

THE EFFECT OF EIGHT WEEKS HIGH INTENSITY INTERVAL TRAINING (HIIT) ON SERUM AMOUNTS OF FGF21 AND IRISIN IN SEDENTARY OBESE WOMEN

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Abstract

Background & Aims: Transforming white adipose tissue to brown adipose tissue is considered a solution to overcome undesirable effects of obesity in human beings. Thus, the aim of this research was to investigate the effect of eight weeks of high intensity interval training (HIIT) on serum amounts of fibroblast growth factor 21 (FGF21) and Irisin in sedentary obese women.

Materials & Methods: Twenty sedentary obese women (with the average age of 30.15 ± 2.96 and BMI of 30.34 ± 1.27) were selected and randomly categorized into HIIT group and control group each consisting of 10 members. HIIT program was conducted for 8 weeks, 3 sessions in each week and with 90 percent of target heart rate. Blood samples were taken from both HIIT and control groups (while fasted) before and after the training to measure Irisin, FGF21 and lipid profile. The research data were analyzed by independent and dependent t-statistic tests in the significant level of $P < 0.05$.

Results: The results of the statistical analysis showed HIIT program increased serum amounts of FGF21 and Irisin in sedentary obese women ($P < 0.05$). Also, the results of independent t test showed there was a significant difference between HIIT group and control group in terms of FGF21 and Irisin amounts.

Conclusion: According to the results of this research, it seems HIIT program with increasing amounts of Irisin and FGF21 may lead to the transformation of white adipose tissue to brown adipose tissue and therefore, it has a key role in preventing obesity and its negative consequences.

Keywords: High Intensity Interval Training (HIIT), Irisin, FGF21, Sedentary obese women

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Introduction

The prevalence of obesity has highlighted the need for preventing its metabolic consequences through new treatment methods. It has recently been reported that transforming white adipose tissue (WAT, calorie storage) to brown adipose tissue (BAT, heat and calorie

usage) is a solution for overcoming undesirable consequences of obesity in human beings (1, 2). White adipose tissue and brown adipose tissue play a key role in energy balance in the mammals. White adipose tissue is widely distributed in the body and is the first place in which fat is stored and burned. Brown adipose tissue is

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relatively few and is responsible for preserving body temperature through thermogenic adaptability (3,4). Heat production in brown adipose tissue is mostly related to adrenergic activity due to lipolysis and free fatty acids release through uncoupling protein 1 (UCP1) (5,6). Studies show white adipose tissue cells are capable to distinguish themselves from brown ones and vice versa. Once exposed to coldness, some of white adipose tissue cells change into brown ones to increase heat production. Being exposed to diet, brown adipose tissue changes to white adipose tissue to store energy (7).

Fibroblast growth factor 21 (FGF21) is amino acid hormone 181 from FGF family which functions as hyperglycemia, anti-fat, and heat producer (8). FGF21 is express in adipocytes, liver, skeletal muscles, and pancreas (9). Transcription of FGF21 is done in the liver under direct control of peroxisome proliferator-activated receptor alpha (PPAR α) and by peroxisome proliferator-activated receptor gamma (PPAR γ) in adipose tissue (9). FGF21 has a positive and significant relation with UCP1 in BAT and through UCP1 and ACC1⁴ leads to the activation and motivation of WAT (10). This process is adjusted by FGF21 through increasing the level of PGC-1 α ⁵ protein (11). Also, FGF21 adjusts energy hemostasis in adipocytes by activating AMP-activated protein kinase (AMPK) and sirtuin (SIRT). The result is an increase in the function of mitochondrial oxidative (11).

It is shown that FGF21 is a metabolic hormone which affects on energy balance and fat and glucose metabolism. The omission of FGF21 causes disturbance in glucose hemostasis and leads to fatness (12). The studies show that the overweight have more amount of FGF21 (13). However, it seems that FGF21 has anti-fat

effects which lead to losing weight through the increase in using fat and reduction in fat mass (13). FGF21 is involved in metabolic disturbances pathogens such as insulin resistance, diabetes type 2, and fatty liver disease (14). It is shown that it can lead to natural revival of blood sugar level and triglyceride and improve insulin sensitivity regardless of weight in overweight and diabetic samples (13).

It is also believed that exercise may lead to improve metabolism of body by stimulating FGF21. Huwan Kim et al. (2013) showed that acute exercise for 30 minutes can increase FGF21 in both humans and animals. This increase is in tandem with an increase in free fatty acids and Glycerol (15). Also, Tanimura et al. (2016) showed that doing exercises with 75% VO₂max for 60 minutes increased serum amounts of FGF21 (13). Other studies, however, don't confirm these results. In this regard, Slusher et al. (2015) showed that 30 minutes of submaximal aerobic exercises creates less FGF21 in the obese people compared with people with natural weight (14).

Also, Kong et al. (2016) showed that 5 weeks of HIIT didn't make any significant changes in FGF21 (1⁷). Birjandi et al. (2016) showed that 6 weeks of HIIT didn't make any significant changes in FGF21 in overweight and obese men (1⁸). Since findings are not consistent, it is necessary to do more researches with longer durations and different intensities.

Irisin is a myokine derived from skeletal muscle which originates from fibronectin type III domain-containing protein 5 (FNDC5) and is dependent on PGC-1 α for thermogenic activation of white adipose tissue and glycemic improvement (1⁸). In fact, Irisin, through expression of UCP1, affects white adipose tissue and transforms it to brown adipose tissue and thus

⁴. *acetyl-CoA carboxylase 1 (ACC1)*

⁵. *Peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1 α)*

leads to the increase in calorie consumption and heat production. This, finally, results in losing weight and improving obesity (19, 20). Moreover, Irisin leads to the oxidation of fatty acids and glucose by activating AMPK which shows the role of Irisin in glucose hemostasis and fat (21). In this regard, Miyamoto et al (2015) showed that endurance training creates more Irisin in old men and reduces their stomach fat (22). Yang (2016) studied the effect of short-term and long-term swimming with moderate intensity in obese rats and showed that Irisin increased in both cases. The short-term exercise (8 weeks) resulted in gradual progression of obesity and long-term exercise (16 weeks) prevented its progression (23). Though a lot of studies support the stimulating effect of exercise on irisin level, Benedini (2017) showed that serum amounts of Irisin in professional and occasional athletes are the same as physically inactive individual (24). Given these results, it seems exercise type, exercise duration, and exercise intensity are influencing factors on Irisin secretion.

HIIT has recently been considered a proper exercise choice not only for athletes but for overweight and obese individuals who have limited time (25). HIIT uses exercise intensity as an effective factor instead of exercise volume. In exercises with low volume and high intensity, fat mass is lost in a more desirable way than consistent low-intensity exercises. For instance, it is shown that HIIT increases VO_2 max and reduces fat percentage and BMI (26). In general, different studies have been conducted in recent years on brown adipose tissue as an effective factor in energy hemostasis and preventing obesity. It has been shown that FGF21 and Irisin increase heat and energy consumption by transforming white adipose tissue to brown adipose tissue and activating UCP1 (27).

Nevertheless since a large number of studies deal with resistance and intensive exercises and HIIT exercises are less used, and given the time consuming

nature of traditional resistance exercises, HIIT exercises can increase motivation of individuals to take part in the exercises. Also, given limited and contradictory results, more studies are required. As a result, this research aims to investigate the effect of eight weeks of high intensity interval training (HIIT) on serum amounts of FGF21 and Irisin in sedentary obese women.

Materials and Methods

The present research is quasi-experimental, pre-test and post-test with control group. The population of the research includes sedentary obese women in Naghade from 25 to 35 years old. Following the initial announcement for participation in the research, 40 sedentary obese women were selected. Then, 20 of them were randomly qualified to fill in the letter of satisfaction and healthy questionnaire and they were categorized in two groups: HIIT group (10 persons) and control group (10 persons).

The criteria for entering the research were as the following: $BMI \geq 30 \text{ kg/m}^2$, not having disease records, not being under medical treatment during the research, not having a regular exercise program. The criteria for leaving the research were: lack of interest, taking medicines and nutritional supplements, not taking part in exercise sessions regularly, and being hurt (14).

Participants were asked to take part in a briefing session. They filled in the letter of satisfaction and healthy questionnaire in this sessions and received necessary information for the following sessions. In the next session, they learned how to do HIIT exercise. Then, 5 cc blood was taken from their arm vein by a lab expert. Sampling was done 24 hours before exercise intervention while participants were fasted.

Next, the anthropometric measurements including height (by Wall stadiometer with 0.5 cm accuracy) and weight (by CAMRY, EF551BW digital scale with 0.1 kg accuracy) were done while participants were wearing light clothes and no shoes. BMI was measured by

squared body weight (kg) divided by height ² (meter), fat percentage by Jackson/Pollock Caliper Method (28). Participants' heart rate was measured and their aerobic capacity (VO₂max) by modified Bruce protocol (In the standard Bruce protocol, the starting point (ie, stage 1) is 1.7 mph at a 10% grade (5 METs). Stage 2 is 2.5 mph at a 12% grade (7 METs). Stage 3 is 3.4 mph at a 14% grade (9 METs). This protocol includes 3-minute periods to allow achievement of a steady state before workload is increased. The modified Bruce protocol has 2 warm up stages, each lasting 3 minutes. The first is at 1.7 mph and a 0% grade, and the second is at 1.7 mph and a 5% grade (29).

Exercise Program:

In the briefing sessions, participants were acquainted

with research aims, the way exercises should be done and research time schedule.

High Intensity Interval Training (HIIT):

Participants exercised on treadmill for 8 weeks, 3 sessions in each weeks, with 90 percent of target heart rate (THR = Resting heart rate + Exercise intensity (Resting heart rate – Maximum heart rate)) (30).

The training program for each session included: 10 minutes for stretching and active warm-up, main exercise (repeating bouts for 4 minutes with 90 percent target heart rate and 2 minutes active recovery between bouts with 50-60 percent of target heart rate), and finally 5 minutes cool-down. These are shown in Table 1 (31). The heart rate of the participants were controlled. If increasing or decreasing exercise intensity was required, sufficient feedback was given to the participants.

Table 1. HIIT program (3 sessions per week)

weeks	Exercise intensity (percentage of THR)	No. of bouts (repetition)	Duration of each bout (min)	Duration of recovery between bouts (min)	Active recovery intensity (percentage of THR)
1st week	90	3	4	2	50-60
2nd week	90	4	4	2	50-60
3rd week	90	5	4	2	50-60
4th week	90	6	4	2	50-60
5th week	90	7	4	2	50-60
6th week	90	8	4	2	50-60
7th week	90	6	4	2	50-60
8th week	90	5	4	2	50-60

Measuring Biochemical Variables:

Blood samples were taken after 12 hours of fasting and in 2 stages. In the first stage, according to the instructions, participants were asked to avoid doing any heavy physical activity, being in stressful conditions, and taking medicines 3 days prior to sampling. The resulting serums were frozen in -70c until the second experiment. The second sampling was done 48 hours

after the last exercise session to remove the effects of this session from exercise and control groups. Serum amounts of FGF21 and Irisin were measured by ELISA Reader and Zellbio kit (made in Germany). Triglyceride, cholesterol, HDL-C and LDL-C were measured by photometric method using Pars Azmoon Co. kit.

Statistical Method:

The analysis of data was done using descriptive statistics for measuring central indexes and dispersion, K-S test for normal dispersion of data, leven test for determining the heterogeneity of variances, dependent t test for studying the pre-test and post-test changes made in dependent variables, and independent t test for studying differences between groups. Data were

analyzed by SPPSS version 22 and significance level was $p < 0.05$.

Results

Table 2 shows anthropometric and physiological characteristics of participants.

Table 2. Anthropometric and physiological characteristics of participants, before and after 8 weeks of exercise program

Variable	group	Pre-test	Post-test	Inter-Group changes		Intra-group changes	
				t	p	t	p
Age (years)	Control	30.5±2.27					
	Exercise	29.8±3.32					
Height (cm)	Control	165.88±3.01					
	Exercise	164.10±4.10					
Body mass (kg)	control	83.05±5.99	82.88±5.41	0.531	0.608	1.140	0.269
	Exercise	81.41±6.27	79.86±6.37	7.05	# 0.001		
BMI (kg/m ²)	control	30.24±1.19	30.02±1.36	2.075	0.068	1.121	0.277
	Exercise	30.48±1.54	29.38±1.183	3.432	# 0.0007		
Fat (%)	control	41.26±3.35	40.86±3.25	0.604	0.143	2.724	* 0.014
	Exercise	39.22±6.8	36.70±3.56	2.743	# 0.023		
WHR (cm)	control	0.92±0.45	0.91±0.05	0.840	0.432	2.202	* 0.041
	Exercise	0.91±0.33	0.87±0.20	4.260	# 0.0002		
VO ₂ max (ml.kg.min ⁻¹)	Control	36.1±3.52	36.21±3.55	-1.129	0.288	-1.363	0.190
	Exercise	37.75±3.29	39.61±2.98	-6.811	# 0.001		

Significance as compared to pre-test amounts

* Significance as compared to HIIT group. The amounts are indicated as standard deviation ± average.

Table 3 shows mean and standard deviation of dependent variables, before and after 8 weeks of exercise program. It also shows the results of dependent t test and independent t test.

The results of dependent t test showed that 8 weeks of HIIT increased serum amounts of Irisin ($p=0.001$) 7.87%, FGF21 ($p=0.000$) 6.11%, HDL-C ($p=0.031$) 7.97%, and VO₂max ($p=0.000$) 4.92% in sedentary Obese women as compared with basic conditions. It also

reduced weight ($p=0.000$) 1.90%, BMI ($p=0.0007$) 3.6%, body fat percentage ($p=0.023$) 6.42%, WHR ($p=0.002$) 4.93%, triglyceride ($p=0.007$) 4.31%, Total cholesterol ($p=0.020$) 3.03%, and CO/HDL ($p=0.017$) 10.22% as compared with basic conditions. No significant difference was observed in the control group ($P > 0.05$). Also, the results of independent t test showed that there is a significant difference in Irisin ($p=0.000$), FGF21 ($p=0.000$), body fat percentage ($p=0.014$), WHR

($p=0.014$) and triglyceride ($p=0.029$) between HIIT and control groups. However, there was no significant difference between two groups in weight, BMI,

$VO_2\max$, HDL-C, LDL-C, cholesterol, LDL/HDL, and CO/HDL ($p>0.05$) (Table 3).

Table 3. Results of Independent and Dependent t-statistic tests in determining the difference between research variables

Variable	Group	Pre-test	Post-test	Inter-group changes		Intra-group changes	
				t	p	t	p
Irisin (ng/ml)	Control	162.03±2.45	162.29±4.36	-0.147	0.865	-5.945	* 0.000
	Exercise	162.73±4.45	175.55±7.22	-4.692	# 0.001		
FGF21 (pg/ml)	Control	249.73±2.67	249.90±3.35	-0.141	0.891	-5.155	* 0.000
	exercise	250.85±4.44	265.93±6.62	-6.232	# 0.000		
WHR (cm)	Control	0.92±0.45	0.91±0.05	0.840	0.432	2.202	* 0.041
	exercise	0.91±0.33	0.87±0.20	4.260	# 0.0002		
$VO_2\max$ (ml.kg.min-1)	Control	36.1±3.52	36.21±3.55	-1.129	0.288	-1.363	0.190
	Exercise	37.75±3.29	39.61±2.98	-6.811	# 0.000		
Triglyceride (ml/dl)	Control	128.92±4.73	127.93±3.87	1.371	0.204	2.374	* 0.029
	exercise	129.56±3.63	129.56±3.63	3.475	# 0.007		
Total cholesterol (mg/dl)	Control	169.46±5.31	168.64±4.31	1.155	0.287	1.389	* 0.182
	exercise	170.94±3.28	165.76±4.96	2.827	# 0.020		
LDL-C (mg/dl)	Control	103.56±12.04	103.21±13.89	0.210	0.839	1.338	0.167
	exercise	102.28±7.149	95.40±12.15	1.594	0.146		
HDL-C (mg/dl)	Control	43.91±4.03	44.64±4.72	-1.017	0.335	-0.799	0.435
	exercise	42.99±4.82	46.42±4.80	-2.55	# 0.031		
CO/HDL (mg/dl)	Control	3.87±0.368	3.81±0.393	0.958	0.363	0.085	0.933
	Exercise	40.01±0.44	3.60±0.452	2.913	# 0.017		
LDL-C/HDL-C (mg/dl)	Control	2.32±0.45	20.25±0.411	0.510	0.622	0.603	0.554
	exercise	2.48±0.43	2.12±0.54	1.44	0.182		

Significance as compared to pre-test amounts

* Significance as compared to HIIT group. The amounts are indicated as standard deviation ± average.

Discussion

The importance of exercise in preventing and curing metabolic and cardiovascular diseases is clear today. However, cellular-molecular and other mechanisms through which these activities exert their positive influence are not fully comprehended yet. Different studies have been done on brown adipose tissue as the effective tissue in energy hemostasis in recent years.

These studies show that FGF21 and Irisin create heat, energy expenditure, and prevent obesity by transforming WAT to BAT and activating UCPI (27). Thus, the aim of this research is to investigate the effect of eight weeks of high intensity interval training (HIIT) on serum amounts of FGF21 and Irisin in obese, sedentary women.

The results showed that 8 weeks of HIIT exercise increased serum amounts of FGF21 in Obese, sedentary women significantly and there was a significant difference between two groups. There are limited and contradictory studies about the effect of HIIT on FGF21. Dlouhy et al. (2016) showed that 8 weeks of aerobic exercises with 60 to 75 maximum heart rate for 30 to 45 minutes increase FGF21 serum amounts significantly but there was no significant change in the control group (32). Cuevas et al. (2012) showed that 2 weeks of aerobic training for 5 days a week can increase FGF21 serum amounts in healthy women (33). Kong et al. (2016) showed that 5 weeks of HIIT training including sixty 8-second repetitions with 12-second intervals, didn't make a significant difference in serum amounts of FGF21 in obese women (16). Also, Birjandi et al. (2016) showed that 6 weeks of HIIT (3 sessions per week) didn't make a significant difference in serum amounts of FGF21 in obese women (17). The existing differences can be due to the difference in groups, number of participants, intensity and duration of the exercise, gender, age, and the time between the last exercise session and blood sampling. For instance, in Slusher et al. (2015) study, blood sampling was done right after the last exercise session while in this research it was done 48 hours after the last session (14). One can compare the study by Kong (5 weeks of training) with that of Birjandi (6 weeks of training) about the effect of exercise duration on the results (16,17).

Mechanisms through which HIIT can increase FGF21 are unknown but the following mechanisms are suggested for justifying increase in FGF21 and its physiologic effects:

AMPK's activation while doing intensive exercise can be one of the factors. AMPK is the key modifier for fat metabolism and energy balance. AMPK gets activated with the increase in inter-cellular AMP/ATP and its activation leads to the activation of metabolic pathway producing ATP (33). Thus, AMPK's activation

activates peroxisome proliferator-activated receptor alpha (PPAR α) and accordingly increases FGF21 (33).

Glucagon ratio to insulin is considered FGF21 modifier and glucagon's content increases by sport activity (34). Insulin motivates FGF21 in skeleton muscles. Muscular FGF21 increases significantly a few hours after insulin injection. Insulin increases FGF21 by activating P13/AKT signaling pathway. In addition to insulin, muscular contraction activates AKT signaling pathway and increases muscular FGF21. Therefore, insulin and skeletal muscle contraction are two main activators of AKT in skeletal muscle. During the training, insulin decreases but skeletal muscle contraction continues which can be a mechanism for activating AKT pathway independent from insulin (15).

It is shown that FGF21 amounts have a direct link with BMI, visceral adiposity, and pericardial adiposity (35). Also, there is a direct link between FGF21 and physical fitness. So, those with low physical fitness have low amounts of FGF21 (36). As a result, HIIT training in this research increases FGF21 by activating AMPK, P13K/AKT, and increasing VO₂max.

FGF21 shows its anti-obesity effects in the following mechanisms:

FGF21 control the body weight by activating mTORC1 and increasing the absorption of glucose, oxidation of free fatty acids and glycolysis (15).

FGF21 increases insulin signaling significantly. It also increases glucose absorption by releasing adipokines sensitive to insulin like adiponectin. In fact, FGF21 increases adiponectin and adiponectin increases β -oxidation through ceramide-adiponectin-FGF21. It also increases energy consumption, decreases glucose, decreases ceramide acid in liver and adipose tissue, improves insulin sensitivity and reduces weight (37).

FGF21 controls liver lipogenesis and increases FFA lipolysis when the person is hungry (38). In response to hunger, FFA is used by activating free fatty acids oxidation and lipolysis in liver tissue (33). Similar to

hunger, in exercise training lipolysis and FFA oxidation are increased as main fuels for ATP production in liver and skeletal muscles tissues and FFA increases the activity of PPAR α in the liver which leads to FGF21 increasing (33).

FGF21 increases energy consumption by modifying AMPK activity and SIRT1 through LKB1 in adipose tissue. Change in NAD⁺ amounts in a cell activates SIRT1, PGC-1 α , and histone-3. Their activation increases the function of mitochondrial oxidative and reduces weight (11).

One of the other effective FGF21 mechanisms is the conversion of white adipose tissue to brown one (33). FGF21 increases UCP1 and other heat producing genes in adipose tissue. An increase in PGC-1 α protein modifies this process. PGC-1 α produces heat by activating UCP1 in the brown adipose tissue (39). In this research, HIIT reduced fat mass, BMI and WHR. Although FFA and UCP1 were not measured in this research, it seems fat mass reduction is an indirect index for FFA increase which shows lipolysis of fat tissue.

Also, the results of this research showed that 8 weeks of HIIT can increase serum amounts of Irisin in obese, sedentary women significantly. There was a significant difference between HIIT and control groups. These results support those of the study done by Molaei et al. (2015) and Khalafi et al. (2016) which showed the increase in irisin in rats after 10 weeks of HIIT and its increase after 8 weeks of HIIT and aerobic training with low intensity respectively (40,41). Miyamoto et al. (2015) showed that 8 weeks of endurance training with 60 to 70 percent of VO₂max increases Irisin in the middle-aged and elderly significantly (22). Yang et al. (2016) showed that swimming training for 8 to 16 weeks in rats fed with high fat diet, increases Irisin. This increase in 8 weeks reduces obesity progression while in 16 weeks it prevents its progression (23). Regardless of Irisin's increase in response to different exercise, some results are contradictory. Benedini et al. (2017) showed

that there is no significant difference in serum amounts of irisin in professional athletes, occasional athletes, and sedentary people (14). Hakimi et al. (2015) showed that 8 weeks of aerobic training with 60 to 80 percent of heart rate in male overweight university students didn't make any changes in their Irisin serum levels (42).

Irisin is a hormone motivated by exercise and secreted in mice and human beings by skeletal muscle. It stimulates white adipose tissue conversion to brown adipose tissue which improves energy consumption rate (18). In this research, it seems AMPK's activation during HIIT training is one of the factors for increasing PGC-1 α and irisin. AMPK's activation leads to the phosphorylation of PGC-1 α as FNDC5's modifier and irisin secretion (43). Also, PGC-1 α activates PPAR γ . PPAR γ is involved in energy metabolism and stimulates FNDC5 and irisin increase (44). It is shown that there is a relation between irisin amounts and precursor of FNDC5 and PGC-1 α (43). Gene expression of FNDC5 in skeletal muscle is related to obesity in human. The amount of expression increases with obesity. This relation can be a compensatory mechanism (18,45). In line with this theory, another research showed gene expression of FNDC5 in skeletal muscle has a positive relation with BMI (18,45). Some studies reveal that irisin has a reverse relation with BMI, glucose and triglyceride (46). Also, some studies show that irisin amounts have a positive correlation with muscular mass and a negative correlation with fat mass (47). Nevertheless, it seems HIIT training in this study causes energy consumption and heat production by increasing muscular tissue ratio to fat tissue, decreasing BMI, changing fat type (converting WAT to BAT), and increasing UCP1. Thus, it paves the way for an increase in PGC-1 α , FNDC5, and irisin.

Obesity comes with metabolic disturbances such as increase in cholesterol serum amount, LDL-C, VLDL-C, TG, and decreasing HDL (47). The results of this research didn't show a significant reduction in LDL-

C and HDL/LDL but showed significant reduction in TG, total cholesterol, cholesterol on HDL, BMI, WHR, and fat percentage. It also showed significant increase in HDL-C amounts. In this regard, Elmer et al. (2016) showed 8 weeks of HIIT compared with moderate intensity aerobic training increased TG but didn't change HDL-C and cholesterol significantly (4[^]). Peterson et al. (2015) showed that 6 weeks of HIIT (each session including 2 thirty-minute intervals of indoor cycling) decreased LDL, cholesterol, and TG while change in HDL was not significant (4[^]). Hassani et al. (2016) showed that 8 weeks of HIIT decreased TG significantly but made no significant change in cholesterol, LDL, and HDL (5[^]). Paahoo. (2015) showed that 12 weeks of HIIT decreased TG, LDL-C and increased HDL-C in sedentary obese boys significantly (5[^]). The differences can be due to the difference in the used HIIT protocols. Using different durations, intensities, and types of HIIT make it possible to create unlimited number of protocols.

An intensity that optimizes fat oxidation as the main energy source during exercise is important. Research has shown that obese individuals have an impaired utilization of free fatty acid in the skeletal muscle (5[^]). Endurance training is an effective strategy for obesity prevention and weight loss because it enhances lipolysis and fatty acid oxidation in the skeletal muscle. This enhancement is known to be intensity-dependent, as the absolute rate of fat oxidation (g.min⁻¹) increases from low to moderate intensity and then decreases as exercise becomes more intense (5[^]). Nevertheless, studies show six weeks of HIIT for 2 weeks can increase skeletal muscle oxidative capacity, endurance performance and change metabolic amount (54). Storina et al. (2013), showed that 2 weeks of HIIT (6 sessions of 4 to 6 repeats of 30s Wingate with 4-5 min recovery) increased resting fat oxidation in overweight/obese sedentary men (5[^]).

Mechanisms through which HIIT leads to increase in fat oxidation and losing weight are unknown. However, it seems HIIT training in this research leads to fat and weight loss and fat oxidation through the following mechanisms:

It seems that fatty acid transport proteins have been linked to enhanced fat oxidation. The increases in fatty acid translocase (FAT/CD36) and plasma membrane fatty acid-binding protein (FABPpm) both found in the sarcolemma, the mitochondrial membrane, and in a cytoplasmic pool in skeletal muscle, could contribute to the enhanced fat oxidation by increasing the rate of free fatty acid transfer across the muscle and mitochondrial membrane (54). It has been shown that Six weeks of HIIT (ten 4-min cycling bouts at 90% VO₂peak separated by 2-min of rest) increased fatty acid transport protein content in whole muscle (FAT/CD36 and FABPpm), sarcolemmal (FABPpm) and mitochondrial (FAT/CD36) membranes in the skeletal muscle of 10 untrained females, suggesting that increases in skeletal muscle fatty acid oxidation following exercise training at high intensity are related in part to changes in fatty acid transport protein content (56).

Exercise training increases lipoprotein type A and LPL, a key enzyme in fat metabolism. The increase in LPL activity can be due to increase in catecholamine, discharge of energy loads, increase in free radicals, and decrease in cellular PH (57). Although LPL activity is not measured in this study, it is probable that its increase has a reverse relation with TG which leads to TG breaking (57). Also, LPL has a direct relation with HDL and leads to the evolution of HDL molecules (5[^]). Moreover, HDL increase can be attributed to factors such as LCAT increase (59). It seems that HIIT increases LPL enzyme, LCAT and HDL activity but decreases LDL, TG, and cholesterol (59).

Exercise training can be an important intervention for losing weight. Exercise represents an important intervention for weight loss as it has the potential to

reduce body mass, increase fat-free mass, and maintain or elevate resting metabolic rate (55). A number of studies have demonstrated that HIIT may induce weight loss in sedentary overweight/obese individuals. For example, a significant reduction in waist circumference and subcutaneous adipose tissue was found after 2 weeks of HIIT in overweight/obese sedentary men (55).

Although the mechanism responsible for fat and weight loss after HIIT is unclear, one possible reason is an increase in post-exercise metabolism (60). Excess post-exercise oxygen consumption (EPOC) response to HIIT may have a role in elevating post-exercise fat oxidation through the increased levels of catecholamine generated during acute HIIT (60). Increase in plasma epinephrine and norepinephrine at the end of HIIT could increase lipolysis and the availability of free fatty acids, resulting in increased overall fat oxidation during and after HIIT (61). Moreover, HIIT significantly increases muscle mitochondrial beta-hydroxyacyl-CoA dehydrogenase, which may enhance fat loss (61). Also, the need to remove lactate and H^+ and to resynthesize glycogen during and after HIIT also increases fat oxidation (53).

Decreased post-exercise appetite is another possible mechanism underlying HIIT-induced fat loss. Although the effect of HIIT on appetite suppression has not been investigated in overweight/obese individuals, a single bout of intense exercise has been found to suppress hunger immediately following cessation of the exercise (53). In investigating the effects of HIIT (6×30s Wingate tests) and endurance exercise (60 min exercise at 68.1% of VO_2max) on appetite. The men reported higher appetite perceptions in the hours after an acute bout of HIIT than after the endurance exercise (62). There is no clear mechanism that explains why hunger level is suppressed after high-intensity exercise. However, there is evidence to suggest a marked effect of intense exercise on subjective hunger based on the reports of exercise-induced anorexia (53). Also,

decreasing appetite after HIIT can be explained through by the considerable redistribution of blood flow away from the splanchnic circulation into the working muscles (53). While acute exercise reduces liver and muscle glycogen stores, which may result in an immediate increase in hunger, chronic exercise training may induce more adaptations that may lead to more stable levels of metabolic fuels, resulting in a suppression of hunger (63). In summary, weight loss and fat oxidation resulting from HIIT can be explained by lipolytic enzymes, fatty acid transport proteins (FAT/CD36, FABPpm, appetite decrease and negative energy balance through EPOC.

Different compatibility responses of individuals to the exercise, lack of some participants' control over their emotions, sleep rate, and lack of control on participants' foods are among the limitations of this study.

To sum up, this study shows that HIIT can cause an increase in FGF21 and Irisin and thus transform white adipose tissue to brown adipose tissue and preventing obesity and its negative consequences. Therefore, it is suggested that obese individuals take part in HIIT - As a time-efficient training method - and overcome their obesity-related problems.

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References

1. Wu j, Bostrom P, Sparks LM, Ye L, Choi JH, et al. Beige Adipocytes are a Distinct Type of Thermogenic Fat Cell in Mouse and Human. *Cell* 2012; 150(2): 366–76.
2. Saito M, Okamatsu-Ogura Y, Matsushita M, Watanabe K, Yoneshiro T, et al. Brown Fat in Humans: Turning up the Heat on Obesity. *Diabetes* 2009; 58(7):1482–4.
3. Klaus S, Ely M, Encke D. Functional assessment of white and brown adipocyte development and energy

- metabolism in cell culture Dissociation of terminal differentiation and thermogenesis in brown adipocytes. *J Cell Sci* 1995;108, 3171-80.
4. Gregoire FM, Smas CM & Sul HS. Understanding adipocyte differentiation. *Physiol Rev* 1998; 78(3): 783-809.
 5. Lowell BB, Spiegelman BM. Towards a Molecular Understanding of Adaptive Thermogenesis. *Nature* 2000; 404:652-60.
 6. Feldmann HM, Golozoubova V, Cannon B, Nedergaard J. UCP1 Ablation Induces Obesity and Abolishes Diet-induced Thermogenesis Inmice Exempt from Thermal Stress by Living at hermoneutrality. *Cell Metab* 2009; 9(2):203-9.
 7. Valeria A, Cathryn M, Stella P, et al. Obesity, insulin resistance and comorbidities – Mechanisms of association. *Arq Bras Endocrinol Metab.* 2014;58: (6):600-9.
 8. Xiaoyu Yang, Lufeng Hu, Yimin Kang, Jinzhong Xu, et al. Positive correlations between and prediction of FGF21, adiponectin, leptin and NPY concentrations in the cerebrospinal fluid of Chinese subjects using back propagating artificial neural networks. *Int J Clin Exp Med* 2016;9(2):2121-9.
 9. Kharitononkov A, B Shanafelt A. FGF21: A novel Prospect for the Treatment of Metabolic Diseases. *Current Opinion in Investigational Drugs* 2009; 10(4):359-64.
 10. Jia Liu, Yuan Xu, Yanjin Hu, Guang Wang. The role of fibroblast growth factor 21 in the pathogenesis of non-alcoholic fatty liver disease and implications for therapy. *Metabolism* 2015;64(3):380-90.
 11. Mary D, Jiaping G, Qing Y, Zhidan W. Fibroblast Growth Factor 21 Regulates Energy Metabolism by Activating the AMPK–SIRT1– PGC-1 α Pathway. *PNAS* 2010; 107 (28):12553-8.
 12. Badman MK, Koester A, Flier JS, Kharitononkov A, Maratos-Flier E. Fibroblast growth factor 21-deficient mice demonstrate impaired adaptation to ketosis. *Endocrinology* 2009;150(11):4931-40.
 13. Tanimura Y, Wataru A, Takanami Y, et al. Acute exercise increases fibroblast growth factor 21 in metabolic organs and circulation. *Physiol Rep*2016; 4 (12):e12828.
 14. Slusher AL, Whitehurst M, Zoeller RF, et al. Attenuated Fibroblast Growth Factor 21 Response to Acute Aerobic Exercise in Obese Individuals. *Nutr Metab Cardiovasc Dis* 2015; 25(9), 839-845.
 15. Kook Hk, Seong HK, YoungKM, et al. Acute Exercise Induces FGF21 Expression in Mice and in Healthy Humans *PLoS ONE*2013; 8(5): e63517.
 16. Kong Z, Sun Sh, Liu M,et al. Short-Term High-Intensity Interval Training on Body Composition and Blood Glucose in Overweight and Obese Young Women. *J Diabetes Res* 2016; 4073618, 9.
 17. Birjandi s,Saghebjo M, Hedayati M. Effect of High Intensity Interval Training and L-Arginine Supplementation on Serum levels of Fibroblast Growth Factor 21 and Atrial Natriuretic Peptide in Overweight and Obese Young Men. *J Birjand Univ Med Sci* 2016; 23 (3): 211-21.
 18. Bostrom P, Wu J, Jedrychowski MP, Korde A, Ye L, Lo JC, et al. A PGC1-alpha-dependent myokine that drives brown-fat-like development of white fat and thermogenesis. *Nature* 2012; 481(7382):463-8.
 19. Caldwell S, Lazo M. Is exercise an effective treatment for NASH? Knowns and unknowns. *Ann Hepatol* 2009; 8(1): 60-6.
 20. Seale P, Conroe HM, Estall J, Kajimura S, Frontini A, Ishibashi J, et al. Prdm determines the thermogenic program of subcutaneous white adipose tissue in mice. *J Clinic Investigation* 2011; 121(1): 96-105.
 21. Xin C, Liu J, Zhang J, Zhu D, Wang H, Xiong L, et al. Irisin improves fatty acid oxidation and glucose utilization in type 2 diabetes by regulating the AMPK signaling pathway. *Int J Obesity* 2016; 40(3):443-51.
 22. Miyamoto E, Sato K, Kurihara T, et al. Endurance Training-Induced Increase in Circulating Irisin Levels Is Associated with Reduction of Abdominal Visceral Fat in

- Middle-Aged and Older Adults. *PLOS ONE* 2015; 10(3): e0120354.
23. Yang X, Yuan H, J LI. swimming intervention mitigates HFD-induced obesity of rats through PGC-1 α -irisin pathway. *Eur Rev Med Pharmacol Sci* 2016; 20(10):2123-30.
 24. Benedini S, Dozio E, Invernizzi PL et al. Irisin: A Potential Link between Physical Exercise and Metabolism - an Observational Study in Differently Trained Subjects, from Elite Athletes to Sedentary People. *J Diabetes Res* 2017; 2017:1039161.
 25. Azuma K, Osawa Y, Tabata Sh, et al. Decrease in regional body fat after long-term high-intensity interval training. *J Phys Fitness Sports Med* 2017; 6 (2): 103-10.
 26. Avazpor S, Kalkhoran J, et al. The Comparison of the Effects of Two Types of High Intensity Interval Training (HIIT) on Body Mass and Physiological Indexes in Inactive Female Students. *J Sports Sci* 2016; 4:219-25.
 27. Blair M. Segsworth. Acute Sprint Interval Exercise Induces a Greater FGF-21 Response in Comparison to Work-Matched Continuous Exercise. Western University; 2015.
 28. Jackson AS, Pollock ML, Ward A. Generalized equations for predicting body density of women. *Med Sci Sports Exerc* 1980; 12(3): 175-81.
 29. Azizan Sh, Siahkhouhian M, Nakhostin Roohi B. A new approach for the determination of anaerobic threshold: methodological survey on the modified Dmax method. *J Hum Sports Exerc* 2012; 7(2): 599-607.
 30. de Abreu Camarda SR, Tebexreni AS, Páfaró CN, Sasai FB, Tambeiro VL, Juliano Y, et al. Comparison of maximal heart rate using the prediction equations proposed by Karvonen and Tanaka. *Arq Bras Cardiol* 2008; 91: 285-8.
 31. Gurd BJ, Perry CG, Heigenhauser GJ, Spriet LL. Highintensity interval training increases SIRT1 activity in human skeletal muscle. *Appl Physiol Nutr Metab* 2010; 35: 350-7.
 32. Dlouhy A, Hedayat S, Abdi A, et al. Effects of aerobic training on serum levels of FGF21, apolipoprotein A-1 and the ratio of LDL-C to HDL-C in obese women. *J Physiol Sport Manag* 2016; 8(1): 77-8.
 33. Cuevas Ramos DC, Valde PA, Arana CE, et al. Exercise Increases Serum Fibroblast Growth Factor 21 (FGF21) Levels. *PLoS One* 2012; 7(5): e38022.
 34. Hansen JS, Pedersen BK, Guowang Xu, et al. Exercise-Induced Secretion of FGF21 and Follistatin Are Blocked by Pancreatic Clamp and Impaired in Type 2 Diabetes. 2016; 101(7):2816-25.
 35. Dushay J, Chui P.C, Gopalakrishnan G.S, Varela-Rey M, Crawley M, Fisher FM, et al. Increased fibroblast growth factor 21 in obesity and nonalcoholic fatty liver disease. *Gastroenterology* 2010. 139:456e463.
 36. Taniguchi H, Tanisawa K, Sun X, et al. Cardiorespiratory Fitness and Visceral Fat Are Key Determinants of Serum Fibroblast Growth Factor 21 Concentration in Japanese Men. *J Clin Endocrinol Metab* 2014; 99: E1877–E1884.
 37. Christine M. Kusminski, Perry E. Bickel, Philipp E. Targeting adipose tissue in the treatment of obesity-associated diabetes. *Nat Rev Drug Discov Nat Rev* 2016;15(9):639-60.
 38. Zhang Y, Lei T, Huang J.F, Wang SB, Zhou LL, Yang XQ, et al. The link between fibroblast growth factor 21 and sterol regulatory element binding protein 1c during lipogenesis in hepatocytes. *Mol Cell Endocrinol* 2011:342 (1-2):41–7.
 39. ffolliott M. Fisher, Sandra Kleiner, Nicholas Douris et al. FGF21 regulates PGC-1 α and browning of white adipose tissues in adaptive thermogenesis. *Genes Develop* 2012:26(3):271-81.
 40. Molaei S, Soori R, et al. Effect of High Intensity Interval Training (HIIT) on Level of Irisin and Insulin Resistance Index Rate. *J Physiol Sport Physical Activity* 2015; 15:1167-74.
 41. Khalafi M, Shabkhiz F, Alamdari k, Bakhtiyari A. Irisin Response to Two Types of Exercise Training in Type 2 Diabetic Male Rats. *AMUJ* 2016; 19(111): 37-45.

42. Hakimi M, Hosseini SR. The Changes of Irisin Serum Levels and Lipid Profile of Overweight Male Students after Eight Weeks of Aerobic Training. *J Shahid Sadoughi Univ Med Sci* 2016; 23(12): 1189-201.
43. Huh JY, Mougios V, Kabasakalis A, Fatouros I, Siopi A, Douroudos II, et al. Exercise-induced irisin secretion is independent of age or fitness level and increased irisin may directly modulate muscle metabolism through AMPK activation. *J Clin Endocrinol Metabol* 2014;99(11):E2154-E61.
44. Seale P, Conroe HM, Estall J, Kajimura S, Frontini A, Ishibashi J, et al. Prdm16 determines the thermogenic program of subcutaneous white adipose tissue in mice. *J Clin Invest* 2011; 121(1): 96-105.
45. Hee Park K, Zaichenko L, Brinkoetter M, Thakkar B, Sahin-Efe A, Joung KE, et al. Circulating irisin in relation to insulin resistance and the metabolic syndrome. *J Clin Endocrinol Metabol* 2013;98(12):4899-907.
46. Hecksteden A, Wegmann M, Steffen A, Kraushaar J, Morsch A, Ruppenthal S, et al. Irisin and exercise training in humans - results from a randomized controlled training trial. *BMC Med* 2013;11:235.
47. Jae KIM H, Joo LEE, SO B. Effect of Aerobic Training and Resistance Training on Circulating Irisin Level and Their Association With Change of Body Composition in Overweight/Obese Adults: a Pilot Study. *Physiol Res* 2016; 65(2): 271-9.
48. Elmer DJ, Laird RH, Barberio MD, Pascoe DD. Inflammatory, lipid, and body composition responses to interval training or moderate aerobic training. *Eur J Appl Physiol* 2016; 116(3):601-9.
49. Petersen B, Hastings B, Gottschall J. High Intensity Interval Cycling Improves Physical Fitness In Trained Adults. *J Fitness Res* 2015; 5(10): 2201.
50. Mohamadhasani F, EsfandyarInezhad. Effect of two training on orexin A and lipid profile in obese adolescent boys. *Bulletin de la Societe Royale des Sciences de Liege* 2016; 85: 653-7.
51. Paahoo A, Tadibi V, Behpoor N. The effect of 12 weeks high intensity interval training (HIIT) on testosterone, cortisol and lipid profile levels in obese and overweight boys. *Metabol Exercise Bioannual J* 2015; 15(1):85.
52. Colberg SR, Simoneau JA, Thaete FL, Kelley DE. Skeletal muscle utilization of free fatty acids in women with visceral obesity. *J Clin Invest* 1995;95(4): 1846-53.
53. Mohammad A. Alahmadi. High-intensity Interval Training and Obesity. *Nov Physiother* 2014.4(3).
54. Gibala MJ Short-term sprint interval versus traditional endurance training: similar initial adaptations in human skeletal muscle and exercise performance. *J Physiol* 2006; 575(3): 901-11.
55. Astorino TA, Schubert MM, Palumbo E, Stirling D, McMillan DW, et al. Effect of two doses of interval training on maximal fat oxidation in sedentary women. *Med Sci Sports Exerc* 2013; 45(10): 1878-86.
56. Talanian JL, Holloway GP, Snook LA, Heigenhauser GJ, Bonen A, et al. Exercise training increases sarcolemmal and mitochondrial fatty acid transport proteins in human skeletal muscle. *Am J Physiol Endocrinol Metab* 2010;299(2): E180-188.
57. Taylor, A.j., Watkins, T. physical activity and the presence and extent of calcified coronary atherosclerosis. *Med Sci Spo Exer* 2002; 34(2): 438-44.
58. Lewis GF, Rade DJ. New insight into the regulation of HDL metabolism and reverse cholesterol transport. *Circ Res* 2005; 96:1221-32.
59. Aki Vniiofa, Korperainen R.A. Effects of impact exercise on physical performance and cardio vascular risk factors. *J Med Sci* 2007; 39: (5): 756-63.
60. Trapp EG, Chisholm DJ, Boutcher SH. Metabolic response of trained and untrained women during high-intensity intermittent cycle exercise. *Am J Physiol Regul Integr Comp Physiol* 2007;293(6): R2370-2375.
61. Talanian JL, Galloway SD, Heigenhauser GJ, Bonen A, Spriet LL. Two weeks of high-intensity aerobic interval training increases the capacity for fat oxidation during

exercise in women. *J Appl Physiol* 2007; 1985: 102(4): 1439-47.

62. Deighton K, Barry R, Cannon CE, Stensel DJ. Appetite, gut hormone and energy intake responses to low volume

sprint interval and traditional endurance exercise. *Eur J Appl Physiol* 2013; 113(5): 1147-56.

63. Elder SJ, Roberts SB. The effects of exercise on food intake and body fatness: a summary of published studies. *Nutr Rev* 2007; 65(1): 1-19.