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Comparison of 6 weeks of high intensity interval training and continuous training on Desnutrin, Adiponectin and Adiponectin Receptor1 genes expression in two subcutaneous adipose tissue and quadriceps muscle tissue of obese male rats

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ABSTRACT

Background: Obesity disrupts the regulation of desnutrin, adiponectin and AdipoR1. The purpose of the present study was to compare 6 weeks of HIIT and continuous training on the expression of desnutrin, adiponectin and Adipo R1 genes in two subcutaneous adipose tissues and quadriceps muscle tissue in obese male rats. *Methods:* 30 obese Wistar rats were randomly divided into 3 groups: control (n=10), continuous Training (n=10) and HIIT (n=10). Continuous training and HIIT were performed 6 weeks and 6 sessions per week. Forty-eight hours after the last training session, the adipose tissue and muscle tissue were extracted, and the expression level was assessed using the RT-PCR method. *Results:* The results of showed that there was a significant difference in the expression of desnutrin (P=0.011) and adiponectin genes (P=0.040) in the adipose tissue between the continuous training and HIIT groups. However, the expression of desnutrin (P=0.855) and Adipo R1 genes (P=0.565) of the muscle tissue was not significant between the two groups. *Conclusions:* It was found in the adipose tissue, continuous training has a greater effect on the expression of Desnutrin and Adiponectin genes than HIIT. However, in the muscle tissue, there is no difference between the HIIT and continuous training in the expression of Desnutrin and AdipoR1 genes. for weight loss HIIT is a better way.

Keywords: Obesity; subcutaneous adipose tissues; quadriceps muscle tissue; Continuous training; High intensity interval training

1. INTRODUCTION

Today, obesity is associated with dangerous diseases such as noninsulin-dependent diabetes (Type 2 diabetes), increased blood lipids, hypertension, cancer and cardiovascular diseases, and it is a major health problem. For this reason, it has attracted attention clinically (Bweir et al., 2009) and in 2004, in mouse, a new lipolyticenzyme called desnutrin/ Adipose triglyceride lipase, was found to be responsible for the lipolysis of triacylglycerol to diacylglycerol and fatty acid (Knapp et al., 2017). The main expression of the desnutrin gene is in the cytoplasm of the white adipose tissue and brown adipose tissue. Of course, there are also body tissues, including the liver and skeletal muscle tissue (Ahmadian et al., 2017). Studies have shown that the levels of the desnutrin enzyme and its gene expression are inversely related to obesity, and its low level is associated with increased body fat, followed by increased triglyceride stores and increased insulin resistance (Villena et al., 2004). Meanwhile, the high expression of this enzyme is related to the amount of brown adipose tissue. Hence, the increase in the expression of desnutrin gene leads to an increase in the expression of uncoupling protein 1 and the oxidation of fatty acid in adipocytes, and is effective in maintaining the phenotype of the brown adipose tissue (Ahmadian et al., 2017). On the other hand, there is a direct and positive relationship between desnutrin and fat oxidation markers (Carnitine palmitoyltransferase Ⅰ, peroxisome proliferator-activated receptor γ coactivator 1, UCP-1 and cytochrome oxidase c) in the skeletal muscle. This indicates that the increase in intramuscular desnutrin can result to the oxidation of more fatty acids in the muscle tissue (Larsen et al., 2015), Morton et al., 2016).

Adiponectin is an anti-inflammatory adipokine that is produced, in particular, from white adipose tissue and a small amount of liver cells (Beltowski et al., 2003). There are two main receptors (AdipoR1, Adiponectin Receptor 2) in the skeletal muscle and the liver (Beltowski et al., 2003, Kadowaki et al., 2005). Unlike other Adipokines, there was a decrease in the expression and plasma concentration of adiponectin in obese and overweight humans and animals. The plasma concentration of adiponectin has an inverse relationship with Body Max Index, body fat percentage, waist to hip ratio, fasting insulin concentration, and plasma triglyceride (Beltowski et al., 2003). Adiponectin plays an important role in the removal and oxidation of fatty acids by skeletal muscle through its receptors (AdipoR1 and AdipoR2). Hence, it reduces the mass of white adipose tissue and reduces blood lipids (Beltowski et al., 2003, Beltowski et al., 2005, Kadowaki et al., 2003). AdipoR1 is the most prevalent adiponectin receptor in skeletal muscle. In fact, its expression is much higher than AdipoR2 in the skeletal muscle (Beltowski et al., 2005). Studies have shown that the expression of Adipo R1 in the skeletal muscle also decreases due to the high-fat diet associated with reduced adiponectin expression in adipose tissue. This results to a decrease in the oxidation of fatty acids and increased insulin resistance in the skeletal muscle. Also, inhibiting

the gene expression of both adiponectin receptors in skeletal muscle suppresses glucose uptake by the tissue(Beltowski et al., 2005). Studies have suggested that there is a positive and direct correlation between adiponectin and desnutrin in adipose tissue and between desnutrin and Adipo R1, and other fat oxidation indicators such as CPT-1 in skeletal muscle (Ding et al., 2012, Li et al., 2009, Yao-Borengasser et al., 2011). For example, Borengasser et al. showed that in the skeletal muscle of non-diabetic subjects and in response to aerobic exercise training, the expression of desnutrin gene has a positive and strong correlation with the expression of the AdipoR1 gene (Yao-Borengasser et al., 2011). Also, Ding et al. showed that caloric restriction in rats resulted to an increase in desnutrin and adiponectin in adipose tissue (Ding et al., 2012). Therefore, Desnutrin, Adiponectin and AdipoR1 are important indicators of fat metabolism in adipose and muscle tissues. They also play a role in maintaining the phenotype of brown adipose tissue (Ahmadian et al., 2017). HIIT is a form of exercise that includes repetitive extreme exercise and active or passive rest, and today it is considered as an effective method in fat burning (Hoshino et al., 2013). Research suggests that HIIT such as traditional continuous training produces comparable effects, but with less time spent on muscle oxidative capacity, the ability to access substrate and fat burning the entire body (Hoshino et al., 2013, Zhang et al., 2017).

Until now, the effect of HIIT has been rarely studied in comparison with traditional continuous training on adiponectin, AdipoR1 and desnutrin. But separately, the effects of any type of training have been studied. For example, Larsen et al. showed that in 6 weeks and 3 sessions per week of HIIT, there was no effect on the level of ATGL and mitochondrial fat oxidation in muscle tissue and subcutaneous fat tissue of overweight inactive subjects (Larsen et al., 2015, Morton et al., 2016). On the other hand, Pierard et al. while investigating the effect of the interaction between exercise training and high-fat diet on the expression of adiponectin in adipose tissue and its receptor in the skeletal muscle, showed that 8 weeks of aerobic exercise training on a treadmill with a high-fat diet or standard increased adiponectin and Adipo R1 gene expression in subcutaneous adipose tissue and skeletal muscle in the training group, and in the group that only consumed high-fat diet, resulted in a decrease in gene expression (Pierard et al., 2016).

Considering that so far, less research has examined the effect of HIIT alone or in comparison with continuous training on the expression of the gene of the mentioned proteins. On the other hand, according to researches, the amount of fat burning in a HIIT is at least equal with traditional continuous training, but with less time, which emphasizes the effectiveness of HIIT in fat burning. Therefore, this research attempts to answer the following question: Is there a difference between the 6 weeks of HIIT and the continuous training with standard diet on the expression of desnutrin, adiponectin and AdipoR1 genes after a period of high fat diet in both subcutaneous adipose tissue and quadriceps muscle tissue in obese male rats?

Effect of two Exercise training on fat metabolism

- examines the effect two types of training protocols on effective proteins (desnutrin, adiponectin and Adiponectin Receptor 1) in fat metabolism on obese society for the first time
- Identify the Suitable method for weight loss (HIIT or continuous training or diet)

2. MATERIALS AND METHODS

The present study utilized a developmental and experimental method. In this study, 40 rats 10 weeks old with an average weight of 220 ± 5 gm were purchased from the Pasteur Institute of Iran. After a brief familiarization with the environment, the obese process lasted for 12 weeks. In order to ensure that the rats were obese in 12 weeks, of the 40 rats, 30 rats received high-fat diet while the remaining 10 rats received standard diet. Thereafter, 30 obese rats were divided into 3 groups based on the average body weight, so that the average weight of the three groups at the onset of the intervention was approximately the same:

First Group

10 rats, for 6 weeks and 6 sessions per week of continuous training and received a standard diet (continuous training).

Second group

10 rats, for 6 weeks, and 6 sessions per week of HIIT and received a standard diet (HIIT).

Third group

10 rats, did not participate in any exercise as a control group (control).

Continuous and HIIT groups ran for 6 weeks and 6 sessions per week on the treadmill. Rats in the control group did not exercise at all. The rats were first exposed to the treadmill for a week (10 minutes at a speed of 10 m/min and five days a week). Continuous training and HIIT was performed with the overload principle in mind (Table 1) (Afzalpour et al., 2015).

Table 1 Continuous training and HIIT protocols

Week	Day	Continuous training	HIIT	
			Odd day	Even day
Week 1	1	20 min,27m/min	2 intervals,40m/min,3 min	3 intervals,54m/min,30 s
	2	22 min,27m/min		
	3	24 min,27m/min	2 intervals,40m/min,3 min	5 intervals,54m/min,30 s
	4	26 min,27m/min		
	5	28 min,27m/min	2 intervals,40m/min,3 min	7 intervals,54m/min,30 s
	6	30 min,27m/min		
Week 2	1	32 min,27m/min	3 intervals,40m/min,3 min	9 intervals,54m/min,30 s
	2	34 min,27m/min		
	3	36 min,27m/min	3 intervals,40m/min,3 min	11 intervals, ,54m/min,30 s
	4	38 min,27m/min		
	5	40 min,27m/min	3 intervals,40m/min,3 min	13 intervals, ,54m/min,30 s
	6	42 min,27m/min		
Week 3	1	44 min,27m/min	4 intervals,40m/min,3 min	15 intervals, ,54m/min,30 s
	2	46 min,27m/min		
	3	48 min,27m/min	4 intervals,40m/min,3 min	17 intervals, ,54m/min,30 s
	4	50 min,27m/min		
	5	52 min,27m/min	5 intervals,40m/min,3 min	19 intervals, ,54m/min,30 s
	6	54 min,27m/min		
Week 4	1	56 min,27m/min	5 intervals,40m/min,3 min	19 intervals, ,54m/min,30 s
	2	58 min,27m/min		
	3	60 min,27m/min	6 intervals,40m/min,3 min	20 intervals, ,54m/min,30 s
	4	60 min,27m/min		
	5	60 min,27m/min	6 intervals,40m/min,3 min	20 intervals, ,54m/min,30 s
	6	60 min,27m/min		
Week 5-6	1-12	60 min,27m/min, to end of 6th week	6 intervals,40m/min,3 min , to end of 6th week	20 min, ,54m/min,30 s, to end of 6th week

Overload was increased by increasing the time in the continuous training group and increasing the intervals in the HIIT group. At the beginning and the end of the continuous training program, as well as the HIIT, there was warming and cooling for 60 seconds at a speed of 16 m/min. This intensity is 68% VO2 max. Moreover, the intensities of the continuous and HIIT program were respectively 80% VO2 max and 95%-100% VO2 max. In the HIIT group, there were 60 seconds of active rest between the intervals, with a speed of 16 m/minute (Afzalpour et al., 2015). The rats were monitored during the training sessions, using a weak electrical shock (0.5 mA intensity) that did not cause stress in the animal. After which the rats were placed behind a treadmill or manipulated with a sponge to encourage continuous running.

Each animal was assigned a fixed line, hence all its activities in the training program on this special line, were used to minimize the factors that could confuse the rat. The rats were maintained in the animal lab at the University of Medical Sciences in Dezful, under controlled conditions of light (12 h of light and 12 h of darkness, start lighting 6 am and start off at 6 pm) and temperature (22°C ± 3°C) and humidity (about 45%). Through the use of standard diet analysis and according to the guidelines for high-fat diet, and with the opinion of livestock and poultry experts, high-fat diet was prepared as a compact plate. High-fat diet content: 59% of energy was from fat (soybean oil), 21% total energy from protein, and 20% energy from carbohydrates (Storlien et al., 1986). 21% total energy from protein, and 20% energy from carbohydratesA group of rats received a 12-week high-fat diet. This diet contained 5.2 kilocalories per gram of rat body weight. Also, in the same 12 weeks, another group of rats consumed a standard diet (Chow), to measure the effect of high-fat diet and standard diet on rat weight. On the other hand, rats in the training and control groups consumed a standard diet in the 6-week protocol. The rats were individually kept in Plexiglass cages with a lid and dimensions of 25 by 27 by 43 cm, which were free to access water and food. Rat weighing was performed once a week throughout the study, including the stage of obesity and the protocol.

Texture extraction

In order to avoid misinterpretation of the data due to the remaining effects of the last session of exercise, 48 h after the last exercise session using 60-80 mg of ketamine per Kg of body weight and 8 mg of Xylazine per Kg of body weight under deep anesthesia and then extirpated by cutting off the head. Samples of subcutaneous adipose tissue and the central part of the quadriceps muscle tissue were performed. Subcutaneous adipose tissue, as compared with visceral fat, provides the highest amount of fatty acids for active muscles during exercise. The quadriceps muscle also shows the highest response to exercise in lower muscles, which is why the two tissues were selected in this study. Each rat was removed in less than 5 minutes from the target tissue and washed properly with normal saline solution to remove excess blood from the tissue and weighed to a precision of 0.0001 in a digital scale. Thereafter, they were frozen immediately using nitrogen and were frozen at -80°C for subsequent measurements.

RNA Extraction and RT-PCR

RNA extraction was conducted using trizol solution and a tissue homogenizer device. For the quantitative measurement of extracted RNA, a Nano-Drop device having a wavelength range of 260-280 nm was used. The average ratio of OD in wavelength range of 260 to 280 nm was 1.90, which indicates the proper quality and purity of the extracted RNA. To make cDNA, the Takara Kit instruction was used. The samples were then loaded into a Thermal cycler for 1 h at 37°C and 5 min at 85°C. For quantitative RT-PCR, the samples were first incubated for 10 min at 95°C. Then, for 40 times the three steps were repeated; 30 s of 95°C for denaturation, 30 s of 58°C for primers connection, and 30 s for 72°C for expansion; and finally, the melting reaction occurred. The Ct related to reactions was extracted by the software of RT-PCR and finally, Ct mean was recorded twice. Table 2 presents the primer used in this research. To quantify the levels of the target gene expression, the formula 2^(- ΔΔct) was used.

Table 2 Primers used in the research

Gene	Host	Forward Primer	Reverse Primer
Desnutrin	Rat	GACAGCTCCACCAACATCCA	AAGTCCATCTCGGTAGCCCT
Adiponectin	Rat	AACCCCTGGCAGGAAAGGA	CCTACGCTGAATGCTGAGTGAT
AdipoR1	Rat	GACTGGCTGAAAGACAATGACTACC	GAAATAGCACAAAACCCAAGCAAATGTG
β-Actin	Rat	CACGGCATTGTCACCAACTG	GCTGGGGTGTTGAAGGTCTC

Statistical Method

Descriptive statistics were used for raw data categorization and description. The Kolmogorov-Smirnov test (KS) was used to verify the natural distribution of the data, as well as the Levene Test to verify the equality of variances. One-way ANOVA test and Tukey's post hoc test were used to compare inter group changes. A significance level $p<0.05$ was provided for all statistical tests. Statistical analysis was conducted using SPSS 16 software and charts were drawn using Excel 2007 software.

3. RESULTS

At first, the early weight changes of rats after 12 weeks of high-fat and standard diet and after 6 weeks of standard diet along with continuous training and HIIT have been presented in (Tables 3 and 4) respectively.

Table 3 Average and standard deviation of rats weight after 12 weeks of high-fat and standard diet

Group	Initial weight (g)	Final weight (g)	Percentage Change
High fat diet	220±5	396±97	+34.43
standard diet	220±5	310±33	+ 27.53

Table 4 Average and standard deviation of rats weight after 6 weeks of standard diet along with continuous training and HIIT

Group	Initial weight (g)	Final weight (g)	Percentage Change
control	395±97	345±9	-7.81
continuous training	394±97	349±30	-6.71
HIIT	394±97	320±5	-15.70

The results of Desnutrin and Adiponectin in Adipose Tissue

In the present study, the expression level of desnutrin gene in both continuous training (P=0.001) and the HIIT groups (P=0.001) was higher compared to the control group and was statistically significant. On the other hand, the level of expression of the Desnutrin gene in the continuous training group was higher than the HIIT group and was statistically significant (P=0.011) (Figure 1).

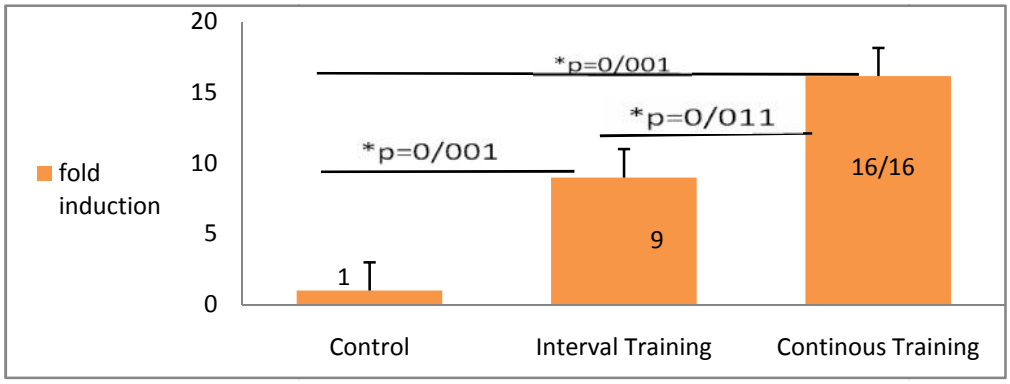

Figure 1 Desnutrin mRNA multi-fold variation ratio of the abdominal subcutaneous adipose tissue in the training groups compared to the control group. The data is expressed as mean ± SEM. Each column is for each group including 10 rats

The expression level of adiponectin gene was significantly higher in both continuous training (P=0.004) and HIIT groups (P=0.033) compared to the control group and statistically significant. On the other hand, expression level of adiponectin gene was higher in the continuous training group than in the HIIT group, and there was a significant difference between both groups (P=0.040) (Figure 2).

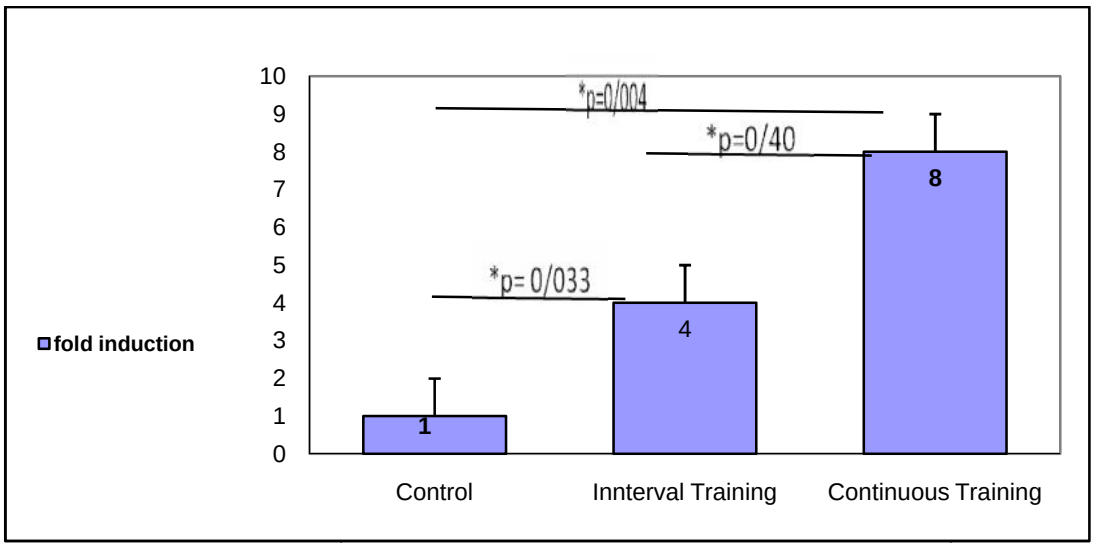

Figure 2 Adiponectin mRNA multi-fold variation ratio of the abdominal subcutaneous adipose tissue in the training groups compared to the control group. The data is expressed as mean ± SEM. Each column is for each group including 10 rats

The results of Desnutrin and AdipoR1 in Muscle Tissue

In the present study, the expression level of desnutrin gene in the continuous training group was higher than the control group, but was not statistically significant (P=0.651). On the other hand, the expression level of desnutrin gene in the HIIT group was higher than the control group and was statistically significant (P=0.016). Also, the expression level of desnutrin gene in the HIIT group was higher than the continuous training group, but was not statistically significant (P=0.855) (Figure 3).

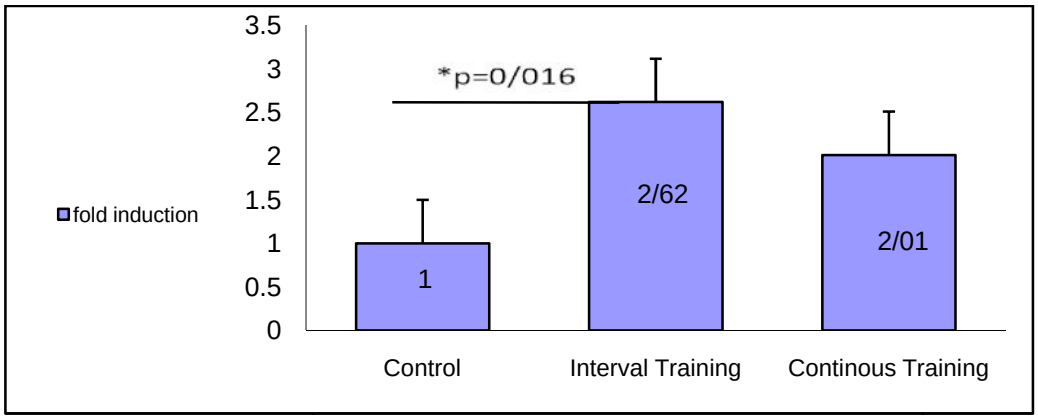

Figure 3 Desnutrin mRNA multifold variation ratio of the quadriceps muscle tissue in the training groups compared to the control group. The data expressed as mean ± SEM. Each column is for each group including 10 rats.

On the other hand, the expression level of AdipoR1 gene in the continuous training group was higher than the control group, but was not statistically significant (P=0.435). The expression level of AdipoR1 gene was higher in the HIIT group compared to the control group and was statistically significant (P=0.03). Also, the expression level of AdipoR1 gene was higher in the HIIT group than the continuous training group, but it was not statistically significant (P=0.565) (Figure 4).

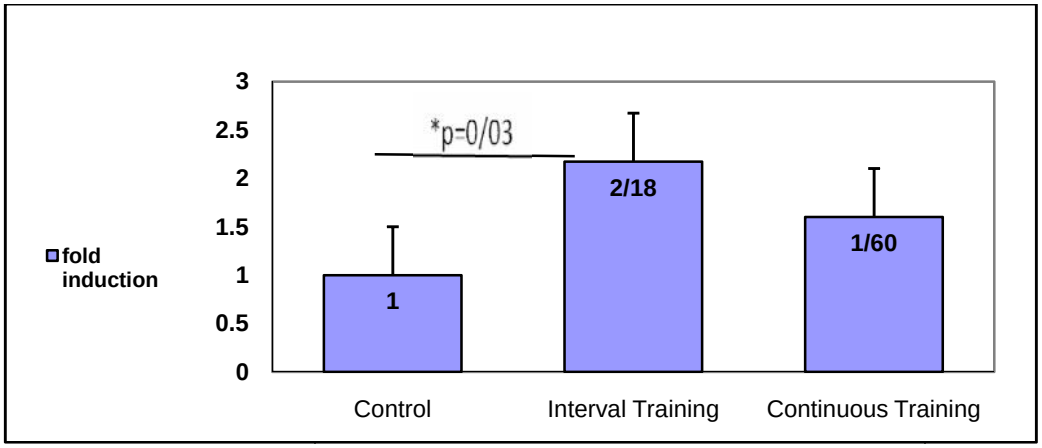

Figure 4 AdipoR1 mRNA multifold variation ratio of the quadriceps muscle tissue in the training groups compared to the control group. The data expressed as mean ± SEM. Each column is for each group including 10 rats.

4. DISCUSSION

In the present study, the expression level of desnutrin gene subcutaneous fat tissue in the continuous training group was significantly higher than the group (approximately 2 fold) and had statistically significant differences. So far, there has not been much research on the effect of HIIT on the expression of the desnutrin gene, and research has shown that aerobic exercise training can increase expression of the desnutrin gene in adipose tissue (Yao-Borengasser et al., 2011, Ogasawara et al., 2012). Here, the cause of an approximately two-fold increase in desnutrin gene expression of the subcutaneous adipose tissue in the continuous training group compared to the HIIT group could be due to the intensity and duration of the training. Since the use of fat increases at lower intensities and over an increased duration of training, and its main source is the triglyceride of adipose tissue Therefore, it is expected that a further increase in desnutrin of the subcutaneous adipose tissue in the continuous training compared to the HIIT can be justified. Moreover, high weight loss in the HIIT group compared to the continuous training group may have resulted to a

decrease in fat tissue mass, and as a result, there has been a decrease in lipolysis and the source of adipose tissue fatty acids in this group (HIIT) and caused this difference in the gene expression. It is also likely that the total amount of energy consumed in the continuous training is more compared to the HIIT. Therefore, with higher energy consumption, the metabolic rate is likely to be higher, and there will be increased desnutrin in the continuous training group compared to the HIIT group.

On the other hand, in the present study, continuous training was associated with a 2-fold increase in adiponectin subcutaneous adipose tissue in comparison with HIIT and was statistically significant. The results of this study are not consistent with the results of Kraemer et al. and Trapp et al. They showed that there is no difference in adiponectin levels between continuous training and HIIT (Kraemer et al., 2003, Trapp et al., 2008). The reason for the difference can be the duration, intensity, training protocol and the training subjects. Kraemer al. examined the effect of short-term exercise training on adiponectin in human subjects, but in the present study, the long-term effects of exercise training were investigated in rats. This could be a reason for the difference in outcome. On the other hand, the inconsistency between the results of Trapp et al. And our results, despite the long-term effects of HIIT and the continuous training on human sample adiponectin, can be in the type of exercise protocol. The number of training sessions in the study of Trapp et al. was carried out three times a week, as well as in the human specimen. Therefore, according to 6 sessions of weekly practice in our research; the total amount of energy expended is likely to be higher, thereby resulting in this contradiction. Consequent upon the lack of scientific literature on comparing two types of HIIT and the continuous training of adiponectin gene expression of subcutaneous adipose tissue, another mechanism that has resulted to this significant difference has been achieved. The reliance on most HIIT is on the uses of intramuscular fat, especially in the thigh (Koichiro et al., 2017, Turnbull et al., 2016), which is likely to result in less use of subcutaneous adipose tissue in comparison to continuous training. This results in less gene expression of adiponectin in the subcutaneous adipose tissue. In the present study, there was a positive correlation between the expression of desnutrin and adiponectin genes in the subcutaneous adipose tissue. So that, a significant increase in adiponectin gene expression in the continuous training group was observed compared to the HIIT group. It was accompanied with a significant increase in the expression of the desnutrin gene in the continuous training group compared with the HIIT group. However, so far, no research has compared the effect of two continuous training and HIIT on the adiponectin and desnutrin gene expression, but the results of the present study are consistent with the results of Ding et al. They also observed an increase in the simultaneous expression of adiponectin and desnutrin genes, but with the caloric restriction alone in the subcutaneous adipose tissue (Ding et al., 2011). In fact, the increase in the expression of desnutrin and adiponectin genes in the subcutaneous adipose tissue due to caloric restriction or exercise training is due to body weight and body fat loss, since there is an inverse relationship between desnutrin and adiponectin with body fat stores (Villena et al., 2004, Beltowski et al., 2005). This indicates the positive relationship between desnutrin and adiponectin as effective factors in fat metabolism in adipose tissue. On the other hand, in the present study, it is possible that the use of fat deposits of subcutaneous adipose tissue is higher due to the lower intensity of continuous training compared with HIIT (Bae et al., 2017, Louche et al., 2013). Therefore, it has a significant effect on continuous training compared with HIIT, on the expression of desnutrin and adiponectin genes.

Also, in the present study, the expression level of desnutrin and AdipoR1 genes in the quadriceps muscle was higher in the HIIT group than in the continuous training group, but there was no statistically significant difference. In this study, HIIT showed a slight increase in the expression of desnutrin and AdipoR1 genes of the quadriceps muscle compared to the continuing training group. Due to adaptive exercise training, the use of intra-muscle triglyceride reserves increases during exercise (Morton et al., 2016, Turnbull et al., 2016). On the other hand, the energy of localized muscle cost in the hip during exercise is more intense than other parts of the body. As a result, there is an increase in the intake of intramuscular fat in an extremely intense exercise in the hip and quadriceps muscle (Koichiro et al., 2017, Turnbull et al., 2016). This results to a further increase in lipolysis and fat oxidation, possibly leading to a greater expression of the desnutrin and AdipoR1 genes muscle. Perhaps the manipulation of intensity and the duration of HIIT could make this difference significant. However, HIIT showed a 1.3 fold increase in the gene expression of desnutrin and 1.5-fold in the AdipoR1 gene expression of the quadriceps muscle compared to the continuing training group. Since there is a positive relationship between desnutrin and AdipoR1, and other markers effective in intra-muscle fat oxidation (CPT-1) Borengasser et al. showed that in the skeletal muscle of non-diabetic subjects and in response to aerobic exercise training, the expression of the desnutrin gene has a positive and strong correlation with the expression of the AdipoR1 and CPT-1 genes (Yao-Borengasser et al., 2016).

5. CONCLUSION

In conclusion, there is a direct and positive relationship between the expression of desnutrin and adiponectin genes in subcutaneous adipose tissue and the expression of desnutrin and AdipoR1 genes in the quadriceps muscle tissue. Also, continuous training, as

compared to HIIT, has a greater impact on the expression of desnutrin and adiponectin genes of the subcutaneous adipose tissue. However, in the quadriceps muscle tissue, there is no difference between the two types of training protocols in the expression of desnutrin and AdipoR1 genes. On the other hand, for weight loss, HIIT is a better way.

List of Abbreviations

AdipoR1= Adiponectin Receptor 1, HIIT= High Intensity Interval Training, ATGL= Adipose triglyceride lipase, UCP-1= uncoupling protein 1, CPT-1= Carnitine palmitoyltransferase I, AdipoR2= Adiponectin Receptor 2, RT-PCR= Real-time Polymerase

conflicts of interest statement

The authors declares that they have no competing interests.

Ethics approval and consent to participate

All stages of rat maintenance and sacrificing were performed according to the rules of the Animal Ethics Committee of Dezful University of Medical Sciences (IR.DUMS.REC. 1397.008). also confirm that the Animal Ethics Committee of Dezful University of Medical Sciences approved this stydy.

Acknowledgements

Saeed Rahmaty carried out the molecular genetic studies, participated in the sequence alignment and drafted the manuscript. Reza Ghaffaripour carried out the immunoassays. Abbas Ali Gaeini participated in the sequence alignment. Mojtaba Dolatshahi participated in the design of the study and performed the statistical analysis. Gholam Reza Salvand conceived of the study, and participated in its design and coordination and helped to draft the manuscript. All authors read and approved the final manuscript. This research was sponsored by Dezful University of Medical Sciences and Health Services. Sincerely grateful to Mr. Hallaj, Mr. Sadi and Mr. Hassani, who helped in keep the animals in the present study.

Conflict of interest

There are no conflicts of interest in this study.

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