EXPERIMENTAL STUDY

The effect of fundus resection on weight loss and ghrelin levels in rats

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ABSTRACT
BACKGROUND: Ghrelin is a 28-amino acid peptide isolated mainly from the oxyntic glands of the stomach, especially the fundus. Administration of ghrelin either centrally or peripherally increases food intake and body weight both in rodents and humans. This study evaluates the effects of fundus resection and sclerosing agent injection on ghrelin level and weight loss.

MATERIAL AND METHODS: Thirty rats were divided into three groups. In group 1, NaCl was injected into the submucosal space at the gastric fundus while in Group 2, a sclerosing agent was injected into the latter site. In group 3, gastric fundus was resected. Ghrelin levels and weight were recorded.

RESULTS: In group 1, rats continued gaining weight and ghrelin levels remained stable. In group 2, rats' weight and ghrelin levels stayed stable. In group 3, while weight stayed stable, ghrelin levels decreased significantly.

CONCLUSION: In rats, the resection of fundus stabilizes weight gain and decreases ghrelin levels. However, in sclerotherapy, although weight gain was stabilized, there was no decrease in ghrelin levels. In humans, the effect of fundus resection on weight gain can usher in a new era of investigation (Tab. 2, Ref. 16).

KEY WORDS: fundus resection, sclerotherapy, ghrelin.

Introduction
Ghrelin is a 28-amino acid peptide that is isolated mainly from the oxyntic glands of the stomach, especially the fundus (1). This peptide hormone is a natural ligand of the growth hormone (GH) secretagogue receptor and stimulates GH release and food intake. Ghrelin has orexigenic effects and is associated with several body systems. Ghrelin administration, either centrally or peripherally, increases food intake and body weight both in rodents and humans (2). These effects of ghrelin are independent of GH stimulation and are mediated by modulating the expression of various hypothalamic peptides via an interaction with the hypothalamic neuromodulator pathways. Ghrelin stimulates the activity of neurons expressing NPY, AgRP, and orexin. The appetite stimulation by peripheral ghrelin is probably due to its action via the afferent vagal nerve. Also, ghrelin is produced locally in the hypothalamus and it may directly affect various hypothalamic nuclei. This peptide hormone affects the regulation of energy balance (3, 4). The secretion of ghrelin by the stomach depends largely on the nutritional state. Ghrelin levels show a diurnal variation; pre-prandial increases and postprandial decreases. In addition, ghrelin levels seem to be influenced by age, gender, BMI, growth hormone, glucose, and insulin (4, 5).

Reports showed that in rats and humans, weight is lost and ghrelin levels are decreased after sleeve gastrectomy (6, 7, 8). This study evaluates the effects of fundus resection and sclerotherapy on ghrelin level and weight loss.

Material and method
Ethical committee approval was obtained from Selçuk University Application and Research Center for Experimental Medicine Ethical Committee. Thirty Wistar albino rats of 250–350 g of weight, 4–6 months of age were divided into three groups as 10 rats in each group. Oral intake of the rats was stopped one day before the procedure. The operation was performed under general anesthesia by giving intraperitoneal ketamine hydrochloride (80 mg/kg) (Ketalar, Pfizer Ilac, Istanbul, Turkey) and xylazine (10 mg/kg) (Rompun, Bayer, Germany). The area was cleaned with povidone iodine after shaving the hair. In all rats, laparotomy was performed with an incision of 3 cm at the midline. Before the procedure, 0.5 ml of blood was obtained from all rats.

In group 1, after laparotomy, the stomach was opened on the anterior surface at the fundus-corpus junction. A volume of 1.5 ml of 0.9% NaCl solution was injected to submucosal space as to bloat the fundus. Then gastric wall was repaired by 4/0 silk suture (Dogsan, Trabzon, Turkey) and abdominal wall was closed by 3/0 silk suture.

In group 2, the same procedure was performed. The only difference was that instead of NaCl solution, 1 cc solution of 1%
polydocanol (Aethoxysklerol 1%, Cem Farma Lap İlac Medikal End Tic ve Paz Ltd Şti, Turkey) was used as a sclerosing agent.

In group 3, after laparotomy, gastric fundus was resected by LigaSure (Force Triad Energy Platform, Covidien, USA) LS1200 probe. The remaining gastric wall was repaired by 4/0 silk suture and the abdominal wall was closed in the same way as in the other groups.

After the procedures, the rats were put into cages and they were given standard food. Rats were weighted once a week. At the 4th week, 0.5 ml blood sample was obtained under general anesthesia and rats were decapitated.

Blood samples were centrifuged at 3,200 rpm for 10 minutes (Nüve-NF 1000 R). Serum samples were stored at −80 degrees Celsius. At the end of the study all ghrelin levels were measured by commercially available rat ghrelin kit (Ghrelin Rat Kit, Phoenix Pharmaceuticals Inc, USA). Results were given in pg/ml.

In statistical analysis IBM SPSS version 20 statistical package was used. In quantitative variables student T test, one-way ANOVA and in subgroup analysis, Tukey’s multiple comparison tests were used. Statistical significance was accepted at the level of 0.05.

### Findings

In group 1 (control group), at weekly weights, rats’ mean weight was significantly higher than in the previous week (p < 0.05). Rats continuously gained weight every week. Moreover, ghrelin levels did not change at the 4th week (p > 0.05). Rats’ weights and ghrelin levels are given in Tables 1 and 2, respectively.

In group 2 (sclerotherapy group), the rats stopped gaining weight at the second week and their weight stayed stable at the end of the 4th week (p > 0.05). Likewise, their ghrelin levels stayed stable (p > 0.05).

In group 3 (resection group), there was a small weight loss at every week. However, this loss was not statistically significant (p > 0.05). Although, rats’ weight stayed stable during the study, their ghrelin levels decreased significantly at the end of the 4th week (p < 0.05).

### Discussion

As it has a positive relation to mortality, obesity is one of the most important healthcare problems in developed countries (9). Leptin, secreted from adipose tissue, has an anorectic effect on hypothalamus, whereas ghrelin has an orexigenic effect (4).

### Tab. 1. Weekly weight gain according to index weight.

| Group 1 | Weight* | p*** |
|---------|---------|------|
| 1st week | 233.14±27.9 | 0.004 |
| 2nd week | 239.14±29.1 | 0.001 |
| 3rd week | 248.43±27.8 | 0.001 |
| 4th week | 257.42±30.4 | 0.001 |
| 5th week | 267.56±25.6 | 0.001 |

| Group 2 | Weight*** | p*** |
|---------|----------|------|
| 1st week | 257.77±43.9 | 0.011 |
| 2nd week | 262.62±43.6 | 0.003 |
| 3rd week | 266.74±46.1 | 0.026 |
| 4th week | 265.77±43.5 | 0.244 |
| 5th week | 263.48±43.6 | 0.24 |

| Group 3 | weight | p*** |
|---------|--------|------|
| 1st week | 315.4±43.5 | 0.111 |
| 2nd week | 311.79±41.8 | 0.851 |
| 3rd week | 314.81±42 | 0.623 |
| 4th week | 313.25±40 | 0.161 |
| 5th week | 306.27±38.5 | 0.161 |

* weight is given in grams ± standard deviation, ** One-way ANOVA

Ghrelin is secreted as preproghrelin (117 aa) and transformed into 28 aa ghrelin. Its halflife is 60 minutes and it is transformed from active N-octanoyl into inactive desacyl N-octanoyl form by plasma esterase.

Several studies showed that ghrelin promotes growth hormone release, stimulates appetite and has several other effects on several systems. Briefly, ghrelin hormone is a main factor in the regulation of feeding behavior and energy homeostasis (3, 10).

Ghrelin secreted from several tissues like placenta, testis, kidney, brain and intestines. However, majority of ghrelin (90 %) is secreted from the gastric fundus (3). In our study, sclerotherapy caused a little and non-significant decrease in ghrelin levels. A significant ghrelin decrease could only be achieved by fundus resection.

In histopathological examination of sleeve gastrectomy materials, Gündogan et al reported that fundus and proximal corpus contains significantly more ghrelin immunopositive cells compared to distal corpus (11). Additionally, the number of Ghrelin immunopositive cells in the gastric mucosa in females was significantly higher compared to males.

Tsoli et al reported sleeve gastrectomy and biliopancreatic diversion procedures to be similar in terms of weight loss and recovery in types 2 diabetes. Although, there was no decrease in ghrelin levels in the diversion group, ghrelin levels decreased significantly in sleeve gastrectomy group (6). Moreover, other studies show a significant decrease in ghrelin levels in gastric resections including fundus (12). In patients with total gastrectomy, ghrelin levels found to be 50 % lower than in healthy individuals (13).

Those reports show that to decrease ghrelin level, the fundus resection is a must. In our study, sclerotherapy has no significant effect, neither on weight gain nor on ghrelin level.

Wren et al evaluated food intake and weight gain on rats after administration of ghrelin (2). After chronic systemic or intracerebroventricular administration of ghrelin for 7 days, cumulative food intake was increased. Also ghrelin administration was associated with excess weight gain and adiposity. These findings are in keeping with the physiological role for circulating ghrelin in the regulation of food intake. As such, ghrelin would be an important

### Tab. 2. Ghrelin levels.

| Group   | Before the procedure | 4th week | p  |
|---------|---------------------|----------|----|
| Group 1 | 0.8643              | 0.8884   | 0.343 |
| Group 2 | 0.8589              | 0.7346   | 0.392 |
| Group 3 | 0.8015              | 0.2456   | 0.008 |

*Student T test, Ghrelin levels are given in pg/ml
new target for the development of treatments for obesity. Ghrelin administration induces dose-dependent contractions in the antrum and increases the motility in rodents and humans (14, 15).

Also the potential of the ghrelin system as a therapeutic target for obesity treatment is still under discussion. As it has been demonstrated that circulating ghrelin levels increase when obese humans lose weight, and because obese mice show an increase in sensitivity to ghrelin upon weight loss, the blockage of ghrelin could prevent weight regain after weight loss (15). In a recent study with rats, it was demonstrated that anti-ghrelin (Spiegelmer NOX-B11) blocks the ghrelin-induced increase in food intake after ghrelin injection (16). This biological agent suppresses ghrelin-induced food intake and ghrelin-induced neuronal activation in the arcuate nucleus.

In rats, the resection of fundus stabilizes weight gain and decreases ghrelin levels. In sclerotherapy however, weight gain was stabilized despite the fact that ghrelin levels did not decrease. In humans, the effect of fundus resection on weight gain can usher in a new era of investigation.

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