International Journal of Sport, Exercise and Health Research

Research Article

IJSEHR 2020; 4(2): 91-95

www.sportscienceresearch.com
Received: 20-08-2020
Accepted: 12-10-2020

The Effect of 8 Weeks of Carob Supplementation and Resistance Training on Lipid Profile and Irisin in Obese Men

Hassan Jaffari[1], Bahram Abedi[2], Hoseyn Fatolahi[3]

1 *Department of Physical Education and Sport Sciences, Mahallat Branch, Islamic Azad University, Mahallat, Iran*

2 *Associate professor, Department of Physical Education and Sport Sciences, Mahallat Branch, Islamic Azad University, Mahallat, Iran*

3 *Assistant Professor, Department of Physical Education, Pardis Branch, Islamic Azad University, Pardis, Iran*

Abstract

Dietary supplements and exercise stimulate metabolic control signaling pathways. The aim of this study was to evaluate the effect of eight weeks of resistance training and carob supplementation on lipid profile and irisin in obese men. 40 obese men were randomly and equally divided into four groups including: control, carob supplementation, resistance training, resistance training + carob supplementation. The study included pre-test-intervention and post-test stages. Resistance training was performed for eight weeks and three sessions each week with an intensity of 55 to 75% 1-RM with gradual overload. The group of carob supplements also received three capsules of 500 mg per day of carob seed powder daily for eight weeks. In the third group, both interventions were performed. The hypotheses were tested using two-way analysis of variance with repeated measures. Carob supplement alone had no significant effect on irisin and lipid profile ($p \leq 0.05$). However, resistance training group and the combination of resistance training + carob supplementation group showed a significant effect on increasing the irisin and lipid profile ($p \leq 0.05$). The findings of this study emphasize that regular physical activity is still the main factor in stimulating signaling pathways in the body. However, modification of herbal supplements and changes in their dosage require further studies.

Keywords: Carob Supplement, Resistance Training, Obesity, Lipid Profile, Irisin.

INTRODUCTION

Obesity and overweight are one of public health challenges, and the health sector in most countries of the world is involved in the problems and complications caused by the increasing incidence of obesity. Adipose tissue is considered as an active endocrine organ that is involved in regulating the body's metabolism by secreting various hormones called as adipokine [1, 2]. Adipose tissue also influences the association of overweight and obesity with insulin resistance, diabetes, and cardiovascular disease by affecting lipid profile, metabolic and inflammatory [3, 4]. In contrast, high levels of brown adipose tissue are associated with resistance to metabolic diseases [5, 6]. In this regard, Van Marken et al. (2009) showed that the amount of brown fat is significantly lower in obese people and there is a negative relationship between brown adipose tissue and fat percentage and body mass index in inactive people [7].

In addition, muscle cells have a high capacity to secrete cytokines [8]. Bostrom et al (2012) identified a new membrane protein, a type of myokin that acts on adipose tissue. This membrane protein which has been shown to break down from cell membranes and is secreted into the bloodstream is known as irisin [5]. Irisin is secreted into the bloodstream by increasing UPC1, converting white adipose tissue to brown fat, which leads to increased heat, increased energy expenditure and ultimately weight loss [5, 6]. In addition, physical activity leads to the secretion of irisin from skeletal muscle and circulation in the blood [5, 6, 9-14]. As a result of exercise and under the influence of PGC1-α, the FNDC5 molecule, which is present in the membrane of muscle cells, is broken down and part of it is secreted into the plasma as an iris molecule [5, 6]. Irisin secreted into the bloodstream leads to the expression of the UCP1 protein and increases mitochondrial contents [6]. UCP1 is one of the genes associated with brown adipose tissue. The adipose tissue turns brown [5, 6, 12, 14, 15]. This effect of irisin improves tissue metabolism and increases energy consumption in the body, which can be considered as a new role in the treatment of metabolic diseases [6, 15].

***Corresponding author:**
Dr. Bahram Abedi
Associate professor, Department of Physical Education and Sport Sciences, Mahallat Branch, Islamic Azad University, Mahallat, Iran
Email: abedi@iaumahallat.ac.ir

Through physical activity, the expression of PGC1α gene is increased as the main activator of irisin secretion [6]. Therefore, the role of physical activity in increasing this myokine is considered, which ultimately has a negative relationship with overweight and obesity [5]. New evidence suggests that myocytes secreted by physical activity play a major role in pancreatic beta cell metabolism and insulin secretion, and that their effects on peripheral insulin sensitivity through AMPK activation also increase glucose uptake [16]. Depending on the type of exercise and signaling pathways, related myokines regulate processes within skeletal muscle as well as other tissues [6]. Resistance training resulted in a two-fold increase in UCP1 gene expression in visceral adipose tissue and a 25-fold increase in abdominal subcutaneous adipose tissue [6, 17].

In addition, new research has suggested the use of herbs as a low-cost method with minimal side effects in terms of weight control. It has been reported that taking various supplements can improve body composition in obese people and poisoned animals [18, 19]. One of these supplements is carob supplement and this supplement has antioxidant properties and is rich in fiber and vitamins [20-24]. Carob (Ceratonia siliqua) is a medicinal plant of the carob genus. Today, health-conscious consumers are increasingly realizing that high-fiber foods contribute to a balanced diet, with carob fiber helping to increase the amount of fiber in our daily diet. It has been reported that eating snacks containing carob can reduce the symptoms of metabolic syndrome [25].

According to the above, one of the most important factors in the secretion of irisin from the muscle source is regular physical activity. Resistance training increases the likelihood of muscle irisin secretion due to its direct effect on the muscles. In addition to the role of physical activity, today the use of nutritional supplements to help treat obesity is increasing due to the prevalence of obesity and the tendency of obese people to seek treatment faster. On the other hand, due to its antioxidant and anti-inflammatory properties, carob supplement has received less attention in the field of exercise. Also, the simultaneous effect of resistance training with this supplement has not been studied. Therefore, the aim of the present study was to evaluate the effect of 8 weeks of resistance training and carob supplementation on the lipid profile and irisin in obese men.

MATERIALS AND METHODS

In a quasi-experimental study, using available and voluntary sampling, 40 obese men were selected and randomly divided into 4 groups: resistance training, carob supplementation, combined training and supplementation, and control group. The research consisted of two stages: pre-test, interventions and post-test. Interventions included 8 weeks of resistance training, carob supplementation and combination of both). The purpose and program of the research were explained to the subjects. After collecting the initial data, the training program began for 8 weeks. Anthropometric and laboratory indices were collected again 48 hours after the last training session in the same environmental and temporal conditions as the initial tests.

A physician examined the variables of age, weight, height and blood pressure. Then the participants came to the gym for the initial measurement of anthropometric indicators such as height, weight, waist circumference, hip circumference, and fat percentage. The 1-RM of the subjects was taken to measure the maximum weight of each participant. Biochemical indices were collected after 12 hours of overnight fasting at 8:00 am. A primary blood sample of 10 cc was taken from the anterior arm vein. Blood sampling was performed in two stages 24 hours before the start of the first training session (pre-test) and 48 hours after the last training session (end of the eighth week). The collected blood was placed in sterile tubes and then the plasma was separated by centrifugation (for 5 minutes at 5000 rpm) and frozen at minus 30 °C until measurement. A similar blood sampling exercise was performed after 8 weeks. All blood samples were taken out of the freezer on the same day and the experiments were performed to measure the variables by special kits.

Exercises (8 weeks 3 sessions per week) started in three shifts (3 sets) with 12-14 repetitions in the first week with 55% of maximum repetition (1 RM) and increased to 75% of maximum repetition in the final week. Because the participants in the present study were obese, this exercise pattern was chosen to exert moderate intensity pressure. 90 seconds of rest was considered after each movement. Exercises were performed in a circle. Exercise devices include: front of the car (thigh extension), back of the car (thigh flexion), machine with trefoil, chest press machine, forearm, arm flexion, back of the arm (arm extension), armpit traction device (boat). A maximum repetition was measured by the standard method [26]. This is a safe and effective way to determine 1RM without having to lift the heaviest weight.

$$1\text{-RM} = \text{Lifted weight (kg)}/[1.0278/(0.0278*re)]$$

In order to do the supplementation, after preparing carob seed powder in the carob supplement groups, the participants consumed 1.5 g of powder in three capsules (500 mg) in three meals per day during 8 weeks [27]. In the combined group, both aerobic exercise intervention and carob supplementation were performed. In the control group (placebo), the participants took three dextrin capsules in three meals during 8 weeks of fasting.

In order to measure the levels of irisin, the human kit of Kozabio Biotech Company of Japan (Human Irisin, CUSABIO, Japan) was used by sandwich ELISA method. The sensitivity of this method was 0.76 ng / ml. Plasma triglyceride levels were measured by enzymatic calorimetric method and fabrication kit of Pars Azmoon Company (Iran)(Sensitivity: 1 mg / dl, Intraassay CV%: 6.1). Total plasma cholesterol levels were measured by photometric enzymatic method and fabrication kit of Pars Azmoun Company (Iran) (Sensitivity: 3 mg / dl, Intraassay CV%: 4.1). Plasma HDL levels were measured by enzymatic photometric method and fabrication kit of Pars Azmoon Company (Iran) (Sensitivity: 1 mg / dl, Intraassay CV%: 1.5). Plasma LDL levels were calculated by conventional calculations.

$$LDL = TC - HDL - TG/0.5$$

In order to analyze the data, descriptive statistical methods were used to calculate the mean and standard deviation. The Shapiro-Wilk test was used to check the normality of the data. Two-way analysis of variance (ANOVA) with repeated measures was used to compare the groups, followed by Tukey's *post hoc* test. Data analysis was performed using SPSS software version 22.

RESULT

The results showed that the interventions had a significant effect on TG ($F = 5.68$, $P = 0.001$, $\eta = 0.28$), cholesterol ($F = 4.78$, $P = 0.001$, $\eta = 0.25$), HDL ($F = 6.39$, $P = 0.001$, $\eta = 0.35$), LDL ($F = 5.9$, $P = 0.001$, $\eta = 0.3$) and plasma irisin ($F = 5.145$, $P = 0.001$, $\eta = 0.29$). The results of Tukey's *post hoc* test showed that the resistance training group and the combination group of resistance training and carob supplements had a significant effect on improving the irisin index and lipid profile ($p \leq 0.05$). Carob supplement alone had no significant effect on irisin index (Table 2, Table 3 and Figure 1).

DISCUSSION

The results of the present study showed that a period of resistance training and a combination of resistance training and consumption of carob supplements increases irisin and improves lipid profile in obese people, but consumption of carob supplements alone did not show a significant change.

Table 1: Resistance training protocol

Week	1	2	3	4	5	6	7	8
Set	3	3	3	3	3	3	3	3
Repetition	12-14	12-14	10-12	10-12	8-10	8-10	6-8	6-8
Rest	60s	60s	80s	80s	100s	100s	120s	120s
1-RM Percent	55-60	55-60	60-65	60-65	65-70	65-70	70-75	70-75
Session per week	3	3	3	3	3	3	3	3

Table 2: Mean and standard deviation of anthropometric and physiological indices measured in the present study

Groups	RT + Carob		resistance training (RT)		Carob		Control	
Time	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Age	27.1±2.14	------	25.8±2.53	------	27.9±2.2	------	27.1±2.61	------
Height	172.2±4.16	------	168.9±3.28	------	170.8±6.53	------	169.8±5.23	------
Weight (kg)	90.6±3.66	87.9±2.93	90.9±3.45	89.2±3.17	91.6±3.93	89.7±3.78	88.7±4.35	86.9±4.49
BMI (kg/m^2)	30.56±0.62	29.67±1.46	31.91±1.93	31.24±1.78	31.48±2.12	30.83±2.7	30.88±0.62	30.24±0.86
TC (mg/dl)	190.2±4.78	183.95±4.29	189.29±4.65	183.45±4.54	189.9±4.32	184.7±4.39	189.9±4.19	190.1±4.22
TG (mg/dl)	159.29±4.14	153.90±4.99	160.24±4.78	154.90±4.29	160.9±4.66	155.1±4.29	160.09±0.52	159.9±4.61
HDL(mg/dl)	54±3.87	59±3.54	54.5±3.54	58.9±3.36	54.35±3.61	57.85±3.29	54.2±3.42	54.4±3.19
LDL(mg/dl)	109.9±3.61	104.5±3.49	110.25±3.86	104.85±3.78	110.5±3.9	105.69±3.34	110.21±3.43	109.29±3.17
Irisin(ng/ml)	4.57±0.76	5.83±0.81	4.54±0.55	5.79±0.75	4.49±0.69	4.69±0.64	4.5±0.58	4.51±0.73

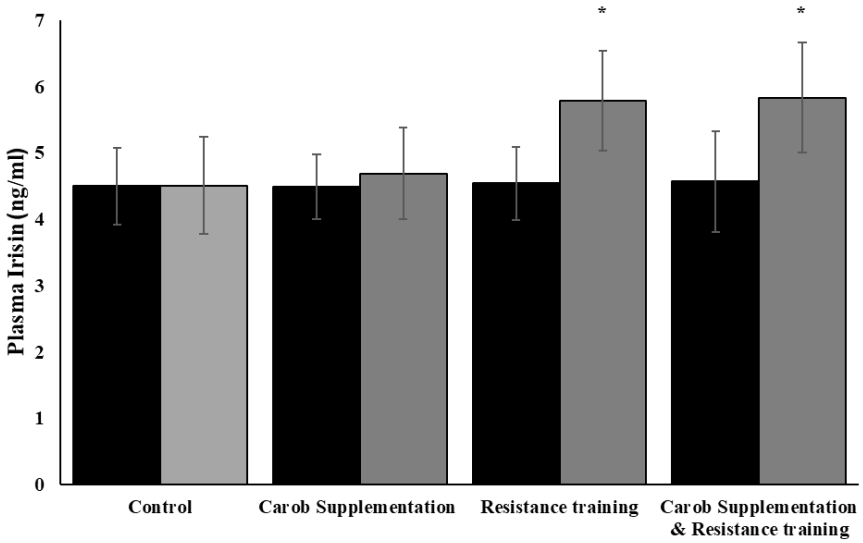

Figure 1: Changes in irisin in the study groups. The training groups have shown the most significant effect

Raisi et al. (2016) showed that plasma irisin protein levels increased significantly after one session of resistance training. Resistance training is likely to improve the body composition by increasing the conversion of white to brown fats by secreting myokines such as irisin [14]. Irisin is one of the myokines that has been shown to increase following exercise. Thus, following exercise and physical activity, PGC-1a expression increases as a transcriptional activating molecule and stimulates the expression of FNDC5 membrane protein in muscle cells. The FNDC5 molecule released from the membrane of muscle cells is broken down and part of it enters the bloodstream called irisin. The irisin molecule produced binds to PPAR-α receptors on the surface of white adipose tissue and converts white adipose tissue to brown adipose tissue by increasing the expression of these receptors. On the other hand, the irisin molecule can increase the mitochondrial content of white adipose tissue and increase its conversion to brown adipose tissue by increasing the expression of UCP-1 molecule on the surface of white adipose tissue. This function of irisin is associated with increased metabolic activity in the body and increased energy consumption in the body, which is considered as a new role of irisin in the treatment of metabolic diseases [9]. Irisin can also cause brown adipose tissue to brown by inhibiting PI3K enzyme signaling and increasing PTEN expression [15]. Depending on the type of exercise and the signaling pathways that are activated, the myokines associated with those targets regulate processes within skeletal muscle as well as other tissues. Also, the importance of the regulatory role of irisin on the insulin resistance index and the lack of available data on the response of irisin to short-term exercise is evident.

Irisine increases the expression of UCP1 gene in the white adipose tissue, which is characteristic of brown adipose tissue and increases fatty acid oxidation and thermogenic conditions. Thus, irisin converts white adipose tissue to brown adipose tissue, which leads to increased heat and energy consumption, and ultimately weight loss [5]. Resistance training may have a significant effect on adipose tissue phenotype due

to its direct effect on muscle, one of the main factors being the activation of the FNDC5 and irisin pathways. It has also been shown that in mice obese on a high-fat diet, irisin injection results in increased oxygen consumption, weight loss, decreased fasting insulin, and increased UCP1 gene expression [5]. In addition, there is ample evidence that resistance training in most studies, including this study, can improve body composition by increasing lean mass or reducing body fat, leading to weight loss and increased oxygen consumption [5, 9-12, 14, 17].

Studies have shown that resistance training leads to a reduction in the body fat percentage, without altering abdominal and central obesity [28] or a reduction in fat percentage, waist-to-hip ratio and weight [11]. It is possible that the observed changes in the body fat percentage because the subjects did not change their diet could be due to increased energy demand from the muscles involved in physical activity, and that after exercise the energy consumption is still high and balance may occur. Negative balance between energy consumption and intake is a reason for this [29, 30]. Evidence also suggests that resistance training increases muscle mass, muscle strength, and resting metabolic rate, and thus favorably stimulates the lipophysis of subcutaneous and visceral adipose tissue, thereby reducing adipose mass [31]. In addition, the weight of individuals and its changes and body composition affect lipoproteins. Some research suggests that if exercise changes body composition and changes the percentage of fat, it can affect lipoproteins, although beneficial changes in lipoproteins without weight loss have been observed [32].

Plasma triglyceride depletion may be due to increased triglyceride catabolism through increased lipoprotein lipase [33]. Increased activity of the enzyme lipoprotein lipase leads to the breakdown of triglycerides and lipoproteins rich in it and increases the absorption of triglycerides from the blood. Triglyceride changes occur even without changes in the body composition. Some research has shown that antioxidants also increase LPL in muscle. Although the amount of this enzyme was not measured in this study, a significant increase in HDL and a significant decrease in triglycerides could be a sign of this. On the other hand, the removal of fatty acids from the blood can lead to a reduction in total cholesterol, although research suggests that cholesterol levels are only affected by antioxidants in high-risk conditions. The biological mechanism that could potentially improve fat profile with exercise is not well understood. Researchers suggest that changes in LDL may be due to increased LPL and decreased hepatic apoprotein B lipase [4], and that changes in HDL may be related to activation of LPL enzymes [34] and increased LCAT, decreased CETP. Regular activity increases LPL gene expression and muscle activity [33]. Changes in these enzymes can provide muscle with the ability to oxidize fatty acids and lower triglycerides [28].

CONCLUSION

In general, the findings of the present study indicate an improvement in the lipid profile of obese men in the resistance training group, resistance training group + carob supplementation. However, there was no significant difference between the training groups in each of the lipid profile indices. In addition, the results showed a significant increase in irisin levels due to resistance training and a combination of resistance training and carob supplementation. Nevertheless, taking carob supplements alone has no effect on obese men. It is recommended to use resistance training and a combination of resistance training and carob supplementation to increase the level of iris in obese people and lose weight. Although in the present study, carob supplement did not directly affect the weight and variables of the study, however, the antioxidant and anti-inflammatory properties of plant compounds, including carob, may reduce oxidative stress and inflammation caused by exercise stress.

Acknowledgments

The authors of this paper would like to express their thanks to all the participants in this study.

Conflict of Interests

All authors of this paper have equally contributed to, read, and approved the final version submitted. The contents of this manuscript have not been copyrighted or published previously. Currently, the contents of this manuscript are not under consideration for publication elsewhere. The authors declare that they have no conflict of interest and there was no funding to this study.

Ethical approval

The experimental protocol in the present study (based on M.S.c thesis) was approved by the ethics committee of Islamic Azad University, Mahallat Branch, Iran. The researchers' Ethics Committee initially approved the experimental procedures and the study protocols, which were fully explained to all the participants. In addition, a written consent form was signed after reading and understanding the details of the experiments. The research was also conducted in terms of the principles stated in the Declaration of Helsinki.

Funding

Not applicable.

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