Irisin administration modifies ovary mast cell numbers, ovarian angiogenesis, and oxytocin levels in obese rats

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ABSTRACT

Exercise hormone irisin, a thermogenic adipo-myokine, promotes energy expenditure by white adipose tissue browning. Considering that irisin improves the white adipose tissue metabolic profile and increases whole-body energy expenditure, it is thought that irisin may be a potential new therapeutic target for the treatment of the growing epidemic of obesity. The roles of mast cells and angiogenesis in the pathogenesis of obesity are known, and the importance of loss of mast cell function and antiangiogenic agents in the treatment of obesity has gained prominence in recent years. In addition, the therapeutic efficacy of oxytocin for obesity, by inducing browning and stimulating thermogenesis, is also noteworthy. To understand better the mechanisms involved in the therapeutic effect of irisin on obesity, the present study evaluated the effects of irisin treatment on high-fat diet-induced, obese female rats, focusing on the number of ovary mast cells, and ovarian angiogenesis and serum oxytocin levels. Our findings showed that ovary mast cell numbers, corpus luteum angiogenesis, and vascular endothelial growth factor (VEGF) immunoreactivity in ovarian tissues increased with obesity and then significantly decreased with irisin administration. Also, it was determined that the increased serum oxytocin levels with obesity, increased markedly depending on irisin administration in obese female rats. Taken together, our findings revealed that irisin can well be considered as a potential therapeutic agent in the treatment of obesity by reducing mast cells and angiogenesis, and promoting oxytocin secretion.

Key words: irisin; obesity; mast cell; angiogenesis; oxytocin

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Introduction

Mast cells are inflammatory, highly granulated and long-lived cells, and have a pivotal role in inflammatory and allergic reactions (ANAND et al., 2012). Although mast cell activities are often associated with allergic responses, these cells have also been implicated in additional disorders, including autoimmune disorders, atherosclerosis, pulmonary hypertension, cancer, diabetes mellitus, male infertility, anxiety, nociception, and diet-induced obesity (LIU et al., 2009; ANAND et al., 2012). Mast cells are known to be widely distributed in the female reproductive organs of many species, including rats (HAMOUZOVA et al., 2017). Moreover, the presence of mast cells in the ovary during each stage of the estrous cycle of rats has also been demonstrated, but it has been revealed that the number of mast cells is higher, especially in the estrus, metestrus, and diestrus phases, compared to the proestrus phase (JONES et al., 1980; GAYFAN et al., 1991; KARACA et al., 2007; BATTH and PARSHAD, 2000).

It has been suggested that mast cells may be cellular players involved in the pathogenesis of obesity (DIVOUX et al., 2012). In obesity, there is an accumulation of mast cells in expanding adipose tissues, and accordingly, it has been reported that the number of mast cells is higher in the white adipose tissue of obese humans and animals compared to lean subjects (ALTINTAS et al., 2011). In addition to white adipose tissue, it has been shown that the number of mast cells is high in several tissues, such as the kidneys, uterus, and ovaries in obesity (KAUR et al., 2016; NIÑO et al., 2020). On the other hand, it is known that obesity decreases due to the loss of mast cell function as a result of the use of mast cell-stabilizing agents or genetically induced deficiency of mast cells in mouse obesity (LIU et al., 2009). Mast cells also contribute to browning (convergence of white adipose tissue to brown adipose tissue), which plays an important role in energy expenditure in obesity, and their functional inactivation may raise systemic energy expenditure by promoting browning and thermogenesis (AUGER et al., 2019; ZHANG et al., 2019a). Similarly, increased expression of uncoupling protein 1 (UCP1), a marker of energy expenditure, in brown fat has been shown in mouse obesity due to mast cell deficiency or inactivation (LIU et al., 2009).

Irisin, a recently identified hormone, is an adipo-myokine that is primarily produced by skeletal muscles in response to exercise. Irisin provides for the browning of white adipose tissue by enhancing energy expenditure via UCP1 expression (BOSTRÖM et al., 2012; OZCELIK et al., 2018). In our recent studies, it has been shown that irisin improves sexual dysfunction caused by antidepressant use or obesity (CANPOLAT et al., 2022; YARDIMCI et al., 2022). Besides this effect of irisin, recent studies have suggested that irisin has been shown to have potential therapeutic potential for obesity and diabetes, and anti-inflammatory, anti-apoptotic, and anti-oxidative properties (AYDIN et al., 2010; ASKARI et al., 2018). In parallel, irisin improves both obesity, by increasing total energy consumption, decreasing body weight, whole-body fat mass and insulin resistance, and inflammation status by decreasing the levels of inflammatory cytokines such as tumor necrosis factor (TNF)-α and interleukin (IL)-6 in obese animals (BOSTRÖM et al., 2012; NIRANJAN et al., 2019). Additionally, it is stated that the anti-inflammatory properties of irisin may play a role in its potential protective effects against the development of obesity (MAZUR-BIALY et al., 2017).

Angiogenesis occurs in both physiological (e.g., ovarian cycle and especially corpus luteum formation) and pathological conditions (e.g., several inflammatory disorders such as obesity and tumors) (RIBATTI and CRIVELLATO, 2012). Angiogenesis is thought to be a key progenitor in the advancement of obesity. Angiogenesis is stimulated by the expansion of adipose tissue via vascular endothelial growth factor (VEGF), the most apparent angiogenic factor, and so VEGF levels have been found to be higher in obese patients (MIYAZAWA-HOSHIMOTO et al., 2003; NIJHAWANS et al., 2020). Mast cells are also actively involved in angiogenesis, and increase angiogenesis by releasing several potent pro-angiogenic mediators, such as VEGF, fibroblast

growth factor, TNF-α, and IL-8 (NORRBY, 2002; RIBATTI and CRIVELLATO, 2012). Consistently, angiogenesis has been reported to be lower in obese mice with mast cell deficiency or inactivation (LIU et al., 2009). In addition, irisin has been shown to increase angiogenesis, and trigger or decrease VEGF secretion. In particular, the therapeutic effect of irisin against myocardial infarction injury has been associated with its pro-angiogenic effect (LIAO et al., 2019; ZHANG et al., 2019b; HAN et al., 2022). However, the role of the pro-angiogenic function of irisin, and the importance of irisin-mast cell interaction in the therapeutic efficacy of irisin on obesity are not fully known.

As another important part of energy balance, oxytocin plays a crucial function in the regulation of energy balance in a variety of ways, including food intake, adiposity levels, and energy expenditure (KUTLU et al., 2010; HO and BLEVINS, 2013). Especially, the activation of brown fat tissue thermogenesis and browning have been thought to be the two main factors in oxytocin-induced energy expenditure. In this regard, oxytocin is also involved in the differentiation of precursor adipocytes into brown adipocytes. A study in obese mice indicated that oxytocin is a novel therapeutic agent for the treatment of obesity by inducing browning and stimulating thermogenesis in obese mice (YUAN et al., 2020). However, the relationships between irisin, which is an anti-obesity hormone and inducer of browning, and the oxytocin hormone, which is important in obesity and energy expenditure, is not fully understood.

Therefore, this study aimed to evaluate the effects of irisin on the number of mast cells, corpus luteum angiogenesis, VEGF expression in ovarian tissues, and serum oxytocin levels in high-fat diet-induced obese female rats. In addition, the effects of irisin on all these parameters were also analyzed in lean female rats.

Materials and methods

Animals. Forty 2-3 month old female Sprague-Dawley rats (200-250 g) were purchased from the Firat University Experimental Research Unit (Elazig, Turkey) and kept on a 12-h light/dark cycle with consistent temperature (21±1℃) and humidity (50-60%). The experimental protocols were approved by the Animal Experimental Ethics Committee of Firat University (31.01.2018, number 19). All animal experimental procedures were carried out in accordance with the governmental guidelines for the care and use of laboratory animals at Firat University.

Experimental design. All the rats were randomly divided into 4 groups (n=10 rats per group): (1) sham-operated control (SOC) group; (2) irisin group; (3) a high-fat diet (HFD) group; (4) HFD+irisin group. Rats in the SOC and irisin groups were fed with a standard, commercial laboratory food, while rats in the HFD and HFD+irisin groups were fed with D12492 (Research Diets, USA; 60% energy derived from fat) during the experiment (for about 16 weeks). All rats in the experimental groups had free access to food and water. In all rats in the HFD and HFD+irisin groups, the development of obesity was accepted by measuring the Lee index (BERNARDIS and PATTERSON, 1968) at the end of the 12th week of feeding with a high-fat diet based on our previous study (DILSIZ et al., 2020; YARDIMCI et al., 2022).

After the occurrence of the obesity model, between 13-16 weeks of experimental studies (for about 28 days), deionized water was given subcutaneously with Alzet mini-osmotic pumps, model 2004 (reservoir volume: 200 µL, releasing rate: 0.25 µL/h (6 µL/day), Durect Corporation, Cupertino, CA, USA) in all rats in the SOC group. Similarly, all rats in the irisin and HFD+irisin groups were subcutaneously implanted with Alzet mini-osmotic pumps, model 2004 filled with irisin (# SRP8039, Sigma-Aldrich, 0.0001 mg/kg/day) dissolved in deionized water. On the other hand, the rats in the HFD group did not receive any treatment during this period. For insertion of the mini-osmotic pumps, all animals in the SOC, irisin, and HFD+irisin groups were anesthetized with an intraperitoneal injection of ketamine (6 mg/kg) and xylazine (5 mg/kg). Afterward, a mini-osmotic pump was surgically implanted in the interscapular region for continuous release of deionized water or irisin under sterile conditions. By measuring the volume of the mini-osmotic pumps before

implantation and after their removal at the end of the experiment, it was confirmed that all mini-osmotic pumps were able to provide a fixed dosage of irisin or deionized water for about 28 days.

From the 25th day of infusion of deionized water/irisin, a vaginal smear was performed (YILMAZ et al., 1996; MARCONDES et al., 2002) in all rats, and accordingly, all rats were sacrificed by decapitation at the same stage of the cycle (i.e. diestrus phase). After decapitation, trunk blood was collected and centrifuged at 4500 rpm for 5 min at 4°C. The serum was separated and stored at –20°C until hormonal analysis. The excised ovaries were fixed using a 10% formalin solution for histological and immunohistochemical examinations. Fig. 1 depicts the flowchart of the study.

Histological examination. Following fixation, the ovaries were submitted to a routine histological procedure, and then they were embedded in paraffin blocks. At 30 μm intervals, 10 series of sections of 5 μm thickness were taken from the ovarian tissue blocks of all experimental groups. All the preparations were examined under an examination microscope (Nikon Eclipse 50i) after all histochemical and immunohistochemical staining.

To calculate the number of mast cells, ovarian sections taken at a thickness of 5 μm were stained with toluidine blue (0.5%, pH:0.5; Sigma-Aldrich, CAS 92-31-9) for 10 min (ENERBÄCK, 1966). Mast cell counts were performed in 10 randomly selected areas of the ovarian sections in all the experimental groups, and the arithmetic mean of the results was taken. For this, a 100 square ocular micrometer (eyepiece graticule) was used to count mast cells, with a magnification of 40×, and all the data were converted into mast cell numbers within a 1 mm^2 unit area (TÜTÜNCÜ et al., 2020).

For corpus luteum angiogenesis, Crossman's triple staining was performed on ovarian sections taken at a thickness of 5 μm in all the experimental groups (CROSSMON, 1937). Corpus luteum angiogenesis was semi-quantitatively graded as severe (3), moderate (2), mild (1), or none (0) on the basis of the histopathological findings by examining 10 randomly selected areas under 40× magnification. The average histological score of corpus luteum angiogenesis was calculated for each rat, and mean values were calculated for each experimental group (BAĞCI et al., 2020).
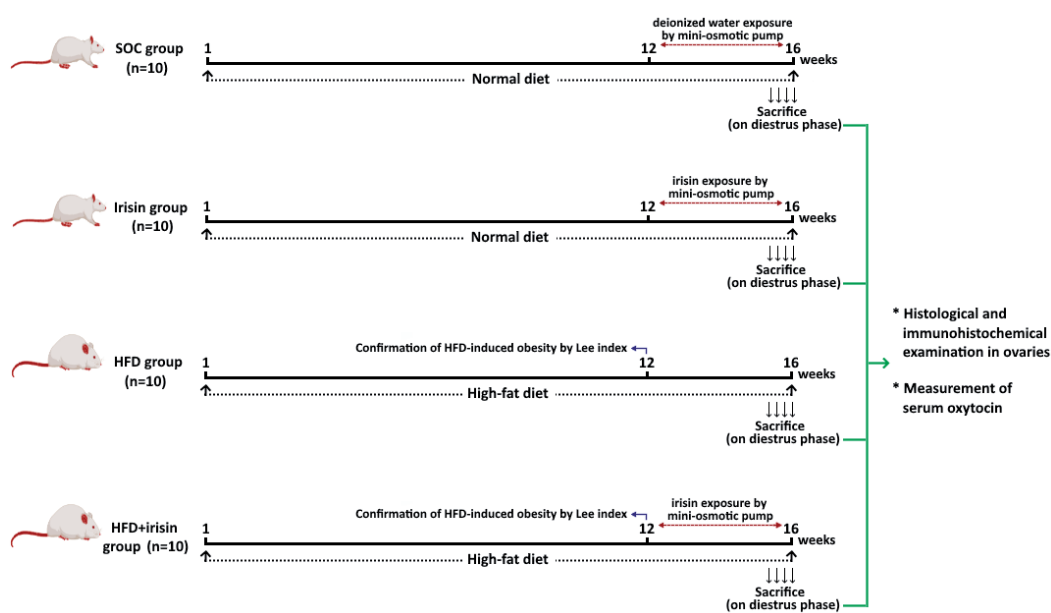

Fig. 1. Flowchart of the experimental procedure. SOC: sham-operated control and HFD: high-fat diet

Immunohistochemical examination. Ovarian tissue sections (5-μm-thick) were stained immunohistochemically with mouse monoclonal VEGF (1/500 dilution, Santa Cruz Biotechnology, sc7269) primary antibody using the Streptavidin biotin complex method (TRUE, 1990). Histostain Plus (Zymed kit: 85-6743, USA) was used as the secondary antibody. Firstly, ovarian sections were deparaffinized and then heated in a microwave oven at 700 W for antigen retrieval in a citrate buffer solution (pH:6). To inhibit endogenous peroxidase activity, the ovarian tissues were incubated in a 3% hydrogen peroxide solution. Serum from the kit was instilled after washing with phosphate buffer solution (PBS) to prevent nonspecific protein binding in the sections. After applying the primary antibody, the sections were kept at +4 °C overnight. The PBS solution was used alone in the negative control group. Following washing, the sections were instilled with the biotinylated secondary antibody and incubated in the streptavidin horseradish peroxidase complex. In the final stage, 3,3'-diaminobenzidine (DAP) was used as a chromogen, and then the sections were coated with entellan after hematoxylin counterstaining. Under 40× magnification, 10 randomly selected fields were evaluated from each section. The intensity of positive staining in the immunohistochemical examination of VEGF expression was assessed semi-quantitatively using a standard four-point scoring scale, with intensity being scored as negative (0), weak (1), moderate (2), or strong (3) (ERTUĞRUL and SEVILGEN, 2022).

Measurement of serum oxytocin. The serum oxytocin levels were measured in duplicate by Enzyme-Linked Immuno Sorbent Assay (ELISA) using a commercially available rat-specific ELISA kit (Catalog No: E-EL-0029, Elabscience, USA) according to the manufacturer's instructions. The assay sensitivity of oxytocin was 9.38 pg/mL and the detection range of oxytocin was 15.63-1000 pg/mL. On the automatic ELISA plate reader (Multiskan FC, Thermo Scientific, USA), the ELISA plate was read at 450 nm.

Statistical analysis. SPSS 22.0 software was used to perform all statistical procedures. Results are expressed as mean ± standard error of the mean (SEM). The Shapiro-Wilk test was used to confirm the normality of the data distribution, and all the data were found to be normally distributed. Differences between groups were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's *post-hoc* test. P<0.05 was considered statistically significant.

Results

Effects of irisin administration and/or high-fat diet exposure on mast cell histochemistry in rat ovaries. To examine the effect of irisin on mast cells in both lean female rats and obese female rats, the mast cell population was identified using toluidine blue staining. When the ovarian tissue sections of all the experimental groups were examined, it was determined that mast cells determined to be prominently round or oval and showing metachromasia were generally located in the medullary region of the ovary, especially close to the blood vessels. On the other hand, the number of mast cells was lower in the cortical region than in the medullary region. In the cortical region, mast cells were found in the connective tissue beneath the germinative epithelium, as well as near the follicles, at different stages of development, at the periphery of the corpus luteum, and between the follicles. Mast cells were more common in the hilum region of the ovary than in the medulla and cortex (Fig. 2).

As shown in Fig. 3A, it was determined that irisin administration did not change the number of mast cells in the lean female rats compared with the SOC group. The mast cell number in the HFD group was significantly higher than in the SOC group (P<0.05). Additionally, irisin exposure significantly decreased the number of mast cells in the HFD+irisin group compared to the HFD group (P<0.05). However, there was no significant difference in the number of mast cells between the SOC and HFD+irisin groups.
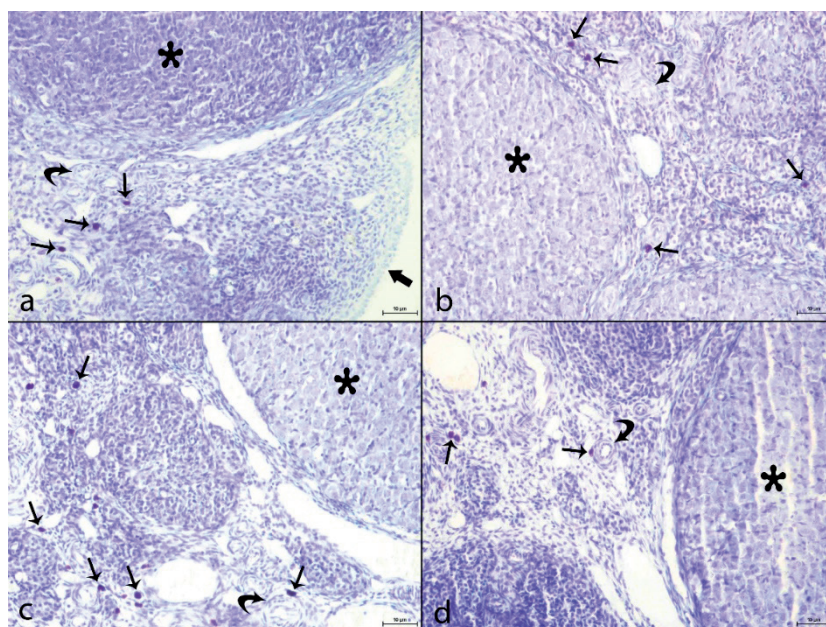
Fig. 2. Toluidine blue staining for ovarian mast cells

a) sham-operated control group. b) irisin group. c) high-fat diet (HFD) group. d) HFD+irisin group. Mast cells (thin arrow), the germinative epithelium (thick arrow), blood vessels (curved arrow), and corpus luteum (star). Original magnification: 20× and range bar: 10 μm

Effects of irisin administration and/or high-fat diet exposure on corpus luteum angiogenesis in rats. The histological view of angiogenesis in the corpus luteum in all experimental groups is presented in Fig. 4. The angiogenesis score in the corpus luteum in the irisin, HFD, and HFD+irisin groups was significantly higher when compared to the SOC group (P<0.001, Fig. 3B). Strikingly, it was observed that corpus luteum angiogenesis increased in lean rats but decreased in obese rats due to irisin exposure. In other words, when compared to the HFD group, angiogenesis in the corpus luteum was significantly lower in the HFD+irisin group depending on irisin administration (P=0.001, Fig. 3B).

Effects of irisin administration and/or high-fat diet exposure on VEGF expression in rat ovaries. Immunohistochemical staining for VEGF in ovarian tissues is illustrated in Fig. 5. Regarding the localization of the brown-colored VEGF immunoreactivity in the ovaries of all the experimental groups, VEGF expression was found in stromal cells, granulosa lutein cells, and germinal epithelium. However, there was no or only weak VEGF immunoreactive positivity in the follicular cells of the Graafian follicle, while weak VEGF immunoreactive positivity was observed in the granulosa cells of the primary follicle.

The obtained data revealed that VEGF expression in ovarian tissues did not change with irisin administration in lean female rats compared with female rats receiving deionized water (Fig. 3C). When compared to the SOC group, VEGF immunoreactivity was significantly more intense in female rats exposed to a high-fat diet in both the HFD group (P<0.001) and the HFD+irisin group (P<0.05) (Fig. 3C). Increased expression of VEGF due to a high-fat diet was significantly decreased by irisin exposure in the HFD+irisin group compared to the HFD group (P<0.001, Fig. 3C), as in corpus luteum angiogenesis.
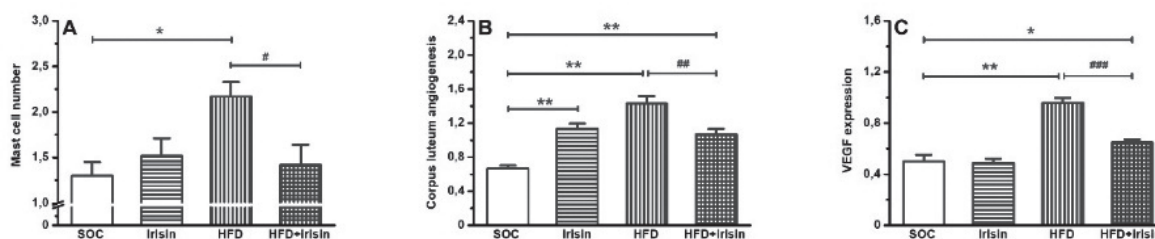
Fig. 3. Effects of irisin administration and/or high-fat diet exposure on mast cell number and angiogenesis in the ovaries of rats

A) Number of ovary mast cells. B) Angiogenesis in the corpus luteum. C) VEGF expression in the ovarian tissues. The results are expressed as mean ± SEM (n = 10 rats per group). *P<0.05 and **P<0.001 compared with the SOC group. #P<0.05, ##P=0.001, and ###P<0.001 compared with the HFD group (One-way ANOVA followed by the Tukey's *post-hoc* test). SOC: sham-operated control group, HFD: high-fat diet group, and VEGF: vascular endothelial growth factor
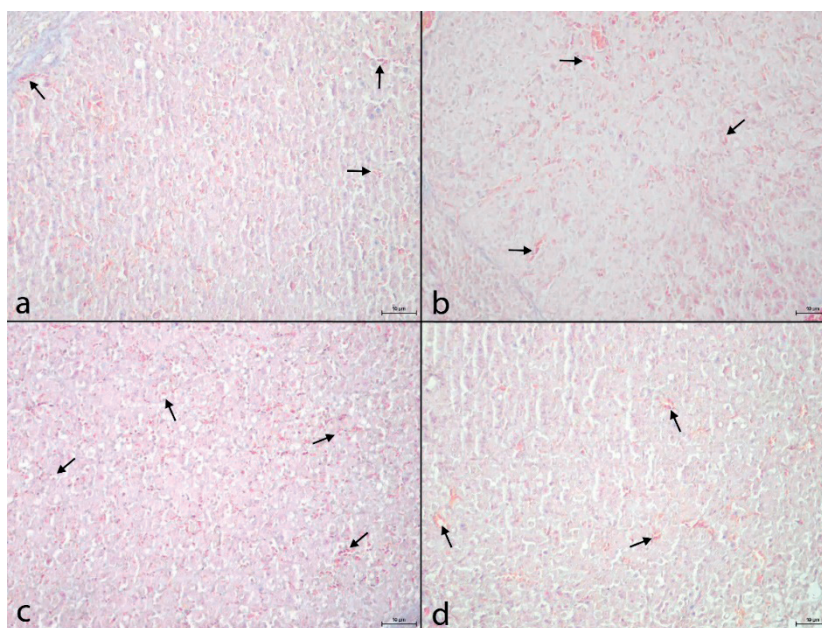

Fig. 4. Microscopic image of corpus luteum angiogenesis in a) sham-operated control group, b) irisin group, c) high-fat diet (HFD) group, and d) HFD+irisin group

Angiogenesis in the corpus luteum (arrow) (Crossman's triple staining). Original magnification: 20× and range bar: 10 μm

Effects of irisin administration and/or high-fat diet exposure on serum oxytocin levels in female rats. Compared to the SOC group, serum oxytocin levels were significantly increased depending on irisin exposure and/or high-fat diet exposure in female rats (P<0.05 for the irisin group, P=0.001 for the HFD group, and P<0.001 for the HFD+irisin group, Fig. 6). In addition, it was determined that the serum oxytocin levels were significantly higher in the HFD+irisin group compared to the HFD group, due to irisin administration (P<0.05, Fig. 6).
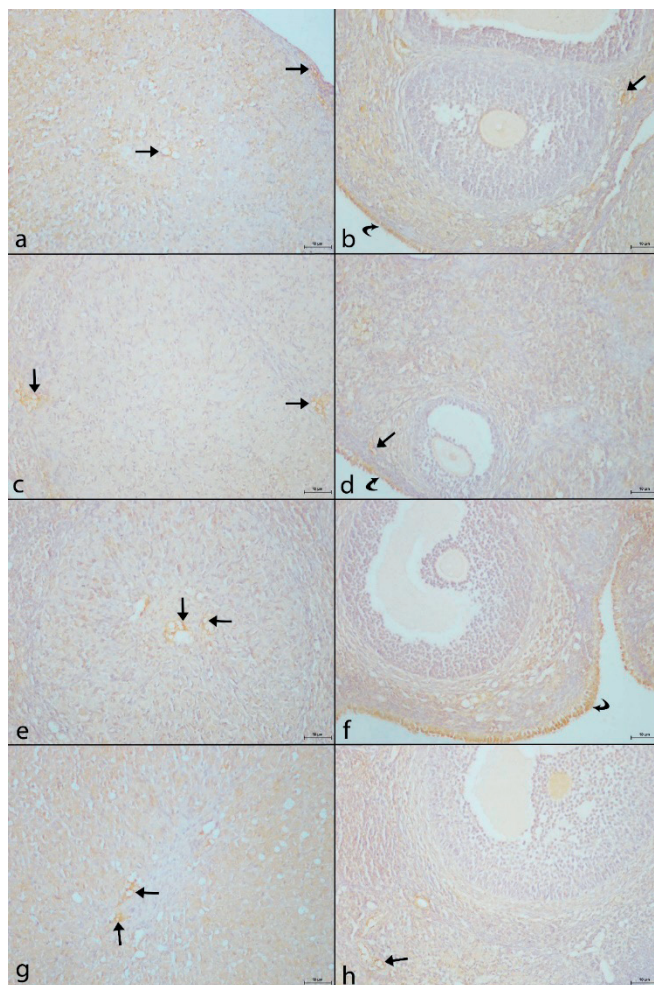
Fig. 5. Immunohistochemical images of vascular endothelial growth factor (VEGF) expression in rat ovaries

a-b) sham-operated control group. c-d) irisin group. e-f) high-fat diet (HFD) group. g-h) HFD+irisin group. VEGF expression in the blood vessels (thin arrow) and VEGF expression in the germinative epithelium (curved arrow). Original magnification: 20× and range bar: 10 μm
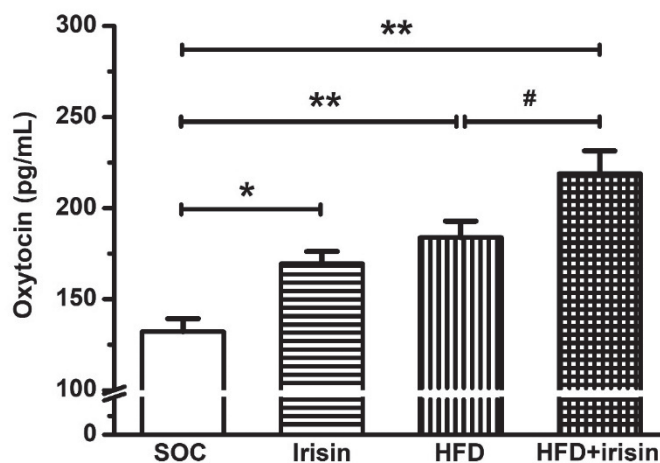

Fig. 6. Effects of irisin administration and/or high-fat diet exposure on serum oxytocin levels in female rats

The results are expressed as mean ± SEM (n = 10 rats per group). *P<0.05 and **P<0.001 compared with the SOC group. #P<0.05 compared with the HFD group (One-way ANOVA followed by the Tukey's *post-hoc* test). SOC: sham-operated control group and HFD: high-fat diet group

Discussion

In the present study, we examined the effects of irisin on mast cell numbers, corpus luteum angiogenesis, and VEGF immunoreactivity in the ovaries of both lean and obese female rats, as well as serum oxytocin levels. It was shown that increased mast cell numbers, corpus luteum angiogenesis, and VEGF expression in ovarian tissues decreased, whereas serum oxytocin levels increased with irisin administration in obese female rats. Accordingly, in the present study, we reported for the first time that irisin can exert its therapeutic effects on obesity through mast cells, angiogenesis, and oxytocin hormone. Other important findings of the present study were that irisin exposure increased angiogenesis in the corpus luteum and serum oxytocin levels in lean female rats, but did not affect the mast cell number in the ovaries.

Mast cells can contribute to immune processes such as allergies and autoimmunity, as well as play a role in the pathogenesis of cancer and diabetes (NAKAMURA et al., 2013). Moreover, recent studies have shown that mast cell numbers increased in several tissues such as white adipose tissue, kidney, uterus, and ovary in obese rats, thus suggesting that mast cells may be cellular actors involved in the pathophysiology of obesity, and may be effective in the development of obesity (ALTINTAS et al., 2011; DIVOUX et al., 2012; KAUR et al., 2016; NIÑO et al., 2020). Consistent with previous findings, we determined that mast cell numbers increased in the ovaries of obese rats in this study. Pharmacologically, mast cell inactivation has been shown to prevent obesity and reduce pre-established obesity, revealing a potential therapy for obese people (LIU et al., 2009). In the current study, our data showed that irisin administration caused a marked reduction in increased mast cell numbers in the ovarian tissues of obese rats. These data suggest that irisin, as a mast cell stabilizing agent, may have therapeutic potential in obesity.

Moreover, several studies in animal models show that improvement of the function of brown adipocytes, beige adipocytes, or both, in humans can be very effective in the treatment of obesity (HARMS and SEALE, 2013). The inhibitory role of mast cells on white adipose tissue browning is known (ELIEH ALI KOMI et al., 2020), and it has been shown that functional inactivation of mast cells can increase systemic energy expenditure by inducing browning and thermogenesis, thereby ameliorating obesity (ZHANG et al., 2019a). Consistently, we speculate that the therapeutic effect of irisin on obesity may have occurred as a result of enhanced browning and thermogenesis due to decreased mast cell numbers, depending on irisin exposure in the ovarian tissues of obese rats.

Recently, the anti-inflammatory potential of irisin with respect to various diseases, including obesity, has been revealed. The anti-inflammatory activity of irisin in obesity appeared to be possibly mediated by reducing inflammatory markers (TNF-α and IL-6) (MAZUR-BIALY et al., 2017; ASKARI et al., 2018). For these reasons, we think that a second explanation for the therapeutic efficacy of irisin in obesity may be related to the anti-inflammatory properties of irisin. Additionally, it can be assumed that the possible decrease in TNF-α and IL-6 expression produced from mast cells as a result of the reduction in the mast cell numbers depending on irisin exposure in obese female rats may also have been related to this situation.

Physiological angiogenesis is essential for wound healing, the menstrual cycle, and pregnancy, but disruption of physiological angiogenesis-related mechanisms can cause many diseases, including obesity (CANPOLAT et al., 2010; TAHERGORABI and KHAZAEI, 2013). Hence, angiogenesis is thought to be a key progenitor in the progression of obesity and a critical factor in the expansion of white adipose tissue. Modifying angiogenesis has the potential to slow the progression of obesity, and current research suggests that antiangiogenic agents may be beneficial in the treatment of obesity (NIJHAWANS et al., 2020). In particular, VEGF is an angiogenic agent that is involved in the angiogenic process, and it is widely assumed that VEGF is responsible for the majority of angiogenic activity in adipose tissue. Also, VEGF plays a crucial role in both normal and pathological angiogenesis (LIJNEN, 2008). Previous studies

have shown that serum VEGF levels were higher in obese patients (MIYAZAWA-HOSHIMOTO et al., 2003; SILHA et al., 2005). In another study, it was shown that both ovarian immunohistochemical staining for VEGF and VEGF levels in ovarian tissues increased with obesity in rats (ABDEL-MOTTALEB et al., 2022). Consistent with these findings, in the present study we observed that high-fat diet-induced obesity caused an increase in VEGF expression in the ovarian tissues of rats. In addition to the VEGF expression in ovarian tissue, ovarian angiogenesis was also evaluated by angiogenesis in the corpus luteum in all experimental groups in the present study. Accordingly, we observed that high-fat diet-induced obesity promotes angiogenesis in the corpus luteum in rats, just like the increase in VEGF expression in ovarian tissue.

In the present study, we found that angiogenesis in the corpus luteum increased, but VEGF expression in the ovaries did not change due to irisin administration in lean rats. In line with our results, several studies have shown that irisin promoted angiogenesis in the heart, human umbilical vein endothelial cells, zebra fish embryos, and human dental pulp cells, thus showing that irisin has a pro-angiogenic effect (WU et al., 2015; LIAO et al., 2019; SON et al., 2021). On the other hand, in terms of the effects of irisin on ovarian angiogenesis in obesity, we observed that irisin exposure induced a decrease in both angiogenesis in the corpus luteum and VEGF immunoreactivity in the ovaries of obese rats treated with irisin when compared to untreated obese rats. In conclusion, it could be speculated that irisin may be considered to have an antiangiogenic effect by targeting ovaries in obesity since irisin administration caused the recovery of ovarian angiogenesis in obese rats. Meanwhile, in obese rats, it was shown that ovarian angiogenesis decreased in parallel with the decrease in mast cell numbers in ovarian tissue with irisin exposure in the present study. This finding is consistent with a previous study showing that mast cell deficiency or inactivation reduced angiogenesis in diet-induced obese mice (LIU et al., 2009). Overall, our findings uncover a new aspect of irisin in relation to ovarian angiogenesis in both lean rats and high-fat diet-induced obese rats.

Oxytocin, crucial in controlling energy homeostasis and body weight balance, is synthesized both centrally and peripherally (e.g. hypothalamus, kidneys, heart, thymus, testis, and ovaries) (ATES et al., 2019). Oxytocin is involved in central and peripheral processes, such as reproductive events, adipogenesis, osteogenesis, glucose homeostasis, metabolic syndromes, and obesity (GIMPL and FAHRENHOLZ, 2001; KUTLU et al., 2004; YUAN et al., 2020). In humans, exercise has been reported to increase plasma oxytocin levels (LANDGRAF et al., 1982; IRIANTI et al., 2017). Another study showed that plasma oxytocin levels are enhanced by cold exposure in mice (YUAN et al., 2020). In addition, Lawson et al. (2014) demonstrated that oxytocin correlates positively with irisin, which causes the browning of white adipose tissue, UCP1-mediated thermogenesis, and energy expenditure in amenorrheic athletes. In conclusion, considering that study (LAWSON et al., 2014) and the fact that irisin hormone is an exercise-induced myokine (BOSTRÖM et al., 2012), and its expression increased due to cold exposure (LEE et al., 2014), irisin has been shown to increase oxytocin levels. Consistent with previously published works, we found that irisin exposure increased serum oxytocin levels in lean female rats in the present study.

Studies have emphasized that oxytocin influences the factors affecting energy balance rather than food intake in reducing body mass (HO and BLEVINS, 2013). In obesity, it has been demonstrated that oxytocin induces the browning of white adipose tissue, stimulates thermogenesis in brown fat tissue, and thus oxytocin fights obesity by promoting thermogenesis (YUAN et al., 2020). In the present study, we observed that irisin administration increased serum oxytocin levels in obese female rats in the HFD+irisin group, and this increase was higher than in the obese female rats. These data suggest that activation of the oxytocin by irisin may be important in promoting thermogenesis in obese female rats, thereby the therapeutic efficacy of irisin in obesity may also be mediated by oxytocin.

Conclusions

On the whole, our findings support the assertion that irisin has a therapeutic effect on obesity. These effects were determined through the reduction in the number of ovary mast cells and ovarian angiogenesis, and the increase in serum oxytocin levels by irisin exposure in obese rats. In line with our results, it is suggested that one of the underlying mechanisms in the therapeutic efficacy of exercise on obesity is probably the effect of the irisin hormone.

Ethics approval

The experimental protocols were approved by the Animal Experimental Ethics Committee of Firat University (31.01.2018, number 19). All animal experimental procedures were carried out in accordance with the governmental guidelines for the care and use of laboratory animals at Firat University.

Financial support statement

Declaration of Competing Interest

The authors declare no conflict of interest.

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SAŽETAK

Hormon vježbanja irizin, termogeni adipomiokin, potiče potrošnju energije posmeđivanjem bijelog masnog tkiva. S obzirom na to da irizin poboljšava metabolički profil bijelog masnog tkiva i povećava potrošnju energije cijelog organizma, smatra se da bi on mogao biti potencijalni novi terapijski cilj u liječenju rastuće epidemije pretilosti. Poznate su uloge mastocita i angiogeneze u patogenezi pretilosti, a posljednjih je godina naglašena i važnost gubitka funkcije mastocita i antiangiogenih lijekova u liječenju pretilosti. Osim toga važna je i terapijska učinkovitost oksitocina kod pretilosti, poticanjem tamnjenja i termogeneze. Kako bi se bolje razumjeli mehanizmi uključeni u terapijski učinak irizina kod pretilosti, u ovom je istraživanju procijenjen učinak liječenja irizinom na pretile ženke štakora koje su hranjene obrocima s visokim udjelom masnoća, pri čemu je naglasak bio na broju mastocita, ovarijskoj angiogenezi i razinama serumskog oksitocina. Naši su rezultati pokazali da se broj mastocita jajnika, angiogeneza žutog tijela i imunoreaktivnost vaskularnog endotelnog faktora rasta (VEGF) u tkivu jajnika povećala s pretilošću i zatim znakovito smanjila primjenom irizina. Ustanovljeno je također da su se serumske razine oksitocina s pretilošću znatno povećale, ovisno o primjeni irizina u pretilih ženki štakora. Sveukupni rezultati pokazuju da irizin doprinosi smanjenju broja mastocita i angiogeneze te potiče lučenje oksitocina. Navedeno ovaj hormon čini potencijalnim terapijskim sredstvom u liječenju pretilosti.

Ključne riječi: irizin; pretilost; mastociti; angiogeneza; oksitocin