of PDK4 in current study [30]. It seems that the combination of carnitine-HIIT by reducing PDK4 expression, tends to increase the conversion of lactate to pyruvate and increase PDC activity [34].

CPT1 gene expression was increased in the 3 treatment groups compared to the control group. Jiang and coauthors and Ishikawa and coauthors showed that carnitine supplementation increased CPT1 expression [35, 36]. The results of Aguiar and coauthors and Oh and coauthors studies [30, 37] are in contrast to the findings of the present study because in these two studies exercise did not affect CPT1 expression,

whereas we showed that HIIT increased CPT1 expression. Performing 10 weeks of HIIT in rats fed a high-fat diet significantly increased CPT1 expression in skeletal muscle [19] that confirmed our finding over CPT1 expression in the liver. Regarding the administration of carnitine, the results obtained from the present study are consistent with previous studies [35, 36]. In the carnitine-HIIT group, we did not see any additive or synergistic effect of carnitine and HIIT (each one alone increased CPT1 expression), and it appears that carnitine supplementation and HIIT may be used same pathway to increase CPT1 expression. But this needs further consideration in future studies.

HIIT significantly increased SCD1, but not SREBP-1c expression. Carnitine did not show any significant effect on the expression of these two lipogenic genes. Interestingly, the combination of carnitine-HIIT caused a significant decrease in the expression of SCD1 and SREBP-1c. The study by Costa and coauthors (2021) showed that HIIT reduces hepatic SREBP-1c expression [25]. Another study in obese individuals showed that HIIT reduced the lipogenic genes SREBP-1c and ACC [37]. Shen and coauthors also showed that HIIT had no significant effect on muscle SCD1 expression [19]. The findings of those studies are contrary to the findings of the present study. On the other hand, another study showed that HIIT increases muscle lipogenesis by increasing the expression of FAS, ACC and SCD1, and despite the fact that the target tissue was muscle, the findings are consistent with the results of our study [38]. In general, according to the above mentioned studies, there is no general agreement on the effect of HIIT on lipogenesis. Carnitine significantly increased MCAD expression and HIIT did not show any significant effect on MCAD and SCAD expression. However, the combination of carnitine-HIIT reduced the expression of MCAD and SCAD in the liver compared to the carnitine group. Ishikawa and colleagues showed that carnitine increased MCAD expression in the liver which confirmed our findings [36]. In HIIT, the need for energy increases which is met by the anaerobic glycolytic system that leads to an increase in blood lactate. When HIIT is continued the lactate production will no longer be at higher and will decrease over time due to metabolic adaptations [38]. On the other hand, lactate increases the expression of PGC-1α and in general, lactate is involved in HIIT-induced adaptations [40].

CONCLUSION

Administration of carnitine and HIIT is very useful and conduct this by reducing the expression of lipogenic genes such as SREBP-1c and SCD1 as well as increasing the expression of CPT1. The higher content and natural configuration of mitochondria performed by HIIT increase the rate of FA oxidation and ultimately reduce the overall lipid deposition in the liver which confirmed by the weight of animal at the final day of the study.

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