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# The effects of exercise on skeletal and heart muscle citrate synthase and carnitine palmitoyltransferase mRNA expressions in high-calorie fed rats

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**Abstract.** In this study, the effect of high-calorie feeding and aerobic exercise on skeletal and cardiac muscle citrate synthase (CS), carnitine palmitoyltransferase-I (CPT-I), and -II (CPT-II) mRNA expressions were evaluated. Genetically non-obese rats were grouped as normal-high calorie and sedentary-exercising. Gastrocnemius-soleus and heart muscles' CS, CPT-I, and CPT-II expressions and skeletal muscles' histopathological characteristics were evaluated. High-fat diet had increased body weight by 10% and aerobic exercise did not make any difference. Skeletal muscle CS expression was increased significantly in normal-calorie exercising group. Exercise and high-fat diet did not change CPT-I and CPT-II expressions in both heart and skeletal muscle. Histopathological evaluations demonstrated increased cytoplasmic lipid droplets in high-calorie fed sedentary rats, and exercise had reduced lipid droplets in skeletal muscle. Also, both mitochondria and nuclei distribution were impaired in high-calorie groups. In conclusion, aerobic exercise without food restriction was not enough to make significant changes in fat transportation mechanism into skeletal and heart muscle.

**Key words:** Aerobic exercise — High-fat diet — Mitochondria — Lipid droplets

## Introduction

Energy homeostasis and maintenance of body weight are possible by adjusting the balance between food intake and calorie consumption. The increase in the ratio of food intake to the energy consumed changes this balance positively and causes weight gain. Besides genetic factors, weight gain and obesity, especially in industrialized societies, have a direct relationship with a sedentary lifestyle and easy access to high-calorie foods (Gale et al. 2004; Romieu et al. 2017; Dragano et al. 2020).

The relationship between weight gain and obesity with metabolic and cardiovascular diseases is stated in the literature (Mokdad et al. 2003; Grundy 2004). On the other hand, obesity is associated with skeletal muscle mitochondrial dysfunction, lipid metabolism dysregulation, abnormal protein turnover (Koves et al. 2008; Heo et al. 2017), and the decrease in citrate synthase (CS) levels (Bonnard et al. 2008; Heo et al. 2017). Besides, due to the enhanced oxidative stress and the increase in intramyocellular triglyceride levels, skeletal muscle mitochondrial content and oxidative capacity decrease in obesity (Koves et al. 2008; Dahlmans et al. 2016).

On the other hand, a fat-rich diet negatively affects mitochondrial functions, and this type of nutrition causes an increase in proteins associated with mitochondrial damage and apoptosis (Dungan et al. 2016; Heo et al. 2017), reduces the ratio of mitochondrial DNA (mtDNA) to non-coding DNA (ncDNA) and causes mitochondrial dysfunction (Bonnard et al. 2008; Heo et al. 2017).

Citrate synthase (CS) is a citric acid cycle enzyme, and the activity of this enzyme is one of the most important

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biomarkers of mitochondrial content and muscle oxidative capacity (Wiegand Remington 1986; Larsen et al. 2012). On the other hand, carnitine palmitoyl transferase-I (CPT-I) (Petrick Holloway 2019) and carnitine palmitoyltransferase-II (CPT-II) are located in the outer and inner membrane of mitochondria, respectively (Lehmann et al. 2017). These enzymes have a crucial role in transporting long-chain fatty acids into mitochondria for beta-oxidation. In skeletal muscle, CPT-I activity (Kelley et al. 1999; Kim et al. 2000) decreases in addition to CS enzyme activity due to obesity (Kim et al. 2000; Bonnard et al. 2008; Heo et al. 2017). On the other hand, the mitochondrial CPT-I activity level is very high in the heart muscle (McGarry et al. 1983; Power et al. 1997), as fats are the primary energy resource (Lopaschuk Gamble 1994). The regulation of CPT-I activity in the heart and skeletal muscle may change depending on the fatty acid content of the foods (Power et al. 1997). Some studies indicate fat-rich diet may increase the amount of CPT-I in the left ventricle (Modrego et al. 2013), but conversely, some publications state that the expression does not change with nutrition (Zhang et al. 2016).

It is known that lipid oxidation rate changes depending on many factors such as hormones, nutrition, gender, duration, and modality of the exercise (Spriet 2014; Jahansouz et al. 2018). Aerobic exercise intensities below 65% of maximal oxygen uptake are described as low-moderate, and lipid oxidation is acknowledged higher during these types of physical activities (Achten Jeukendrup 2004; Bogdanis et al. 2008). On the other hand, regular aerobic training triggers many adaptive responses in the body. The increase of capillary density, muscle fibre type changes, increased oxidative enzymes, and changes in substrate utilization are the mentioned cardiovascular and pulmonary adaptive responses (Fiuza-Luces et al. 2013). In addition, transportation and oxidation of fatty acids to skeletal muscles, migration of fatty acids to mitochondria, increased volume and number of mitochondria, and increased expression of mitochondrial enzymes are also described as adaptive metabolic responses (Lundby Jacobs 2016; Granata et al. 2018; Maunder et al. 2018). Besides, studies indicate that an increase in CPT-I (Schenk Horowitz 2006; An et al. 2016) and CS activity and expression(Siu et al. 2003) may occur according to increased oxidative capacity in skeletal muscle as a response to exercise. Contrary to these studies, it was reported that skeletal muscle CPT-I and CPT-II expressions do not change with exercise (Carnevali et al. 2012). On the other hand, CS and CPT-II expressions can increase in the left ventricle (Iemitsu et al. 2003; Siu et al. 2003), but CPT-I expression does not change with exercise (Iemitsu et al. 2003). These results point out that the cardiac mitochondrial enzyme responses to exercise are controversial. A limited number of publications in the literature evaluate the metabolic responses of exercise and nutrition on the left ventricle and skeletal muscle at enzymatic perspective.

This study aims to evaluate the effect of high-calorie feeding and regular aerobic exercise on the skeletal and cardiac muscles CS, CPT-I, and CPT-II enzyme expressions in genetically non-obese rats.

**Table 1.** Nutrient contents of the chow

| | Standard chow | High calorie chow |
|---|---|---|
| Carbohydrate (%) | 55 | 55 |
| Protein (%) | 23 | 23 |
| Fat (%) | 6 | 6 (min) |
| Cellulose (%) | 7 | 7 (max) |
| Ash (%) | 8 | 8 (max) |
| Salt (%) | 1 | 1 (max) |
| Total calorie (kcal/kg) | 2800 | 3100 |

## Materials and Methods

The study was performed in Cukurova University Faculty of Medicine, Department of Physiology. Ethics committee approval (5; 6; 2015) was obtained from the Animal Experiments Local Ethics Committee of Çukurova University for Animal Experiments.

### *Animals*

Wistar-Albino male rats were housed four *per* cage at the laboratory under conditions: 23 ± 1°C, 40–60% humidity, 12-hour light/dark cycle, and fed chow and water *ad libitum* throughout the study. The animals were weighed once a week and before the exercise for the exercising animals.

In this study, two types of chow, normal calorie and high calorie, were used. The nutrient contents of the chow were presented in Table 1. High-calorie chow had 10% higher calories than standard chow. There were no findings suggest that general health conditions are affected in animals fed with high-calorie chow.

### *Experimental design*

Four-week-old rats were randomly divided into four groups: normal-calorie fed-sedentary (NC-S), normal-calorie fed-exercising (NC-E), high-calorie fed-sedentary (HC-S), and high-calorie fed-exercising (HC-E). The animals were fed according to their nutrition groups for the following eight weeks without any exercise training. At the age of 12 weeks NC-E and HC-E groups started

exercise training, and NC-S and HC-S groups remained sedentary lifestyles.

*Exercise protocol*

The first day of the adaptation period to treadmill running started with 5 minutes of inactive phase on the treadmill, and rats completed 40 min running at 15 m/min. The adaptation period was completed in 5 days and each day running speed and duration increased gradually. Following the adaptation period, rats performed running exercises 1 h a day, five consecutive days a week for 8 weeks. The running speed was 20 m/min, and the treadmill's slope was fixed at 8%. This running speed has been reported as an average of 70% levels of the maximal aerobic capacity of rats (Brooks White 1978; Hoydal et al. 2007).

*Surgical protocol*

The rats were sacrificed on the day following the end of the protocol (twenty-week-old for both groups). Rats were anesthetized with an intraperitoneal mixture of ketamine 2.0 ml, xylazine 1.0 ml, and saline 1.0 ml (0.2 ml/100 g weight) (Arango-Gonzalez et al. 2012; Mitsunaga Junior et al. 2012). The gastrocnemius-soleus (GC/S) muscle group was removed from the inguinal region by detaching it from the surrounding tissues (Ergen et al. 2005), weighed with a scale (Shimadzu; 0.0001 g sensitivity), and frozen in liquid nitrogen. The heart was rapidly excised from the rats and weighed. The left ventricle was dissected, weighed, and immediately frozen in liquid nitrogen. All samples were stored at −80°C until later analyses.

*Histopathological protocol*

Open muscle biopsy was done from the right GC/S muscles. Muscle biopsy was frozen in isopentane precooled in liquid nitrogen for cryosection. Muscle sections were stained with hematoxylin and eosin (H&E), periodic acid Schiff (PAS), Kongo red, Oil Red O stains for histochemistry. Muscle sections were also stained with nicotinamide adenine dinucleotide (NADH)-tetrazolium reductase (NADH-TR), cytochrome oxidase (COX), COX–SDH, adenosine triphosphatase (ATPase), Sudan black for enzyme histochemistry, and fast myosin, slow myosin, and neonatal myosin stains for immunohistochemistry. Muscle fibers were transversely oriented and evaluated under a light microscope (Olympus BX51), and the images of stained slides were captured using a camera raw 70 Olympus E330.

*Quantitative Real-Time PCR analysis*

Total RNA was isolated from rat GC/S and left ventricle muscles using Trizol protocol (according to the manufacturer's instructions, Invitrogen, Carlsbad, California, USA). The RNA concentration was measured by spectrophotometric (Shimadzu UV-1280) determination using 4 μl of isolated RNA for each muscle sample. Complementary DNA (cDNA) was obtained using High-Capacity cDNA Reverse Transcription Kit (Thermo Fischer) from purity-checked RNA samples. Primer sequences used for amplification were given in Table 2.

The reverse transcriptase and real-time PCR methods were used to estimate the concentrations of the mRNA of β-actin, CS, CPT-I, and CPT-II. The protocol was arranged as 2 min at 50°C, 10 min at 95°C, and 15 s of denaturation at 95°C, and 1 min at 60°C in the form of 40 cycles (Real Time Thermal Cycler, Light Cycler 480 Roche).

The number of cycles in which the samples exceed the minimum value required to observe the fluorescent signal amount in the PCR is expressed by the cycle threshold (Ct) value. For each sample, a ΔCt value was obtained by subtracting β-actin values from the gene of interest. The ΔCt value of the control group was subtracted from the sample to derive a ΔΔCt value. The change in gene expression was calculated by the formula $2^{-\Delta\Delta Ct}$ (Livak Schmittgen 2001).

*Statistical analysis*

SPSS 21 program was used for statistical evaluations. Test of normality was evaluated by the Shapiro-Wilk test. Normally distributed data were evaluated using One-way ANOVA. *Post hoc* differences between the means from groups were determined *via* Bonferroni test. Non-normally distributed data were evaluated using the Mann Whitney U test. Results were given as mean ± SEM. Significance was set at $p < 0.05$. Changes in mRNA expression levels were expressed as multiple increases (Livak Schmittgen 2001).

**Table 2.** Real-time PCR primer sequences

| Gene | Forward Primer (Sense) | Reverse Primer (Antisense) |
|---|---|---|
| β-actin | 5'-GAAGATCCTGACCGAGCGTG-3' | 5'-AGCACTGTGTTGGCATAGAG-3' |
| CS | 5'-CCGTGCTCATGGACTTGGGCCTT-3' | 5'-CCCCTGGCCCAACGTAGATGCTC-3' |
| CPT-I | 5'-GCCTCAACACAGAACACTCATG-3' | 5'-GTACTTGGAGACGATGTAGAGG-3' |
| CPT-II | 5'-GAGCCCCTAGTAGGCCCTTA-3' | 5'- AGGCTTCTGTGCATTGAGGT-3' |

CS, citrate synthase; CPT, carnitine palmitoyltransferase.

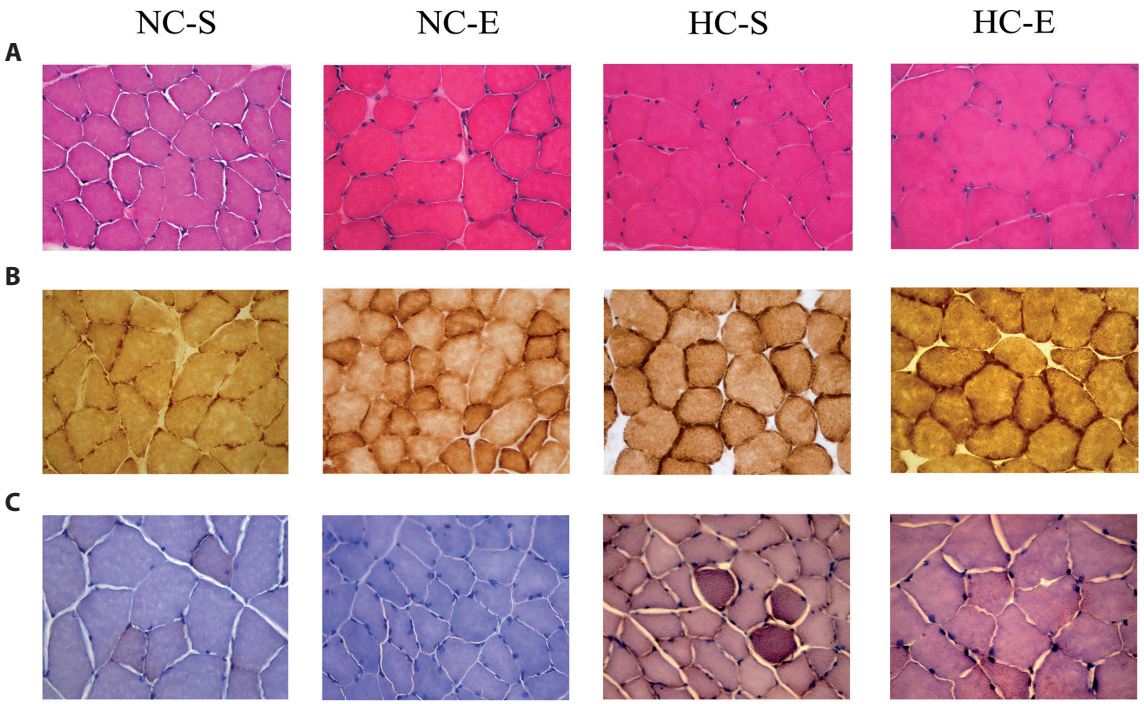


**Figure 1.** Histopathological changes in skeletal muscle. Muscle sections were stained with hematoxylin and eosin (**A**; magnification ×400), cytochrome oxidase (**B**; magnification ×400), Oil Red O (**C**; magnification ×400). NC-S, normal-calorie fed-sedentary group; NC-E, normal-calorie fed-exercising group; HC-S, high-calorie fed-sedentary group; HC-E, high-calorie fed-exercising group.

## Results

Rats were weighed once a week. Initial and final body weights, GC/S muscle weights, heart weights, and heart/final body weight ratios of rats were presented in Table 3.

*Histopathology observation of skeletal muscle*

The results of pathological observation are shown in Figure 1. In H&E staining, subsarcolemmal nuclei were observed with a slight size difference in muscle fibers in the exercising groups compared to the NC-S. In the HC-S, less frequent internal nuclei were detected. In COX staining, intramyofibrillar and subsarcolemmal mitochondrial distribution is remarkable in the NC-E. In the HC-E, although the vision is similar to the NC-E group, a mild mitochondrial enlargement is observed in type 1 fibers. In Oil Red O staining, lipid droplets in type 1 fibers are scattered and less frequent in the NC-E than the NC-S. A more generous amount of intracellular lipid droplets has been observed in animals fed with high calories. On the other hand, lipid droplets in the HC-S group were more prominent than in the other groups. In the HC-E, lipid droplets were reduced compared to the HC-S.

**Table 3.** Effect of exercise and diet on body weight, GC/S muscle weight and heart weight

| | NC-S | NC-E | HC-S | HC-E |
|---|---|---|---|---|
| Initial body weight (g) | 66.20 ± 12.56 | 73.53 ± 7.24 | 67.13 ± 9.40 | 75.73 ± 8.49 |
| Final body weight (g) | 255.07 ± 3.59 | 266.13 ± 5.78 | 294.93 ± 7.69$^{*\#}$ | 291.27 ± 6.61$^{*\#}$ |
| GC/S weight (g) | 1.50 ± 0.04$^{‡}$ | 1.72 ± 0.04 | 1.72 ± 0.05 | 1.84 ± 0.05 |
| Heart weight (g) | 0.75 ± 0.01 | 0.79 ± 0.01 | 0.81 ± 0.02 | 0.87 ± 0.03$^{*\#}$ |
| Heart/final body weight ratio | 0.0030 ± 0.0002 | 0.0030 ± 0.0002 | 0.0028 ± 0.0002 | 0.0030 ± 0.0002 |

$^{*}$ $p < 0.01$ *vs.* NC-S group, $^{\#}$ $p < 0.01$ *vs.* NC-E group, $^{‡}$ $p < 0.01$ *vs.* all groups. GC/S, gastrocnemius-soleus; NC-S, normal-calorie fed-sedentary group; NC-E, normal-calorie fed-exercising group; HC-S, high-calorie fed-sedentary group; HC-E, high-calorie fed-exercising group.

*Citrate synthase*

In the gastrocnemius-soleus muscle, the NC-S group had the lowest CS enzyme expression. In the NC-E group, CS mRNA expression was increased by approximately 65% compared to the NC-S group ($p < 0.05$). In the HC-E group, there was a 32% increase observed compared to the HC-S group. However, this difference did not express statistical significance (Fig. 2A). Left ventricular CS enzyme expression did not change depending on the exercise. Citrate synthase expressions decreased by 32% among sedentary groups and 14% among exercising groups in terms of nutrition with high calories ($p > 0.05$) (Fig. 2B).

*Carnitine palmitoyl transferase-I*

No significant differences were observed for CPT-I enzyme expressions in skeletal muscle (Fig. 3A) and left ventricle (Fig. 3B) in response to aerobic exercise and high-calorie nutrition. Left ventricular CPT-I expression of the HC group increased by 50% due to aerobic training, but this change was not statistically significant because of the variation between rats. On the other hand, the same enzyme expression was suppressed by 50% in the HC-S group than the NC-S group ($p > 0.05$).

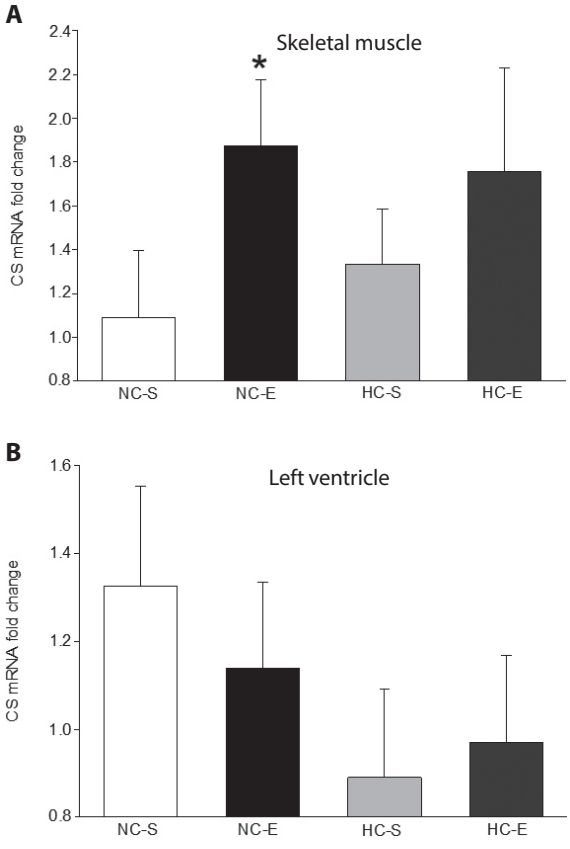


**Figure 2.** Citrate synthase (CS) enzyme expression levels in skeletal muscle (**A**) and in the left ventricle (**B**). * $p < 0.05$ significantly higher than NC-S group. For abbreviations, see Fig. 1.

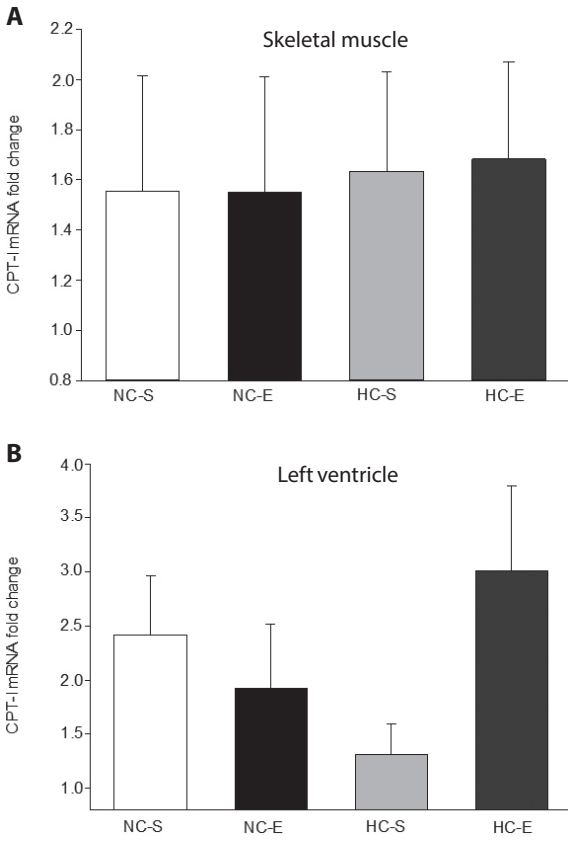


**Figure 3.** Carnitine palmitoyltransferase-I (CPT-I) enzyme expression levels in skeletal muscle (**A**) and in the left ventricle (**B**). For abbreviations, see Fig. 1.

*Carnitine palmitoyl transferase-II*

The CPT-II expression of skeletal muscle in the NC-E group increased by 41% compared to the sedentary group ($p > 0.05$). In addition, there were no significant differences between the HC-S and HC-E groups (Fig. 4A). On the other hand, neither exercise nor high-calorie diet models induced any significant difference in CPT-II enzyme expressions of the left ventricle (Fig. 4B).

## Discussion

In this study, only 10% excess calorie consumption was sufficient for significant weight gain in genetically non-obese rats with similar initial body weight. In sedentary rats fed with high-calorie chow for eight weeks, final body and GC/S

muscle weights increased significantly compared to their normal calorie-fed equivalents. Moraal et al. (2012) stated that *ad libitum* feeding may increase the possibility of variation in body weight in rats. Since *ad libitum* calorie intake did not cause body weight heterogeneity in our study, we may interpret that animals were fed equally in their cages. It was also important to find that exercise without any food restriction did not cause weight loss, which is in agreement with the data presented by Kawanishi et al. (2018). Our finding indicates that calorie intake is one of the most important factors to determine body weight. Since the nutrition style of this study caused weight gain, our model may represent a group of overweight people in the world whose calorie intake is slightly higher than the others. In addition, low-moderate intensity aerobic exercise caused a significant increase in skeletal muscle CS expression in the NC-E group. Contrary to the literature, NC-S and HC-S groups' skeletal muscle CS mRNA enzyme expressions were not significantly different. Even though Koves et al. (2008) had shown a decrease in CS enzyme activity, the fat content of their chow and the gain in the body weight of their animals were higher than in our study. These disparities may explain the non-significant difference in CS enzyme expression. This study is one of the unique researches that analyze left ventricular enzyme expressions, and our results indicate that high-calorie diet and exercise did not change CS, CPT-I and CPT-II enzyme expressions in rat left ventricle muscle. Another important finding of our study is that heart weight did not increase in proportion to body weight. This finding suggests that our exercise intensity and exercise duration was not enough to activate signal pathways that trigger cardiac hypertrophy (de Souza Cordeiro et al. 2019).

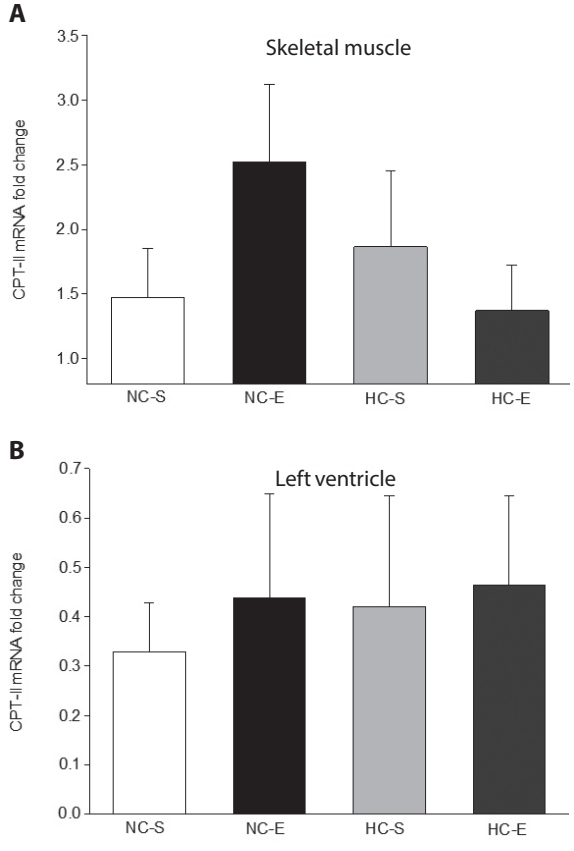


**Figure 4.** Carnitine palmitoyltransferase-II (CPT-II) enzyme expression levels in skeletal muscle (**A**) and in the left ventricle (**B**). For abbreviations, see Fig. 1.

Since all the rats used in this study were at the same age, the increase in GC/S muscle weight may be associated with both exercise and food consumption. Histopathological images showed that mitochondrial distribution was compatible with exercise and fat droplets tend to decrease in the exercising groups. However, in the high-calorie sedentary group, intracellular fat droplet content was increased, together with the impaired distribution of skeletal muscle nuclei and mitochondria. Fatty acid accumulation in skeletal muscle may impair mitochondrial dynamics (Chen et al. 2018), which disrupt fuel homeostasis together with beta-oxidation (Galgani et al. 2008; Koves et al. 2008; Heo et al. 2017). Different exercise modalities may regulate cellular signal pathways and improve the lipid oxidation enzyme activity and/or expression (Shen et al. 2015; Granata et al. 2018). Our histopathological findings indicate that high-fat nutrition and sedentary lifestyle may cause fat droplet accumulation within the skeletal muscle as expected. Moreover, GC/S muscle weight gain of high calorie fed sedentary rats was found to be related with an increase in adipose tissue mass together with intramyocellular lipid droplet accumulation (Gopalan et al. 2021). However, aerobic exercise reduces fat droplet content in the skeletal muscle without significant differences in CPT-I and II mRNA expression levels. These results indicate that aerobic exercise may induce adaptive metabolic changes by different mechanisms other than CPT-I and II mRNA expression.

### *Skeletal muscle enzyme expression*

Citrate synthase is one of the main regulatory enzymes of energy metabolism and is accepted as an essential biomarker of muscle oxidative and respiratory capacity (Wiegand Remington 1986; Larsen et al. 2012). Many studies reported that weight gain and obesity cause a decrease in oxidative capacity and CS enzyme activity together with mitochondrial dysfunction in skeletal muscle (Bonnard et al. 2008; Koves et al. 2008; Dahlmans et al. 2016; Heo et al. 2017). On the other hand, both human and animal studies indicate that 8–12 weeks of moderate exercise intensities are sufficient to increase aerobic capacity, skeletal muscle CS expression, and

activity (Abdelmalki et al. 1996; Dyck et al. 2000; Leek et al. 2001; Siu et al. 2003; Galbes et al. 2008; Farenia et al. 2019). Aerobic exercise-induced long-term adaptive responses may upregulate the oxidative enzyme activities and expressions in skeletal muscle (Granata et al. 2018). Indeed, it is also known that low-intensity regular exercise increases CS enzyme activity and gene expression in the skeletal muscle of rats. In this study, similar to the literature, it was found that exercise had increased skeletal muscle CS enzyme expression significantly compared to the sedentary group in animals fed with normal calorie chow. Many studies in the literature, the exercise intensities which increased CS enzyme expressions were higher than in our study (Sanchez et al. 1983; Abdelmalki et al. 1996; Dyck et al. 2000). On the other hand, the significant change in CS enzyme expression showed that our relatively low-intensity training model was sufficient to activate metabolic adaptive response pathways.

In the literature, although many studies were analysing the effects of high-calorie nutrition, to our knowledge, there was no study analysing CS expression in skeletal muscle in response to weight gain and exercise due to high-calorie nutrition in genetically non-obese rats. However, CS enzyme expression of the HC-E group, which were fed with high-calorie and gained weight, increased about 32%, but this difference was not significant due to inter-individual variabilities. The possible effects of weight gain after feeding with high calorie on skeletal muscle may induce intracellular lipid accumulation in sedentary animals, which may negatively affect the CS enzyme expression pathway (Kelley et al. 2002; Christe et al. 2013). The exercise intensity, which activates the adaptation process in normal-weight animals, may have been insufficient in some of the high-calorie fed animals. Indeed, the variation between gene expression values suggests that this response may differ among animals.

Human and animal studies indicate a decrease in CPT-I enzyme activity, which is the rate-limiting enzyme in fatty acid oxidation in skeletal muscle, due to obesity (Kelley et al. 1999; Simoneau et al. 1999; Koves et al. 2008). In this study, although high-calorie feeding caused a significant increase in body weight both in the sedentary and exercise groups that did not make a significant difference in the enzyme expression. In the literature, similar to our findings, there was no increase in CPT-I enzyme expression, although weight gain was observed in groups fed with high-fat diet (Frier et al. 2011; Shen et al. 2015). On the other hand, regardless of the nutrition type, exercise did not affect CPT-I enzyme expressions, and the difference between HC-E and NC-E groups was not statistically significant. Shen et al. similar to our study, reported that CPT-I expression level did not change in rats according to moderate running exercise and high-fat feeding (Carnevali et al. 2012; Shen et al. 2015). The low-to-moderate exercises, which were thought to be more effective in fat oxidation, could not generate sufficient signals to increase CPT-I and CPT-II enzyme expressions. Indeed, although we have not evaluated the enzyme activity in our study, it has been stated that similar training model may not change the enzyme expression; however, the enzyme activity may increase (Carnevali et al. 2012). The increase in the enzyme activity without any change in the enzyme expression level claims that enzyme activity may have a different metabolic pathway than gene expression.

A limited number of studies evaluated the effect of exercise and high-calorie consumption on CPT-II enzyme expression. Yan et al. (1995) stated that 14 days of moderate-intensity treadmill exercises increase rat skeletal muscle CPT-II mRNA level by approximately 25%; however, this result was not statistically significant, in accordance with our study. On the other hand, Carnevalli et al. (2012) found that one hour of moderate-intensity continuous swimming training for eight weeks caused a non-significant change in CPT-II expression and activity. These results suggest that the CPT-II enzyme may be regulated by more complex mechanisms than CS and CPT-I enzymes, and these enzyme expressions are not affected by weight gain and low-moderate exercise intensities.

*Left ventricular enzyme expression*

Heart muscle utilizes lipids as the main energy resource. Therefore, CS, CPT-I, and CPT-II enzyme expressions and activities are arranged most efficiently in heart muscle (Siu et al. 2003). A limited number of studies in the literature research the relationship between left ventricular CS and CPT enzyme complex, nutrition, and exercise. In our study, it was found that training did not cause a significant change in left ventricular CS expression, which indicates that the moderate exercise intensity did not cause cardiac strain. However, it was stated that the higher exercise intensities (28 m/min) increase CS enzyme expression in the rat heart muscle without causing any change in enzyme activity (Siu et al. 2003). Another study indicates that the amount of CS decreases in the heart muscle similar to skeletal muscle due to the high-fat diet and obesity, which is compatible with our results (Heo et al. 2017).

In our study, the expressions of CPT-I and CPT-II enzymes did not change in rats, suggesting that myocardial lipid metabolism was not affected by 10% increase in calorie consumption and low-moderate training. Previous studies stated that nutrition models and exercise did not increase left ventricular CPT-I expression in accordance with our results (Iemitsu et al. 2003; Zhang et al. 2016). In contrast, Iemitsu et al. (2002) showed that left ventricular CPT-I enzyme expression increased in 5 weeks of swimming exercise in elderly (21 months-old) and normal-weight rats without any alteration in left ventricular CPT-II expression. On the other hand, another study presented an increase in the level of left ventricular CPT-II expression

in rats due to swimming exercise for 15 weeks (Iemitsu et al. 2003). The long exercise durations of both studies are another result that suggests sufficient signal could not be generated in low-moderate exercise intensities for the stimulation of the regulatory system of gene expression in the heart. This study shows that low-moderate exercise intensities could not increase gene expression levels of CS, CPT-I, and CPT-II enzymes in younger rats' left ventricle with dietary-induced weight gain.

## Conclusion

This study had shown that only ten percent high-calorie consumption was enough to gain body weight in Wistar-Albino rats. Our findings indicate that aerobic exercise without food restriction may not be enough to reduce body weight. Aerobic exercise caused a significant increase in skeletal muscle CS enzyme expression in the normal-calorie group. However, exercise and the high-calorie diet did not cause any significant difference in skeletal and left ventricular muscle CS, CPT-I, and CPT-II enzyme expressions. Although the body weights of the animals were normally distributed, the heterogeneity of the enzyme expression levels among animals may be considered as an inter-individual difference. Due to the inter-individual differences, increasing the number of animals may be a strategy to reduce the deviations in enzyme expressions to show possible significance. Future studies are required to show the effect of different exercise modalities and nutrition types in genetically non-obese animals on mentioned enzyme activities and the expressions.

**Conflict of interest.** The authors declare no conflict of interest.

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