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Research article

Exercise as a physiotherapy potentiates thermogenesis and obesity management through elevation of myokine "irisin"

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Abstract

The present study was designed to study the possible effect of exercise on serum irisin level in both healthy and obese rats and also, to explore the relations between irisin and some atherogenic risk factors. The obtained data revealed significant increases in serum levels of irisin and FT4 in both healthy and obese groups in response to exercise, while FT3 values showed insignificant change. Also, a significant increase in serum irisin and a significant decrease in FT4 were observed obese group compared to normal group. Exercise significantly improved lipid profile, glucose, insulin level and HOMA-IR that rose in obese group. In conclusion, irisin was found to increase after moderate intensity exercise training; therefore, irisin could be a potential therapeutic target for human metabolic diseases and other disorders that are improved with exercise.

Introduction

Obesity is a chronic disease that has become one of the most serious health problems [1]. Life style factors, including reduced physical exercise and a high calorie intake, are responsible for the increase of obesity in industrialized countries [2]. High fat diet produces a consistent and significant increase in body fat content that is dependent on both the amount of fat in the diet and the duration of feeding [3].

Over the past decades, studies have shown a protective effect of regular physical activity on morbidity and all-cause mortality [4]. For example, exercise can help control blood lipid abnormalities, diabetes, and obesity; in addition, aerobic exercise adds an independent modest blood pressure-lowering effect in certain hypertensive cases [5].

Evidence has been provided, in recent years, that skeletal muscles produce a variety of molecules, denominated "myokines", which act in an autocrine, paracrine, or endocrine hormone-like fashion [6]. Myokines released by contracting skeletal muscles, by creating a systemic anti-inflammatory environment and exerting endocrine effects on visceral fat and glucose and lipid metabolism, may be, at least partially, responsible for the beneficial effects of exercise [7]. Exercise, diet and other non-pharmaceutical interventions,which are considered the cornerstone of obesity and T2DM treatment, have proved wide variations of effects on both adipokines and myokines levels [8].

Irisin is a new myokine that is secreted by myocytes during exercise, and plays a role in creating the beneficial effects of exercise on metabolism [6]. Irisin is composed of 112 amino acids and has a molecular weight of 12 KDa. It has been first isolated from muscle tissue. It promotes a brown-phenotype switching in white adipose tissue, that results in enhanced thermogenesis and increased energy expenditure [10]. Some clinical studies observed higher serum irisin level following a 12-week combined strength and endurance training with higher elevation in a group with pre-diabetes and overweight compared to normoglycemic and normal weight [11]. Therefore, molecular mechanisms underlying irisin and increasing the amount of the brown fat may lead to the discovery of the basis of physical exercise benefits on different conditions [12]. However, contradictory results have been published in several studies in humans, as it is reported that irisin serum levels remained stable after acute exercise or endurance training [13]. So, Irisin regulation seems quite complicated and there are conflicting data concerning the effects of physical activity on its levels. In light of previous data, the present study was designed to study the possible effect of exercise on serum irisin level in both healthy and obese rats and also, to explore the relations between irisin and some atherogenic risk factors.

Materials and methods

Animals

Adult male Wistar albino rats (*Rattusnorvegicus*) weighting 150-170g were obtained from Animal Colony, National Research Centre, Giza, Egypt. The animals were housed in suitable plastic cages for one week before the experiment for acclimation. Excess tap water and standard rodent food pellets [20.3% protein (20% casein and 0.3% DL-Methionine), 5% fat (corn oil), 5% fibers, 3.7% salt mixture and 1% vitamin mixture [14] were always available. All animals received human care in compliance with the standard institutionals' criteria for the care and use of experimental animals as cited by animal ethical committee number FWA00014747, National Research Centre.

Animal grouping

This study was carried out on healthy and obese adult male rats. The animals were made obese by feeding high fat diet for 12 weeks; this high fat diet consisted of corn oil (25.5%), lard (20.5%), 24% carbohydrates [corn starch (6%) and sucrose (18%)], 20.3% proteins [casein (20%) and DL-Methionine (0.3%)], 5% Fiber, 3.7% salt mixture, and 1% vitamin mixture. The weight and nose-anus length of high fat diet fed rat groups were measured. Body mass index (BMI) was calculated. Animals with BMI greater than 0.68 g/cm^2 were considered obese as previously described [14]. Rats that recorded BMI values below 0.68 g/cm^2 were excluded from the study. However, all rats of the obese group attained the target BMI and were all included.

After 12 weeks, both healthy and obese rats were divided into 4 groups for another 4 weeks:

Group (I): control (n=10), healthy animals in this group were fed on normal laboratory chow diet and not practicing exercise.

Group (II): exercised group (n=10), healthy rats in this group were fed on normal laboratory chow diet and trained by swimming exercise training.

Group (III): obese group (n=10): obese rats in this group were fed a high-fat chow diet and remained sedentary in their cages.

Group (IV): obese exercised group (n=10), obese rats in this group were fed a high-fat chow diet and trained by swimming exercise training.

Swimming exercise program

The rats in exercised groups were assigned to perform moderate intensity swimming exercise, one hour/day, and five days a week for four weeks. Swimming was performed in a cylindrical tank of 80 cm high, 45 cm diameter and filled with tap water 45 cm deep. The temperature of water was electrically controlled at 25±2°C [15].

Swimming rats were initially trained for 15 minutes/day and duration was gradually increased such that the rats were able to perform exercise for one hour/day, which was achieved in one week. Exercise was performed between 9:30-10:30 am, at the end of each exercise session; the animals were kept to dry and kept in a warm environment. The animals that practiced exercise were sacrificed 48 h after the end of the last training session to minimize the acute effects of the exercise [16].

Anthropometric measures

Weight (by the digital balance), length (nose to anus length) and BMI were calculated for each animal. BMI was calculated by the equation below; this index can be used as an indicator of obesity where the cut off value of obesity BMI is more than 0.68 gm/cm^2 [17].

BMI (g/cm^2) = body weight (g) / length^2 (cm^2)

Blood samples

Following diethyl ether anesthesia blood samples were immediately withdrawn from the retro-orbital plexus using heparinized and sterile glass capillaries. Blood samples were allowed to clot at room temperature before centrifuging for 20 minutes at approximately 3000 rpm. The separated sera were stored at -20° C until analysis

Analytical determinations

Estimation of serum irisin level was carried out by competitive Enzyme Linked-Immunosorbent Assay (ELISA) using rat irisin ELISA kit obtained from BioVendor-Laboratornimedicina, (U.S.A.). The serum level of insulin hormone was evaluated using insulin ELISA using reagent kit purchased from DRG International, Inc. USA. Serum glucose, total cholesterol and triglycerides levels were determined photometrically using the instruction manual of DiaSys reagent kits purchased from DiaSys Diagnostic System GmbH, Germany. Serum high density lipoprotein cholesterol (HDL- cholesterol) and low density lipoprotein cholesterol (LDL- cholesterol) levels were determined according to the CHOP-PAP method by photometric system according to the instruction manual of Centronic reagent kits purchased from DiaSys Diagnostic System GmbH, Germany. Serum free triiodothyronine (FT3) and free thyroxine (FT4) levels were determined by ELISA technique using rat ELISA Kits Cat. 1650 for quantitative determination purchased from ALPHA DIAGNOSTIC international, 6203 Woodlake Center Drive, San Antonio, Texas78244, USA.

Calculation of homeostasis model assessment (HOMA)

The results of glucose and insulin measurements are introduced into equation to measure the homeostasis

model assessment (HOMA), as a measure of insulin resistance [HOMA-IR = fasting serum insulin (MIu /mL) x fasting serum glucose (mmol/L) / 22.5] [18]; where 1mmol/L glucose= 1mg/dl glucose/18.

Statistical analysis

The obtained data are presented as mean ± SD after they were subjected to one way analysis of variance (ANOVA) followed by (Tukey) post hoc test at level of $p \leq 0.05$ according to Steel &Torrie [19] using statistical analysis system (SAS) program software; copyright (c) 1998 by SAS Institute Inc., Cary, NC, USA.

Results

In the current study, we found significant increase in serum irisin level in groups II (normal exercised group), III (obese group) and IV (obese, exercised group) when compared with that of the first group (control group). BMI showed significant increase in groups III and IV but showed insignificant change in group II when compared with group I. On comparison with the obese group (group III), exercise significantly increased irisin level and significantly decreased BMI in group IV (Figure 1).

Serum FT3 did not significantly affected by exercise in both healthy and obese groups. In obese rats (Group III), FT4 level decreased significantly compared to control group. In group IV, FT4 level was significantly increased by exercise compared to group III (Figure 2).

Data in table 1 show significant increases in the values of glucose, insulin and HOMA-IR in groups III and IV compared to control group. The elevations in these values decreased significantly by exercise in group IV when compared with that of group III but still significantly higher than those in group I.

The effects of exercise on some parameters of lipid profile are depicted in table 2. The data show that exercise induced insignificant change in the levels of total cholesterol, LDL-cholesterol, HDL-cholesterol and triglycerides in serum of group II compared with group I. While in group IV, exercise induced significant decreases in the serum levels of total cholesterol, LDL-cholesterol and triglycerides, but induced significant increase in HDL-cholesterol compared with their corresponding values in group III.

Tables 3 and 4 illustrate the correlation coefficient between irisin and other parameters. There was significant positive correlation between irisin and BMI in groups I, II and IV. Another significant positive correlation was found also between irisin and FT3 in groups I and IV. It is found also significant positive correlation between irisin and total cholesterol in groups I and II; significant positive correlation between irisin and triglycerides in groups III and IV; significant positive correlation between irisin and LDL-cholesterol in groups II and III. Where as HDL-cholesterol in groups I and III negatively correlated with irisin.

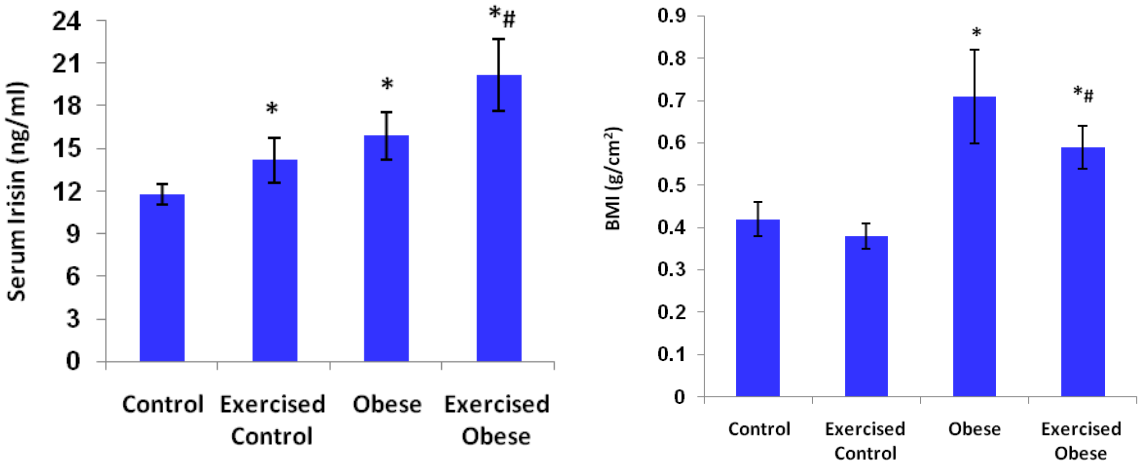

Figure 1. Serum irisin and BMI levels of all studied groups; Data are presented as mean±SD; *is significant from control group, while # is significant from obese group at $p \leq 0.05$.

Table 1. Serum glucose, insulin and HOMA-IR in all studied groups.

	Group I	Group II	Group III	Group IV
Glucose (mmol/L)	4.8 ± 0.42	4.7 ± 0.35	13.2 ± 0.89*	9.7 ± 0.66*#
Insulin (MIu/ml)	2.2 ± 1.2	1.4 ± 1	7.5 ± 0.6*	4.3 ± 1.8*#
HOMA-IR	0.48 ± 0.26	0.29 ± 0.20	4.37 ± 0.33*	1.9 ± 0.18*#

Data are presented as mean±SD (n=10). Groups I (normal group), II (normal exercised group), III (obese group) and IV (obese exercised group). * Significant difference compared to group I, p<0.05; # significant difference compared to group III, p<0.05.

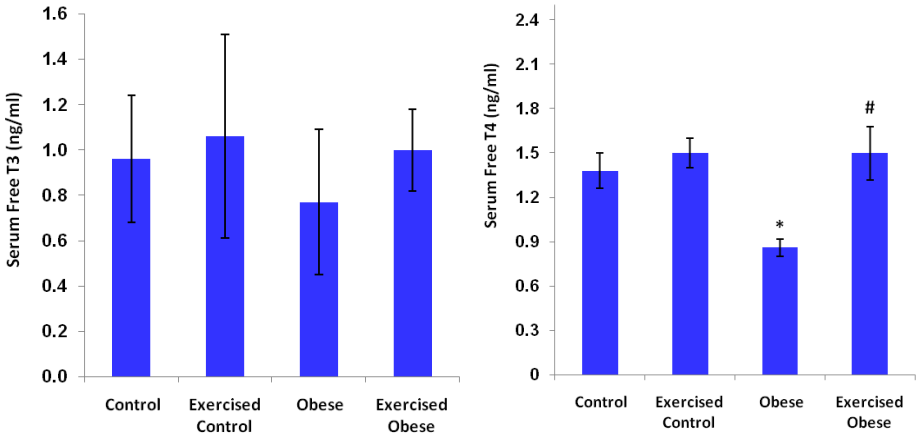

Figure 2. Serum FT3 and FT4 levels of all studied groups; Data are presented as mean±SD; * is significant from control group, while # is significant from obese group at $p \leq 0.05$.

Table 2. Serum lipid profile in all studied groups.

	Group I	Group II	Group III	Group IV
Cholesterol (mg/dl)	120±22.3	111.6±30.5	301.7±17.7*	255.8±42.1*#
Triglycride (mg/dl)	112 ± 13.7	101.5 ± 17.9	230.9 ± 39.2*	186.1 ± 47.4*#
LDL (mg/dl)	77.6 ± 26.6	60.2 ± 14.5	226.3 ± 22.9*	184.9 ± 39.6*#
HDL (mg/dl)	34.8 ± 4	36.7 ± 2.5	29.2 ± 4.4*	33.6 ± 2.2#

Data are presented as mean±SD (n=10). Groups I (normal group), II (normal exercised group), III (obese group) and IV (obese exercised group). * Significant difference compared to group I, p<0.05; # significant difference compared to group III, p<0.05.

Table 3. Correlation coefficient between irisin and other parameters.

		Group I	Group II	Group III	Group IV
BMI	r	0.8	0.8	0.5	0.3
	p value	0.004*	0.004*	0.4	0.04*
FT3	r	0.7	0.3	0.3	0.7
	p value	0.02*	0.4	0.4	0.02*
FT4	r	- 0.1	0.3	-0.6	-0.4
	p value	0.9	0.3	0.09	0.3
Glucose	r	- 0.3	0.4	- 0.5	-0.4
	p value	0.4	0.3	0.1	0.3
Insulin	r	- 0.3	-0.01	- 0.1	-0.02
	p value	0.4	0.9	0.9	0.9
HOMA-IR	r	- 0.3	-0.01	- 0.1	-0.02
	p value	0.4	0.9	0.9	0.9

Pearson's correlation test, * Significant at p<0.05.

Table 4. Correlation coefficient between irisin and other parameters.

		Group I	Group II	Group III	Group IV
Cholesterol	r	0.6	0.7	-0.5	-0.1
	p value	0.05*	0.01*	0.1	0.7
Triglycerides	r	-0.2	-0.5	0.7	0.8
	p value	0.6	0.2	0.02*	0.001*
LDL	r	0.4	0.6	0.7	- 0.1
	p value	0.2	0.04*	0.03*	0.7
HDL	r	-0.8	0.2	-0.7	0.2
	p value	0.001*	0.6	0.03*	0.7

Pearson's correlation test, * Significant at p<0.05.

Discussion

In the present study, the exercise produced a statistically significant increase in irisin level in normal exercised group (II) when compared with normal sedentary group (I) and in obese exercised (IV) group when compared with obese sedentary group (III). Also Significant increase in obese sedentary group when compared to control group. The significant increase in serum irisin level with exercise when compared to non-exercised groups is in analogue with Boström *et al.* [10] who found a two fold increase in serum level of irisin after ten weeks of endurance exercise on non-diabetic adults. Also, Huh *et al.* [20] reported induction of serum irisin 30 minutes after 2 sets of 2 × 80 m sprints in healthy adult males.

In addition, the study of Timmons *et al.* [21] confirmed a 30% increase in "the irisin precursor" FNDC5 mRNA expression in muscles of trained subjects compared to sedentary group. Lecker *et al.* [11] also observed higher expression of FNDC5 mRNA in muscles of patients with high aerobic performance versus low aerobic performance. Moreover, Kazeminasab *et al.* [22] reported that endurance training increased the protein content of FNDC5 in the skeletal muscle of obese C57BL/6 mice compared to control group.

Some of the best-recognized effects of exercise on muscle are mediated by the transcriptional coactivator PGC1α. It has been shown that PGC1α expression in muscle stimulates an increase in the expression of FNDC5, a membrane protein that is cleaved and secreted as irisin. Irisin acts on white adipose cells in culture and in vivo to stimulate UCP1 expression and a broad program of brown fat-like development. Irisin is induced with exercise in mice and humans, and mildly increased irisin levels in blood cause an increase in energy expenditure in mice [10]. On the other hand, other authors [23, 24] reported no change in muscle FNDC5 mRNA after 8 weeks of endurance training in obese non diabetic volunteers.

Also we found in this study a significant increase in serum irisin level in high fat diet group when compared to normal diet group with positive correlation with BMI in Group I, II and group IV; and these results come in agreement with previous studies which have reported that FNDC5 mRNA expression was positively correlated with BMI in both healthy adults and in obese subjects [20, 21]. However, these results are in conflict with other studies which found that plasma irisin level was negatively correlated with BMI in overweight subjects with Type II diabetes [25] and in obese in pre-pubertal children with metabolic syndrome [26]. The significant increase in irisin level in the exercised versus non exercised groups and the positive correlation with BMI can be explained by the reports of Roca-Rivada *et al.* [27] which revealed that irisin is secreted from both adipose tissue and muscle tissue so it is considered both a myokine and an adipokine. In the current study, we found no statistically significant difference in FT3 values among groups, while there was a significant increase in serum level of FT4 in group II and Group IV in response to exercise and a significant decrease on high fat diet in group III, in comparison to control group. The significant increase of FT4 in response to exercise is in agreement with previous studies of Ciloglu *et al.* [28] who reported that maximal aerobic exercise greatly affect thyroid hormones by increasing FT4. We also found in this study a positive correlation between irisin level and FT3 in group 1 and group 4 and this come in line with studies that demonstrated that there is a positive correlation between irisin and thyroid hormones [29].

The correlation between irisin and thyroid hormones may be explained by Bocco *et al.* [30] who reported that physical exercise and thyroid hormone mediate the peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1a) expression in skeletal muscle that is crucial to skeletal muscle mitochondrial function. PGC1α expression in muscle stimulates an increase in the expression of FNDC5, a membrane protein that is cleaved and secreted as irisin [10]. Bocco *et al.* [30] also reported that the expression of type 2 deiodinase (D2), which activates thyroid hormone in skeletal muscle, is up-regulated by acute treadmill exercise through a β-adrenergic receptor-dependent mechanism.

In group II (normal diet with exercise) compared to control group (normal diet with no exercise) there was a slight decrease in serum levels of insulin, glucose and HOMA-IR but it didn't reach statistical significance. In group III (high fat diet, no exercise) there was a significant increase in glucose, insulin and HOMA-IR compared to control group. In group IV (high fat diet with exercise), animals recorded a significant decrease in glucose, insulin level and HOMA-IR in response to exercise compared to group III. These results are supported by previous researches which reported that aerobic exercise training produces beneficial improvements in glucose tolerance and insulin response to glucose and may even normalize glucose levels in impaired individuals and diabetics [12] and in obese mice [18]. Irisin levels didn't correlate with insulin, glucose and HOMA-IR. This comes in agreement with Timmons *et al.* [21] and Pekkala *et al.* [24] who reported that irisin level was not correlated with glucose homeostasis. However, irisin was found to be positively correlated with insulin by other investigators and they reported decreased plasma irisin level in type II diabetic patients [31, 32].

Increased irisin expression in mice was described to lead to decrease weight and improve glucose tolerance [33]. The adipose tissue of obese rats synthesize more irisin than those of control groups , Thus, irisin is considered as a thermogenic agent that serves antiobesitic and antidiabetic functions and plays its role through cell surface receptors [27]. Irisin decreases insulin quantity

and insulin resistance while enhancing mRNA and oxygen consumption. It also causes a rise in the expression of Elov13, Cox7a, and Otop1 genes, are molecules influencing energy expenditure [34].

Considering lipid profile, in this study we found that exercise could significantly decrease the values of serum total cholesterol, LDL-cholesterol and triglyceridesthat rose in obese rats. Exercise improved also the level of HDL-cholesterol that decreased by high fat diet in obese rats. In group I serum irisin levels correlated positively with cholesterol and negatively with HDL. In group II (normal diet, exercise) a positive correlation was found between irisin and both total cholesterol and LDL, while in high fat diet group (group III), irisin level correlated positively with triglycerides and LDL-cholesterol and negatively with HDL-cholesterol. In group IV serum, irisin level correlated positively with triglycerides only. In accordance with these findings, de la Iglesia *et al.* [35] found that the changes in irisin level paralleled with the variation in the atherogenic parameters (triglycerides, LDL-cholesterol, triglycerides / HDL-cholesterol and Apo B) after weight-loss therapy and suggested possible involvement of irisin in fat metabolism and lipid disorders.

On the other hand, Huh *et al.* [36] observed a negative association between irisin and triglycerides while, Wen *et al.* [37] reported that irisin level is independently associated with high-density lipoprotein cholesterol level in chronic kidney disease patients. While, Oilman *et al.* [38] detected significant inverse associations between irisin and circulating levels of total cholesterol, LDL-cholesterol and triglycerides for both males and females. The marked variability in the reports about lipid profile might be attributed to differences in study design, species, and sample size or diet regimens.

Conclusion

Irisin was found to increase after moderate intensity exercise training and was significantly correlated with some metabolic parameters as lipid profile and thyroid hormones as well as BMI. Irisin could be a potential therapeutic target for human metabolic diseases and other disorders that are improved with exercise. However, further studies are required for better understanding of its metabolic effects and their possible mechanisms.

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