The Effect of Estrogen Hormone on Leptin Receptor in Small Intestine of Ovariectomized Rats

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ABSTRACT

Objective: Leptin, a 16 kDa hormone encoded by the obese (Ob) gene, is known for its role in regulating food intake, body composition, and energy expenditure. Leptin receptor expression has been demonstrated in several tissues, including the small intestine. Weight gain may occur in humans after menopause or in animals following ovariectomy. Estrogen affects leptin and leptin receptor expressions. In this study, we aimed to contribute to the etiology of obesity by investigating the effects of E2 on leptin receptors in the small intestines of ovariectomized rats as a model of postmenopausal conditions.

Materials and Methods: Bilateral ovariectomy was performed on 6-month-old Sprague-Dawley female rats. Ovariectomized rats (Ovx) were injected with 0.2 ml of sesame oil/rat/day or E2 (25 µg/rat/day) and euthanized at the 18th, 90th, or 162nd hours. Duodenum, jejunum, and ileum samples were fixed and embedded in paraffin using standard methods. The expression of leptin receptors were detected in the small intestine through immunohistochemistry.

Results: Leptin receptor expression was found in the villi and crypt epithelium of the small intestine and in Brunner’s gland of the duodenum. E2 administration increased the leptin receptor expressions on the epithelium of villi and crypt in the duodenum and jejunum at the 90th hour (p<0.05); ileum at the 18th hour (p<0.05); and also on the epithelium of villi in the duodenum at the 162nd hour (p<0.05).

Conclusion: Our results indicate that E2 may upregulate the expression of leptin receptors in the small intestine, where glucose and other nutrients are absorbed after food intake and digestion, depending on the timing.

Keywords: Estrogen, Leptin receptor, Ovariectomized, Rat, Small intestine.

INTRODUCTION

Leptin (Ob) is produced by adipocytes (1, 2) and is transported via the blood to the hypothalamus, where it binds to the leptin receptor (Ob-R). The hormone functions primarily in the regulation of energy expenditure of the hypothalamus, food intake (3, 4), and prevention of obesity (5).

Leptin has been identified in many peripheral tissues where it is often transported from the blood via transcytosis and paracellular transport (6-9). Recent data suggest leptin could also be considered a gastrointestinal hormone (10, 11, 12). Leptin is expressed by some cells in the gastric mucosa (12, 13). One of these cells is an exocrine cell called the chief (principal) cell (12, 13) which secretes leptin and pepsinogen into the digestive juice. In addition to that, a small amount of exocrine leptin is also expressed by Parietal cells (13). Another leptin-secreting cell is small endocrine cells localized between the gastric glands. From these cells, which are in close proximity to the capillaries, leptin is released into the bloodstream (12). After food intake, leptin is secreted into the gastric lumen by Chief cells and thus carried to the intestine (14).

It is suggested that the role of leptin in the short–term control of food intake (12) and may role locally gastrointestinal tract affect intestinal functions such as sugar and peptides absorption and lipid handling (15, 16, 17) via its receptor (18). Leptin receptors are located in the apical and basolateral membranes of chief and parietal cells in the gastric mucosa (11) and enterocytes in the intestinal mucosa (12, 19, 20, 21). It was reported leptin reaches the brush border (apical membrane) binds to its receptor via gastric juice, and arrives at the basolateral membrane through the blood (19).
They show paracrine effects by binding endocrine leptin to leptin receptors localized on the basolateral membrane of intestinal epithelial cells (19). Studies have shown that the leptin hormone increases the length and mucosal thickness of the small intestine, like a growth factor; shows that it plays a role in intestinal absorption functions (22, 23).

In the control of nutrition and weight gain, steroid hormones and, therefore gender differences are of great importance. Many studies have investigated the relationship of leptin or its receptor with estrogen (24, 25, 26, 27, 28). Estrogen causes changes in the production of leptin hormone by affecting the obesity (ob) gene transcription through its receptors in fat cells (29). It has been reported that in the premenopausal period have higher serum leptin levels than men and postmenopausal women (27). Similarly, administration of estrogen hormone to postmenopausal women has been found to cause more leptin synthesis from omental adipose tissue (30, 31). Menopausal women experience an increase in body weight and fat mass (32), along with alterations in body fat distribution (33). Similarly, in cats (34) and dogs (35), there is an increase in body weight, mostly in body fat, due to increased food intake following ovarioectomy. For this reason, ovarioectomized rats are a frequently used method to model the postmenopausal period in studies (21, 24, 26).

In addition, estrogen hormone deficiency also affects the level of leptin receptors in some tissues (24, 25, 26, 36). In poultry, administration of estrogen increased the expression of cOb-R mRNA in the intestines. This indicates that estrogen is also associated with the regulation of leptin receptor expression in the poultry small intestine (37). It was also reported that leptin receptor expression decreased in ovarioectomized rat colon, and 17β-estradiol (E2) administration normalized the leptin receptor level (21).

The small intestines play an important role in the etiology of obesity seen in ovarioectomized animals. As a result of the disappearance of the effect of steroid hormones, especially estrogen hormone, the degree of expression and therefore the sensitivity of leptin receptors localized in the small intestines decreases. Normal bowel functions and nutritional intake may be affected by this condition. While it is established that leptin and its receptor play a crucial role in regulating gastrointestinal system functions, there is a scarcity of studies demonstrating the relationship between estrogen hormone and leptin receptor in the regulation of these functions in mammals.

In this study, our aim was to contribute to the understanding of obesity etiology by investigating the impact of E2 on leptin receptors in the small intestine of ovarioectomized rats, which serve as a model for postmenopausal conditions.

## MATERIAL and METHODS

**Ethical Statement:** All procedures were approved by the Ethics Committee at Afyon Kocatepe University, Türkiye (AKÜHEK. 49-07; 25.01.2008).

**Animals:** Six-month-old Sprague-Dawley female rats were supplied from Department of Laboratory Animals, Gülhane Military Medical Academy Research Centre, Ankara, Türkiye were used in the present study. All animals used in the study were kept in Afyon Kocatepe University Experimental Animal Research and Application Center under standard control conditions and were fed commercial rat feed ad libitum.

Briefly, rats (total n = 36) were anesthetized by ketamine (Alfamime, Alfasan Int. BV3440 AB, Voerden Holland) (21.2 mg/kg i.p.) and xylazine (Rompum® inj, Bayer Türk Kimya San. Ltd. Sti. Istanbul) (4.2 mg/kg i.p.), and underwent bilateral ovarioectomy (Ovx). Small incisions were made bilaterally on the sides of the back to expose the ovaries retroperitoneally. The ovaries were clamped and removed, and the uterine tubes were ligated. The muscle and skin were then sutured.

Ovariectomized rats were administered 60 000 IU/day Penicillin G (Pfizer) for 5 days. One week after the operation, the rats were first separated into control (C, n = 18) and 17β estradiol (E2, n =18) groups then randomly divided into six subgroups as C18, C90, C162, E218, E290, E2162 (according to the hour of the experimental period on which the animals were euthanized). The experimental protocol applied in the groups is summarized in Table 1.

**Immunohistochemical Staining:** Duodenum, jejunum, and ileum samples were fixed immediately in 10% buffered formalin and washed with tap water, dehydrated through 70, 80, 95, and 100% alcohol, cleared in two baths of xylene and embedded in paraffin. The paraffin sections (5μ thick) were used for immunohistochemical staining. After deparaffinization and hydration, an antigen-retrieval procedure was performed to unmask the antigens by treating the samples three times in a microwave oven at 700 W for 5 min each time in 10 mM citrate buffer (pH 6).

### Table 1. The experimental procedures of the control group and estrogen group in the study.

<table>
<thead>
<tr>
<th>Group</th>
<th>Subgroup</th>
<th>Application</th>
<th>Termination of Experiment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>C18: (euthanasia at 18th hour)</td>
<td>0.2 ml/rat Sesame oil, intramuscular (i.m.)</td>
<td>18th hours after the application</td>
</tr>
<tr>
<td></td>
<td>C90: (euthanasia at 90th hour)</td>
<td>0.2 ml/rat/day Sesame oil, (i.m)</td>
<td>At 90th hour</td>
</tr>
<tr>
<td></td>
<td>C162: (euthanasia at 162nd hour)</td>
<td>0.2 ml/rat/day, Sesame oil, (i.m.)</td>
<td>At 162nd hour</td>
</tr>
<tr>
<td>17β estradiol</td>
<td>E218: (euthanasia at 18th hour)</td>
<td>25 µg/rat/day E2 (Sigma E8875), (i.m.)</td>
<td>18th hours after the application</td>
</tr>
<tr>
<td></td>
<td>E290: (euthanasia at 90th hour)</td>
<td>25 µg/rat/day E2, (i.m.)</td>
<td>At 90th hour</td>
</tr>
<tr>
<td></td>
<td>E2162: (162nd hour)</td>
<td>25 µg/rat/day E2, (i.m.)</td>
<td>At 162nd hour</td>
</tr>
</tbody>
</table>

Rats were euthanized by cervical dislocation under anesthesia at the 18th, 90th, or 162nd hours respectively.
After cooling at room temperature, slides were treated with 3% hydrogen peroxide (H2O2) in methanol for 10 min and then washed with PBS for 3 x 5 min. The sections were incubated in blocking reagent for 10 min, and incubated overnight at 4 °C with Ob-R goat polyclonal primary antibody at a 1:25 dilution (Santa Cruz Biotechnology M-18, sc-1834). Staining was completed with a Histostain Plus Kit (Universal Dakocytomation LSAB® + Kit, Peroxidase K0690) according to the manufacturer’s instructions. The color reaction product was developed with 3,3’-diaminobenzidine (DAB) (Zymed, 00-2020). All sections were counterstained with hematoxylin (21).

**Immunohistochemical Evaluation**

Immunohistochemical evaluation was done by investigating several aspects such as whether the target tissue was stained or not, the character of the staining (nuclear or cytoplasmic), which parts of the target tissue structures were stained, and the staining intensity. The evaluations were made by two independent observers, giving values from 0 to 3 for unstained (-), weak staining (+), moderate staining (++), and severe staining (+++) according to the characteristics (38).

**Statistical Analysis**

The mean and standard error values of the data obtained from the immunohistochemical evaluation were assessed, and it was determined whether the differences between the groups were statistically significant. The data of the estrogen and control groups were compared with a non-parametric Mann-Whitney U test, and the statistical differences were analyzed (*: p≤0.05). Statistical analyses of the study were performed using the SPSS 10.0 (Statistical Package for Social Sciences) program.

**RESULTS**

**Leptin Receptor Immunoreaction in Small Intestine**

Leptin receptor immunoreaction was observed in the apical cytoplasm of duodenum villi, crypt epithelium, Brunner’s gland epithelial cells, connective tissue cells, and a small number of goblet cells. In the jejunum, the positive reaction for leptin receptor was observed in the apical cytoplasm of the villi and crypt epithelium, connective tissue cells, and a small number of goblet cells. Lastly, in the ileum, the positive reaction for the leptin receptor was observed in the apical cytoplasm of the villi and crypt the epithelium of the ileum, connective tissue cells, and a small number of goblet cells. In terms of leptin expression intensity, within-group variations were observed in both groups.

**Duodenum**

In the control group, a negative reaction was observed in the villus epithelium of the duodenum along with a negative or weak reaction in the epithelial cells at the bottom of the crypts at the 18th hour (Figure 1 A, B). In the estrogen group, a weak reaction was detected in the villus epithelial cells and the epithelial cells in the middle and upper portions of the crypts, while a moderate reaction was seen in some of the epithelial cells in the lower parts (Figure 1 C, D). At the 18th hour, a weak to moderate immunoreaction was observed in the Brunner’s glands of the duodenum in the control and the estrogen groups.

In the control group, a negative reaction was observed in the villus epithelial cells of the duodenum at the 90th hour and the immunoreaction in the crypt epithelial cells were mostly negative apart from a few samples showing a very weak reaction (Figure 2 A, B). In the estrogen group, a weak immunoreaction was detected in the villus epithelial cells and a moderate reaction was observed in the crypt epithelial cells at the 90th hour. A weak to moderate expression was observed in the Brunner’s glands of the duodenum both in the control and estrogen groups at the 90th hour (Figure 2 C, D).

In the control group, an immunoreaction ranging from negative to very weak was observed in the villus epithelial cells of the duodenum at the 162nd hour, while a weak reaction was detected in the villus epithelial cells in the estrogen group. In the control group, a negative to weak reaction was detected in the epithelial cells in the middle and upper portions of the duodenal crypts, while a weak reaction with a few samples showing moderate immunoreaction was observed in the epithelial cells at the bottom portion (Figure 3 A, B). In the estrogen group, some epithelial cells at the bottom of the crypts stained moderately, while the epithelial cells in the middle and upper portions of the crypts were negatively or weakly stained. Weak to moderate leptin receptor expression was detected in Brunner’s gland epithelial cells (Figure 3 A, B).
**Jejunum**

In the control group, negative immunoreactions were observed both in the villus epithelial cells of the jejunum and the crypt epithelial cells at the 18th hour along with some samples showing weak reactions (**Figure 4 A, B**). In the estrogen group, negative immunoreactions were observed in the villus epithelial cells and negative or weak immunoreactions were determined in the cells of the middle and upper parts of the crypt epithelium. In addition to that, a moderate immunoreaction was seen in the lower parts of the crypt epithelium (**Figure 4 C, D**).

While a weak reaction was observed in the villus epithelial cells of the jejunum at the 90th hour of the control group, a negative or weak immunoreaction was detected in the epithelial cells of the crypts, and a moderate immunoreaction in some epithelial cells was observed in the bottom parts (**Figure 5 A, B**). In the estrogen group, a varying range of immunoreactions was observed from weak to severe reactions in the villus epithelial cells, while a moderate reaction was observed in the middle and upper epithelial cells of the crypts, and a severe reaction was observed in some epithelial cells in the lower parts (**Figure 5 C, D**).

In the control group, the villus epithelial cells of the jejunum were stained negatively, while a weak to moderate reaction was detected in the villus epithelial cells of the estrogen group at the 162nd hour (**Figure 6 A, C**). Leptin receptor immunoreaction was also detected in connective tissue cells (**Figure 6 A, C**). A weak reaction was observed in the crypt epithelial cells of the control group, while a moderate expression was detected in the crypt epithelial cells in the estrogen group (**Figure 6 B, D**).
Figure 5. The leptin receptor immunoreaction in the duodenum at the 90th hour. A, B: Control group; C, D: E2 group. A: Weak leptin receptor immunoreaction in villus epithelial cells (arrow); B: Moderate leptin receptor immunoreaction in crypt epithelial cells (arrow); C: Moderate leptin receptor immunoreaction in villus epithelial cells (arrow); D: Moderate leptin receptor immunoreaction in crypt epithelial cells (arrow). Bar =100 μm.

Figure 6. The leptin receptor immunoreaction in the jejunum at the 162nd hour. A, B: Control group; C, D: E2 group. A: Negative immunoreaction for leptin receptor in villus epithelial cells (arrow) and leptin receptor immunoreaction in connective tissue cells (arrowhead); B: Weak leptin receptor immunoreaction in crypt epithelial cells (arrow); C: Moderate leptin receptor immunoreaction in villus epithelial cells (arrow) and leptin receptor immunoreaction in connective tissue cells (arrowhead); D: Moderate leptin receptor immunoreaction in crypt epithelial cells (arrow) Bar =100 μm.

Figure 7. The leptin receptor immunoreaction in the ileum at the 18th hour. A, B: Control group; C, D: E2 group. A: Negative immunoreaction for the leptin receptor in villus epithelial cells (arrow) and the leptin receptor immunoreaction in Goblet cells (arrowhead); B: Negative immunoreaction for the leptin receptor in crypt epithelial cells (arrow) and the leptin receptor immunoreaction in Goblet cells (arrowhead); C: Moderate leptin receptor immunoreaction in crypt epithelial cells (arrow) and the leptin receptor immunoreaction in Goblet cells (arrowhead); D: Moderate leptin receptor immunoreaction in crypt epithelial cells (arrow) and the leptin receptor immunoreaction in Goblet cells (arrowhead) Bar =100 μm.

### Ileum

A negative reaction was observed in the villi and the crypt epithelial cells of the ileum at the 18th hour of the control group (Figure 7 A, B). In the estrogen group, a moderate reaction in was observed in the villus epithelial cells, while a weak immunoreaction was detected in the crypt epithelium cells in general, and a moderate immunoreaction was seen in some epithelial cells at the bottom (Figure 7 C, D).

In the ileum tissue, an immunoreaction ranging from negative to weak was observed in the villus epithelial cells of the control and estrogen groups at the 90th hour. In the control and estrogen groups, a moderate to severe reaction was detected only in the epithelial cells at the bottom of the crypts (Figure 8).

In the control group, the villus epithelial cells of the ileum tissue showed a weak immunoreaction but, in some samples, the reaction was moderate at the 162nd hour, while a very weak immunoreaction was detected in the crypt epithelium (Figure 9 A, B). In the estrogen group, the immunoreaction in the villus epithelial cells was either negative or very weak, while a moderate immunoreaction was only detected in epithelial cells lining the bottom parts of the crypts (Figure 9 C, D).
Effect of E2 on Leptin Receptor in the Small Intestine
In the ovariectomized rats, E2 administration increased the leptin receptor expression on the epithelium of villi and crypt in duodenum (villi, C90: 0.00±0.00 and E290: 0.80±0.20; crypt, C90: 0.33±0.33 and E290: 1.90±0.40) and jejunum (villi, C90: 0.50±0.28 and E290: 1.70±0.30; crypt, C90: 0.62±0.23 and E290: 2.00±0.31) at 90 hours (p<0.05) (Table 2,3); and ileum (villi, 0.00±0.00 and 2.00±0.40; crypt, C18: 0.00±0.00 and E218: 1.50±0.28) at 18 hours (p<0.05) (Table 4); and also, on the epithelium of villi in duodenum (C162: 0.25±0.25 and E2162: 1.00±0.00) at 162 hours (p<0.05) (Table 2, 3, 4).

Table 2: Effect of E2 on duodenum leptin receptor expression at 18th, 90th and 162nd hours in ovariectomized (Ovx) rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Villi</th>
<th>Crypt</th>
<th>Brunner’s gland</th>
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<tbody>
<tr>
<td></td>
<td>0.00±0.00</td>
<td>0.83±0.60</td>
<td>1.50±0.28</td>
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<tr>
<td>C18</td>
<td>0.66±0.33</td>
<td>2.16±0.60</td>
<td>1.66±0.33</td>
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<tr>
<td>E218</td>
<td>0.217</td>
<td>0.127</td>
<td>0.637</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.00±0.00</td>
<td>0.33±0.33</td>
<td>1.50±0.28</td>
</tr>
<tr>
<td>C90</td>
<td>0.80±0.20</td>
<td>1.90±0.40</td>
<td>1.50±0.50</td>
</tr>
<tr>
<td>E290</td>
<td>0.040*</td>
<td>0.046*</td>
<td>0.817</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.25±0.25</td>
<td>0.50±0.28</td>
<td>1.50±0.28</td>
</tr>
<tr>
<td>C162</td>
<td>1.00±0.00</td>
<td>1.10±0.10</td>
<td>1.87±0.12</td>
</tr>
<tr>
<td>E2162</td>
<td>0.025*</td>
<td>0.079</td>
<td>0.317</td>
</tr>
</tbody>
</table>

Statistical differences were analyzed with a Mann–Whitney U-test(* P<0.05).
In this study, 17β-estradiol is the most abundant and the most effective (39). In addition, in studies on estrogen, it has been determined that the effects of estrogen in tissues occur approximately in the 18th hour (40). In light of these reports, in this study, 17β-estradiol was administered in ovariectomized rats at a dose of 25 μg/rat/day, and at the same time, to determine the effect of the application time, leptin receptor expressions in the small intestines were observed at the 18th, 90th and 162nd hours.

Leptin is secreted by the chief, parietal, and endocrine cells of the stomach and binds with the leptin receptor that is localized in different parts of the small intestine. Immunohistochemical techniques could be used to confirm the presence of leptin receptors in the mucosa of small intestinal segments. In our study, only intracytoplasmic staining was observed in enterocytes of both villi and crypts by immunohistochemistry. In previous studies, leptin receptors especially had been reported in apical and basolateral membranes (12, 19, 41), in addition to Barrenetxe et al.’s work which showed a lining of intracytoplasmic staining in the intestinal villi and crypts (19).

To investigate the impact of estrogen hormone on the leptin receptor, we employed an experimental model of ovariectomized rats. Ovariectomized rat model is widely used in experimental studies on female sex hormones. Although, more than 20 estrogens have been identified in mammals, estradiol is the most abundant and the most effective (39). In addition, in studies on estrogen, it has been determined that the effects of estrogen in tissues occur approximately in the 18th hour (40). In light of these reports, in this study, 17β-estradiol was administered in ovariectomized rats at a dose of 25 μg/rat/day, and at the same time, to determine the effect of the application time, leptin receptor expressions in the small intestines were observed at the 18th, 90th and 162nd hours.

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Our study detected only intracytoplasmic expression, not membranous expression in villi and crypt epithelium. This case results from after leptin binding to receptors, endocytosis, and vesicle traffic in the cell. Nevertheless, variations in fixatives, species, and antibodies are also crucial factors that can contribute to disparities between different studies. For instance, Barrenetxe et al. observed divergent leptin receptor expressions within the same species, and the fixatives used for tissue fixation varied. Additionally, the utilization of antibodies targeting either the N or C-terminal leptin receptor is a pivotal factor that may also lead to discrepancies. Contrary to our findings, despite using the same primary antibody as our study, Buyse et al., reported discrepancies. Contrary to our findings, despite using the same primary antibody as our study, Buyse et al., reported leptin expressions in the apical membranes in frozen sections (17). It is assumed that this localisation difference might have arisen due to using paraffin or frozen sections.

In this study, the reaction was strong at the deeper layer of crypts but was weaker in villi and the upper layer of crypts. It is known that the deeper layer of crypts contains the proliferative matrix cells. This may suggest that leptin could also be a proliferation factor in the small intestine. Alavi et al. also suggested that leptin may be a growth factor for the small intestine. On the contrary, it was claimed by other researchers that leptin has no effect on proliferation in small intestine and colon crypts (42).

### Table 3: Effect of E2 on jejunum leptin receptor expression at 18th, 90th and 162nd hours in ovariectomized (Ovx) rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Villi</th>
<th>Crypt</th>
</tr>
</thead>
<tbody>
<tr>
<td>C18</td>
<td>0.00 ± 0.00</td>
<td>0.33 ± 0.33</td>
</tr>
<tr>
<td>E218</td>
<td>0.00 ± 0.00</td>
<td>1.33 ± 0.33</td>
</tr>
<tr>
<td>P</td>
<td>1.000</td>
<td>0.099</td>
</tr>
<tr>
<td>C90</td>
<td>0.50 ± 0.28</td>
<td>0.62 ± 0.23</td>
</tr>
<tr>
<td>E290</td>
<td>1.70 ± 0.30</td>
<td>2.00 ± 0.31</td>
</tr>
<tr>
<td>P</td>
<td>0.039*</td>
<td>0.022*</td>
</tr>
<tr>
<td>C162</td>
<td>0.00±0.00</td>
<td>0.75 ± 0.25</td>
</tr>
<tr>
<td>E2162</td>
<td>1.60 ± 0.40</td>
<td>1.70 ± 0.30</td>
</tr>
<tr>
<td>P</td>
<td>0.180</td>
<td>0.059</td>
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</table>

Statistical differences were analyzed with a Mann–Whitney U-test (* P<0.05).

### Table 4: The effect of E2 on ileum leptin receptor expression at 18th, 90th and 162nd hours in ovariectomized (Ovx) rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Villi</th>
<th>Crypt</th>
</tr>
</thead>
<tbody>
<tr>
<td>C18</td>
<td>0.00 ± 0.00</td>
<td>0.00±0.00</td>
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<tr>
<td>E218</td>
<td>2.00±0.40</td>
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<tr>
<td>P</td>
<td>0.026*</td>
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<tr>
<td>C90</td>
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<tr>
<td>E290</td>
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<tr>
<td>P</td>
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<tr>
<td>C162</td>
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<tr>
<td>E2162</td>
<td>0.33±0.33</td>
<td>2.00±0.00</td>
</tr>
<tr>
<td>P</td>
<td>0.138</td>
<td>0.128</td>
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Statistical differences were analyzed with a Mann–Whitney U-test(* P<0.05).

### DISCUSSION

To investigate the impact of estrogen hormone on the leptin receptor, we employed an experimental model of ovariectomized rats. Ovariectomized rat model is widely used in experimental studies on female sex hormones. Although, more than 20 estrogens have been identified in mammals, estradiol is the most abundant and the most effective (39). In studies on both leptin hormone and other agents in ovariectomized rats, 10-50 μg/rat/day doses of 17-B estradiol were used. (25, 26, 36). In addition, in studies on estrogen, it has been determined that the effects of estrogen in tissues occur approximately in the 18th hour (40). In light of these reports, in this study, 17β-estradiol was administered in ovariectomized rats at a dose of 25 μg/rat/day, and at the same time, to determine the effect of the application time, leptin receptor expressions in the small intestines were observed at the 18th, 90th and 162nd hours.

Leptin is secreted by the chief, parietal, and endocrine cells of the stomach and binds with the leptin receptor that is localized in different parts of the small intestine. Immunohistochemical techniques could be used to confirm the presence of leptin receptors in the mucosa of small intestinal segments. In our study, only intracytoplasmic staining was observed in enterocytes of both villi and crypts by immunohistochemistry. In previous studies, leptin receptors especially had been reported in apical and basolateral membranes (12, 19, 41), in addition to Barrenetxe et al.’s work which showed a lining of intracytoplasmic staining in the intestinal villi and crypts (19).
Leptin receptor expression was showed in Brunner’s glands of the duodenum and goblet cells of the intestine. In addition, epidermal growth factor, one of the important growth factors, is produced in Brunner's glands (43). Leptin and the epidermal growth factor in Brunner’s glands can regulate proliferation and nutritional status. Studies in the colon showed that luminal leptin increases mucin secretion from goblet cells (44, 45). It has been emphasized that the effect of leptin on mucus secretion is initiated by binding to leptin receptors expressed in the apical and basolateral parts of the colonic cell membranes of luminal and systemic leptin (44). Our data showed that the increase of mucin secretion is not only related to leptin receptors in enterocytes, but leptin receptors expressed in goblet cells also may regulate mucin secretion.

We observed positive reactions in a few connective tissue cells mainly in the jejumum. Leptin is found in immune cells like granulocytes and macrophages in lamina propria of rats jejumum (15). Therefore, leptin may have a role in immune function in the mucosa of the small intestine.

Hypothalamus has a significant role in revealing the specific effects of leptin. Previously, leptin receptors were localized in estrogen receptors α and β (ER α and β) expressing cells in the hypothalamus, and it was suggested that these receptors were acting in coordination (26). Leptin receptor’s colocalization with ER α is reported to be 100% but with ER β the colocalization rates were found only 15% in dorsal root ganglion neurons. It is declared that estrogen increases leptin receptor mRNA expressions through ER α mediation (25). Estrogen receptors are known to exist in small intestinal epithelial cells. This data suggests that estrogen may also affect the leptin receptor expression of small intestinal epithelial cells. (46). In chicken, cOb-R mRNA did not change during sexual maturation in most tissues whereas mRNA levels increase in the intestine. However, estrogen treatment enhances Ob-R mRNA expressions in the intestine of chicken. In addition, while estrogen administration increases the expression of cOb-R mRNA in the intestines, it does not change its expression in other tissues. It has been claimed that estrogen is also associated with the regulation of leptin receptor expression in the poultry small intestine and that there may be a correlation between gonadal and metabolic functions (37).

In this study, we observed that estrogen causes an increase in leptin receptor expressions in all small intestine crypts and villi except at the 18th hour in the jejumum and 162nd hours in the ileum. We determined statistically significant increased reaction intensities between groups only crypt and villi of ileum at the 18th hours, jejumum and duodenum at the 90th hour, and villi of the duodenum at the 162nd hours. The fact that significant effects occurred earlier in the ileum than in other parts of the intestine suggests that estrogen may affect the intestinal parts differently. On the other hand, it appears that application times also play a role in the different effects of estrogen on the leptin receptor. Alonso et al. (2007) reported that the short-term administration of estrogen in adipocytes of ovariectomized rats initially reduced leptin receptor expression, followed by an increase in expression in the subsequent days, and long-term (chronic) administration ultimately led to a decrease in expression (24). In the short-term estrogen administration, we observed increased leptin receptors expressions in the ileum, and at 90th hour expression levels raised in the jejumum and duodenum. Estrogen can modulate the leptin receptors in the small intestine in a time and tissue-dependent way. Previous studies have also reported that the effects of estrogen vary depending on duration (46, 47, 48).

While estrogen significantly increased the expression of the leptin receptor in the villi and crypt earlier, these effects appeared later in the duodenum and jejumum.

In summary, E2 may upregulate the expression of leptin receptors in the small intestine, where glucose and other nutrients are absorbed after food intake and digestion, depending upon the time. The results of this study show that the effects of estrogenic agents on the leptin receptor in the small intestine vary according to intestinal segments and time-dependent

CONCLUSION

Our findings may offer a new field of investigation for the effects of estrogen on leptin and its receptor in the gastrointestinal tract. However, applications at different time intervals are needed to determine the time-dependent effects of estrogen on the regulation of leptin receptor expression in the small intestine.

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