Effects of Irisin Compared with Exercise on Specific Metabolic and Obesity Parameters in Female Mice with Obesity

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Abstract

Objective: We aimed to analyze the changes in metabolic parameters after administration of irisin to obese female mice.

Materials and Methods: Sixty mice aged 5–6 weeks were randomized into three groups as irisin, exercise, and control. The control and irisin group remained sedentary, whereas the exercise group started free wheel exercising 6 weeks after the start of the study. The irisin group received irisin after 20 weeks. All mice were sacrificed at the 22nd week of the study, and obesity-related metabolic parameters were analyzed.

Results: There was no significant difference between the irisin and exercise groups in weight gain ($P > 0.05$). By contrast, weight gain in the control group was significantly higher compared with the irisin and exercise groups ($P < 0.05$). Serum bone morphogenetic protein (BMP), ghrelin, insulin, kisspeptin, leptin, and visfatin levels were statistically lower in the irisin and exercise groups compared with the control group, but no significance was detected between the irisin and exercise groups ($P < 0.05$ for all parameters).

Conclusion: Similar to the effect of exercise, irisin injections resulted in the amelioration of certain obesity-related parameters such as the concentration of adipokines, BMP4, insulin, and ghrelin. Its role as a potential alternative to exercise needs to be further studied.

Keywords: irisin, obesity, adipokines, BMP4, ghrelin

Introduction

OBESITY, OFTEN REFERRED to as “the plague of the new age,” is becoming an increasingly prevalent global issue. According to a recent analysis, incidences of obesity are twice as common now as they were 20 years ago.¹ Individuals with obesity face numerous co-morbidities, among them diabetes, hypertension, cardiac diseases, dyslipidemia, mood disorders, and even some cancers. Immobility is one of the most significant consequences of the disease, resulting in metabolic changes and modifications to adipose tissue.

Adipose tissue does not function exclusively as a protective and insulative layer. It is also an endocrine organ and secretes a variety of hormones or peptides, known as adipocytokines, into the regulatory system; leptin, resistin,

adiponectin, visfatin, and kisspeptin are among the most prominent adipocytokines produced by adipose tissue.^{2,3} Adipose tissue consists of important molecules that help organize the structure of fat tissue, such as a gastrointestinal system peptide, ghrelin, bone morphogenetic proteins (BMPs), and irisin (an exercise-induced myokine). BMPs are well-known members of the transforming growth factor- β superfamily. In the case of obesity, BMP2, BMP4, and BMP7 are well-studied molecules, and are understood to aid in the transformation of adipose tissue.⁴ The BMP family is known to stimulate fat tissue alteration. Studies have shown that exogenous administration of BMP4 and BMP7 cause the transformation of fat tissue into a more active variant.⁵

Irisin is a muscle-secreted peptide known as fibronectin type III domain-containing protein 5 (FNDC5). The secretion

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FIG. 1. Experimental model.

mechanism of irisin occurs through cleavage from FNDC5 by the induction of peroxisome proliferator-activated receptor gamma coactivator 1- α as a response to physical exertion or any activity that activates myokines.^{6,7} It is hypothesized that irisin induces transformation of fat tissue, meaning it may mimic the effects of exercise.⁸ Several studies show that diet-induced obesity and its related co-morbidities such as endothelial dysfunction can be reversed with irisin overexpression, thus drawing attention to irisin as a possible target for obesity treatments.⁹

The purpose of this investigation was to compare adipokines, BMP4 levels, and weight changes after intravenous administration of irisin and physical exercise on sedentary obese mouse populations.

Material and Methods

Experimental design for mouse population

This study is a complementary analysis of an experimentally designed female mouse population with high-fat diet-induced obesity and the effect of irisin compared with exercise.¹⁰

The experiment was conducted in the Institute of Experimental Medicine, Istanbul University (Istanbul, Turkey), using 60 female C57BL/6J mice aged 5–6 weeks. Five mice were designated to each cage, maintained on a 14-hr light/10-hr dark cycle, and allowed access to food and water *ad libitum* for the duration of the experiment. The mice were fed a high-fat diet (60% kcal, cat. no. 58Y1; Test Diet, St. Louis, MI) and maintained on the same diet until they were euthanized. Protocols of the Turkish Legislation for the Use and Care of Laboratory Animals were adhered to and ethics approval was received from the Experimental Animal Use Ethics Committee of Istanbul University.

The mouse population was divided into three groups: the irisin group ($n=20$), exercise group ($n=20$), and control group ($n=20$), and were randomized as shown in Fig. 1. Mice in the irisin group remained sedentary and were injected with 10^{10} FNDC5-expressing adenovirus (cat. no. 041429A; Applied Biological Materials Inc., Richmond, British Columbia, Canada) beginning at the 20th week of the study until they were euthanized. The age of the mice, dosing of adenovirus, and timing were selected according to our previous study and as described by Bostrom et al.^{6,10} At

TABLE 1. CHARACTERISTICS OF FEMALE C57BL/6J MICE WITH HIGH-FAT DIET-INDUCED OBESITY

	Irisin	Exercise	Control	P
Weight change (grams)	9.47 ± 0.89^a	7.75 ± 0.86^b	$11.66 \pm 1.19^{a,b}$	<0.05
HOMA-IR	7.54 ± 0.77^a	6.65 ± 0.67^b	$9.31 \pm 1.23^{a,b}$	<0.05
Irisin (ng/mL)	742.02 ± 282.07^a	830.96 ± 303.56^b	$631.95 \pm 201.99^{a,b}$	<0.05
E2 (pg/mL)	$2,441.50 \pm 1,062.62$	$2,568.58 \pm 1,174.19$	$3,080.54 \pm 1,887.93$	NS
LH (mIU/mL)	85.00 ± 32.21^a	70.95 ± 9.46	59.78 ± 32.73^a	<0.05
FSH (ng/mL)	117.48 ± 39.78	111.50 ± 17.74	108.72 ± 28.63	NS

Similar superscript letters indicate a statistically significant difference ($P < 0.05$).

E2, estradiol; FSH, follicle-stimulating hormone; HOMA-IR, homeostatic model assessment of insulin resistance; LH, luteinizing hormone; NS, not significant at $P > 0.05$.

TABLE 2. COMPARISON OF METABOLIC PARAMETERS BETWEEN IRISIN, EXERCISE, AND CONTROL GROUPS

	<i>Irisin</i>	<i>Exercise</i>	<i>Control</i>	P
Adiponectin (mg/L)	105.7 ± 34.5	90.4 ± 51.7	76.6 ± 43.7	NS
ANP (ng/L)	979.3 ± 267.6	1,136.5 ± 600.5	847.7 ± 348.5	NS
BMP (ng/L)	2,927.5 ± 712.4 ^a	3,034.1 ± 1,144.4 ^b	12,210 ± 740.9 ^{a,b}	<0.05
BNP (ng/L)	549.0 ± 223.9	680.7 ± 374.0	541.1 ± 48.9	NS
FGF21 (pg/mL)	1,176.6 ± 376.8 ^a	1,473.6 ± 819.6	1,633 ± 551.4 ^a	<0.05
Ghrelin (pg/mL)	5,314.3 ± 2,710.9 ^a	5,906.0 ± 1,504.9 ^b	15,055.9 ± 2,762.2 ^{a,b}	<0.05
Insulin (mIU/L)	49.5 ± 19.7 ^a	44.2 ± 17.8 ^b	138 ± 16.9 ^{a,b}	<0.05
Kisspeptin (pg/mL)	2,509.2 ± 895.9 ^a	2,117.6 ± 642.7 ^b	3,340.4 ± 313.8 ^{a,b}	<0.05
Leptin (ng/L)	2,789.2 ± 1,362.1 ^a	2,572.5 ± 1,087.5 ^b	5,599.9 ± 321.0 ^{a,b}	<0.05
RBP4 (ng/mL)	398.8 ± 132.8	339.8 ± 87.3	432.2 ± 221.5	NS
Resistin (ng/mL)	131.0 ± 65.2	115.7 ± 39.5 ^b	158.8 ± 22.7 ^b	<0.05
Visfatin (ng/mL)	482.1 ± 155.4 ^a	443.7 ± 175.4 ^b	1,388.8 ± 226.7 ^{a,b}	<0.05

Similar superscript letters indicate a statistically significant difference ($P < 0.05$).

ANP, atrial natriuretic factor; BMP, bone morphogenetic protein; BNP, brain natriuretic factor; FGF21, fibroblast growth factor 21; RBP4, retinol-binding protein 4; NS, not significant at $P > 0.05$.

the 6th week of the study, at ~12 weeks of age, the mice in the exercise group began exercising using freewheel running until the end of the study, when they were euthanized at the 22nd week. The controls remained sedentary from the beginning of the experiment until they were euthanized. One of the exercise mice died of an unknown cause.

Physical activity assessment

Free wheel running systems were installed and all mice in the exercise group used wheels for 6 weeks from the beginning of the study (Fig. 1).

Definition of obesity

The mice were weighed every 2 weeks and their weights were documented. To standardize the results, the homeostatic model assessment insulin resistance (HOMA-IR) index was determined at the end of the study using the following formula: (fasting insulin [mIU/L] fasting glucose (nmol/L))/22.5. All HOMA data are presented in Table 1.

Adipokine and hormone levels

Blood samples were collected after 12 hr of fasting. Mouse blood was obtained through cardiac puncture immediately after euthanasia. After centrifugation, serum was separated into 15-μL vials and stored at -80°C until required for evaluation. At the time of testing, serum was used for enzyme-linked immunosorbent assay (ELISA) analysis. Ghrelin, insulin, kisspeptin, visfatin, adiponectin, atrial natriuretic peptide (ANP), BMP, estradiol, follicle-stimulating hormone, luteinizing hormone, irisin, retinol-binding protein 4 (RBP4), and resistin were measured using commercially available ELISA kits according to the manufacturer's instructions, keeping inter- and intra-assay coefficients of variations <10% (Merck Millipore, Darmstadt, Germany). Serum BMP4 levels were measured using a BMP-4 ELISA kit (cat. no.# ab99982; Abcam, Cambridge, MA). Adipokines (leptin and resistin) were measured using a mouse gut hormone magnetic bead panel (Millipore, Billerica, MA) according to the manufacturer's instructions.

Statistical analysis

The mean value was used to present continuous variables (standard deviation). All variables were distributed normally in accordance with histograms. The comparison of two groups was accomplished using Student's *t*-test, and three groups were analyzed using one-way analysis of variance (ANOVA), followed by *post hoc* Tukey Tests. Pearson's correlation coefficient was chosen for pairwise correlations of hormonal and selected mediators. Analyses were performed using version 20.0 of the Statistical Package for the Social Sciences software (SPSS, Inc., Chicago, IL) and version 6 of GraphPad Prism software (GraphPad Software, Inc., La Jolla, CA). A $P < 0.05$ was considered significant.

Results

In this investigation we found no significant difference between the irisin and exercise groups (9.47 vs. 7.75 g; $P > 0.05$) with respect to weight change. The weight change in the control group was significantly greater compared with the irisin and exercise groups ($P < 0.05$). The HOMA-IR, which was used to check the risk of metabolic syndrome, showed no difference between the irisin and exercise groups ($P > 0.05$), although it was significantly higher in the control group ($P < 0.05$). The characteristics of the female mice with high-fat diet-induced obesity are listed in Table 1.

Serum adiponectin, ANP, BNP, RBP4, and resistin levels showed no significant difference between the irisin and control groups ($P > 0.05$). The serum levels of the same molecules showed a similarity in the exercise group, whereas serum resistin levels were significantly reduced in the exercise group compared with the control group (115.7 vs. 158.8 ng/mL, $P < 0.05$). In contrast, resistin levels were not significantly different between the irisin and exercise groups (131.0 and 115.7 ng/mL, respectively; $P > 0.05$). FGF21 levels were lower in both the irisin and exercise groups compared with the controls, showing a statistical difference in the irisin group ($P < 0.05$). Serum BMP, ghrelin, insulin, kisspeptin, leptin, and visfatin concentrations were statistically lower in the irisin group compared with the control group (2,927.5 vs. 12,210 ng/L, 5,314.3 vs. 15,055.9 pg/mL, 49.5 vs. 138 mIU/L, 2,509.2 vs. 3,340.4 pg/mL, 2,789.2 vs. 5,599.9 ng/L, 482.1 vs.

1,388.8 ng/mL, $P < 0.05$ for all parameters, respectively). Similarly, serum BMP, ghrelin, insulin, kisspeptin, leptin, and visfatin concentrations were significantly lower in the exercise group in comparison with the controls. Moreover, there were no statistical differences between BMP, ghrelin, insulin, kisspeptin, leptin, and visfatin serum concentrations in the irisin and exercise groups (Table 2).

Discussion

In the study, irisin injection resulted in exercise-mimicking effects on metabolic parameters related to obesity. The mice in the irisin group gained only a little more weight than the exercise group, even when not exercising under sedentary conditions. All metabolic parameters were positively altered in the irisin group similar to the exercise group.

Abdominal obesity, determined by fat tissue, is a risk for metabolic syndrome and is known to cause cardiovascular disease and type 2 diabetes. Today, it is believed that the underlying factors for the adverse effects of fat tissue arise from the excess release of adipokines from fat tissue cells into the blood system.^{11,12} The adipokines secreted from enlarged adipocytes (e.g., leptin, ghrelin, kisspeptin, and resistin), and cytokines from the newly arrived inflammatory cells, cause systemic inflammation and metabolic syndrome.^{13–15} These findings led to hypotheses involving lowering adipokine levels to find a solution to obesity. Abu-Farha et al. reported that groups with elevated levels of more than two proinflammatory adipokines had higher rates of metabolic syndrome compared with those with less than two proinflammatory adipokines.¹⁶ According to Meiliana et al., the prevalence of obesity and metabolic syndrome changed with the levels of adipokines between the obese group and control group.¹⁷

The effects of exercise on fat tissue have been well documented, showing an increase in lipolysis and glucose exchange. Stanford et al. claimed that transplantation of fat tissue from exercising mice into sedentary mice helped the recipients improve their glucose homeostasis.¹⁸ The mechanism of the molecular changes caused by exercising is still controversial; however, one of the strongest candidates is elevated quantities of irisin molecules.

Irisin is a polypeptide cleaved from FNDC5A. Boström et al. showed an increase in serum irisin levels after 8 weeks of voluntary wheel exercising mice.⁶ They explained the mechanism as PGC1- α expression in muscle, which stimulated an increase in the expression of FNDC5. Recent studies also showed the role of irisin in myoblast proliferation and muscle development.¹⁹ These findings encouraged hypotheses of exercise-mimicking effects produced by irisin administration, as seen in the recent study by Ye et al., which defined irisin as an agent reversing insulin.²⁰

Our results show that both exercise and irisin administration produce similar outcomes on serum BMP, ghrelin, insulin, kisspeptin, leptin, and visfatin concentrations. Recently, a human study showed that administration of exenatide, an inhibitor of BMP4, to obese patients resulted in an improvement in obesity-related diseases and changes in body fat distribution.²¹ Although there are no investigations that demonstrate the relation between BMP4 levels and irisin administration, our results are in correlation with BMP decrease-based outcomes from previous studies.

The predominant molecules associated with obesity are adipokines (leptin, visfatin, and kisspeptin), and a gastrointestinal system peptide, ghrelin.²² Leptin levels in the blood system usually correlate with adipocyte production, which represents the amount of adipose tissue.²³ Serum leptin concentrations are higher in individuals with obesity compared with normal weight individuals. Our results showed a decrease in leptin and insulin levels both with irisin administration and exercise.

Visfatin and kisspeptin are also derived from adipose tissue. Visfatin is known to capture glucose into adipocytes and prevent its release resulting in an increase in glucose-to-lipid translation.²⁴ Until recently, it was thought that kisspeptin was only derived from the brain and had functions in reproduction, but it is now known that it has other sources and targets such as liver, adipose tissue, and pancreas, suggesting a role in metabolism and metabolic syndrome.^{25,26} Thus, the serum levels of kisspeptin seem to be a possible target for next-generation obesity treatments.

Ghrelin is a gastrointestinal system-secreted peptide that has a unique structure with 28 amino acids and an *n*-octanoyl ester at its third serine residue. It is a well-known orexigenic hormone that stimulates food intake.²⁷ The effect of precipitating hunger is increased in a dose-dependent manner. Hence, the amount of ghrelin in circulation seems related to obesity caused by excess food intake. Exercise appears to break the chain by decreasing the amount of ghrelin, as irisin application does, or change the appetite.

There were several limitations on our investigation. The number of available animal subjects for use in the study was relatively small. Furthermore, the techniques we used to measure the quantity of molecules can be differentiated by using different assay modalities.^{28,29} It is our hypothesis that our findings might be reproducible in humans, but the data cannot be transferred directly; only through human testing can this be confirmed.

Conclusion

In conclusion, obesity is still the greatest battle of our age, and the only proven tool we have against obesity is exercise. Similar to the effect of exercise, irisin injections resulted in the amelioration of obesity-related parameters such as the concentration of adipokines, BMP4, insulin, and ghrelin. Its role as a potential alternative to exercise needs to be further studied.

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