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Suppression of the thermogenic response in a diet-induced obesity model: The role of irisin and uncoupling protein 1

Elif Şahin*[1], Hüseyin Çınar Zihni[2], Ahmet Alver[1]

[1]*Department of Medical Biochemistry, Karadeniz Technical University, Faculty of Medicine, Trabzon, Türkiye*

[2]*Department of Medical Biochemistry, Karadeniz Technical University, Graduate School of Health Sciences, Trabzon, Türkiye*

ABSTRACT

Aim: To evaluate irisin and uncoupling protein 1 (UCP1) levels and oxidative stress parameters in an obesity model induced by a cafeteria (CAF) diet that mimics human dietary habits.

Methods: A total of 12 male Wistar rats were divided into two groups: control (n=6) and cafeteria diet (n=6). The control group was fed a standard laboratory diet, while the CAF group was fed a CAF diet consisting of high-calorie foods for 16 weeks. At the end of the experiment, retroperitoneal white adipose tissues (WAT) and brown adipose tissues (BAT) were removed. Tissue irisin and UCP1 levels were determined by ELISA, and total oxidant (TOS) and antioxidant (TAS) status levels were determined by colorimetric methods.

Results: In the CAF group, final body weight, retroperitoneal fat, and brown adipose tissue levels increased significantly compared to the control group (*p=0.006, p=0.035, p=0.030, respectively*). Irisin and UCP1 levels in BAT were significantly reduced in the CAF group (*p=0.001 for both*). TAS levels decreased significantly in the CAF group (*p=0.010*), while the increase in TOS levels was not statistically significant (*p=0.655*).

Conclusion: The decrease in irisin and UCP1 levels in the cafeteria diet-induced obesity model indicates suppressed thermogenic capacity. The decrease in TAS levels suggests weakened antioxidant defense and increased oxidative stress. These findings suggest that the irisin/UCP1 axis and oxidative stress parameters may be important biomarkers for understanding obesity-related metabolic dysfunction.

Keywords: Diet-induced obesity, irisin, UCP1, oxidative stress.

✉ * *Elif Şahin*

Department of Medical Biochemistry, Karadeniz Technical University, Faculty of Medicine, Trabzon, Türkiye

E-mail: elifsahin@ktu.edu.tr

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1. Introduction

Obesity is a multifactorial and chronic metabolic disorder characterized by energy intake exceeding energy expenditure, with devastating health and economic consequences and a steadily increasing global prevalence [1]. Obesity affects nearly all organ systems in the body and is a risk factor for numerous morbid conditions [2]. In developed and developing countries, obesity is associated with serious health problems such as diabetes, cardiovascular diseases, and certain types of cancer, leading to higher morbidity and mortality rates [3]. Although lifestyle changes, particularly diet and physical activity, are fundamental strategies in combating obesity, the effectiveness of these interventions is limited in many individuals, necessitating additional therapeutic approaches [4].

To study the development and risk factors of obesity, researchers use diet-induced obesity (DIO) animal models, which mimic human obesity more reliably than genetic models [5]. Among the dietary protocols used to model obesity in laboratory animals, CAF diet stands out as one of the methods that best mimics human eating habits. This diet consists of high-calorie and highly palatable foods found in the human diet; it causes metabolic disorders in rodents, such as hyperphagia, significant weight gain, hyperinsulinemia, hyperglycemia, and glucose intolerance [6,7].

In addition, inflammation and increased oxidative stress in white adipose tissue have been observed in animals fed the cafeteria diet [8]. It has been indicated that oxidative stress plays a role in both the progression of obesity and the development of obesity-related complications [9]. TAS and TOS levels frequently measured to indicate oxidative status.

Discovered in 2012 by Böstrom et al. [10], irisin is defined as a myokine induced by exercise that increases energy expenditure by promoting the conversion of white adipose tissue to brown adipose tissue (BAT). Irisin, formed by the proteolytic cleavage of fibronectin domain-containing protein 5 (FNDC5), is an adipo-myokine hormone produced during exercise and shows therapeutic potential for conditions such as metabolic disorders, osteoporosis, sarcopenia, obesity, type 2 diabetes, and neurodegenerative diseases, including Alzheimer's disease [11]. Increased irisin levels trigger UCP1 expression, supporting thermogenesis and lipolytic activity. Due to these properties, irisin is considered a potential target molecule for the treatment of obesity and related metabolic disorders [12]. So, it was aimed at evaluating irisin and UCP1 levels and oxidative stress parameters in an obesity model induced by a CAF diet that mimics human dietary habits.

2. Materials and methods

Ethical committee approval was obtained from the Karadeniz Technical University, Faculty of Medicine, Local Ethics Committee for Animal Experiments, protocol number 2024/37.

Four- to six-week-old male Wistar rats used in the study were obtained from the KTU Faculty of Medicine Surgical Application and Research Center. They were housed and fed in steel cages under a 12-hour light-dark cycle at the same location. Food and water were provided ad libitum. After weighing 12 rats, they were randomly divided into 2 groups. The proteins studied in this work have been measured in previous studies. Using these data, the effect size was calculated with the G*Power program. With the entered effect size (0.50), alpha error (0.05), power (0.95), and number of groups (2), the total sample size was calculated to be 12. This was calculated as 6 animals per group.

Group I (Control) (n=6): Subjects in this group were fed laboratory-type rodent feed for 16 weeks.

Group II (Cafeteria Diet) (n=6): Subjects in this group were fed a cafeteria diet for 16 weeks.

The cafeteria diet consisted of a mixture of potato chips, fish crackers, biscuits, fruit cake, chocolate wafers, and 30% laboratory-type rodent feed (for vitamin, mineral, and trace element supplementation). The values indicated on the packaging of the food items used to calculate the energy intake were used as a basis; The diet was adjusted so that 40% of the energy came from fat, 55% from carbohydrates, and 5% from protein. The calculated amounts were then purchased, ground into powder using

laboratory-grade grinders, and formed into pellet feed. Briefly the products used in the feed were ground into powder. They were mixed with a small amount of water to form a paste and pressed into molds. They were dried in an oven at 50°C and turned into pellets. [5]. At the end of 16 weeks, rats in all groups were sacrificed by decapitation. After removal of the retroperitoneal and brown adipose tissues from the rats, they were stored at -80°C for biochemical analysis.

2.1. Measurement of Irisin and UCP1 Levels: To determine irisin levels in tissue, the irisin commercial ELISA kit with product code E6281Ra from BT LAB (Shanghai, China) was used. Tissue UCP1 levels were determined using the UCP1 commercial ELISA kit with product code E1252Ra from BT LAB (Shanghai, China). Measurements were performed in accordance with the manufacturer's recommendations. Sample concentrations were calculated in ng/mL. Results were expressed as ng/mg protein.

2.2. Determination of Total Oxidant Status (TOS) Level: Total Oxidant Status levels in tissue samples were measured using a commercial colorimetric kit (Rel Assay, RL0024, Gaziantep, Turkey). This method is based on the colorimetric measurement principle whereby oxidants in the sample cumulatively oxidize the Fe^{2+}-o-dianisidine complex to Fe^{3+} ions. The Fe^{3+} ions then form a colored complex with 'Xylenol Orange' in an acidic environment. The resulting color intensity is measured spectrophotometrically and increases proportionally to the amount of oxidant present in the sample. Results are given in µmol H_2O_2 equivalent/g protein.

2.3. Determination of Total Antioxidant Status (TAS) Level: The tissue total antioxidant status levels was determined using a commercial colorimetric kit (Rel Assay RL0017, Gaziantep, Turkey). This measurement method is based on the principle that the antioxidants in the sample convert the dark blue-green ABTS radical into the colorless ABTS form. The total antioxidant level of the sample is inversely proportional to the measured color intensity at 660 nm. The standard solution for this method is prepared using trolox (a vitamin E analogue) as the most stable antioxidant. The standard concentration is 1.0 mmol equivalent/L. Results are given as mmol Trolox equivalent/g protein.

2.4. Protein Determination: The protein content of the obtained tissue homogenates was analyzed using the BCA method. This method is based on the reduction of Cu^{2+} to Cu^{1+} by proteins present in an alkaline environment. The colorimetric determination is performed by reacting the formed copper cation (Cu^{1+}) with BCA. The results were calculated in µg/mL.

2.5. Statistical Analysis: The normality of the experimental groups was assessed using the Kolmogorov–Smirnov test. The independent Samples t test is used to compare the meanings of two groups. Values are expressed as the mean ± standard deviation (SD), and $p<0.05$ is considered statistically significant.

3. Results

3.1. Comparison of Total Weight and Fat Tissue Amounts Between Groups: It was noted that there was no statistical difference in the initial weights of the rats included in the experiment. However, a significant difference was observed in their final weights at the end of the experiment ($p=0.006$). A statistically significant increase was also found in WAT and BAT ($p=0.035$ and $p=0.030$, respectively). Based on the results obtained, it can be said that the CAF diet mimics the experimental obesity model (Table 1).

Table 1. Findings related to weight data**.**

Parameters	Control	CAF
Initial weight (g)	211±7,41	213±5,23
Final weight (g)	459±20,44	516±39,81*
WAT(g)	2,75±1,11	4,21±1,05*
BAT(g)	0,31±0,13	0,48±0,12*

*Statistically significant compared to *Control ($p<0.05$). WAT: white adipose tissues; BAT: brown adipose tissues.*

Table 2. TAS and TOS values.

Parameters	Control	CAF
TAS (mmol Trolox equivalent/g protein)	0,32±0,16	0,18±0,03*
TOS (µmol H_2O_2 equivalent/g protein**)**	2,66±0,98	3,04±1,14

*Statistically significant compared to *Control ($p<0.05$). TOS: total oxidant status; TAS: total antioxidant status.*

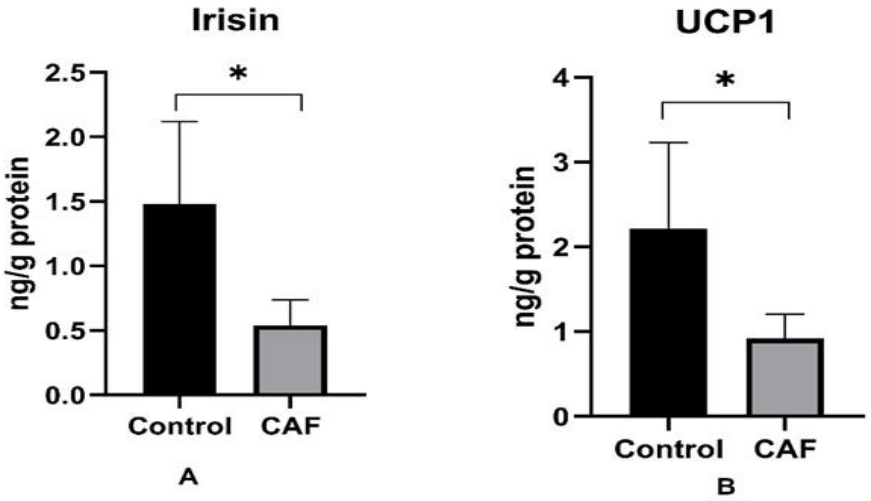

Figure 1. Tissue Irisin and UCP1 levels. Statistically significant compared to *Control group ($p<0.05$). *(UCP: uncoupling protein 1).*

3.2. Comparison of Irisin and UCP1 Levels in Tissue: Irisin and UCP1 levels obtained in the obtained BAT were determined using commercial ELISA kits (Figure 1). Tissue irisin and UCP1 levels were found to be statistically decreased in CAF groups compared to the control group (p=0.001 and p=0.001, respectively).

3.3. Comparison of Tissue TAS and TOS Values: TAS and TOS levels in the obtained BAT were determined using commercial colorimetric kits in accordance with the manufacturer's recommendations. The tissue TAS level decreased statistically in the CAF group compared to the control group (p=0.010). Although an increase was observed in the TOS value, this increase was not found to be significant (p=0.655) (Table 2).

4. Discussion

In this study, changes in the irisin/UCP1 axis and oxidative stress parameters were examined in an obesity model induced by a diet that mimics human dietary habits. The findings show that rats fed the CAF diet had significant weight gain, increased retroperitoneal and brown adipose tissue, decreased irisin and UCP1 levels, and decreased antioxidant capacity. These results provide important clues for understanding the molecular basis of obesity-related metabolic dysfunction.

The observation of significant weight gain and an increase in retroperitoneal/brown adipose tissue mass in rats fed a CAF diet is consistent with previous studies [7,13]. In particular, the increase in retroperitoneal and

brown adipose tissue confirms the obesity-promoting effect of this diet. The data we obtained supports the existing literature (Table 1). Increased adipose tissue in obesity, particularly visceral fat, causes chronic low-grade inflammation. This inflammatory process increases the production of reactive oxygen species (ROS) [14]. Also, excessive energy intake and fatty acid oxidation increase ROS production in mitochondria. This creates oxidative stress in cells [15]. Johnson et al. [8]. reported that oxidative damage in white adipose tissue was triggered by the CAF diet. In this study, the significant decrease in TAS levels in BAT also indicates that antioxidant defense is suppressed and oxidative stress is increased. The upward trend in TOS values supports this finding (Table 2). Increased oxidative stress plays a critical role in the development of obesity-related pathologies such as mitochondrial dysfunction, inflammation, and insulin resistance [16]. Brown adipose tissue is a thermogenic tissue that primarily dissipates energy as heat via UCP1. Although BAT constitutes only 1-2% of body fat, it is important for regulating energy homeostasis and preventing obesity [17].

In this study, it was shown that the significant decrease in UCP1 levels in BAT indicates that energy expenditure is suppressed and thermogenic capacity is weakened (Figure 1). This situation can be considered a fundamental mechanism of energy imbalance in the development of obesity. So, recent years have seen numerous studies conducted on various model organisms, highlighting the importance of BAT's anti-obesity function by increasing energy expenditure [18]. As a product of the post-translational processing of FNDC5, irisin is a newly identified myokine that is upregulated during exercise. This hormone not only promotes the conversion of white adipose tissue to browning but also increases energy expenditure [19]. In the present study, like the decrease in UCP1, a decrease in irisin levels was also demonstrated in the CAF group (Figure1). The significant decrease in irisin and UCP1 levels in the CAF group indicates that the thermogenic response associated with obesity is suppressed [20]. In this context, the decrease in irisin/UCP1 in our study reflects the reduction in energy expenditure and suppression of lipolytic activity associated with obesity. Zhang et al. 12] demonstrated that irisin release from skeletal muscle increases UCP1 expression in adipocytes and promotes lipolysis. This data reveal that the irisin/UCP1 axis plays a critical role in the pathophysiology of obesity and may be considered a therapeutic target.

4.1. Conclusion

The present study demonstrated that the irisin/UCP1 axis is suppressed, antioxidant defense is weakened, and oxidative stress is increased in an obesity model induced by a cafeteria diet. These findings provide significant molecular insights into the mechanisms underlying obesity-related metabolic dysfunctions. Specifically, the decrease in irisin and UCP1 levels indicates suppressed energy expenditure and thermogenic response; the decrease in TAS levels indicates inadequate antioxidant defense at the cellular level. These parameters can be regarded as biomarkers of obesity-related metabolic disorders. It is recommended that future studies investigate the potential effects of pharmacologically activating the irisin/UCP1 axis in the treatment of obesity. In addition, antioxidant strategies aimed at reducing oxidative stress should be evaluated. Interventions targeting this axis may offer a new roadmap for preventing obesity-related complications.

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