Research Article

Eight Weeks of Progressive Resistance Training Increased the Content of UCP1 in Visceral and Subcutaneous Adipose Tissue of Rats

Hani Firooz-zare [1], Alireza Safarzade [1, 2 *], Elahe Talebi-Garakani [1, 2]

[1] *Department of Exercise Physiology, Faculty of Sports Sciences, University of Mazandaran, Babolsar, Iran*
[2] *Athletic Performance and Health Research Center, University of Mazandaran, Babolsar, Iran*

Received 6 March 2024 Accepted 14 October 2024

Abstract

New evidence indicates that exercise training as a stimulant of adipose tissue thermogenesis can play a positive role in preventing obesity. The purpose of this research is to investigate the effect of eight weeks of progressive resistance training on the levels of proteins involved in the thermogenesis of visceral and subcutaneous adipose tissues in sucrose-fed rats. Twenty- four male Wistar rats (222±26g, 4- 6 weeks) were divided into normal control, sucrose control, and progressive resistance training groups. The normal control group was fed only standard food and water. In addition to free access to water and standard food, the other two groups were fed a 10% sucrose solution. The exercise program started after two months of nutritional intervention and continued for 8 weeks, 3 days a week. 72 hours after the last training session and after 4 hours of fasting, the rats were anesthetized, and blood samples and visceral and subcutaneous adipose tissues were taken. Serum glucose and insulin levels and insulin resistance index along with tissue levels of PGC-1α and UCP1 were measured. The results showed that the consumption of sucrose solution significantly increased serum glucose (P≥0.001) and insulin (P≥0.001) levels and insulin resistance index (P≥0.001) and decreased UCP1 levels in subcutaneous fat tissue (P≥0.03) compared to the control group. Also, progressive resistance training caused a significant decrease in insulin (P≥0.007), insulin resistance index (P≥0.025), and increased UCP1 levels in visceral(P≥0.032) and subcutaneous (P≥0.005) adipose tissue compared to the sucrose control group. However, the levels of PGC1α in visceral and subcutaneous fat tissues did not show any significant changes. The results showed that progressive resistance training, in addition to improving insulin sensitivity, can play an effective role in the process of browning white adipose tissue by increasing the level of UCP1 in visceral and subcutaneous adipose tissue, and as a therapeutic method for improving insulin resistance and obesity.

***Keywords*:** Progressive Resistance Training, insulin resistance, UCP1, PGC-1α

Introduction

Carbohydrates are important macronutrients in human dietary patterns that play a key role in vital metabolic pathways and provide the necessary energy for proper body function. However, sugar homeostasis requires complex hormonal and neural control for body energy balance. In recent years, the effect of sugar consumption on health is still a controversial issue. There is much evidence that excessive consumption of sugar directly and indirectly causes metabolic, cardiovascular, and diabetes diseases. The direct path involved includes the hepatic absorption of sugar (sucrose and fructose) and their metabolism, which leads to the accumulation of liver fat, blood lipid disorders, and a decrease in insulin sensitivity. Epidemiological studies show that sugar consumption has a direct relationship with weight gain (Stanhope, 2016). Furthermore, it has been demonstrated that insulin resistance, and possibly diabetes, can increase with a rise in body fat content (as indicated by body mass index [BMI]) in individuals ranging in weight from lean to obese, indicating that body fat levels affect insulin sensitivity in many ways. Insulin resistance in obesity and type 2 diabetes is manifested by impaired insulin-stimulated glucose transport and glucose metabolism in fat cells and skeletal muscles and by disruption of hepatic glucose output (Kahn & Flier, 2000). Considering the obesity epidemic, it is important to try to understand the biology of adipose tissue. Adipose tissue, as an effective tissue in the body's metabolism, responds to changes in food availability and environmental temperature. Mammals have two types of adipose tissue with different morphology and functions: white and brown adipose tissue. According to the classical point of view, the main role of white fat tissue is to

*Corresponding author's e-mail address:
a.safarzade@umz.ac.ir

supply metabolic fuel for long-term periods, especially during physical activities (Wang et al., 2008). However, in recent decades, scientific findings have shown that white adipose tissue can be recognized as an active endocrine organ that can secrete a wide range of different bioactive factors such as adipokines. White adipose tissue in the body is stored in two parts: visceral white adipose tissue (VWAT) and subcutaneous white adipose tissue (ScWAT). Both types of white adipose tissue play a role in functions such as fat storage, hormone production, immune system function, and shaping peripheral tissue (Stanford, Middelbeek, & Goodyear, 2015). Compared to white adipose tissue, brown adipose tissue contains smaller brown adipocytes and multicellular cells with a high number of mitochondria, which are metabolically highly active and use lipids as an energy source in a process called non-vibrational thermogenesis (Reddy et al., 2014). Research has shown that stimuli such as cold can change the characteristics of some cells in white fat tissue and take on the characteristics of brown adipocytes, which are called beige or bright cells (Young, Arch, & Ashwell, 1984). The browning process of white adipose tissue is effective in converting the storage form to the energy consumption form, and by adjusting the energy balance, it can have anti-obesity and anti-diabetic effects. Three basic changes in this process include the increase in UCP1 gene expression, the increase in small fat droplets in an adipocyte, and the increase in the number of mitochondria (Lo & Sun, 2013). Some researchers have investigated the relationship between the level of UCP1 in the white fat tissue of transgenic mice and obesity and concluded that with higher expression of UCP1 in these mice, the resistance to obesity induced by fatty food increases (Kopecky et al., 1995). Also, it has been shown that UCP1 ablation induces obesity and abolishes diet-induced thermogenesis in mice exempt from thermal stress by living at thermoneutrality (Feldmann et al., 2009). Studies have shown that direct induction of UCP1, which ultimately causes cell thermogenesis, is regulated by the PGC-1α protein. In skeletal muscles and white adipose tissue, the transcriptional activity of PGC-1α is responsible for the expression of a network of genes that control glucose consumption, fatty acid oxidation, the tricarboxylic cycle, and oxidative phosphorylation (Sharma, Patil, & Satyanarayana, 2014). Some studies have proposed strategies to promote the browning of white adipose tissue, which could play a role in ameliorating obesity. Exercise can reduce fat mass and improve the metabolism of the whole body, in which the browning of fat tissue is also involved. So, the browning of adipose tissue through exercise is a promising strategy against obesity and metabolic disorders. Browning of white adipose tissue due to exercise includes various mechanisms, the most important of which are reactive oxygen species, metabolites, the nervous system, exerkines, and lipolysis (Mu et al., 2021). Meanwhile, different types of exercise training with different intensities and durations can have different effects on the browning of fat tissue. Some research has shown that interval training is more effective than continuous training for fat tissue browning. This difference in the effectiveness of exercise on fat tissue browning can be attributed to the activated mechanisms of various types of exercise training. Regarding the comparison of the effects of endurance and resistance exercises, similar results have been reported in animal models. The results showed that both endurance and resistance exercise can increase the expression of the UCP1 and PGC-1α genes and induce browning of white adipose tissue (Picoli et al., 2020). Tani Mora and his colleagues (2022) (Tanimura et al., 2022), Khalafi and his colleagues (2020) (Khalafi et al., 2020) showed in their research that high-intensity endurance exercises have a greater effect on fat tissue browning than lower intensities. Nevertheless, it can be said that in addition to the type of exercise, the intensity of exercise can also be an effective factor in the issue of the effectiveness of exercise on the browning of fat tissue. Based on the investigations, few studies have done the effect of high-intensity resistance training on the browning of white adipose tissue. Therefore, the purpose of this research is to investigate the effect of eight weeks of progressive resistance training on the levels of proteins involved in the thermogenesis of visceral and subcutaneous fat tissues in rats fed sucrose solution.

Materials and Methods

Animals and Design Study

A total of 24 male Wistar rats (222±26g, 4-6 weeks) were obtained from Pasteur's Institute, Tehran, Iran, and then transferred to the animal laboratory of the Faculty of Sports Sciences of Mazandaran University. The animals were housed in cages (four rats in each cage) and maintained under controlled light/dark (12/12 h) and temperature (22 ± 2^{o}C) conditions. After one week of acclimation to their living conditions, the animals were initially divided into three groups. 1. Normal control 2. sucrose control, 3. progressive resistance training. During the entire research period, the weight of the

animals was recorded weekly. All the care and ethical principles of working with animals were observed according to the guidelines for the use and care of laboratory animals and were approved by the ethics committee of Mazandaran University under the code of ethics IRMU.IREC.1402.005.

Research nutritional intervention

During the research period, standard food (20 % protein, 4 % fat, 60 % carbohydrate, plus fibre) and water were freely available to the normal control group. In addition to free access to water and standard food, the two groups of sucrose control and progressive resistance training were provided ad libitum with a 10 % (w/v) sucrose solution in tap water presented in a second home-cage bottle, while normal control rats were given a second water bottle. To prepare a 10% sucrose solution, we mixed one kilogram of sucrose with nine liters of water. A dietary intervention period of 10% sucrose solution was implemented for 8 weeks. (Chan et al., 2013).

Progressive resistance training protocol

After the 8-week nutritional intervention period of 10% sucrose solution, the sucrose + progressive resistance training group performed the training program for 8 weeks/3 days. The resistance training program consisted of climbing a ladder with 26 steps at a height of 1 meter, using weights attached to the rats' tails, and the ladder was placed at an angle of 80 degrees. To train and adapt to the training conditions, the rats climbed the ladder without carrying weights for 3 days a week, 4-5 repetitions per session with a 2-minute rest between repetitions. To stimulate the animals to perform the exercises, only touching and rubbing their tails were used. Also, to equalize the stress caused by encountering the tester, at a certain time, the animals in the control group were moved and touched. The first to third sessions of the progressive resistance training program included constant weights, equivalent to 50, 75, and 100 percent of the animal's body weight, respectively, which were performed with eight repetitions and two-minute rests between repetitions. In the fourth session and after that, the maximum carrying capacity of each session, which included the gradual load carried by each animal, was measured. For this purpose, the first to fourth repetitions were performed with a load equal to 50, 75, 90, and 100% of the animal's body weight, respectively. From the fourth to the eighth repetition, in each repetition, 30 grams were added to the load attached to the animals' tails. If the animal was not able to carry a weight of 30 grams, the next repetitions continued with the same load as before. The maximum amount of weight moved in each session was recorded as the maximum carrying capacity of that session, and at the beginning of the next session, the loads of each repetition were performed based on it. To perform the warm-up and cool-down phases, the rats climbed the ladder twice (without load) before and after each training session(Hornberger Jr & Farrar, 2004).

Sample Collection

72 hours after the last training session, to eliminate the acute effect of training and after 4 hours of fasting, the rats were anesthetized with an intraperitoneal injection of a combination of ketamine (50 mg/kg) and xylazine (3 mg/kg). Approximately 6 mL of blood was obtained from the abdominal vena cava and centrifuged (3000 rpm; 4 °C; 15 min). The serum was immediately separated and kept frozen at -20°C for further analysis. After tissue removal and washing of visceral and subcutaneous fat tissues and placing them in microtubes, they were immediately frozen using liquid nitrogen and transferred to a -80°C refrigerator(Narita et al., 2018).

Measurement of biochemical and tissue indices and calculation of insulin resistance index

Serum glucose levels were evaluated by an enzymatic colorimetric method (Pars Azmun Co., Tehran, Iran). Enzyme-linked immunosorbent assay kits were used to measure serum insulin concentration and tissue levels of UCP1 and PGC-1α. Also, to measure tissue levels, 1 mg of visceral and subcutaneous fat tissue was homogenized. Then, using a special kit (Hangzhou Eastbiopharm, China), serum insulin concentration and tissue levels were measured by ELISA method. Homeostasis model assessment of insulin resistance (HOMA-IR) scores were calculated using the following formula: HOMA-IR = [Insulin (mIU/L)× blood glucose(mmol/L)]/22.5(Min et al., 2017).

Statistical Analysis

All the data were e statistically analyzed using SPSS software (version 16.0). The Shapiro-Wilk test was used to determine the normality of the data distribution. Also, one-way analysis of variance (ANOVA) and LSD post-hoc tests were performed to determine the difference of dependent variables among the research groups. The statistically significant differences were considered in the case of a P-value less than 0.05. All data were expressed as mean ± standard deviation (SD).

Results

Weight body of rats in different stages of research

Table 1 shows the mean ± standard deviation of the body weight of rats in the two initial and final stages and the weight of visceral and subcutaneous fat tissue in different study groups.

The effect of sucrose consumption and progressive resistance exercise on serum levels of glucose, insulin, and insulin resistance index

The results of a one-way analysis of variance for serum glucose concentration showed that the serum glucose level in two sucrose control (P≥0.001) and progressive resistance training (P≥0.001) groups had a significant increase compared to the normal control group (Figure 1). Also, the insulin level in the sucrose control group was significantly increased compared to the normal control group (P≥0.001), and a significant decrease in the progressive resistance training group compared to the sucrose control group (P≥0.007) (Figure 2). The insulin resistance index also increased significantly in the sucrose control group (P≥0.001) and progressive resistance training (P≥0.001) compared to the normal control group, but in the progressive resistance training group compared to the sucrose control group (P=0.025) had decreased (Figure 3).

Table 1. Mean ± std deviation of body, fat tissue weights in research stages

Variables	Normal Control	Sucrose control	Progressive resistance training
Initial stage	216/88±24/59	218/25± 25/18	228±31/41
Final stage	343± 41/25	391/88±62/93	357/26±39/16
Visceral fat tissue	3/48± 1/64	7/44± 2/94	2/91± 1/02
Subcutaneous fat tissue	2/95±1/19	5/33± 2/18	2/38± 0/81

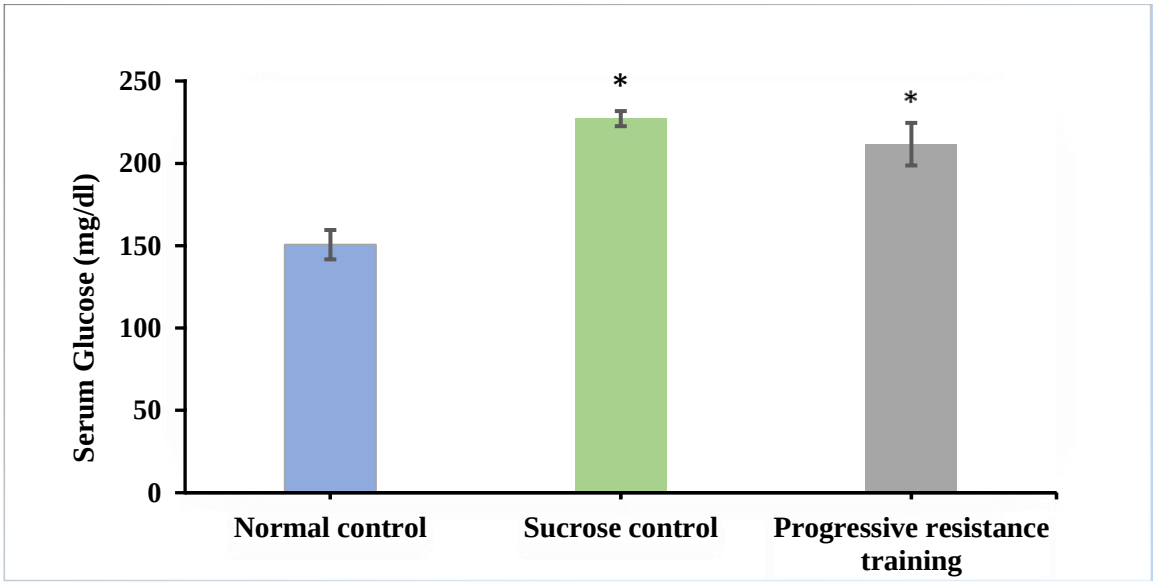

Figure 1. Mean ± std deviation of serum levels of glucose. Significant differences were considered at the significance level of P ≤0.05. * Significant difference with the normal control group.

The effect of sucrose consumption and progressive resistance exercise on UCP1 and PGC-1α protein levels in visceral and subcutaneous fat tissues

The results of a one-way analysis of variance showed that the protein level of visceral adipose tissue UCP1 increased significantly in the progressive resistance training group compared to the sucrose control (P≥0.032) and normal control (P≥0.012) groups (Figure 4). Whereas the UCP1 level in the subcutaneous fat tissue was a significant decrease in the sucrose control group compared to the normal control group (P≥0.03), and was a significant increase in the resistance training group compared to the sucrose control group (P≥0.005) (Figure 4). The results of the one-way analysis of variance showed that there is no significant difference between the research groups in the PGC-1α protein level of visceral and subcutaneous fat tissues (Figure 5).

Discussion

The results of the present study showed that due to the use of sucrose solution, glucose levels, insulin, and insulin resistance index were significantly increased in the sucrose control group. In recent years, many researchers have used artificially sweetened beverages in animal samples to induce insulin resistance or metabolic diseases such as obesity. In the meantime, they mainly use sucrose (a disaccharide composed of glucose and fructose) and high fructose corn syrup (50 to 55 % fructose) to make these drinks (Souza Cruz et al., 2020). Much research has investigated the effects of prolonged use of sucrose solution compared to drinking water on laboratory rats. With the increase in glucose and insulin levels, our results were in line with the findings of Chen G-C et al(Chen et al., 2011), Aguilera AA et al(Aguilera et al., 2004) and EL Hafidi et al(El Hafidi et al., 2001), if Masek T et al (Mašek et al., 2017) and Kawasaki T et al(Kawasaki et al., 2005) reported no change in insulin resistance index due to the use of sucrose solution. The reasons for the contradiction in the results of the research can be used for the duration and type of consumption and the percentage of sucrose used. Some research has shown that even rats fed with sucrose solution have 10 % liver steatosis, which is more advanced than insulin resistance (Souza Cruz et al., 2020). Also, the consumption of sucrose causes fat accumulation in the liver and subsequently causes liver insulin resistance, which is a risk factor for NAFLD. Hyperinsulinemia caused by insulin resistance increases liver fibrosis precursors, reduces FFA beta-oxidation, and increases the production of free radicals (Sun et al., 2018)

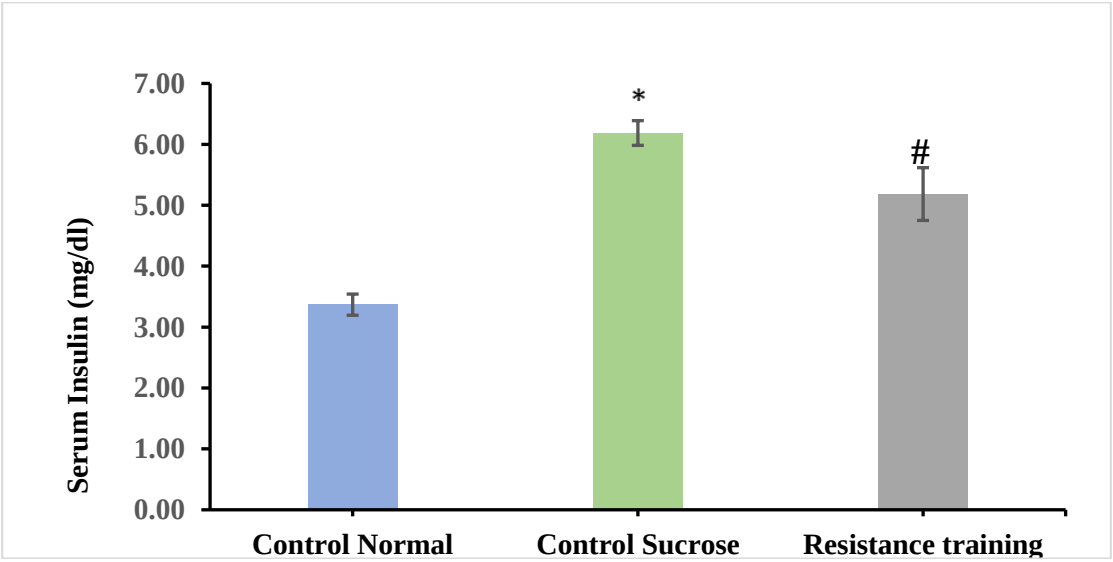

Figure 2. Mean ± std deviation of serum levels of insulin. Significant differences were considered at the significance level of P ≤0.05. * Significant difference with the normal control group. # Significant difference with the sucrose control group.

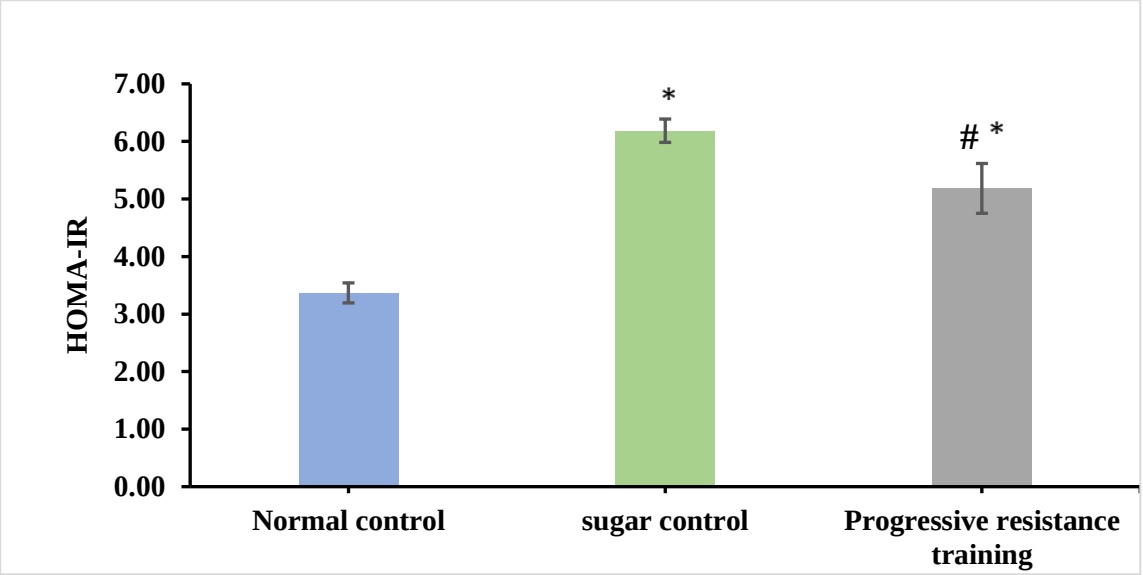

Figure 3. Mean ± std deviation of serum levels of insulin resistance index. Significant differences were considered at the significance level of P ≤0.05. * Significant difference with the normal control group. # Significant difference with the sucrose control group.

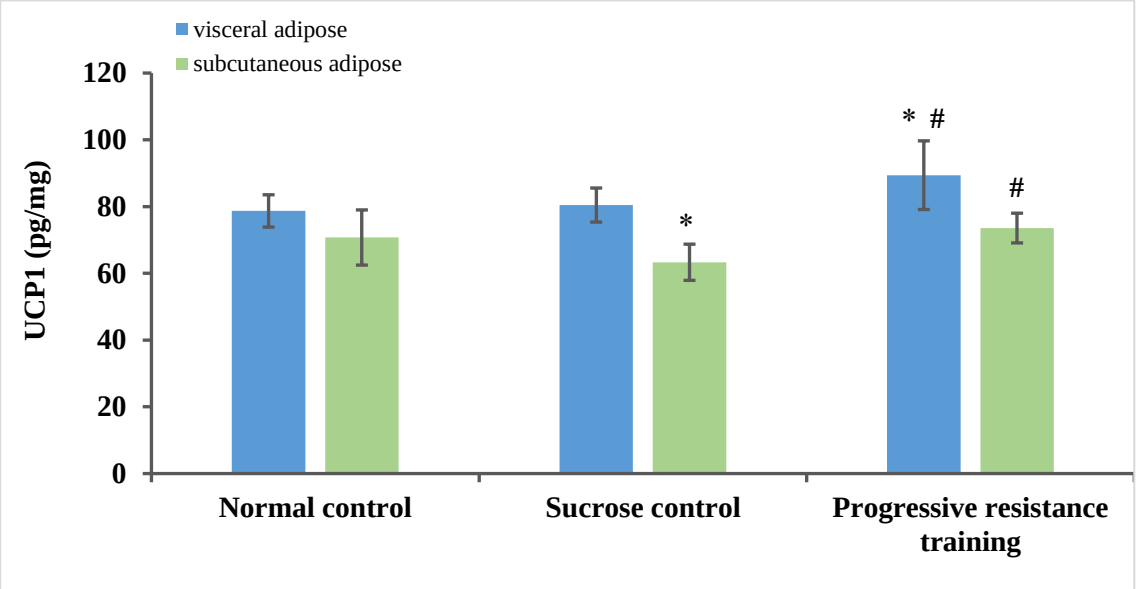

Figure 4. Mean ± standard deviation of UCP1 protein level in visceral and subcutaneous fat tissues. Significant differences were considered at the significance level of P ≤0.05. * Significant difference with the normal control group. # Significant difference with the sucrose control group.

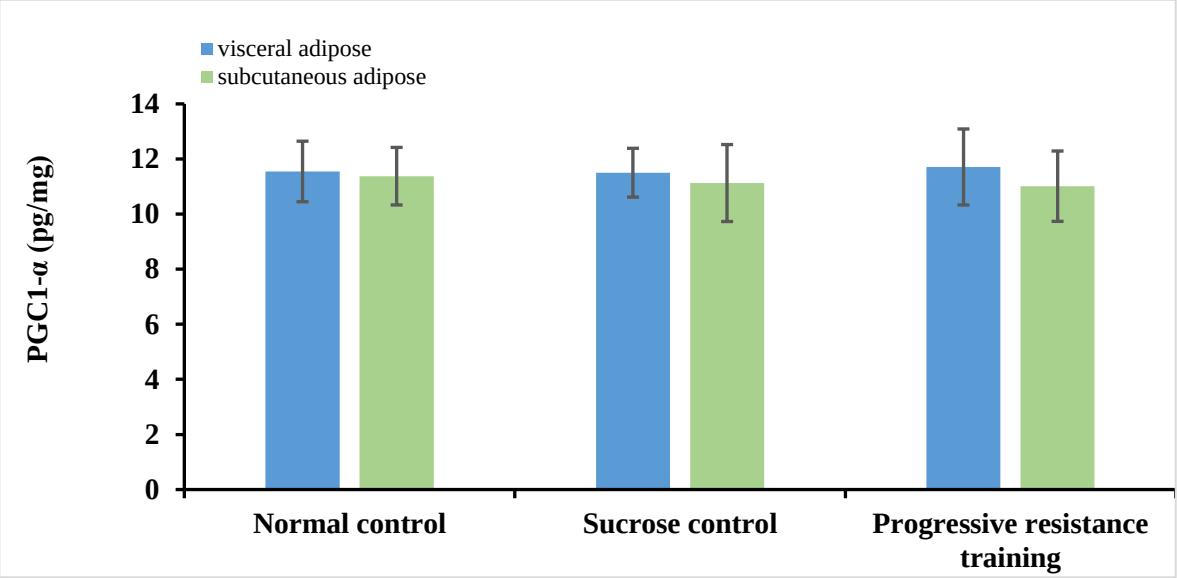

Figure 5. Mean ± standard deviation of PGC-1α protein level in visceral and subcutaneous fat tissues. Significant differences were considered at the significance level of P ≤0.05.

From the results of the present study, it can be concluded that fluid sucrose consumption causes hyperinsulinemia and changes insulin sensitivity indicators. These findings indicate that the use of sucrose can change both the secretion of pancreatic insulin and the sensitivity of peripheral tissues to insulin. Overall, these data support insulin resistance due to the use of sucrose solutions. Recently, many studies on obesity and thermogenics have sought to identify the browning factors of adipose tissue, conditions that strengthen browning reactions, the most important of which is to increase the amount and activity of UCP1 in adipose tissue (Kalinovich et al., 2017). The present study showed that UCP1 activity was significantly reduced due to the use of sucrose in the subcutaneous adipose tissue, while no significant changes were observed in visceral adipose tissue. There have been many studies on the relationship between insulin resistance and obesity with the expression of the UCP1 gene. It has been shown that over-expression of UCP1 and the activation of thermogenesis prevent obesity and insulin resistance due to a genetic or high-fat diet (Michurina et al., 2020). A study showed that inducing insulin resistance to high-fat diets increased UCP1 expression along with other indicators of adipose tissue browning in white adipose tissue, and this is an initial reaction to dealing with obesity(García-Ruiz et al., 2015).While it has been shown that UCP1 decreases in long-term insulin resistance disease, a study on rats with high-fat diet-induced insulin resistance showed that Loss of UCP1 leads to whitening of brown adipocytes, thermogenesis impaired, disruption of glucose metabolism, and liver Steatosis (Winn et al., 2017). Zuriaga et al., (2017) (Zuriaga et al., 2017) and Rockstroh D et al. (2015) (Rockstroh et al., 2015) examined UCP1 changes and other expressions of visceral and subcutaneous adipose brown genes, and in line with our results showed that UCP1 expression in subcutaneous adipose tissue is changing more than visceral fat. It has also been shown that UCP1 in subcutaneous adipose tissue has a higher gene expression (Shirkhani et al., 2021). One of the possible reasons for the difference in UCP1 activity in visceral and subcutaneous adipose tissue in response to insulin resistance (sucrose consumption in this study), is the sensitivity of visceral adipose tissue cells compared to subcutaneous adipose cells to Lipolysis activated with Catocolamins, as well as visceral fat is also less sensitive to anti-insulin effects (Wajchenberg, 2000). The visceral and subcutaneous fat tissue reserves also have distinctive properties, visceral fat has a higher level of macrophages, T cells, and natural fatal cells, and is associated with the release of inflammatory cytokines and helps increase the risk of obesity-related diseases (O'rourke et al., 2009).Therefore, the difference in accumulation location, structural properties, and higher UCP1 level can be the reasons for the decline in UCP1 activity in subcutaneous fat, not in visceral fat. In recent years, many studies on animal and human models have shown that white adipose tissue browning is associated with a decrease in blood glucose levels, improved insulin resistance, and increased resting energy expenditure.

The process of browning is regulated by a complex hormonal interaction and many environmental factors such as genetic manipulation, exposure to cold, exercise, and drug treatment (Bettini et al., 2019). Many studies have reported the effect of exercise on white adipose tissue browning due to increased UCP1 and PGC-1α proteins. It has been shown that PGC-1α is induced by exercise in the muscle and stimulates many of the beneficial effects, including mitochondrial biogenesis and muscle fiber changes in the muscle. Also, PGC1α expression in muscle stimulates an increase in expression of Fndc5, a membrane protein that is cleaved and secreted as a new hormone, irisin. Irisin acts on white adipose cells and stimulates UCP1 expression and a broad program of brown fat-like development and as a result, it improves obesity and glucose homeostasis (Boström et al., 2012). The results of the present study showed that progressive resistance exercise was able to increase the level of UCP1 protein in both visceral and subcutaneous adipose tissue compared to the sucrose control group, while the PGC-1α level had no significant change due to exercise and the use of sucrose solution. Progressive resistance exercises with different mechanisms can increase UCP1 activity. Due to the non-change of PGC-1α level, the mechanisms of independent PGC-1α may have increased UCP1 in visceral and subcutaneous adipose tissue. According to studies, it is also possible that the levels of PGC-1α in the muscles have increased and through plasma irisin, the protein level of UCP1 in visceral and subcutaneous tissue has increased. Therefore, measuring PGC-1α in muscles and plasma irisin, which is one of the limitations of the study, is recommended for other studies. Picoli and his colleagues have reported that endurance and resistance exercises can be increased in similar UCP1 and PGC-1α genes in both inguinal fat and retroperitoneal adipose tissues in training groups compared to the control group in healthy mice (Picoli et al., 2020). Differences in PGC-1α measurement level (gene expression in the Picoli study and protein level in the present study), resistance exercise intensity (constant load and progressive), and subjects (healthy and insulin resistance) can be The reasons for the heterogeneity of the two studies are related to the changes in PGC-1α. Various studies have been conducted in examining the mechanisms of effective exercise on the process of browning of adipose tissue and increasing gene expression or UCP1 activity level, most notably active oxygen species, metabolites, the nervous system, exerkins, and free fatty acids caused by lipolysis (Yang & Kwon, 2020). It has also been shown that the type, intensity, and duration of exercise training have different results (Mu et al., 2021). Meanwhile, the mechanisms by which resistance exercises affect the browning of white adipose tissue have not yet been fully identified. It has been shown that lactate produced by exercise can increase UCP1 through two GPR81/P38/PGC-1/PPARγ/UCP1/UCP1 and MCT/NAD+/UCP1 (Carrière et al., 2020; Yao et al., 2020). Depending on the progressive intensity and the likelihood of increased lactate production without significantly changing the level of PGC-1α activity, it can be stated that the second route may have been involved in increasing UCP1 levels in visceral and subcutaneous adipose tissue. Resistance exercises have been shown to increase muscle hypertrophy by increasing and phosphorylation of the MTOR protein. It has also been shown to play a role in increasing UCP1 activity and the browning of adipose tissue (Ye et al., 2019). Although the level of this protein was not measured in the present study, the type of resistance training in the present study likely increased UCP1 in visceral and subcutaneous adipose tissue. In addition, Betahidroxy Boutirat (BHHIBA) is a ketone body produced by the liver as an active biological metabolite with a wide range of signaling and regulatory effects and has been shown to increase after prolonged exercise and during the recovery period. It can increase UCP1 by activating the MTOR signal in adipose tissue (Evans, Cogan, & Egan, 2017). Another exercise mechanism that probably increased the level of UCP1 with progressive resistance training in the present study is the increase in cAMP in adipocytes, which increases the production of free fatty acids and increases the expression of the UCP1 gene by binding to PPAR-α (Schreiber et al.). One of the debatable topics is the different responses of different anatomical parts of white fat tissue to the exercise-mediated browning process. Although in the present study, an increase in the level of UCP1 was observed in both visceral and subcutaneous fat tissues, it can be said that exercise-induced browning of white fat occurs in visceral and subcutaneous fat tissues through different pathways. The results of the present study showed that progressive resistance training in addition to improving insulin sensitivity can play an effective role in the browning of subcutaneous and visceral adipose tissue by increasing UCP1 and be considered a therapeutic approach in dealing with obesity and insulin resistance in the animals' studies.

Conclusion

The present study showed that progressive resistance training by increasing the level of UCP1 in visceral and subcutaneous adipose tissue can play an effective role in the browning process of white adipose tissue. Also, considering the lack of significant change in the level of PGC-1α, it can be stated that in visceral and subcutaneous tissues, the mechanisms of increasing UCP1 levels are different and independent of PGC-1α. It was also shown that increased resistance training could improve insulin sensitivity in sucrose-fed rats.

Conflict of Interests

The authors declare that there is no conflict of interest.

References

Aguilera, A. A., Díaz, G. H., Barcelata, M. L., Guerrero, O. A., & Ros, R. M. O. (2004). Effects of fish oil on hypertension, plasma lipids, and tumor necrosis factor-α in rats with sucrose-induced metabolic syndrome. *The Journal of Nutritional Biochemistry*, *15*(6), 350-357.

Bettini, S., Favaretto, F., Compagnin, C., Belligoli, A., Sanna, M., Fabris, R., Serra, R., Dal Prà, C., Prevedello, L., Foletto, M., Vettor, R., Milan, G., & Busetto, L. (2019). Resting Energy Expenditure, Insulin Resistance and UCP1 Expression in Human Subcutaneous and Visceral Adipose Tissue of Patients With Obesity. *Front Endocrinol (Lausanne)*, *10*, 548.

Boström, P., Wu, J., Jedrychowski, M. P., Korde, A., Ye, L., Lo, J. C., Rasbach, K. A., Boström, E. A., Choi, J. H., & Long, J. Z. (2012). A PGC1-α-dependent myokine that drives brown-fat-like development of white fat and thermogenesis. *Nature*, *481*(7382), 463-468.

Carrière, A., Lagarde, D., Jeanson, Y., Portais, J.-C., Galinier, A., Ader, I., & Casteilla, L. (2020). The emerging roles of lactate as a redox substrate and signaling molecule in adipose tissues. *Journal of physiology and biochemistry*, *76*, 241-250.

Chan, C. Y.-Y., Kendig, M., Boakes, R. A., & Rooney, K. (2013). Low-volume exercise can prevent sucrose-induced weight gain but has limited impact on metabolic measures in rats. *European journal of nutrition*, *52*, 1721-1732.

Chen, G.-C., Huang, C.-Y., Chang, M.-Y., Chen, C.-H., Chen, S.-W., Huang, C.-j., & Chao, P.-M. (2011). Two unhealthy dietary habits featuring a high fat content and a sucrose-containing beverage intake, alone or in combination, on inducing metabolic syndrome in Wistar rats and C57BL/6J mice. *Metabolism*, *60*(2), 155-164.

El Hafidi, M., Cuéllar, A., Ramírez, J., & Baños, G. (2001). Effect of sucrose addition to drinking water, that induces hypertension in the rats, on liver microsomal Δ9 and Δ5-desaturase activities. *The Journal of Nutritional Biochemistry*, *12*(7), 396-403.

Evans, M., Cogan, K. E., & Egan, B. (2017). Metabolism of ketone bodies during exercise and training: physiological basis for exogenous supplementation. *The Journal of physiology*, *595*(9), 2857-2871.

Feldmann, H. M., Golozoubova, V., Cannon, B., & Nedergaard, J. (2009). UCP1 ablation induces obesity and abolishes diet-induced thermogenesis in mice exempt from thermal stress by living at thermoneutrality. *Cell metabolism*, *9*(2), 203-209.

García-Ruiz, E., Reynés, B., Díaz-Rúa, R., Ceresi, E., Oliver, P., & Palou, A. (2015). The intake of high-fat diets induces the acquisition of brown adipocyte gene expression features in white adipose tissue. *International journal of obesity*, *39*(11), 1619-1629.

Hornberger Jr, T. A., & Farrar, R. P. (2004). Physiological hypertrophy of the FHL muscle following 8 weeks of progressive resistance exercise in the rat. *Canadian journal of applied physiology*, *29*(1), 16-31.

Kahn, B. B., & Flier, J. S. (2000). Obesity and insulin resistance. *J Clin Invest*, *106*(4), 473-481.

Kalinovich, A. V., de Jong, J. M., Cannon, B., & Nedergaard, J. (2017). UCP1 in adipose tissues: two steps to full browning. *Biochimie*, *134*, 127-137.

Kawasaki, T., Kashiwabara, A., Sakai, T., Igarashi, K., Ogata, N., Watanabe, H., Ichiyanagi, K., & Yamanouchi, T. (2005). Long-term sucrose-drinking causes increased body weight and glucose intolerance in normal male rats. *British journal of nutrition*, *93*(5), 613-618.

Khalafi, M., Mohebbi, H., Symonds, M. E., Karimi, P., Akbari, A., Tabari, E., Faridnia, M., & Moghaddami, K. (2020). The impact of moderate-intensity continuous or high-intensity interval training on adipogenesis and browning of subcutaneous adipose tissue in obese male rats. *Nutrients*, *12*(4), 925.

Kopecky, J., Clarke, G., Enerbäck, S., Spiegelman, B., & Kozak, L. (1995). Expression of the mitochondrial uncoupling protein gene from the aP2 gene promoter prevents genetic obesity. *The Journal of clinical investigation*, *96*(6), 2914-2923.

Lo, K. A., & Sun, L. (2013). Turning WAT into BAT: a review on regulators controlling the browning of white adipocytes. *Bioscience reports*, *33*(5), e00065.

Mašek, T., Filipović, N., Vuica, A., & Starčević, K. (2017). Effects of treatment with sucrose in drinking water on liver histology, lipogenesis and lipogenic gene expression in rats fed high-fiber diet. *Prostaglandins, Leukotrienes and Essential Fatty Acids*, *116*, 1-8.

Michurina, S., Stafeev, I., Podkuychenko, N., Sklyanik, I., Shestakova, E., Yah'yaev, K., Yurasov, A., Ratner, E., Menshikov, M., & Parfyonova, Y. (2020). Decreased UCP-1 expression in beige adipocytes from adipose-derived stem cells of type 2 diabetes patients associates with mitochondrial ROS accumulation during obesity. *Diabetes Research and Clinical Practice*, *169*, 108410.

Min, M., Li-Fa, X., Dong, H., Jing, W., & Ming-Jie, B. (2017). Dietary patterns and overweight/obesity: a review article. *Iranian journal of public health*, *46*(7), 869.

Mu, W.-J., Zhu, J.-Y., Chen, M., & Guo, L. (2021). Exercise-mediated browning of white adipose tissue: its significance, mechanism and effectiveness. *International Journal of Molecular Sciences*, *22*(21), 11512.

Narita, T., Kobayashi, M., Itakura, K., Itagawa, R., Kabaya, R., Sudo, Y., Okita, N., & Higami, Y. (2018). Differential response to caloric restriction of retroperitoneal, epididymal, and subcutaneous adipose tissue depots in rats. *Experimental Gerontology*, *104*, 127-137.

O'rourke, R., Metcalf, M., White, A., Madala, A., Winters, B., Maizlin, I., Jobe, B., Roberts, C., Slifka, M., & Marks, D. (2009). Depot-specific differences in inflammatory mediators and a role for NK cells and IFN-γ in inflammation in human adipose tissue. *International journal of obesity*, *33*(9), 978-990.

Picoli, C. d. C., Gilio, G. R., Henriques, F., Leal, L. G., Besson, J. C., Lopes, M. A., Franzoi de Moraes, S. M., Hernandes, L., Batista Junior, M. L., & Peres, S. B. (2020). Resistance exercise training induces subcutaneous and visceral adipose tissue browning in Swiss mice. *Journal of Applied Physiology*, *129*(1), 66-74.

Reddy, N. L., Tan, B. K., Barber, T. M., & Randeva, H. S. (2014). Brown adipose tissue: endocrine determinants of function and therapeutic manipulation as a novel treatment strategy for obesity. *BMC obesity*, *1*(1), 1-12.

Rockstroh, D., Landgraf, K., Wagner, I. V., Gesing, J., Tauscher, R., Lakowa, N., Kiess, W., Bühligen, U., Wojan, M., & Till, H. (2015). Direct evidence of brown adipocytes in different fat depots in children. *PloS one*, *10*(2), e0117841.

Schreiber, R., Reverte-salisa, L., Dong, H., Christensen, D. P., Sun, W., Gnad, T., Karavaeva, I., Nielsen, T. S., Kooijman, S., & Cero, C. Lipolysis drives expression of the constitutively active receptor GPR3 to induce adipose thermogenesis.

Sharma, B. K., Patil, M., & Satyanarayana, A. (2014). Negative regulators of brown adipose tissue (BAT)-mediated thermogenesis. *Journal of cellular physiology*, *229*(12), 1901-1907.

Shirkhani, S., Marandi, S. M., Kazeminasab, F., Ghaedi, K., Esfarjani, F., & Nasr-Esfahani, M. H. (2021). The effect of exercise on beige adipose tissue. *Sport Physiology*, *13*(50), 17-38.

Souza Cruz, E. M., Bitencourt de Morais, J. M., Dalto da Rosa, C. V., da Silva Simões, M., Comar, J. F., de Almeida Chuffa, L. G., & Seiva, F. R. F. (2020). Long-term sucrose solution consumption causes metabolic alterations and affects hepatic oxidative stress in Wistar rats. *Biol Open*, *9*(3). bio047282.

Stanford, K. I., Middelbeek, R. J., & Goodyear, L. J. (2015). Exercise effects on white adipose tissue: beiging and metabolic adaptations. *Diabetes*, *64*(7), 2361-2368.

Stanhope, K. L. (2016). Sugar consumption, metabolic disease and obesity: The state of the controversy. *Critical reviews in clinical laboratory sciences*, *53*(1), 52-67.

Sun, S., Hanzawa, F., Umeki, M., Ikeda, S., Mochizuki, S., & Oda, H. (2018). Time-restricted feeding suppresses excess sucrose-induced plasma and liver lipid accumulation in rats. *PloS one*, *13*(8), e0201261.

Tanimura, R., Kobayashi, L., Shirai, T., & Takemasa, T. (2022). Effects of exercise intensity on white adipose tissue browning and its regulatory signals in mice. *Physiological Reports*, *10*(5), e15205.

Wajchenberg, B. L. o. (2000). Subcutaneous and Visceral Adipose Tissue: Their Relation to the

Metabolic Syndrome. *Endocrine Reviews*, *21*(6), 697-738.

Wang, P., Mariman, E., Renes, J., & Keijer, J. (2008). The secretory function of adipocytes in the physiology of white adipose tissue. *Journal of Cellular Physiology*, *216*(1), 3-13.

Winn, N. C., Vieira-Potter, V. J., Gastecki, M. L., Welly, R. J., Scroggins, R. J., Zidon, T. M., Gaines, T. K. L., Woodford, M. L., Karasseva, N. G., & Kanaley, J. A. (2017). Loss of UCP1 exacerbates Western diet-induced glycemic dysregulation independent of changes in body weight in female mice. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, *312*(1), R74-R84.

Yang, Y. R., & Kwon, K.-S. (2020). Potential roles of exercise-induced plasma metabolites linking exercise to health benefits. *Frontiers in Physiology*, *11*, 1620.

Yao, Z., Yan, Y., Zheng, X., Wang, M., Zhang, H., Li, H., & Chen, W. (2020). Dietary lactate supplementation protects against obesity by promoting adipose browning in mice. *Journal of Agricultural and Food Chemistry*, *68*(50), 14841-14849.

Ye, Y., Liu, H., Zhang, F., & Hu, F. (2019). mTOR signaling in Brown and Beige adipocytes: implications for thermogenesis and obesity. *Nutrition & metabolism*, *16*, 1-14.

Young, P., Arch, J., & Ashwell, M. (1984). Brown adipose tissue in the parametrial fat pad of the mouse. *FEBS letters*, *167*(1), 10-14.

Zuriaga, M. A., Fuster, J. J., Gokce, N., & Walsh, K. (2017). Humans and mice display opposing patterns of "browning" gene expression in visceral and subcutaneous white adipose tissue depots. *Frontiers in cardiovascular medicine*, *4*, 27.