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Effect of Training and Gender on Plasma Irisin, Leptin, and Insulin Levels

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ABSTRACT: The objective of this study is the comparison of the irisin, leptin, and insulin hormone levels of the female and male elite athletes and non-athletes. Elite taekwondo athletes (7 males, 6 females) and university students (8 males, 6 females) between the ages of 16 and 20 years volunteered to participate in this study. In the beginning of the study, the body compositions of the participants were determined and their plasma irisin, leptin, and insulin hormone analyses were determined by the Enzyme-Linked Immuno Sorbent Assay (ELISA) method. After an overnight fast, the blood samples were collected at 8:00 a.m. A two-way ANOVA was performed to examine the effects of gender and training status. There was found no significant main and/or interaction effect of training and gender on the irisin and insulin hormone levels ($p>0,05$). However, training and gender affected the leptin levels significantly ($p<0.05$). The leptin levels in females in both athletes and non-athletes were higher than males to significant extent and the leptin levels of both female and male athletes were significantly lower than non-athletes. As a result, it can be said that exercise training status and gender do not affect the levels of irisin and insulin hormones, but they increased the leptin level.

KEY WORDS Irisin – Leptin – Insulin – Gender – Training status

INTRODUCTION

It is known that regular physical activity increases physical fitness, affects the general health condition positively, and plays an efficient role in the protection from illnesses. The body composition and biochemical parameters may be affected by the type, intensity, and duration of the exercise [1,2].

The irisin hormone is a new myosin and a proteohormone with the weight of 12 kDa consisting of 112 amino acids isolated from the muscle tissue for the first time by Boström et al. [3]. It forms in the skeleton muscles because of the

destruction of the Fibronectin type III Area 5 (FNDC5) protein by an unknown protease. The myokines that are related with energy metabolism and muscle renewal capacity provide support to the active muscles through acute or regular exercises. The during exercise, Peroxisome Proliferator Coactivator Alpha (PGC-1 α) expression within muscles increases and upon contraction of the muscles, the white fat tissues transform into brown fat tissues that have more mitochondria and the production of irisin is started. The mRNA expression of the uncoupling protein-1 (UCP1) gene is increased by FNDC5. The increase of UCP1



prevents ATP synthesis and ensures the augmentation of energy consumption by causing heat formation [3]. It was observed in the studies conducted that, irisin, which was initially demonstrated to be synthesized in the skeleton muscles in relation with exercise, is also synthesized in many other tissues in addition to the skeleton muscles [3,4]. Upon the demonstration of the fact that irisin is related with the increases in energy consumption, it was asserted that it will play a significant part in the prevention of obesity and metabolic disorders but the impact physiology in humans is not yet fully explained [5].

Insulin secreted by the β -cells of the Langerhans islets of the pancreas is a hormone in polypeptide structure [6]. Insulin that has anabolic impact in the metabolism ensures the entry of glucose, particularly to the muscles, liver, and adipose tissues and causes glycogen and fat accumulation in such tissues [7]. It was thought that the glucose entry to the skeleton muscles increased independently from insulin subsequent to a single exercise session; that, such impact with short duration lost after 48 hours; that, however, a permanent increase in the insulin impact in the skeleton muscles was found upon the routine exercises performed by the obese and insulin-resistant individuals; and that such increase of the impact might result from the increase of the expression and activation of the signal proteins that play a part in the entry of glucose in the skeleton muscles [8]. Pedersen and Febbraio [9] pointed out that physical activities increase glucose tolerance and reduce the possibility of having Type 2 diabetes but exercises with very long durations are necessary to observe such positive impacts.

The impacts of leptin, a proteohormone, which was discovered for the first time by Zhang et al. [10] with 16 kDa molecule weight containing 167 amino acids, on the body are defined concisely as the prevention of the development of obesity upon the regulation of the food intake and energy metabolism through the negative feedback mechanism on the central nervous system [11]. It was reported that leptin controls the energy metabolism on the hypothalamus axis by stimulating the food and energy intake and that there is a strong relation between the serum leptin level and body mass index, insulin resistance, and diabetes [12], and it was determined that the body fat mass reductions observed repressed the serum leptin level through regular exercises [13,14,15].

The hormonal response to exercise have attracted the attentions of numerous researchers and the manner of the

insulin and leptin hormones considered directly related with exercise from being affected by acute and chronic exercises has been intensively studied. However, the studies on the impact of exercises on the recently-discovered irisin are limited and the publications that express that only the acutely performed resistance and aerobic exercises increase the irisin concentration [16,17,18], but the long-term exercise programs do not affect the irisin concentration [19,20,21], and recent studies even reduce irisin [22] are encountered as well. It appears that the studies regarding the responses of irisin to acute and chronic exercises have been conducted in different age groups and in the study design with different exercise type and duration (resistance, aerobic etc), in the healthy females and males with different activity levels [23,24,25], in the patients with diabetes [26] or in obese individuals [27]. It was observed that even though the impacts of training and gender have been researched in the studies about irisin, the subjects were not elite athlete. The focus of our study was to investigate the effects of gender and training status on the irisin, leptin, and insulin hormones in elite athletes and non-athletes. The hypothesis is that the levels of irisin, leptin, and insulin hormones is different between elite athlete and non-athletes at rest.

METHODS

Subjects

Total 27 volunteers consisting of elite taekwondo athletes (7 males, 6 females) between the ages of 16 and 20 who were currently actively competing and ranked in the European, World, and Olympic competitions and the healthy individuals (8 males, 6 females) studying at Selçuk University who had no exercise training for at least 18 months participated in the study. The study design was performed in accordance with the Declaration of Helsinki and approved by the local ethics committee at Faculty of Sports Science, Selçuk University. Prior to the study, the subjects were examined by a physician, informed about the study, and their written approvals stating that they agreed to take part in it were received.

General Design of the Study

The body composition measurements and blood samples in the resting periods of the subjects were received in different days, at the same hours of the morning, and under equal conditions after staying hungry at the previous night. It was ensured that the subjects did not perform any heavy physical activities until at least 48 hours and they were also warned not to use any medication or liquid that might affect the values, to determine the impact of training status and

gender on the biochemical variables.

Determination of the Body Composition

The body heights (cm) of the volunteers were measured by using a Seca-brand mechanic weight with height measuring mechanism, as bare feet, feet pressing smoothly on the weighing platform, heels adjoined, knees stretched, and body in upright position, with 1 mm sensitivity and their body weights (kg) were determined at 100 g sensitivity with their outfits of shorts and t-shirts in participants. The body mass index (BMI) was calculated by dividing the body weight (kg) by the body height (m) square [28]. To determine the percentage of the body fat, a Holtain skinfold caliper was used applying 10 g/sq mm pressure at every angle, and their triceps, biceps, and subscapularis and suprailiac skinfold thicknesses were taken and body fat was determined [29].

Male = $1,1631 - 0,0630 * \text{Log} (\text{biceps} + \text{triceps} + \text{subscapularis} + \text{suprailiac})$

Female = $1,1599 - 0,0717 * \text{Log} (\text{biceps} + \text{triceps} + \text{subscapularis} + \text{suprailiac})$

Fat % = $(4,95 / \text{Body Density} - 4,50) * 100$

Biochemical Analyzes

The blood samples received at 8:00 a.m. after an overnight fast, by the health personnel were centrifuged at +40C 3000 cycle/minute for 20 minutes to acquire their

plasmas. The irisin, leptin, and insulin hormone levels in the plasma samples were determined using commercial kits (Abbott) through the Enzyme-Linked Immuno Sorbent Assay (ELISA) method and the plasma LDL, HDL, total cholesterol, triglyceride, and glucose levels were determined with an Abbott-brand (Architect model) device.

Statistical Analysis

Statistical analysis was performed using SPSS for Windows (version 16.0, SPSS Inc. Chicago, IL, USA). A two-way ANOVA was performed to examine the main effects of gender, trained and untrained group (2x2) and interaction on the measured variables. Also, One-way ANOVA with post-hoc Bonferroni test was used to compare group means. The levels of statistical significance were set at $p < 0,05$ and $p < 0,01$. Data are expressed as means \pm Standard Deviation (SD).

STATISTICAL RESULTS

The age and BMI were similar among the groups ($p > 0,05$). It was observed that the body weight and height of males were significantly higher than those of the females ($p < 0,05$). The body fat percentages in males lower than females ($p < 0,05$). The body height of athletes was significantly higher in both the females and males than the non-athlete and their body fat percentage averages were significantly lower only in the active athlete females than non-athlete ($p < 0,05$) (Table 1).

Table 1. Physical characteristics of subjects

		Athlete			Non-athlete			F
		Mean	\pm	SD	Mean	\pm	SD	
Age (years)	F	20,15	\pm	1,81	20,33	\pm	1,86	0,83
	M	19,29	\pm	1,50	20,75	\pm	1,83	
Body Weight (kg)	F	β 57,00	\pm	10,06	β 55,83	\pm	5,78	6,45*
	M	72,71	\pm	12,65	72,13	\pm	7,55	
Height (cm)	F	β 172,67	\pm	3,56	β 162,50	\pm	6,38¥	7,70*
	M	186,43	\pm	8,10	174,63	\pm	4,69¥	
Body mass index (kg/m ²)	F	19,03	\pm	2,66	21,17	\pm	2,12	5,03
	M	20,79	\pm	2,16	23,65	\pm	2,12	
Body fat percentage (%)	F	β 18,40	\pm	3,27	β 24,10	\pm	2,36¥	60,09*
	M	8,91	\pm	1,40	10,74	\pm	2,09	

* $p < 0,05$ significant differences among the groups

β $p < 0,05$; Significant differences between men and women in athlete or non-athlete groups

¥ $p < 0,05$; Significant differences between men and women in athlete or non-athlete groups

F; Female, M; Male

Even though the effects of gender and training on plasma levels of irisin and insulin were not significant ($p > 0,05$), there was a significant interaction effect between gender and

training for the leptin level ($F(4,27)=16,13$; $p < 0,05$), (Table 2). The leptin concentrations were significantly higher in females than males in the both group, and that leptin levels

of the female and male non-athletes were significantly higher than the athlete. On the plasma LDL, total cholesterol and glucose levels that were the other biochemical parameters examined, no significant effect of gender and/or training was observed ($p>0,05$). HDL levels were

significantly higher in both groups of athlete and non-athlete females than males ($p<0,05$). Plasma triglyceride levels were found similar for gender, but both female and male athlete's levels were significantly lower than the non-athletes ($p<0,05$) (Table 2).

Table 2. Effect of gender and the training status on some hormones and biochemical variables

		Athlete		Non-athlete		Group	Gender	Group×Gender
		Mean	± SD	Mean	± SD			
Irisin (ng/mL)	F	57,92	± 22,03	48,60	± 26,22	0,02	0,16	0,83
	M	46,14	± 29,49	53,25	± 13,85			
Leptin (ng/mL)	F	1,32	± 0,52	11,68	± 5,74 ¥	22,82*	20,54*	16,13*
	M	0,71	± 0,11	β 1,61	± 2,58			
Insulin (μIU/mL)	F	9,38	± 8,23	10,17	± 4,08	1,46	0,80	0,72
	M	5,60	± 1,52	10,06	± 6,39			
LDL (mg/dl)	F	70,83	± 14,76	62,33	± 25,16	0,07	0,01	1,31
	M	63,29	± 10,72	68,63	± 9,74			
HDL (mg/dl)	F	β 56,17	± 11,87	52,67	± 9,73	0,75	15,41*	0,08
	M	43,29	± 3,77	41,50	± 4,96			
Glucose (mg/dl)	F	82,50	± 10,45	84,33	± 4,59	0,18	0,21	0,05
	M	84,43	± 6,92	85,00	± 6,37			
Total Cholesterol	F	165,50	± 28,98	159,17	± 25,02	0,02	3,06	0,41
	M	145,57	± 18,64	149,88	± 13,39			
Triglycerides	F	57,83	± 18,61	98,33	± 41,52 ¥	9,69*	2,92	0,00
	M	80,29	± 32,11	119,75	± 32,25 ¥			

* $p<0,05$ significant main and/or interaction effects

¥ $p<0,05$; Significant differences between athlete and non-athlete for both gender

β $p<0,05$; Significant differences between men and women in athlete or non-athlete groups

F; Female, M; Male

DISCUSSION

The main result of this study is that the leptin levels were affected by both training and gender. It was observed that the females had higher leptin hormone level than males and that the hormone levels in athletes were lower than the non-athletes. However, it was seen that training status reduce the leptin levels in females more notable than those of the males as well. The second important result of the study is that the irisin and insulin hormones are not affected by the training status and/or gender.

In the literature, different results have been reported in the studies conducted in the impact of exercise training on leptin that is considered repressive on the food intake and causing the formation of a negative energy balance. In some of the studies, it was observed that acute exercises caused no change on the leptin levels [30,31], while some others found reduction in the leptin levels [32,33]. The studies where athlete and non-athlete groups are compared specify that the leptin levels of young male athletes from different sports branches [13], professional male soccer players [14], and elite male weightlifters [15] were significantly lower than the non-athletes significantly. In a harmonious manner with such studies, it was determined in our study that the leptin levels of the female and male athlete were significantly lower than those of the non-athlete ones. In different studies oriented to the obese female and males, it was reported that the leptin levels reduce to a significant extent at the end of the aerobic and resistance training programs [34,35].

In our study results show that irisin levels at rest are not affected by training status and/or regular exercise training. None of the regular physical activity or physical fitness are associated with resting irisin concentrations in healthy humans [36]. The studies related to the effect of acute and/or chronic exercise on the irisin levels were conflicting. Timmons et al. [37] stated that FNDC5 mRNA levels in the skeleton muscle of the groups 6-week cycling exercise did not exhibit a significant difference from the non-athlete ones. It was expressed by Hecksteden et al [19] that in the irisin levels of the female and males on whom a 26-week aerobic endurance and strength training were applied, no change was determined and by Huh et al [38] that no statistically significant difference took place in the plasma irisin levels of healthy young females after a 6-week training program. Kurdiova et al. [39] determined that long-term chronic exercises do not affect the skeleton muscles and blood irisin levels significantly. Pekkala et al. [40] underlined that the irisin levels subjected to chronic exercise exhibited differences although they were not significant, but

such differences do not mark an absolute connection between the FNDC5 expression and irisin secretion. However, Boström et al. [3] specified that, with a 10-week endurance training applied to healthy adults, their blood irisin levels doubled after training and that the obesity caused by dieting and insulin resistance declined. Arkan et al. [41] reached similar results and stated that the plasma irisin levels of the male soccer players were higher and their insulin resistance was lower than the non-athletes. Norheim et al. [20] reported that in the adult male individuals, upon a 12-week endurance and strength training, the irisin levels in circulation reduced in both the control group and the pre-diabetic individuals as a response to the strength training. Similarly [42], stated that chronic exercise training leads to decreased circulating irisin levels.

In this study, contrary to the results of Norheim et al. [20], Boström et al. [3], and Arkan et al. [41], it was determined that the irisin levels are not affected by training status and gender. We consider that the contradictions between our study and the foregoing studies might have arisen from the participants' training status and study design, the difference between their durations and impacts, diversity of the subject (healthy, ill, obese, athlete, non-athlete, young, old, female, male etc.), and their dieting habits.

Although Gunay et al. [43] specified that the insulin concentrations reduce in line with the secretion catecholamines during exercise, insulin levels were not affected by exercises after long-term aerobic and resistance training programs, by Gerosa-Neto et al. [44] in the overweight/obese females and males, by Nunes et al. [45] in menopausal females, by Timmons et al. [37] in young non-athletes, and by Hecksteden et al. [19] in healthy adult females and males. Similarly, in our study, no difference was found the insulin levels between the elite athletes and non-athletes in both male and female. It was determined that the insulin levels increased following acute exercises applied in different durations and types [16,46]. In their studies on the cell culture and experiment animals, Boström et al [3] observed that irisin, which is secreted from the muscle cells and which ensures the communication between the muscle and fat tissue, assumes efficient role in the regulation of the insulin resistance and energy metabolism, while contradicting results in terms of the presence of a significant relation between irisin and insulin were provided in many publications. In obese youth, irisin increases following an acute bout of aerobic exercise, but not resistance exercise, and this response is related to a greater

improvement in insulin sensitivity in response to chronic resistance training [47]. It was asserted that such contradictions might have resulted from the body compositions and metabolic states of individuals and from the fact that their levels of exercise training are different from each other [38,39,40,41].

This study has some limitations; first, all the participants were informed about their dietary contents a night before the testing days, but they were not checked. In addition, the blood samples were received from the subjects at 8:00 a.m. as a single sample after an overnight fast. However, as the

day-night rhythms of the irisin and leptin hormones vary during the day [48,49,50], the fact that the samples were not taken intervals might have affected the results of the study. Also, certain methodological issues need to be considered here such as the analytical assays used to measure irisin concentration in the circulation [51].

As a result, it can be stated that the irisin and insulin hormone levels in the young adults are not affected from training status and genders, but leptin levels are, and that the leptin concentration is lower in males than females and in elite athlete than non-athletes.

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