

Effects of Irisin on the Reproductive System of Obese Female Rats Induced by a High-fat Diet

Key words

irisin;
obesity;
hormones;
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Abstract: Obesity is becoming more common all across the world, causing a variety of health problems, including reproductive disruption. Although the novel, exercise-induced hormone irisin may affect the hypothalamus-pituitary-gonadal axis and reproductive function control, its impact on obesity-induced damage to the female reproductive system is not fully known. Hence, this study aimed to investigate the potential effects of irisin on reproductive hormones and reproductive organs in female rats with obesity induced by a high-fat diet. Forty female rats were divided into four groups: control, irisin, obese, and obese+irisin (n = 10 in each group). After simulating a high-fat diet-induced obesity model (via 60% kcal fat for 12 weeks) in the obese and obese+irisin groups, irisin (100 ng/kg/day via mini-osmotic pumps for about 28 days) was administered subcutaneously to the irisin and obese+irisin groups. Results showed that subcutaneous irisin perfusion increased serum luteinizing hormone (LH), the LH to follicle-stimulating hormone (FSH) ratio (LH/FSH), and progesterone levels while decreasing the histopathological damage in the ovaries of obese rats. On the other hand, endogenous irisin serum concentrations were similar in lean female rats and obese female rats with reproductive disorders. These results suggest that irisin may affect the reproductive axis in obese female rats. An increase in serum LH levels, which trigger ovarian steroidogenesis, and reducing histopathological changes in ovarian tissue could contribute to this effect.

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Introduction

The prevalence of obesity has rapidly increased worldwide, and obesity causes several health problems, including reproduction. Obesity directly or indirectly negatively affects the hypothalamus-pituitary-ovarian (HPO) axis, resulting in various reproductive disorders by causing hormone imbalances and ovulatory dysfunction. Additionally, it is also known that obesity may have a direct effect on ovarian function, independent of the HPO axis (1, 2). Studies in humans and rodents have shown that obesity-related ovarian dysfunction includes abnormalities in folliculogenesis and ovulation, irregular estrous cyclicity, and depletion of ovarian reserve (3).

Lifestyle modifications, including a healthy diet and exercise, are successful options for treating women with reproductive dysfunctions. Specifically, exercise has a protective effect against obesity-induced impairment in the female reproductive system. Exercise, for example, has been shown to improve menstrual cyclicity, ovulation, and pregnancy rates in obese anovulatory women (4, 5). Similarly, in obese polycystic ovary syndrome (PCOS) rats fed a high-fat diet, swimming training also appeared to recover ovarian morphology indexes such as the numbers of antral follicles and corpora lutea (6).

Irisin was recently discovered as a new hormone-like myokine released into the circulation in response to physical exercise. It is produced by cleavage of its precursor, fibronectin type III domain containing 5 (FNDC5). Following secretion, irisin promotes the browning of white adipocytes and the expression of uncoupling protein 1 (UCP1), leading to enhanced UCP-1-mediated thermogenesis and increased energy expenditure (7, 8). Irisin has a potential role in mammalian growth and regulation of the reproductive axis (9-11). It is documented that irisin deficiency is associated with disordered endocrine metabolism, poor growth, and decreased fertility in female mice (9). Interestingly, it is known that irisin can cause different effects on reproductive function depending on gender in the rat model, such as exhibiting androgenic activity in males and causing reproductive disorders in females (12). Furthermore, recent studies have shown that irisin improves antidepressant-induced sexual dysfunction and obesity-related reproductive disorders. Accordingly, it has been revealed that irisin may mediate the effects of exercise on the reproductive system and that irisin and exercise may also have similar effects on reproductive potential (13-16). However, there are not enough studies on the possible potent effects of the irisin hormone on reproductive system disorders caused by obesity in female rats.

This study was performed to investigate the effects of exogenous irisin on serum levels of reproductive hormones and insulin and on the reproductive organs in obese female rats. Furthermore, endogenous irisin levels were measured in obese female rats to determine whether irisin levels are associated with obesity-induced changes in sex hormones and reproductive organs.

Material and Methods

Animals and diets

All animal experiments were carried out in accordance with the governmental guidelines for the care and use of laboratory animals at Firat University and approved by the Animal Experimental Ethics Committee of Firat University (31.01.2018, number 19). Sprague-Dawley female rats (2-3 months old, 200-250 g) were obtained from the Firat University Experimental Research Unit (Elazig, Turkey). Animals were housed 3-4 per cage and kept in a 12 h light/12 h dark cycle (light on 07:00-19:00), under standard conditions (21 ± 1 °C temperature and 50-60% humidity), with *ad libitum* access to water and food. Forty rats were randomly allocated to four treatment groups as follows: control (control group with rats subjected to vehicle treatment), irisin (irisin-treated rats), obese (obesity-induced rats), and obese+irisin (irisin-treated obesity-induced rats) (n = 10 rats per group). All rats in the control and irisin groups were fed with standard commercial rat food (Korkuteli Yem Gıda Santic A.Ş., Antalya, Turkey), while all rats in the obese and obese+irisin groups were fed a high-fat diet (D12492,

Research Diets, 60% kcal fat) for 16 weeks. After 12 weeks of high-fat diet exposure, the induction of obesity in both obesity-induced treatment groups was confirmed by measuring the Lee index, which is used for experimental validation of obesity as described previously (16-18).

Continuous administration of irisin

After 12 weeks of diet exposure (i.e., after the establishment of obesity due to the high-fat diet), all rats in the control, irisin, and obese+irisin groups were anesthetized with a mixture of ketamine (6 mg/kg) and xylazine (5 mg/kg) anesthetics, then a mini-osmotic pump (Alzet, Model 2004; Durect Corp., Cupertino, CA) was subcutaneously implanted between the scapulae of each animal under sterile conditions. Alzet mini-osmotic pumps were filled with either deionized water (vehicle) for the control group or irisin (SRP8039, Sigma-Aldrich; dissolved in deionized water to deliver at a dose of 100 ng/kg/day) for both irisin and obese+irisin groups as previously described (18). The Alzet model 2004, with a reservoir volume of 200 µL, infused deionized water or irisin at a flow rate of 0.25 µL/h for about 28 days.

Sample collections

The serum hormone levels of female rats fluctuate greatly depending on the estrous cycle, and therefore, all rats were in the same estrous cycle phase (diestrus) to determine the comparability of hormone levels at the end of the experiment. On days 25-28 of deionized water or irisin perfusion, all rats in the diestrus phase as determined by vaginal smears were sacrificed by decapitation at the light phase between 17:00 h and 19:00 h. After decapitation, trunk blood was immediately collected for a serum-based enzyme-linked immunosorbent assay (ELISA) and centrifuged (4500 rpm; 4 °C; 5 min) to obtain serum samples, which were stored at -20 °C. In addition, the uterine horns and ovaries were immediately excised and cleaned of fat, and their wet weights were measured and expressed as mg/100 g body weight (BW). The final body weight of each animal was measured just before decapitation. The mid-portion of the uterine horns and all of the ovaries were fixed in a 10% formaldehyde solution for histopathological investigations. The serum and reproductive organs used in this study belonged to the animals used in the previous study (18).

Hormone measurements

Commercial rat ELISA kits were purchased from Elabscience Biotechnology Inc. (Texas, USA) and Enzo Life Sciences (Switzerland). They were used to assess in duplicate the follicle-stimulating hormone (FSH; E-EL-R0391), luteinizing hormone (LH; ENZ-KIT107), 17β-estradiol (E2; ADI-900-008), progesterone (ADI-900-011), testosterone (ADI-900-065), insulin (E-EL-R3034), and irisin (FNDC5, E-EL-R1104) serum levels according to the manufacturer's instructions

by using an ELISA microplate reader (Multiskan FC, Thermo Scientific, USA). Serum FSH, LH, E2, progesterone, testosterone, and insulin levels were quantified in all groups. On the other hand, serum irisin levels were measured only in the control and obese groups to determine whether obesity-induced damage to the female reproductive system is related to irisin levels.

Histopathological examinations

For light microscopic evaluation, formalin-fixed uterus and ovary tissues were dehydrated through an increasing alcohol series (70%, 80%, 96%, and 100%) and then embedded in paraffin wax. Afterward, sections with a thickness of 5 μ m were cut from uterine and ovarian tissues and stained with hematoxylin and eosin (H&E) and/or Masson trichrome staining. All of the uterine and ovarian tissue sections were evaluated and photographed by a blinded examiner using a Leica DM500 light microscope (DFC295; Leica, Wetzlar, Germany). At 20x magnification, twenty random fields from a section of each ovary and uterus were examined and/or scored.

The follicles (primordial, primary, secondary, and Graaf follicles) and corpus luteum in twenty random fields of each ovarian section were counted. Ovarian follicles were classified according to the morphologic criteria as described by Artaş et al. (2018) (19). Histopathological examination of the uterine and ovarian tissue damage was carried out by a thorough qualitative histologic examination, and fibrosis pathology was semi-quantitatively scored (0, no fibrosis; 1, low fibrosis; 2, intermediate fibrosis; 3, severe fibrosis) (12, 20).

TUNEL Assay

The apoptosis in ovarian tissues was evaluated by the terminal deoxynucleotidyl transferase-mediated deoxyuridine-biotin nick end labeling (TUNEL) method. For the detection of apoptotic cells, the ApopTagPlus Peroxidase in Situ Apoptosis Detection Kit (Chemicon, Lot: 3006560, USA) was used according to the manufacturer's instructions. In the evaluation of the TUNEL assay, the nuclei of healthy cells were blue, and the cells with stained brown nuclei were considered apoptotic cells. A total of 200 cells were counted in randomly selected areas under light microscopy at 20x magnification. Accordingly, the apoptotic index (%) was calculated as the ratio of the number of TUNEL-positive cells to the total number of cells (21).

Statistical analysis

All data are presented as mean $\pm$ standard error of the mean (SEM) and were analyzed using the SPSS 22.0 software. Before analysis, the normality of all data was verified using the Shapiro-Wilk test. For multiple comparisons between groups, a one-way analysis of variance (ANOVA) followed by Tukey's *post-hoc* test was utilized. Comparisons

between the control and obese groups were evaluated by an unpaired student t-test (irisin levels). $p < 0.05$ was considered statistically significant.

Results

Serum sex hormone levels

Serum FSH, LH, LH/FSH ratio, E2, progesterone, and testosterone levels in all experimental groups are shown in Fig. 1. When compared to the control group, serum FSH levels decreased significantly in the irisin group ($p < 0.05$) although they remained the same in both the obese and obese+irisin groups (Fig. 1A). Serum LH levels were found to increase significantly in the obese and obese+irisin groups due to the high-fat diet ($p < 0.001$), but no obvious changes were observed in the irisin group. Additionally, compared to the obese group, serum LH levels in the obese+irisin group significantly increased depending on subcutaneous administration of irisin ($p < 0.001$) (Fig. 1B). The LH/FSH ratio was significantly increased depending on the decreased serum FSH levels and unchanged serum LH levels in the irisin group ($p < 0.01$, Fig. 1C). As a result of increased serum LH concentration due to the high-fat diet, it was determined that LH/FSH ratios increased in both obese and obese+irisin groups ($p < 0.05$ and $p < 0.001$, respectively, Fig. 1C). Moreover, compared with the obese group, the LH/FSH ratio of rats in the obese+irisin group was significantly increased depending on irisin exposure ($p < 0.001$, Fig. 1C).

As seen in Fig. 1D, serum E2 levels were reduced in obese rats when compared to control rats ($p < 0.05$). Serum progesterone levels were significantly increased due to subcutaneous irisin perfusion in the irisin and obese+irisin groups as compared to the control group ($p < 0.01$). This increase in the obese+irisin group was also found to be significant compared to the obese group ($p < 0.01$) (Fig. 1E). In comparison with the control group, serum testosterone levels were substantially increased in the obese+irisin group ($p < 0.01$). On the other hand, it was determined that serum testosterone levels were not affected by subcutaneous irisin perfusion or high-fat diet exposure alone (Fig. 1F).

Serum insulin and irisin levels

Continuous administration of irisin, high-fat diet exposure, or both continuous administration of irisin and high-fat diet exposure in female rats did not cause a change in serum insulin levels compared with female rats treated with a vehicle (Fig. 2A). As shown in Fig. 2B, there was no significant difference in the serum irisin levels in female rats exposed to a high-fat diet in comparison to the vehicle-treated female rats.

Reproductive organ weights

Irisin perfusion had no significant effect on body weight in both lean and obese rats, as determined in our previous study (18). As shown in Table 1, there was a significant reduction in the uterine wet weights normalized for body weights in both irisin and obese+irisin groups compared to

the control group ($p < 0.05$ and $p < 0.001$, respectively). When compared to the control group, high-fat diet exposure significantly decreased reproductive organ weights in female rats ($p < 0.05$ for ovarian wet weights normalized to body weights and $p < 0.01$ for uterine wet weights normalized to body weights). Also, it was determined that subcutaneous irisin perfusion had a statistically insignificant effect on the

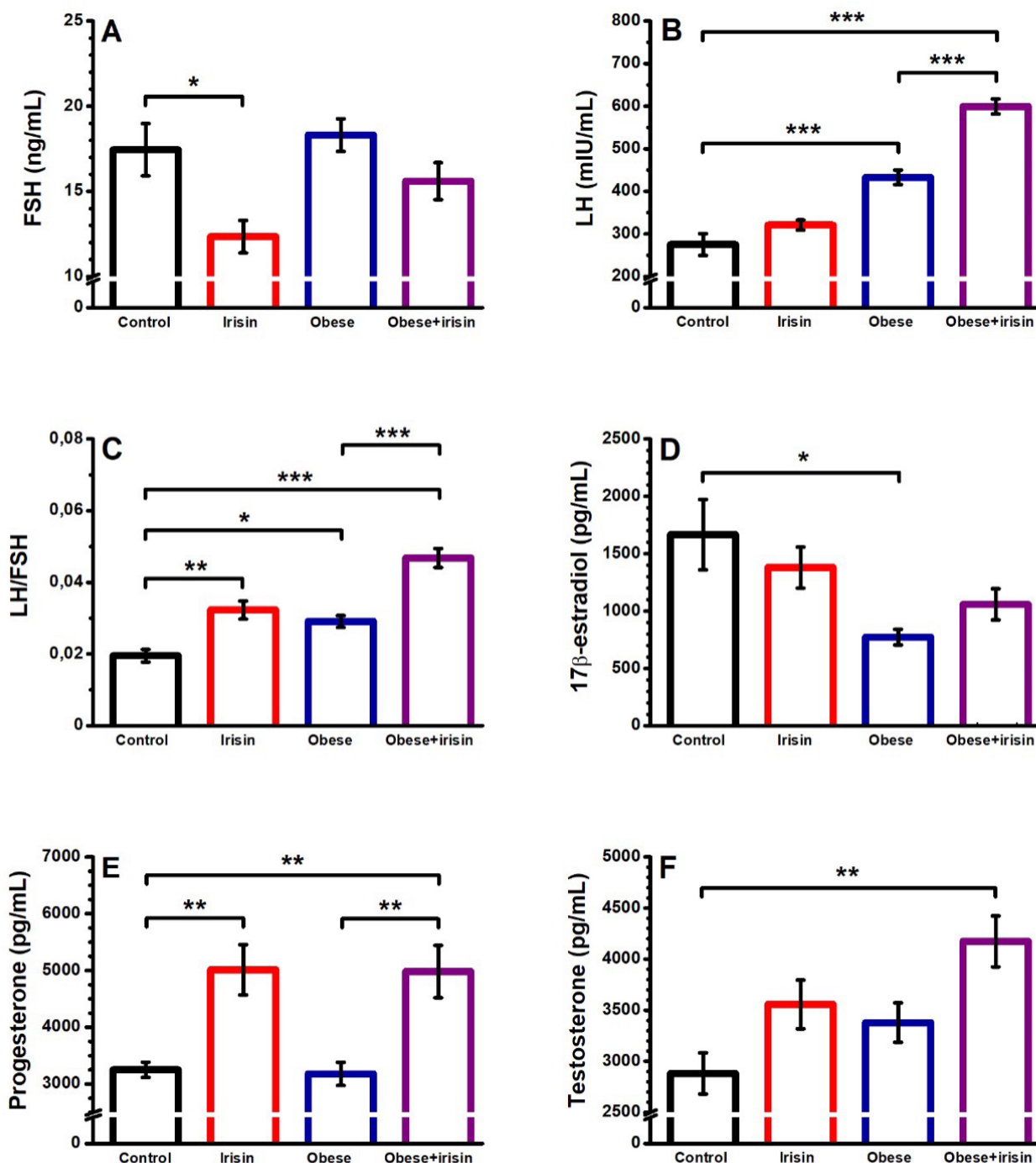


Figure 1: Effects of subcutaneous irisin perfusion and/or high-fat diet-induced obesity on serum reproductive hormones in female rats. A) Serum FSH levels. B) Serum LH levels. C) LH/FSH ratio. D) Serum 17β-estradiol levels. E) Serum progesterone levels. F) Serum testosterone levels. Data were expressed as mean ± SEM. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ (one-way ANOVA followed by the Tukey's *post-hoc* test, $n = 10$ in each group). FSH: follicle-stimulating hormone, and LH: luteinizing hormone

decreased reproductive organ weights of high-fat diet-induced obese rats in the obese+irisin group (Table 1).

Uterine histopathology

As shown in Fig. 3, the uterine epithelium, uterine glands in the endometrium, and endometrial connective tissue fibers showed normal histological structure in the control, irisin, and obese+irisin groups. Unlike other groups, epithelial

degeneration was determined to be more common in the uterine sections of high-fat diet-induced obese rats (Fig. 3).

Ovarian histopathology

Histological features of ovarian sections are shown in Fig. 4. Tissue sections from the ovary of vehicle-treated rats show common ovary histology with germinal epithelium consisting of single-layered cubic cells, numerous corpus luteum,

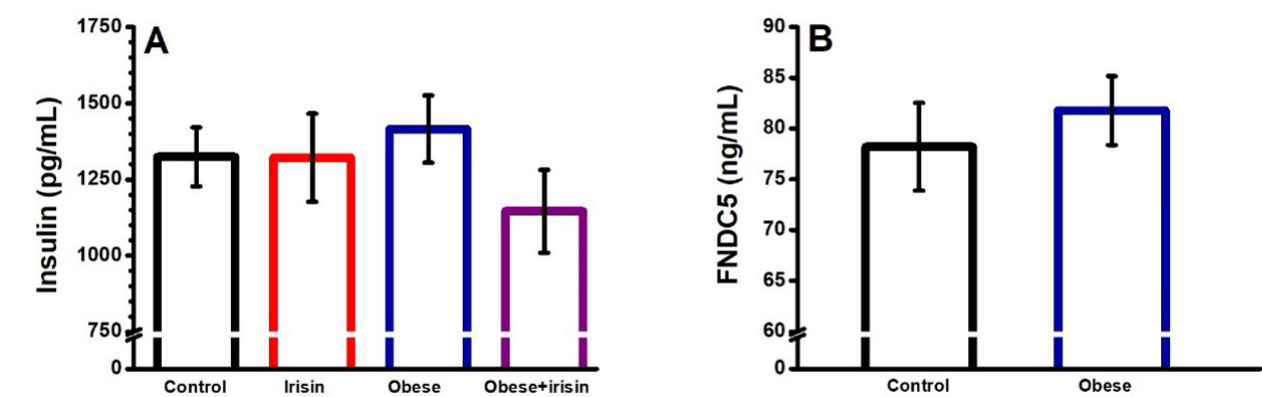


Figure 2: Effects of subcutaneous irisin perfusion and/or high-fat diet-induced obesity on A) serum insulin levels and B) serum FNDC5 levels in female rats. Data were expressed as mean ± SEM (one-way ANOVA followed by Tukey's *post-hoc* test or Student's *t*-test, n = 10 in each group)

Table 1: Effects of subcutaneous irisin perfusion and/or high-fat diet-induced obesity on reproductive organ weights in female rats. Data were expressed as mean ± SEM. *p<0.05, **p<0.01, and ***p<0.001 compared with the control group (one-way ANOVA followed by the Tukey's *post-hoc* test, n = 10 in each group). BW: body weight

Groups	Normalized Ovarian Weight (mg/100 g BW)	Normalized Uterine Weight (mg/100 g BW)
Control	46.01 ± 2.39	165.21 ± 8.61
Irisin	48.47 ± 1.87	132.18 ± 8.38*
Obese	36.25 ± 1.94*	118.89 ± 8.75**
Obese+irisin	41.18 ± 2.09	113.22 ± 5.96***

Table 2: Effects of subcutaneous irisin perfusion and/or high-fat diet-induced obesity on the number of ovarian follicles and the histopathological score of fibrosis in female rats. Data were expressed as mean ± SEM. *p<0.05 compared with the control group (one-way ANOVA followed by the Tukey's *post-hoc* test, n = 6 in each group)

Variable	Control	Irisin	Obese	Obese+irisin
Primordial follicle	9 ± 1.22	8.6 ± 0.74	6.2 ± 1.06	8 ± 0.44
Primary follicle	10 ± 1	7.8 ± 1.77	6.6 ± 1.6	9.6 ± 0.97
Secondary follicle	9.6 ± 1.43	10.2 ± 1.15	8.6 ± 0.5	8.6 ± 0.67
Tertiary follicle	1.6 ± 0.4	1.6 ± 0.4	0.6 ± 0.24	1.6 ± 0.24
Corpus luteum	10.8 ± 1.31	12.2 ± 1.31	8.6 ± 1.32	9.4 ± 0.5
Fibrosis	0.33 ± 0.21	0.66 ± 0.33	1.83 ± 0.40*	1 ± 0.25

and ovarian follicles (primordial, primary, secondary, and tertiary follicles) during different stages of development in the cortex. Similar to the control group, many corpus luteum and ovarian follicles at different developmental stages were detected in the irisin, obese, and obese+irisin groups (Fig. 4 and Table 2). However, unlike the control group, extreme vascular dilatation and congestion in the ovarian cortical stroma and ovarian germinal epithelium degeneration were

observed in the high-fat diet-induced obese rats (Fig. 4). Along with all these histopathological changes in the obese group, a significant increase in fibrosis was also found in the obese group compared to the control group ($p < 0.05$, Table 2). On the other hand, when compared to the obese group, a decrease was observed in vascular congestion and germinal epithelial degeneration in the obese+irisin group depending on irisin exposure (Fig. 4).

Apoptosis in the ovary

Fig. 5A shows that TUNEL-positive granulosa cells were observed in the secondary and graaf follicles, while apoptosis was not detected in the primordial and primary follicles in all experimental groups. Accordingly, we found that the apoptotic index (%) was significantly higher in the irisin, obese, and obese+irisin groups compared with the control group (control group: $1.71 \pm 0.28\%$, irisin group: $5.42 \pm 0.57\%$, obese group: $5.42 \pm 0.61\%$, and obese+irisin group: $5.14 \pm 0.59\%$; $p < 0.05$ for irisin, obese, and obese+irisin groups, Fig. 5B).

Discussion

In this study, it was revealed for the first time that irisin hormone affects reproductive hormones and ovarian histopathology in high-fat diet-induced obese female rats. Accordingly, it was observed that irisin exposure increased serum LH, LH/FSH ratio, and progesterone levels in obese female rats, and the histopathological changes in the ovarian tissues of obese rats decreased with irisin exposure (i.e., vascular congestion and germinal epithelial degeneration).

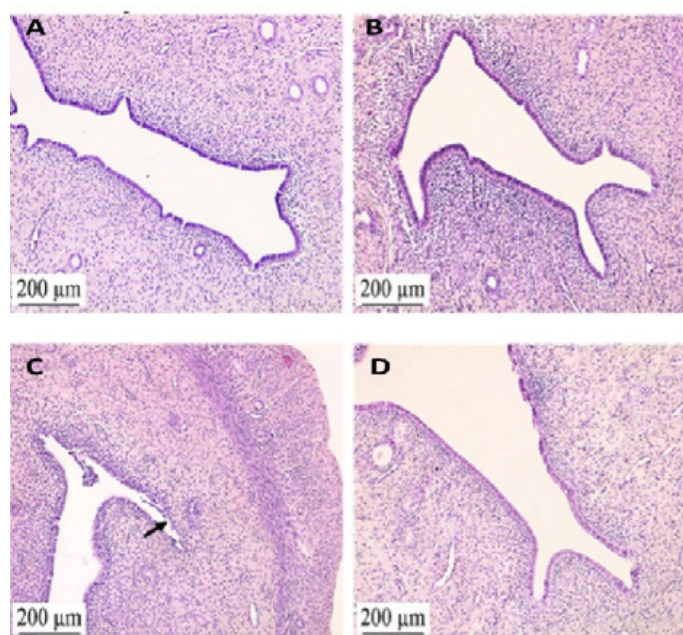


Figure 3: Represents the effects of subcutaneous irisin perfusion and/or high-fat diet-induced obesity on uterine tissue. A) Control group. B) Irisin group. C) Obese group: epithelial degeneration (arrow). D) Obese+irisin group. Stained with hematoxylin and eosin (H&E), Bar: 200 µm

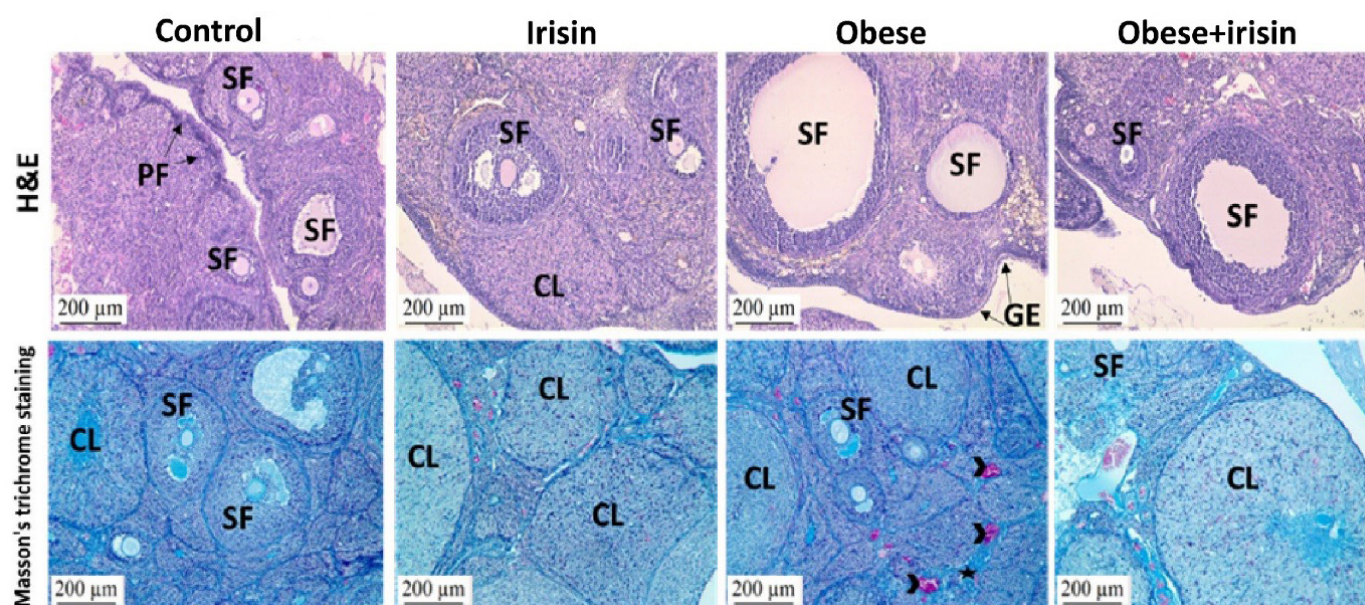


Figure 4: Represents the effects of subcutaneous irisin perfusion and/or high-fat diet-induced obesity on ovary histology. Obese group: vascular dilatation and congestion (arrowhead), fibrosis (star), and germinal epithelial degeneration (arrow). Stained with hematoxylin and eosin (H&E) and Masson trichrome staining, Bar: 200 µm. CL: corpus luteum; GE: germinal epithelium; PF: primordial follicle (in the control group, arrow); SF: secondary follicle

In addition, it was determined that endogenous irisin serum concentration did not change in the obese female rats, and exogenous irisin administration induced apoptosis in the lean rat ovary.

It has been shown in the literature that irisin/FNDC5 is most likely involved in the reproductive system. In female mice, it has been determined that there are various disturbances in the components of the female reproductive system due to the deletion of FNDC5. In some studies conducted in rat models, it was suggested that irisin had a beneficial effect on uterine receptivity or caused disorders in the reproductive system (9, 12, 22). Recent studies have shown that irisin has a potentially positive role against obesity-induced reproductive dysfunctions in male rats (15, 16).

The 'obesity epidemic' in many countries is a serious threat to public health, and reproduction is one of the major health hazards induced by obesity (23). It has been reported that obese women may have three times more reproductive disorders due to disruptions in the HPO axis, resulting in anovulatory cycles, irregular menstruation, and infertility, which are associated with PCOS (24, 25). In previous studies, it has been observed that anterior pituitary gonadotropins (FSH and LH) levels, which are critical regulators of ovarian function and female fertility, can increase in obese female rats. For example, Akamine et al. (2010) found that serum FSH levels did not change but serum LH levels were increased in obese female rats subjected to 120 days of high-fat diet treatment (26). Similar results on the secretion of gonadotropins were also found in female rats exposed to a 16-week high-fat diet in our study. Taken together, our data strongly suggest that obesity increases serum LH

levels in female rats and thus causes an adverse effect on the gonadal axis of female rats.

Irisin either stimulates the expression of FSH and LH or inhibits the secretion of FSH or LH by competing with the gonadotropin-releasing hormone. These dual effects of irisin occur simultaneously and interact with each other, resulting in variations in circulating hormone levels when one activity is dominant (10, 12, 27). In the current study, it was determined that irisin decreased FSH levels in lean female rats by acting at the central inhibitory effect. In our previous study using female rats, it was determined that irisin administration changed serum levels of gonadotropins, resulting in reduced serum FSH levels and elevated serum LH levels (12). Similarly, in the present study, irisin was found to decrease serum FSH levels. However, the unchanged LH levels in the present study are thought to may be related to the application time and method of the irisin (i.e., 4 weeks versus 10 weeks and subcutaneous versus intraperitoneal administration).

On the other hand, when the effects of irisin on the secretion of gonadotropins, which changed due to obesity, were examined, it was shown that FSH levels did not change but LH levels and the LH/FSH ratio increased by irisin exposure in high-fat diet-induced obese female rats in this study. These results are supported by a study of the exogenous administration of irisin in obese female mice induced by a high-fat diet (13). Another possible condition in which LH levels and the LH/FSH ratio can increase in obese women is known to be PCOS (28, 29). Because of this, depending on the results of this study, it is speculated that irisin may have a triggering effect on the formation of PCOS in obesity.

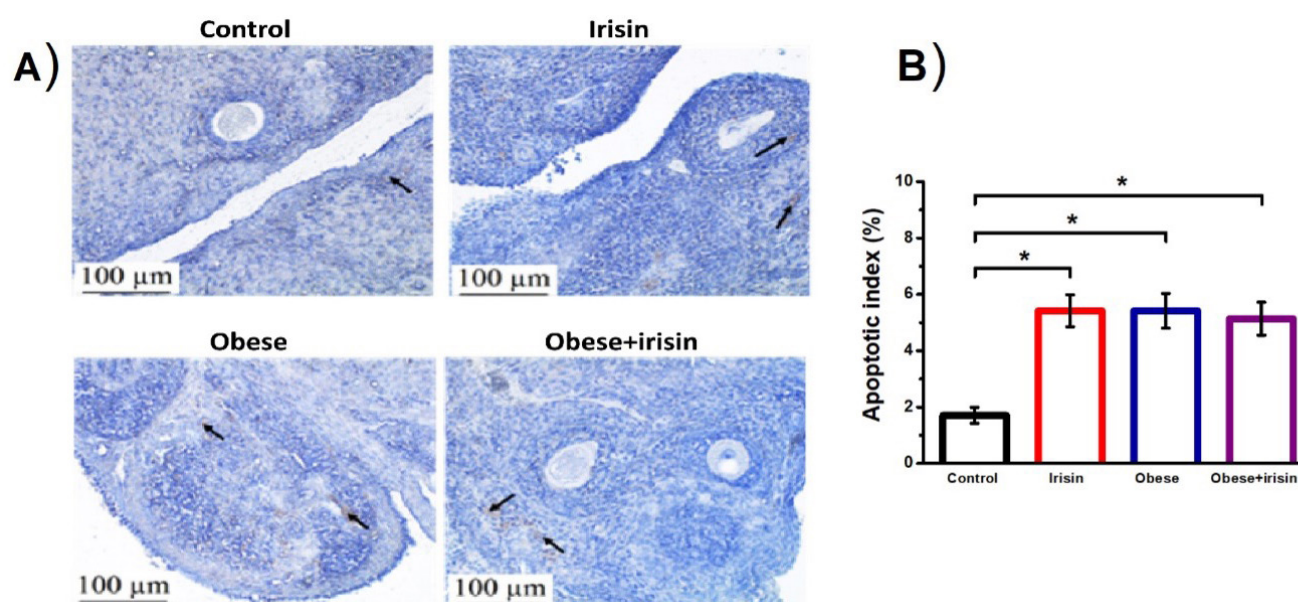


Figure 5: Effects of subcutaneous irisin perfusion and/or high-fat diet-induced obesity on the apoptosis of ovarian granulosa cells in rats. A) Representative images of TUNEL staining show apoptotic granulosa cells in the control, irisin, obese, and obese+irisin groups (arrow), Bar: 100 µm. B) The apoptotic index of each group. Data were expressed as mean $\pm$ SEM. * $p < 0.05$, compared with the control group (one-way ANOVA followed by the Tukey's *post-hoc* test, $n = 6$ in each group)

Serum leptin levels are high in obesity, and an increase in leptin levels impairs ovulation and causes infertility. Moreover, it has been reported that serum leptin levels are negatively correlated with serum estradiol levels in obese female rats, and increased serum leptin levels may reduce estradiol synthesis by direct action on the ovary (30). Our previous study demonstrated that serum leptin levels were increased in obese female rats, and irisin administration decreased the elevated serum leptin levels in these rats (18). Therefore, in the present study, although serum leptin levels were not measured, it is speculated that the possible obesity-related increase in serum leptin levels may affect the ovary in obese rats and cause a decrease in E2 synthesis. A previous study reported that serum estradiol concentration decreased and serum progesterone concentration did not change in cafeteria diet-induced obese female rats (24). Consistently, similar results were also found in obese female rats induced by a high-fat diet in the present study.

On the other hand, it was determined that subcutaneous irisin perfusion increased serum progesterone levels in both lean and obese rats. In addition to the increase in progesterone levels, it was determined that there was also an increase in serum testosterone levels in the obese+irisin group. It is already known that LH is the major factor in ovarian steroidogenesis and triggers steroid production, including estradiol, progesterone, and testosterone (31). Therefore, considering the increasing effect of irisin on the LH levels in obese female rats as stated above, our data suggest that irisin-induced LH increment, independent of insulin, may increase steroidogenic capacity in the obese+irisin group.

In our study, serum insulin levels were unchanged in female rats fed a high-fat diet for 16 weeks compared to vehicle-treated female rats. Parallel to our study, Lu et al. (2016) reported that serum insulin levels did not change in Wistar male rats fed a high-fat diet for 24 weeks (32). Insulin plays an important role in the increase in ovarian steroidogenesis and the process of follicular development (26, 33). Especially, it has been revealed that there is an increase in theca-cell steroidogenesis as a result of the synergistic interaction of LH and insulin (34). In the present study, it was assumed that the changes in ovarian steroidogenesis in all experimental groups (i.e., increased progesterone levels in the irisin group, decreased E2 levels in the obese group, and increased progesterone and testosterone levels in the obese+irisin group) may have occurred independently of insulin. Furthermore, it is the first time, to our knowledge, that obesity-related reproductive dysfunction in a female rat model was found to be independent of serum irisin levels, considering the unchanged serum irisin levels in obesity in the current study.

Different exercise intensities, known to elicit an irisin response, induce different effects on the reproductive system in females. For example, it was observed that there is no change in serum FSH, LH, or estradiol levels as a result of short-time exercise or aerobic exercise in young

women (35, 36), while there is a decline in the plasma levels of FSH, LH, estradiol, and progesterone in women who engage in regular high intensity exercise (37). Depending on the results of the present study and our previous study (12), it was determined that irisin administration at different durations and ways has different effects on reproductive hormones as well as ovarian histology and reproductive organ weights in female rats. In light of the above-mentioned information, our data from our studies suggested that the irisin hormone released into circulation due to exercise may play a pivotal role in the relationship between exercise variables and the reproductive system.

Gaspar et al. (2016) and Benevides et al. (2019) reported that both the weights of the uterus and ovarian tissues were reduced due to obesity in rats (38, 39). Hence, the marked reduction in reproductive organ weights seen in high-fat diet-induced female rats in the present study is consistent with previous literature and provides evidence of the direct adverse effects of obesity on reproductive organs. Besides, for the first time in the current study, it was revealed that irisin did not affect the weight of reproductive organs in obese female rats.

In rodent models, it is also known that obesity causes different histopathological changes in ovarian tissue as well as negative effects on the weight of reproductive organs. Akamine et al. (2010) showed that rats fed a high-fat diet for 180 days had significantly abnormal ovarian morphology (26). In addition, Atteia et al. (2020) revealed that there are several histopathological features such as stromal edema, congestion, and a high degree of fibrosis in the ovarian cortices of the obese group (40). Our findings agree with the aforementioned studies: obese rats showed histopathological changes in the ovaries like extreme vascular dilatation and congestion in the ovarian cortical stroma, ovarian germinal epithelium degeneration, and fibrosis. With regards to the ovarian follicle reserve, obesity did not affect ovarian follicle development in the current study. Benevides et al. (2019) and Hussain et al. (2016) found similar results: obesity did not promote a significant change in the count of ovarian follicles or corpora lutea (39, 41). In particular, similar follicular development in all experimental groups is an expected result due to the unchanged serum levels of insulin involved in follicular development in this study.

It is noteworthy that irisin exposure reduced the changes in ovarian pathology (i.e., vascular congestion and germinal epithelial degeneration) in obese rats, while it did not cause any effect on the ovarian tissues of lean rats in the present study. Accordingly, in this study, it could be speculated that irisin exhibited a curative effect on obesity-induced pathological changes in the ovarian tissues. Taken together, our data suggested that irisin can act directly on the ovaries in obese rats, regardless of the HPO axis.

In the present study, the effects of irisin on ovarian function in rats fed a high-fat diet were also assessed by apoptosis

in addition to hormone assays and histopathological examinations. Our data showed that apoptosis in the ovary was increased, which contributes to ovarian function failure, by irisin exposure alone or obesity alone in rats, but the administration of irisin did not affect granulosa cell apoptosis in obese rats. This effect of obesity on apoptosis of ovarian follicles shown in this study is supported by recent studies demonstrating that diet-induced obesity is known to increase apoptotic ovarian follicles in rodents (13, 24, 42). Irisin has also been demonstrated to increase apoptosis in ovarian cancer cells and breast cancer cells (43, 44). Similarly, to our knowledge, our findings have shown for the first time that irisin induces apoptosis in the ovarian tissue of lean rats. In conclusion, we have demonstrated that reproductive impairments in irisin-exposure rats in the present study may be associated with decreased serum FSH levels, increased serum progesterone levels, decreased uterine weights, and increased granulosa cell apoptosis.

In conclusion, our study revealed that irisin increased serum LH levels in high-fat diet-induced obese female rats, and irisin-induced LH increment may cause an increase in ovarian steroidogenesis in these obese rats. In addition, it was shown that irisin exposure could alleviate ovarian tissue damage in high-fat diet-induced obese rats. Based on these results, we suggest that the irisin hormone may modulate the HPO axis of obese rats at both central neuroendocrine and gonadal levels.

Conclusions

It was determined that λ -cyhalothrin has a toxic effect on *D. polymorpha* and this toxic effect increases depending on the temperature. In our study, it was concluded that the *D. polymorpha* model organism and some of its biochemical parameters (AChE, CAT, SOD, GSH and MDA) are suitable biomarkers for revealing the effect of temperature variable on toxic response. It has also been shown that biochemical parameters such as SOD, CAT, AChE activities and GSH, TBARS levels used are suitable biomarkers for the evaluation of the toxic effects of λ -cyhalothrin.

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interpreted the data. The final version of the manuscript was read and approved by all authors.

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Učinki irisina na reproduktivni sistem debelih samic podgan, povzročeni s prehrano z visoko vsebnostjo maščob

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Izvleček: Debelost je vse pogostejša po vsem svetu in povzroča različne zdravstvene težave, vključno z motnjami reprodukcije. Čeprav hormon irisin, ki se izloča med vadbo, lahko vpliva na hipotalamično-hipofizno-gonadno os in reproduktivno funkcijo, njegov vpliv na z debelostjo povezane poškodbe ženskega reproduktivnega sistema ni povsem znan. Zato je bil namen te študije raziskati morebitne učinke irisina na reproduktivne hormone in reproduktivne organe pri samicah podgan z debelostjo, povzročeno s prehrano z visoko vsebnostjo maščob. Štirideset samic podgan smo razdelili v štiri skupine: kontrola, irisin, debelost, debelost+irisin (n=10 v vsaki skupini). Po 12 tednih simulacije modela debelosti, povzročene s prehrano z visoko vsebnostjo maščob (60 % kcal maščobe), smo v skupinah debelost in debelost+irisin podganam podkožno dajali irisin (100 ng/kg/dan prek mini-osmotskih črpalk približno 28 dni). Podkožna aplikacija irisina je povečala serumski luteinizirajoči hormon (LH), razmerje med LH in folikle stimulirajočim hormonom (FSH) (LH/FSH) in raven progesterona, hkrati pa zmanjšala histopatološke poškodbe v jajčnikih debelih podgan. Vendar pa so bile koncentracije endogenega irisina v serumu vitkih in debelih podgan z reproduktivnimi motnjami podobne. Rezultati kažejo, da bi irisin lahko vplival na reproduktivno os debelih podgan. K temu učinku bi lahko prispevala povečanje serumske koncentracije LH, kar sproža steroidogenezo jajčnikov, ter zmanjšanje histopatoloških sprememb tkiva jajčnikov.

Ključne besede: : irisin; debelost; hormoni; jajčnik; apoptoza; reprodukcija pri samicah