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Effect of Eight Weeks High-Intensity Interval Training on PLIN5 and UCP3 Genes in Skeletal Muscle of Obese Rats

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ABSTRACT

Background: Prescription of sports activity is an effective strategy for treating metabolic disorders, including obesity. The study aimed to determine the effect of 8 weeks of high-intensity interval training (HIIT) on PLIN5 and UCP3 gene expressions in skeletal muscle of obese rats.

Methods: Fifty male Wistar rats were randomly divided into two groups of control receiving standard diet (CSD=20) and those with high-fat diet (HFD=30) for obesity induction (10 weeks). Ten rats from each group were selected to evaluate obesity (target weight: 350±20 g and serum triglyceride). The rest of high-fat diet group were randomly divided into 2 subgroups of obese control (OC=10) and HIIT high-fat diet(HIIT-HFD=10) and 10 in control standard diet rats were applied as the control standard diet in exercise phase. The HIIT protocol on the treadmill included 8 weeks, 5 sessions per week (10-degree incline) with 20 bouts (2 minutes maximum speed), and 1-minute active recovery. PLIN5 and UCP3 gene expressions in the gastrocnemius muscle were measured by real time-PCR and plasma triglycerides by ELISA.

Results: The triglyceride (TG) level was higher in CSD compared to the HFD group. The weight, TG level and PLIN5, and UCP3 gene expressions significantly reduced in the experimental group compared to the CSD and OC groups.

Conclusion: The values of energy intake, body weight, and PLIN5 and UCP3 gene expressions denoted to activated thermogenesis and fatty oxidation. Physical activity resulted in weight loss, and decreased gene expression to deal with this trend.

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Introduction

Obesity is the most common metabolic disorder (1) that has been linked to a variety of metabolic, behavioral, genetic, biological, and environmental factors. Consuming high-fat foods and reducing physical activity can lead to an energy imbalance that ultimately causes obesity (2). Dietary fat can increase intramuscular triglyceride (IMTG), which plays an important role in metabolic diseases such as obesity (3). IMTGs are droplets of lipids stored adjacent to mitochondria and serve as an energy source during exercise (4). The regulation of lipid

metabolism affects the entire body's metabolism, weight, and obesity; therefore, weight loss reduces the accumulation of IMTGs and improves fat oxidation and mitochondrial function (5).

Perilipins (PLINs) are a family of phosphorylated proteins that are associated with lipid droplets, including perilipins 1 to 5 (6). They are responsible for the formation, structure, and destruction of fat droplets in various cells such as lipid cells, steroid-producing cells, liver cells, and heart and skeletal muscles (7, 8). PLIN5 plays a significant role in the synthesis and breakdown of lipid droplets and is expressed in highly oxidative tissues such as brown adipose tissue, skeletal muscles, the heart, and the liver (9). PLIN5 increases the connection between fat droplets and mitochondria in these tissues (10). It acts as a barrier against triglycerides by coveringlipid droplets, thus increasing their storage and balances energy loss. Furthermore, PLIN5 inhibits lipolysis when energy needs are low by regulating enzyme activity and balancing energy consumption (11).

Reports suggest that genetics contribute to obesity by 30-70% (12). The relationship between uncoupled protein 3 (UCP3) and obesity has been confirmed. Previous studies have shown that UCP3 is expressed in skeletal muscle and catalyzes adaptive thermogenesis by increasing proton transfer to the mitochondrial inner membrane (13). UCP3 separates substrate oxidation from ADP to ATP phosphorylation and leads to rapid oxygen consumption and heat production. The metabolic changes after exercise and weight loss may lead to UCP3 mRNA and protein expression. Itseems that UCP3 plays a role in the oxidation of fatty acids and leads to weight loss (14). Gene regulation of UCP3 affects plasma free fatty acid (FFA) levels; hence, UCP3 mRNA changes following exercise may affect plasma FFA levels (15).

Prescription of sports activity is an effective strategy for treating metabolic disorders, including obesity. Skeletal muscle is known as an endocrine gland that can control energy balance, body composition, and adiposity through its paracrine and endocrine products (16). The gastrocnemius muscle contains type I (oxidative) and type II (glycolytic) fibers. PLIN5 genes are present in type I fibers, while UCP3 genes are present in type II fibers (17). The effect of exercise on PLAIN5 and UCP3 gene expression in muscle tissue as regulatory markers of lipolysis is not yet clear. A few studies have reported that high-intensity interval training (HIIT) decreased the amounts of PLIN5 in visceral adipose tissue in rats (18). On the other hand, endurance exercise increased the amounts of PLIN5 in human muscle tissue (17). HIIT training also decreased UCP3 gene expression and protein in skeletal muscle in rats (19), while endurance training increased UCP3 gene expression in rat muscle tissue (20).

Hence, it is unclearhow UCP3 and PLIN5 genes of muscle tissue respond to exercise as lipolysis regulatory indicators? HIIT is considered an effective exercise method for fat loss and leads to metabolic adaptations such as improving oxygen consumption, mitochondrial biogenesis, and fat oxidation (21). Still, the effect of interval training on the expression of PLIN5 and UCP3 genes in muscle remains unknown. Further studies on exercise and the expression of PLIN5 and UCP3 genes are necessary to understand the molecular metabolism of weight loss in patients with obesity. So this study was undertaken to determine the effect of eight weeks HITT on PLIN5 and UCP3 genes in skeletal muscle of obese rats

Materials and Methods

This study employed an experimental design with post-test measures and a control group, utilizing fifty male Wistar rats (age=8 weeks, weight=180±20 g) procured from Kerman Physiology Research Center in Iran. The rats were housed under standard conditions, including 22±2 °C temperature, a light-dark cycle of 12:12 h, and relative humidity of 50%, in conventional cages with free access to food (special field rat pellets, Javane Khorasan Company, Iran) and water. The rats'body weight was recorded three days a week throughout the study. After two weeks of acclimation to the laboratory environment, the rats were randomly divided into two groups for the first phase of the experiment (induction of obesity): a standard diet group (CSD=20) and a high-fat diet group(HFD=30), which lasted for ten weeks. At the end of this period, ten rats from each group were selected based on their target weight (350±20 g) and serum triglyceride levels (≥150 mg/dL) to test the effect of obesity. In the second phase (exercise), the HFD group rats were sorted into two subgroups matched by body mass including an obesity control group (OC=10) and a high-intensity interval training-high-fat diet group (HIIT-HFD=10). The CSD group (CSD=10) followed the study as the standard diet control group. During the exercise protocol, two rats died, thereby leaving 8 rats in each group for data analysis. The CSD consisted of 10% fat, 70% carbohydrates, and 20% protein, while the HFD comprised 60% fat, 20% carbohydrates, and 20% protein**.** In the study, all interventions and experiments on animal subjects were conducted by international guidelines and ethical standards and have been registered by the ethical approval code (IR.UK.VETEMD.REC.1401.001).

The exercise protocol consisted the warming-up phase involved a 2-minute treadmill exercise at 10 m/min. The maximum speedwas determined by increasing the treadmill speed by 1.8 to 2 m/min every two minutes, with a starting speed of 12 m/min, until the rats were unable to continue running; the last recorded speedwas considered the maximum running speed for each rat. During the HIIT exercise protocol, the rats underwent 5 sessions per week on a treadmill set at a 10-degree incline and 20 bouts. Each bout comprised 2 minutes of activity at an intensity of 90% of the maximum running speed, followed by 1 minute of active recovery over an 8-week period. The treadmill speed increased from 15 m/min in the first week to 21 m/min in the sixth week and remained constant at 31 m/minin the final two weeks (Table 1). The cooling down phase consisted of a 2-minute low-intensity exercise at the end of each training session (Table 1) (22).

Blood samples were collected from the rats 24 hours following the last training session and after an overnight fast, between 8:00 am and 12:00 pm. Anesthesia was induced through intraperitoneal injection of a combination of xylazine (10 mg/kg) and ketamine (75 mg/kg). The blood samples were taken from the ventricle (4 mL), and the serum triglyceride levels were measured one day before the exercise protocol and 24 hours following the last training session. The samples were centrifuged for 15 minutes at 4 °C, and the plasma serum was separated and stored at -80 °C for analysis. The gastrocnemius muscle was removed from the lower limb of the rats, washed in physiologic serum, and immediately frozen at -80 °C for further tests.

The quantitative gene expression levels of PLIN5 and UCP3 were evaluated using the relative gene expression real-time quantitative PCR (qPCR) and cyber green fluorescence method. Muscle tissue samples were processed using two kits of total RNA (ALL-In-One Mini-Preps kits, Bio Basic, Canada) and an RNA quantity measurement kit (Master Mix, Ampliquan, Denmark).The cDNA was synthesized using the Easy TM cDNA Synthesis kit (Parstous Company, Iran). The primer sequences were presented in Table 2. qPCR protocol involved an initial incubation step (95°C, 10 min), followed by successive PCR cycles comprising an annealing temperature- dependent 15-second incubation period and a 30-second cycle duration, which were repeated for 40 cycles. The relative quantification strategy used for qPCR data analysis was the 2-ΔΔCT method, starting with the 18S gene as a control.

The descriptive statistics of the data were analyzed using mean and standard deviation. The normality of the data was assessed using the Shapiro-Wilk test, while the homogeneity of variance was tested using Levene's test. The mean difference between variables was evaluated using the one-way analysis of variance test (One-way ANOVA) and the LSD post hoc test ($p \leq 0.05$). Statistical analysis was performed using SPSS software (version 26, Chicago, IL, USA).

Results

Figure 1 illustrates the mean weight changes of rats during the induction of obesity (first phase: 10 weeks) and exercise training (second phase: 8 weeks). The weight of the HFD was higher than the CSD during the induction of obesity phase. All three groups showed an increase in body weight during the 8 weeks of exercise, with the HFD group demonstrated a greater increase than the CSD and HIIT-HFD groups.

Regarding weight findings, there was a significant increase in weight in the OC group compared to the CSD group during the induction of obesity phase (t=8.15; $p<0.001$). The LSD post hoc test revealed a significant difference between the OC group and the HIIT-HFD andCSD groups, as well as a significant difference between the CSD and OC groups in the exercise phase (F=14.07; $p<0.001$, Figure 2).

Table 1: Training protocol of the animals.

Variable	Warm-up	Bout	Recovery	To cooling
Training speed	50%	90%	50%	50%
Training period	2 minutes	2 minutes	1 minute	2 minutes

Table 2: Base primer sequence of studied genes.

Gene	Reverse primer			Forward primer		
PLIN5	1	TGCATATGCTGGATCAGCTC	20	1	CCATCTTGCCTATCAACACTC	21
PL=108	994	…….....	975	887	……..……	907
UCP3	1	GGCGTATCATGGCTTGAAAT	20	1	ATGAGTTTTGCCTCCATTCG	20
PL=184	567	……	548	384	……	
18s	GGCCTCACTAAACCATCCAA			GCAATTATTCCCATGAACG		

PLIN5: Perilipin 5; UCP3: Uncoupling protein 3

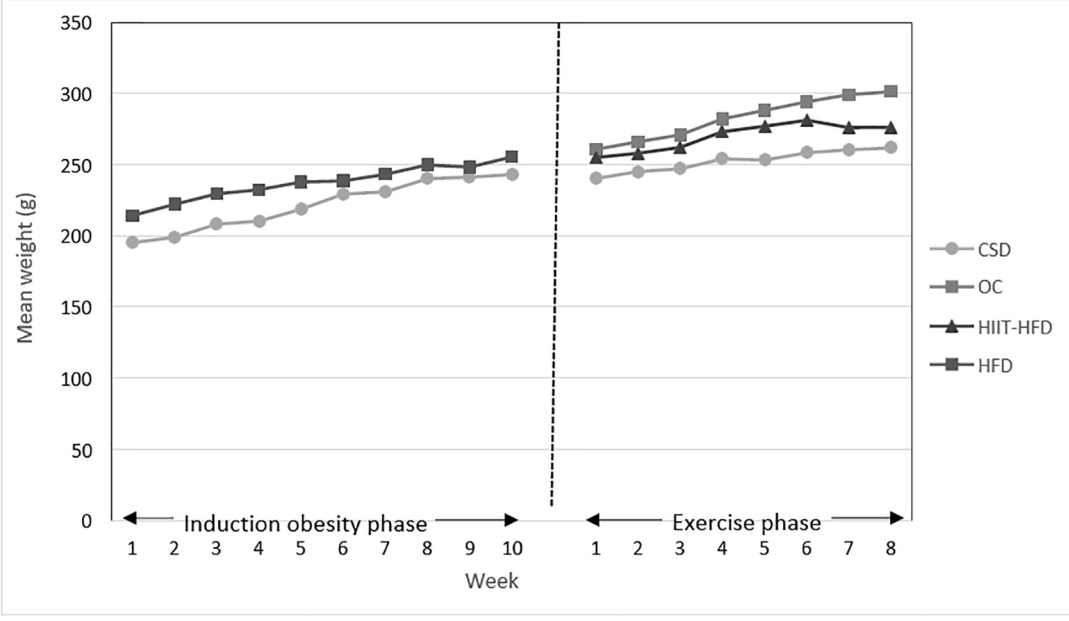

Figure 1: Changes in the weight of rats during the induction of the obesity phase and the exercisephase. CSD=Control standard diet, HFD=High fat diet, OC=Obese control, HIIT-HFD=High intensity interval training-High fat diet.

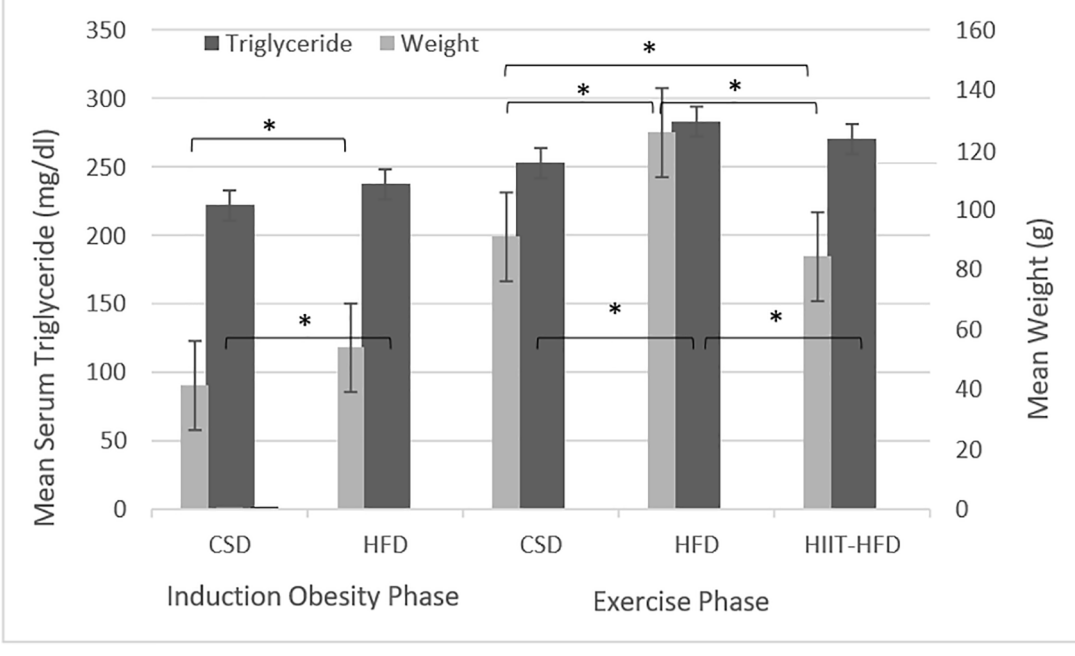

Figure 2: Comparison of serum triglyceride (left) and weight (right) in two groups of CSD and HFD in the induction obesity phase (left) and CSD, OC, and HIIT-HFD groups in the exercise Phase (right) *Significant difference ($p \leq 0.05$). CSD=Control standard diet, HFD=High fat diet, OC=Obese control, HIIT-HFD=High intensity interval training-High fat diet.

Figure 2 shows the average serum triglyceride levels of rats during the induction of obesity phase and exercise. The HFD group had higher mean serum triglyceride levels than the CSD group during the induction of obesity phase. In the exercise phase, the HFD group had higher average serum triglyceride levels than the CSD and HIIT-HFD groups.

The average PLIN5 and UCP3 levels were shown in Figure 3. The HIIT-HFD group had lower levels of these proteins compared to the CSD and HFD groups. The LSD post hoc test showed a significant decrease in PLIN5 levels in the HIIT-HFD group compared to the CSD and HFD groups in the exercise phase ($F=3.78$; $p<0.001$). In addition, a significant decrease was observed in UCP3 levels between the HIIT-HFD group and the CSD group in the exercise phase ($F=3.29$; $p<0.001$).

The Pearson correlation coefficient test results, shown in Figure 4, indicated no significant relationship between PLIN5 and UCP3 in the CSD and HIIT-HFD groups ($p \geq 0.05$). However, positive correlation coefficients were significant in the OC and total groups ($p \leq 0.05$).

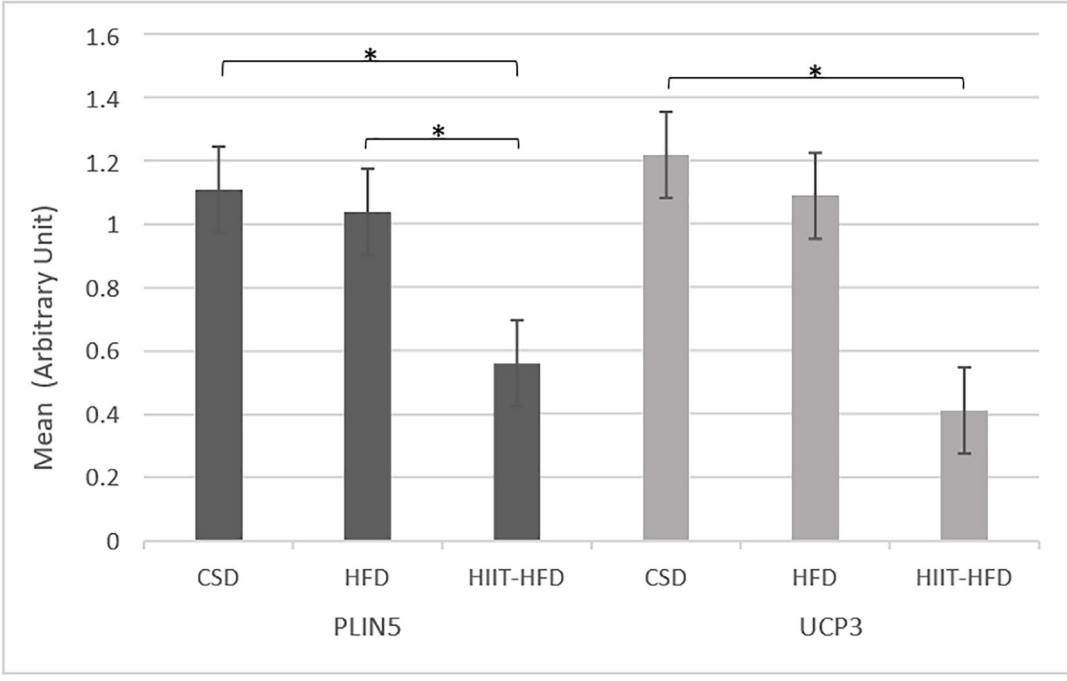

Figure 3: Comparison PLIN5 (left) and UCP3 (right) in CSD, OC, and HIIT-HFD groups. *Significant difference ($p \leq 0.05$). CSD=Control standard diet, HFD=High fat diet, OC=Obese control, HIIT-HFD=High intensity interval training-High fat diet.

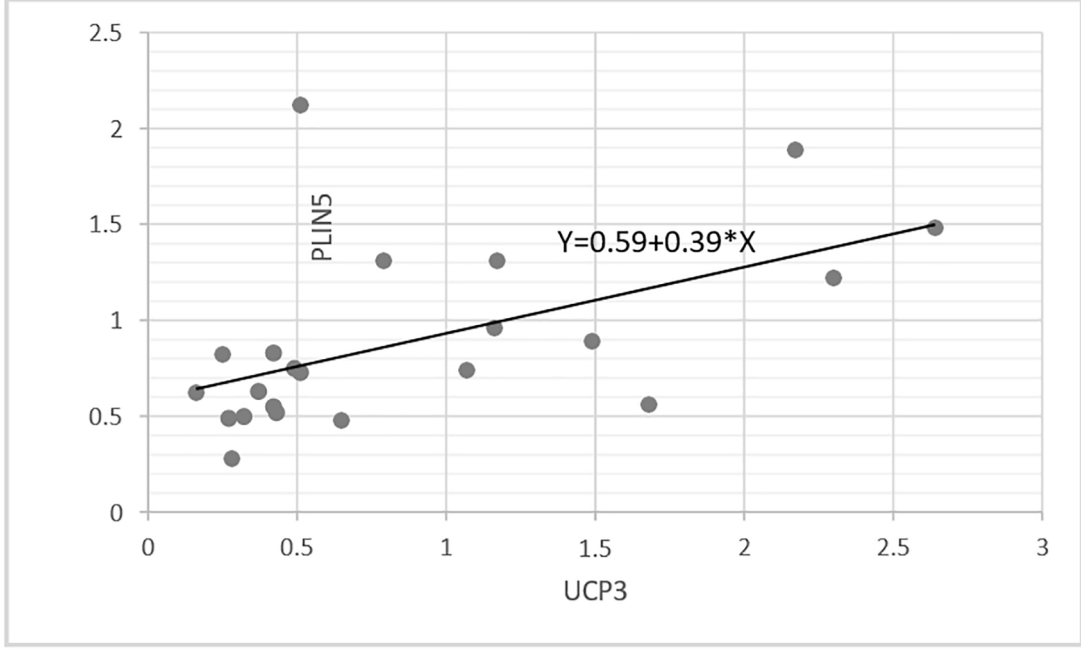

Figure 4: Coefficients correlation between PLIN5 and UCP3 genes.

Discussion

The most significant finding of the study was that the HIIT protocol resulted in a decrease in weight, serum triglyceride levels, as well as a reduction in the expression of PLIN5 and UCP3 genes when compared to the OC and NC groups after completion of the exercise phase. The current study investigated the effects of a high-fat diet on weight gain and serum triglyceride levels in Wistar rats. Results showed that consumption of a high-fat diet led to an increase in body weight and serum triglyceride levels, which are indicators of metabolic syndrome and adipose tissue expansion. Furthermore, six weeks of HIIT resulted in increased weight loss compared to the control group over the last two weeks of the exercise regimen. HIIT training has been shown to induce metabolic adaptations such as increased mitochondrial biogenesis (23), improvements in mitochondrial function, and an increase in the number of mitochondria, particularly in conditions of obesity (24).

Mitochondrial biogenesis is a complex process

that involves several steps ranging from signaling, transcription, protein expression, and post-translational modifications. The activation of various factors including co-factors of transcription like PGC-1α and transcription factors such as P53, NRF-1, NRF-2, and Tfam leads to gene transcription of various proteins involved in mitochondrial biogenesis (25). Studies have suggested that increased content of SIRT1, nuclear PGC-1α, and Tfam may coordinate adaptations related to mitochondrial biogenesis in response to HIIT (26). Similar findings have been reported by Bakhtiari *et al.* where rats exhibited decreased body weight with HIIT exercises for 12 weeks, However, Tabari *et al.* did not report any significant changes in the body weight of rats following HIIT exercises after 12 weeks (23). These findings suggest that weight gain increased mitochondrial biogenesis or muscle-building gene changes that lead to an increase in muscle mass. Further studies are needed to elucidate the molecular mechanisms of HIIT-induced mitochondrial biogenesis and their implications for physical fitness and health. (27).

It appears that there is a significant difference in triglyceride levels between certain groups, but not in others. The duration and intensity of the exercise may have an impact on these findings. Interestingly, the studies by Ghasemi *et al.* and Salimi *et al.* had different results regarding the effect of HIIT training on triglyceride levels in overweight individuals. However, it seems that a minimum training intensity of 75% of maximum heart rate is necessary to affect lipids and fat oxidation (28, 29). Overall, these findings suggest that exercise can have an impact on triglyceride levels, but further research is needed to fully understand the relationship between exercise and lipid metabolism (30).

Based on the information provided, it seems that HIIT can result in a reduction of PLIN5 in skeletal muscle. This is consistent with findings from previous studies on diabetic rats and endurance training (18). Endurance exercise training has been shown to increase skeletal muscle PLIN5 and coordinately up-regulate fat oxidative capacity and lipolytic protein expression in subjects with obesity (31). In contrast, intense endurance training has been found to significantly increase PLIN5 in skeletal muscle among diabetic rats, while low-intensity endurance training has little effect on this index (32). HIIT exercises may increase the adipose triglyceride lipase enzyme through increasing the stimulation of beta-adrenergic receptors, which can increase the lipolysis process. The decrease in triglyceride levels observed in the HIIT group suggests that HIIT may have led to an increase in PLIN5 phosphorylation, although this finding was not investigated in the present study (33). Overall, these findings suggest that HIIT may be an effective exercise regimen for reducing PLIN5 in skeletal muscle and improving lipid metabolism.

The present study found that UCP3 gene expression was affected by the consumption of fatty food, weight gain, and HIIT training. In the CSD and OC groups, which experienced weight gain, there was an increase in UCP3 gene expression. However, in the HIIT-HFD group, where rats were subjected to HIIT and a HFD, there was no significant weight gain, and UCP3 gene expression actually decreased. This suggests that HIIT training may have different effects on UCP3 gene expression compared to other types of exercise. It's worth noting that previous studies have reported conflicting results regarding the effect of HIIT training on UCP3 gene expression. For example, some studies have shown that HIIT training can lead to a decrease in UCP3 mRNA expression, while others have reported an increase in UCP3 expression with intense intermittent exercise and supplementation (34-36). It's possible that differences in study design, duration of interventions, and type of exercise may contribute to these discrepancies.

The effect increase in obesity and weight loss due to endurance training on UCP3 mRNA expression in mice with obesity showed the mean relative expression of UCP3 mRNA in the high-intensity and low-intensity groups was significantly lower than in the control. It was greater in the HITT than in the low-intensity training group (37). In contrast, De Quiros *et al.* reported that endurance training (8 weeks) running on a treadmill, decreased UCP3 mRNA in the quadriceps muscle of rats (38). Overall, the findings of this study suggest that changes in UCP3 gene expression were associated with both dietary intake and exercise, and further research is needed to understand the complex interplay between these factors (38).

Based on the information provided, it appears that plasma-free fatty acids play a role in regulating the UCP3 gene expression. Specifically, obesity caused by consuming HFD leads to an increase in UCP3 mRNA expression, which may be a way to counterbalance the increase in energy intake. In addition, regular eating can increase metabolic rate by 25-40%, known as the thermogenic effect of food or food-induced thermogenesis, and a decrease in UCP3 due to exercise that may be a compensatory mechanism to prevent excessive energy expenditure and heat generation under basal conditions (39). These findings suggest that UCP3 may play a crucial role in regulating energy balance and metabolism in response to dietary and environmental changes (38).

It appears from the research that changes in body weight and UCP3 gene expression are closely related, with increases in energy intake and subsequent weight gain leading to an increase in UCP3 levels, which in turn enhances energy expenditure and fatty acid oxidation. Conversely, weight loss due to increased physical activity has been shown to decrease UCP3 levels, suggesting that this gene may play a role in regulating energy balance and metabolism in response to changes in body weight. Additionally, the intensity of sports activity and plasma free fatty acid levels have been found to impact the regulation of UCP3, indicating that multiple factors may influence the expression of this gene. Overall, these findings underscore the complex interplay between genetics, environment, and behavior in the regulation of energy balance and highlight the potential importance of UCP3 as a key component of this system (37).

It appears that there is a significant relationship between PLIN5 and UCP3 gene expression in the HFD control group. Previous research has suggested that PLIN5 is a protein related to fat droplets and is involved in both their synthesis and breakdown. In addition, PLIN5 expression has been found to be positively correlated with IMTG content, and induction of obesity through consuming a HFD may increase PLIN5 levels (40). Given these findings, it seems likely that the significant relationship observed between PLIN5 and UCP3 expression in the HFD group is related to increased fat intake and storage associated with consuming a HFD (37). Together, these results suggest that both PLIN5 and UCP3 may play important roles in regulating fat metabolism in response to changes in dietary fat intake.

There were research limitations in including diet as subjects used two types of standard and HFD. Also, the physical activity due to the inability or refusal of the animals to run on the treadmill, some of them were removed and replaced in the training course. The amount of physical activity outside the training protocol was controlled by keeping the rats in cages. Some rats were more active in cages during the research period. Finally the stress that despite not creating natural conditions of treadmill stress and moving rats in all groups, theamount of stress caused by training (psychological stress) was not the same in all groups. The methodological limitations of the current research provided the lack of measurementof muscle weight, intramuscular triglycerides, plasma FFA, mitochondrial biogenesis, phosphorylated amounts of PLIN5, and UCP3, as well as factors regulating gene expression and fat droplet size.

Conclusion

Despite these limitations, the study findings suggest that eight weeks of obesity induction or HFD consumption leads to an increase in fat and serum triglyceride levels among subjects. Furthermore, HIIT training appears to have a positive impact on serum triglycerides, PLIN5, and UCP3 in the gastrocnemius muscle, and significantly decreases fat in the rats. The results also suggest that long-term HIIT exercises with different intensities may have even more beneficial effects on obesity. Overall, while further research is needed to fullyunderstand the mechanisms underlying these findings, they underscore the potential benefits of exercise for combating obesity-related health issues.

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Conflicts of Interest

None declared.

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