

Examination of The Relationship Between Intermittent Fasting and Irisine Levels In Rats Fed A High-Fat Diet

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ABSTRACT

In this study, the relationship between intermittent fasting and irisin levels has been examined in rats fed on a high-fat diet. Eighteen Wistar albino rats were randomly divided into three groups as control, high-fat diet (HFD) and, high-fat diet+intermittent fasting (HFD+IF) (n=6). After 8 weeks of study irisin level were measured in serum.

Irisin levels in the HFD group were lower (8.24 ng/ml) than the control (10.50 ng/ml) but in the HFD+IF group was higher (12.25 ng/ml) compared to the HFD group (8.24 ng/ml) ($P<0.05$). It was observed that body weight in the HFD group was higher (385.92 g) than the control group (270.50 g) and was lower (297.71 g) in the HFD+IF group compared to the HFD group (385.92 g) ($P<0.05$).

As a result, it has been seen that intermittent fasting increases the level of irisin and causes weight loss, and it is concluded that it can be evaluated among the methods to be used in the treatment and prevention of obesity.

Keywords: Irisin, Obesity, Intermittent fasting, High-fat diet

Introduction

High-fat diet causes the disruption of the energy balance and an increase in the adipose tissue mass (1,2). Obesity, defined as excessive fat accumulation in an organism, has become a worldwide epidemic (3). Obesity is one of the significant metabolic diseases commonly found in developing and developed countries due to the prevalent sedentary lifestyle and consumption of fast foods that contain high levels of fat and glucose (4,5). Obesity hyperlipidemia in humans is closely related to the incidence of many chronic diseases such as hyperglycemia, diabetes, low-grade inflammation, hypertension, coronary artery disease, metabolic syndrome (6). The composition of the diet, especially high-fat diets (HFD), causes obesity in humans (7) and rats (8). As the average amount of fat in the diet increases, the incidence of obesity increases as well. (9). Studies have shown that dietary fat intake mostly causes an increase in adipose tissue and causes obesity (10,11). High-fat diets are known to cause an increase in blood pressure and heart rate (12). Epidemiological studies have shown a link between dietary fat intake and the risk of obesity and its associated complications (13,14). Intermittent fasting (IF) is a dietary practice that is practiced during regular periods of nutrition, in

the form of energy restriction or a break from eating between certain hours, typically 1 to 3 days a week. By reducing the amount of energy with intermittent nutrition, a negative energy balance is created that causes weight loss (15). Excessive energy intake is associated with an increase in the incidence of many chronic diseases worldwide, including obesity and type II diabetes. Intermittent nutrition and calorie restriction prolong life by reducing the incidence of diseases associated with aging (15,16). Through intermittent fasting practiced between 8 and 12 weeks, significant weight loss is achieved with the increase in insulin sensitivity (17) Significant weight loss is achieved with the control of dyslipidemia, arterial pressure and changes in body composition (18,19). In this study, the relationship between intermittent fasting and irisin levels was investigated in rats fed on a high-fat diet.

Materials and Methods

The study was approved by the Van Yuzuncu Yıl University Animal Experiments Local Ethics Committee (decision date and number 31.03.2022 2022/03-03). This study was conducted according to the Declaration of Helsinki, as revised in 2000.

This study was performed on 18 male Wistar albino rats, which were randomly divided into

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three groups (n=6 per group). Except for the experimental diet, all other conditions were provided within the standards of laboratory animal care. The groups were created as follows. Control group: a standard diet was provided (2.8% crude fat, 23.1% crude protein, 5% crude fiber, 7.1% crude ash, and 12.8% moisture). High-fat diet (HFD) group: 300 g/kg margarine was melted into a standard pellet meal (so 60% of energy is from saturated fat of the diet is saturated fat); it was prepared and provided for 8 weeks daily. High-fat diet + Intermittent fasting (HFD+IF) group: (24 hours break after non-successive diet for 2 days a week, and all food was restricted except water) (20). In the literature surveys, because it was seen that there were 8 weeks of studies in which a high-fat diet was applied (21), our study was planned as 8 weeks in order to be compatible with the literature.

At the end of the study, a blood sample was taken from the heart of rats euthanized with Ketamine + Diazepam (50+8 mg/kg) and the serum irisin level was examined via the ELISA method.

Taking of the blood samples: At the end of the experiment period, with general anesthesia application the abdominal region of the rats (control and experimental groups) was excised in the form of an inverted letter V from the anal (pubis) area to the chest cavity, the abdominal cavity was opened, and the required amount of blood was taken by penetrating the heart with an injector. Intracardiac blood collected at the end of the study was placed in yellow-capped biochemistry tubes and centrifuged at 3.500 g for 10 min. The supernatant serum was transferred to another tube and kept at -80°C until it was studied.

Quantitative measurement of the irisin from the rat serum samples was performed using a commercial enzyme-linked immunosorbent analysis (ELISA) kit (Phoenix Pharmaceuticals Inc, Burlingame, California, USA). The determination range of the kit was 0.781 - 50 ng/ml, and intra-variation coefficients <0.469 and sensitivity and 94% recovery values were measured using commercial enzyme-linked immunosorbent analysis (ELISA) kits (21).

Statistical Analysis: Descriptive statistics of the groups were given as mean and standard deviation. The Shapiro-Wilk test was used to determine whether the data were distributed. For the same parameter, presence of significant differences between the groups was evaluated with the Kruskal-Wallis Test. In order to determine

which group causes the difference, post hoc analysis (Tukey HSD) was performed and results which have a p value of 0.05 or smaller were considered significant. The SPSS (ver.22) statistical package program was used for the calculations.

Results

Serum irisin values were lower in the HFD group compared to the control group, but were higher in the HFD+IF group compared to the HFD group ($p<0.05$) (Table 1, Figure 1). Weight value differences were higher in the HFD group compared to the control group, and lower in the HFD+IF group compared to the HFD group ($p<0.05$) (Table 1, Figure 2).

Discussion

Irisin is an anti-obesity hormone that increases fat burning and prevents the formation of adiposity (22). Irisin, which was first isolated by Boström et al. in 2012, is a new myokine consisting of 112 amino acids (23). Irisin is secreted from liver, adipose tissue and different tissues in varying amounts (24). Irisin secretion increases with exercise in humans and rats (25). Increasing irisin with exercise, browns the white adipose tissue in the organism, enabling the energy to be converted into heat and increased energy consumption (26). Irisin is a heat-increasing hormone and has a significant effect on reducing fat mass (24, 27). Mazur-Bialy et al. reported that plasma irisin levels were significantly reduced in sedentary rats fed on a high-fat diet (HFD) with or without colitis (28). In another study, Jimenez- Aranda et al. noted that there were high levels of irisin in thin rats, compared to obese rats (29).

Kang et al. found that in an 8-week high-fat diet study they conducted, the level of irisin was significantly reduced in the high-fat diet group compared to the control group after 8 weeks (30). Yun Lu et al. reported that fasting a HFD in rats increased body fat mass and decreased serum irisin level (31). Irisin levels have been reported to be lower in obese rats (23). Studies have shown that irisin in men is has a negative correlation with BMI, waist/hip ratio and fat ratio, and that the levels of the irisin were lower in obese and overweight men (32). In another study, it was determined that FNDC5 decreases in rats fed on a HFD, and the level of irisin in skeletal muscle also decreases significantly (33). These results are

Table 1: The mean and Standard Deviation Values of Live Weight and The Irisin In Serum Samples

	Control	HFD	HFD+IF	p
Irisin (ng/mL)	10.50±0.585b	8.24±0.390c	12.25±0.240a	0.001
Live weight (g)	270.50±7.92c	385.92±5.27a	297.71±5.71b	0.001

“a,b,c: Shows the difference between groups (Tukey HSD)”

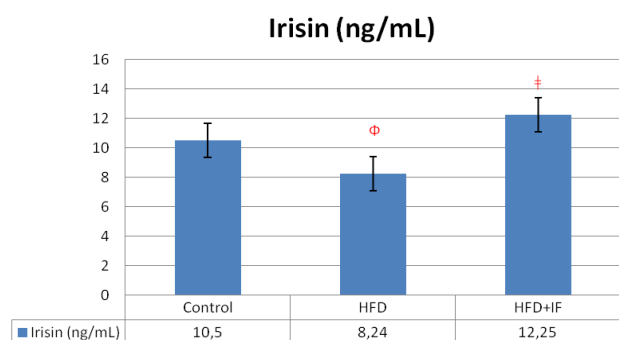


Fig. 1. The mean values of serum irisin among the groups (ng/ml).

Φ p: significant compared with the control group ($p < 0.05$), † p: significant compared with the HFD group ($p < 0.05$)

supported by studies reporting that irisin levels are low in obese rats and humans (34, 35). Stengel et al. reported that there is a relationship between weight and irisin levels in obese patients (36). The results of our current study support the above studies and found that the level of irisin in the group fed with a HFD decreased significantly compared to the control group, the body weight increased significantly in the group fed a HFD, and in the group that applied a HFD+ IF, the weight decreased significantly compared to the group that applied a HFD (Table 1).

In the 7-week intermittent fasting study conducted by Karras et al, they found that the level of irisin increased after 7 weeks compared to the control group (37). In another study conducted by Alzoughool et al. they found that fasting irisin values were higher compared to postprandial values during Ramadan (3). As a result of the eight-week study by Crujeiras et al. aiming to provide weight loss in 93 patients (39), the 10-week study by Huerta et al. aiming to provide weight loss in 79 female patients (40), and the 6-week study of de la Iglesia et al. aiming to provide weight loss, they all reported that in obese patients, with the decrease in the body weight, serum irisin levels were also decreased (41). In the study we carried out, it was observed that the level of the decreased irisin in the group fed on a high-fat diet was increased significantly in the group fed on a HFD+IF. In the literature search, it was

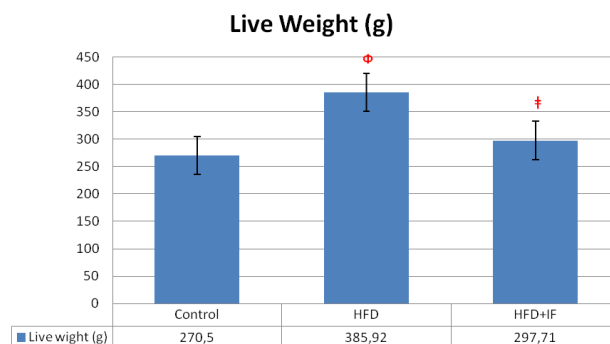


Fig. 2. The mean values of live weight among the groups (g).

Φ p: significant compared with the control group ($p < 0.05$), † p: significant compared with the HFD group ($p < 0.05$)

observed that there are studies with different results on the levels of irisin in obese people. It is believed that the factor that causes inconsistency in irisin results is the biochemical analyzes (42). It has been stated that since irisin is a sensitive molecule different results can be obtained in different kits, and the discrepancy in the study results may also be due to this (43).

In conclusion: In the present study carried out, it was seen that the 8-week intermittent fasting application with 2 days a week, which was applied to the group that was fed on a high-fat diet, increased the level of irisin and causes weight loss. It is contemplated that intermittent fasting can be employed as one of the methods of preventing and treating obesity, under the control of a physician, if necessary, by being supported with further studies on intermittent fasting.

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Conflict of interest: The author declare that they have no conflict of interest.

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