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The impact of laparoscopic sleeve gastrectomy on plasma obestatin and ghrelin levels

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Abstract. – OBJECTIVE: To investigate the effect of laparoscopic sleeve gastrectomy (LSG) on the levels of obestatin and ghrelin hormones and body mass index (BMI) in morbidly obese patients.

PATIENTS AND METHODS: The study included 30 morbidly obese patients who had LSG. Five cc blood samples were taken from the patients preoperatively and at postoperative months 3 and 6. After serum extraction, the levels of obestatin and ghrelin hormones and the levels of fasting insulin and glucose were studied using the enzyme-linked immunosorbent assay (ELISA) method. The homeostatic model assessment of insulin resistance (HOMA-IR) score was calculated. Preoperative and postoperative 3- and 6-month BMI were calculated. Kruskal-Wallis Analysis of Variance, Bonferroni-Dunn Test, Spearman's correlation test, and Pearson's correlation test were used for statistical analysis.

RESULTS: BMI of the patients were statistically significantly reduced at postoperative months 3 and 6 compared to preoperative values, and at postoperative month 3 compared to month 6 values ($p < 0.001$). Ghrelin values were higher at postoperative month 6 compared to the preoperative and postoperative month 3 values ($p < 0.001$). Obestatin values of the patients were lower at postoperative month 6 compared to the preoperative and postoperative month3 values ($p < 0.001$). Insulin and glucose values were statistically significantly lower at postoperative months 3 and 6 compared to preoperative values ($p < 0.001$), whereas there was no difference between months 3 and 6. HOMA-IR score was significantly lower at postoperative month 3 compared to preoperative values ($p < 0.001$).

CONCLUSIONS: LSG enables effective weight loss and glucose regulation in obese patients. LSG has also effects on obestatin and ghrelin hormones, which are coded by the same gene and have opposing effects, and the associated mechanisms of which are still controversial. Obestatin produces a feeling of satiety, whereas ghrelin initiates eating by producing a feeling of hunger. The patients were observed to have increased ghrelin and reduced obestatin postoperatively due to a negative energy balance.

Key Words:
Body mass index, Gastric sleeve gastrectomy, Morbid obesity, Ghrelin, Obestatin, insulin, Glucose.

Introduction

Obesity is a serious chronic disease affecting public health, which develops by the shifted balance of energy intake and consumption toward intake in the human body. It is a complex endocrine and metabolism disorder associated with mortality and morbidity, increasing every day. Medical and surgical methods are used for its treatment[1]. Excess energy intake results in excess fat accumulation in the body and this leads to several comorbidities such as type 2 diabetes, arterial hypertension, cardiovascular diseases, dyslipidemia, fatty liver, depression, and gastrointestinal malignancies. The World Health Organization classified obesity defined body mass index (BMI) as a value derived by dividing weight in kilogram by the square of height in meters (kg/m^2) and classified individuals with a BMI value < 18.5 kg/m^2 as thin; individuals with a BMI between 18.5-24.9 kg/m^2 as normal; individuals with a BMI between 25-29.9 kg/m^2 as overweight; individuals with a BMI between 30-34.9 kg/m^2 as obese (class 1); individuals with a BMI between 35-39.9 kg/m^2 as obese (class 2); and individuals with a BMI > 40 kg/m^2 as morbidly obese (class 3)[2] . For obesity treatment, lifestyle should be organized and the balance between food intake and physical activity should be ensured. As of the late 1990s, surgical treatment (bariatric/metabolic surgery) has become effectively used in obesity treatment[3].

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Ghrelin is a 28-amino acid lipopeptide gut-brain hormone, which was first identified from rat and human stomachs in 1999[4]. It is also synthesized in the duodenum, hypothalamus, pituitary, endocrine pancreatic islets, bowel, breast, kidney, heart, and testicles. It is known as the appetizing hormone and it has the ability to bind to the growth hormone secretory receptors[5,6]. Ghrelin has been shown to have anti-inflammatory effects and to inhibit the production of pro-inflammatory cytokines[7]. It reduces blood pressure through its protective effects on endothelial functions. Some studies reported that obesity is related to low levels of circulating ghrelin.

Obestatin is a 23-amino acid anorectic peptide, which is coded by the same gene as ghrelin and released by the stomach. The new appetite hormone, which was first discovered by Zhang et al[8] from Stanford University, is called obestatin[9]. It is also released by the small bowel, hypothalamus, spleen, and breast tissues. Obestatin has anorectic effects and decelerates gastric emptying. It opposes the effect of ghrelin and produces a feeling of satiety while eating. It is believed that ghrelin initiates eating, whereas obestatin suppresses appetite. There are studies reporting low obestatin levels in obese patients and a negative correlation with BMI, insulin, and glucose values[10].

Ghrelin and obestatin are hormones originating from the same precursor. They have opposing effects and even minor changes in their levels play a major role in energy homeostasis and fat accumulation. Bariatric surgery has important effects on the plasma levels of ghrelin and obestatin hormones. Such hormonal effects play a great role in weight loss and the long-term maintenance of obesity surgery.

The present study aims to examine the changes in obestatin and ghrelin hormones in obese patients undergoing laparoscopic sleeve gastrectomy.

Patients and Methods

The present study included 30 patients (mean BMI = 49.30 ± 7.92) who underwent LSG with the diagnosis of morbid obesity in Antalya Training and Research Hospital General Surgery clinic between January 2014 and December 2014.

The approval for the study protocol was obtained from Antalya Training and Research Hospital Ethics Committee (dated 10/10/2013, No: 26/05). The study was conducted in accordance with the Declaration of Helsinki and all study participants provided written informed consent before the study.

Five cc blood samples were taken from the patients preoperatively and at postoperative months 3 and 6. The WHO formula (kg/m^2) was used to calculate BMIs of the patients. After serum extraction of the blood samples, the levels of obestatin and ghrelin hormones and the levels of fasting insulin and glucose were studied using the enzyme-linked immunosorbent assay (ELISA) method. The HOMA-IR score was calculated. The inclusion criteria were a primary diagnosis of morbid obesity, primary treatment of LSG for morbid obesity, and age ≥ 18. The exclusion criteria were previous bariatric surgery, history of mental impairment, drug or alcohol addiction, recent major vascular event, and/or malignancy.

LSG- Surgical Method

The patient was taken onto the operating table in supine position. The legs were then positioned to be separated from each other. The patients were anti-embolic stockings and a Foley catheter was inserted. The surgeon was situated between the patient's legs with the assistant surgeon on the left and the assistant cameraman on the right side. Five trocars were inserted. Then, the pneumoperitoneum was accessed. A laparoscopic vessel sealer, laparoscopic Ligasure (Covidien, Minneapolis, MN, USA), was used to separate the angle and proximal stomach from the upper section of the spleen. The omental bursa was accessed by dividing the short vessels located on the great gastric curvature. Subsequently, a 32-French orogastric tube was advanced through the mouth and placed in the small gastric curvature. Since the distal part of the stomach was thick, the stomach was split in the direction of the gastroesophageal junction using a different kind of staplers. Tisseel (Baxter International, Inc.) fibrin sealant was used to cover the entire stapler line to the distal part of the stomach. The stomach that was resected was taken off from the trocar hole in the size of 12 mm. Finally, saline was used to clean out the entire stapler line and the remainder fluid was aspirated. 3-0 prolene was used to suture the skin.

Measurement of Ghrelin and Obestatin

Measurements were made by taking blood samples preoperatively and at postoperative months 3

and 6. Blood samples were kept at 25°C for minimum 30 minutes for clotting and then centrifuged at 4°C at 2500 rpm for 15 minutes for serum separation. After aliquoting, the serum samples were kept at -80°C until the obestatin test. A commercial ELISA kit (RayBiotech, Norcross, GA, USA; catalogue no. EIA-OBS) was used to measure obestatin and ghrelin levels in accordance with the instructions provided by the manufacturer. For obestatin, the minimum detectable concentration was 0.1 pg/ml and the test range was 0.1-1,000 pg/ml. For ghrelin, these figures were 0.16 pg/ml and 0.1-1,000 ng/ml, respectively. The inter-test variance coefficient was < 15% and the intra-test variance coefficient was < 10%.

The hexokinase method was used to measure the glucose levels with a commercial kit (Beckman AU5800; Beckman Coulter Diagnostics, Fullerton, CA, USA). Insulin was measured with a chemiluminescent assay (AccessDxI800; Beckman Coulter Diagnostics, Fullerton, CA, USA). HOMA-IR was determined via the following formula: fasting glucose (mmol/L) × fasting insulin (IU/mL)/22.5[11].

Statistical Analysis

Descriptive statistics were expressed in frequency, percentage, mean, standard deviation (SD), median, minimum, and maximum values. The statistical analysis results were presented using frequency, percentage, mean, standard deviation (SD), median, minimum, and maximum values. Normal distribution was determined using Shapiro-Wilk test due to the small sample size (n < 50). Non-normally distributed data were analyzed using the Friedman test in each measurement. Normally-distributed data were analyzed using ANOVA in repeated measures. When there was a significant difference between measurements, post hoc tests, such as the Bonferroni-Dunn test (for nonparametric tests) or Bonferroni test and Fisher's least significant difference test LSD (for parametric tests) tests, were used for paired comparisons. Spearman's correlation test was used to establish the correlations between continuous variables that were non-normally distributed, whereas Pearson's correlation test was used to establish the correlations between those with a normal distribution. $p < 0.05$ was considered significant. SPSS 22.0 package (SPSS, Inc., Chicago, IL, USA) was used for all statistical analyses.

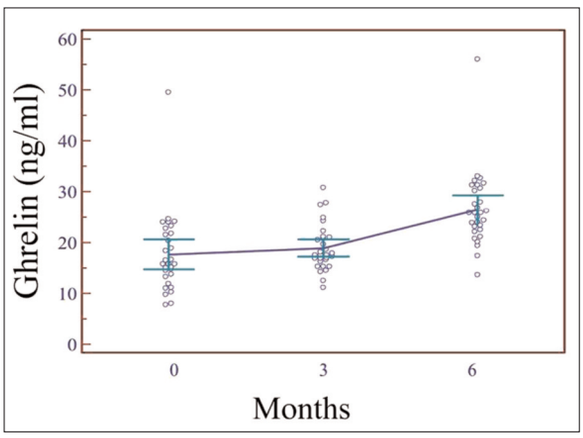

Figure 2. Preoperative, postoperative month 3, and postoperative month 6 plasma Ghrelin levels.

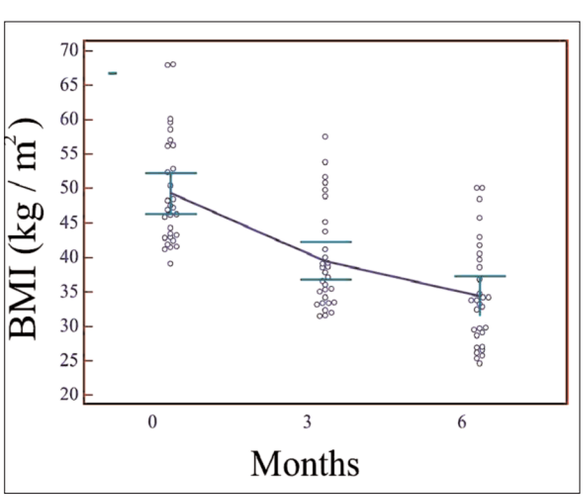

Figure 1. Preoperative, postoperative month 3, and postopertive month 6 BMI values.

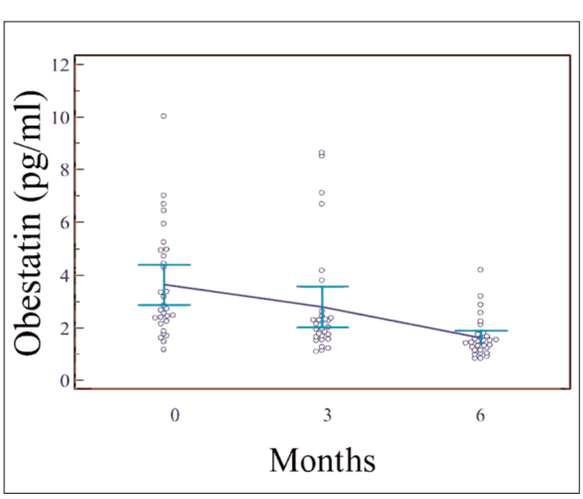

Figure 3. Preoperative, postoperative month 3, and postoperative month 6 plasma Obestatin levels.

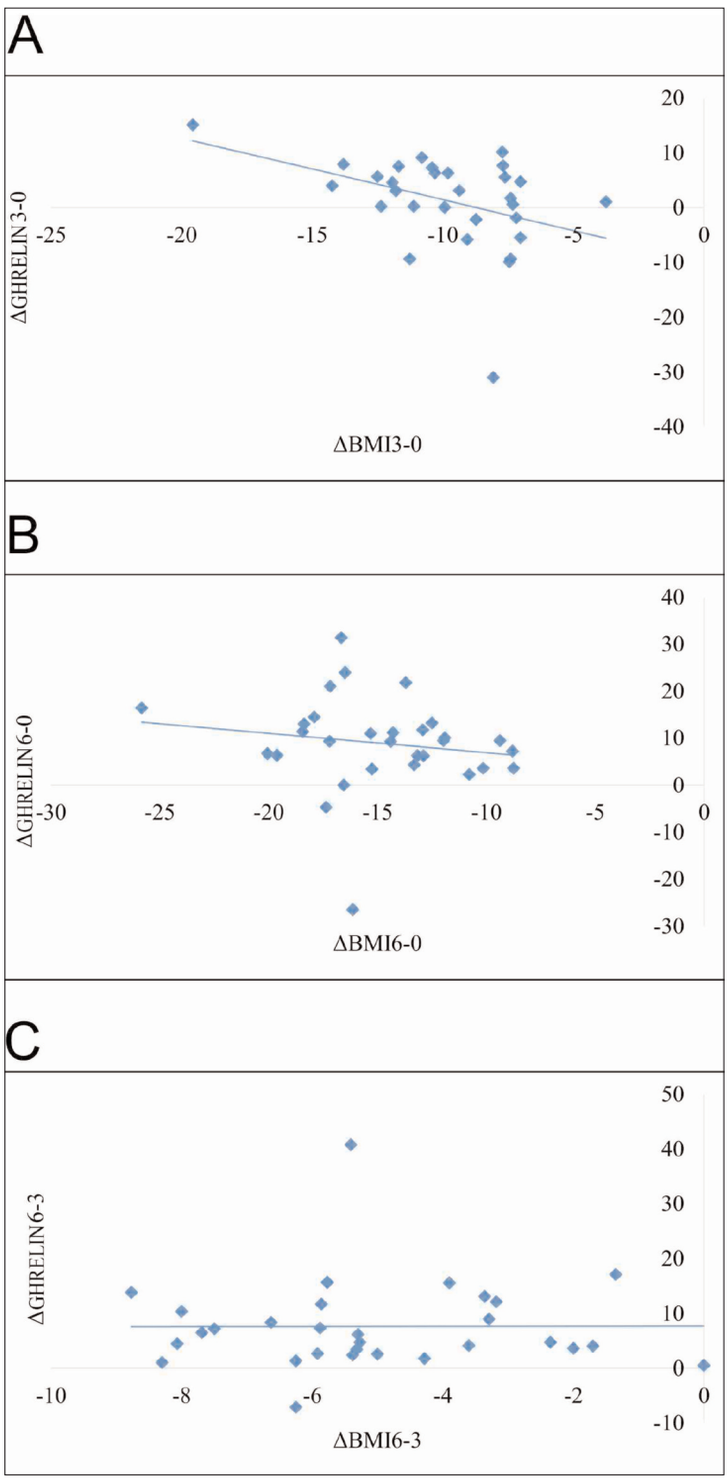

Figure 4. ***A,*** Sequential changes in plasma Ghrelin levels and BMI between preoperative (*baseline*) and postoperative month 3 values. ***B,*** Sequential changes in plasma Ghrelin and BMI between preoperative and postoperative month 6 month values. ***C,*** Sequential changes in plasma Ghrelin levels and BMI between postoperative month 3 and postoperative month 6 month values. Δ: change; ΔGHRELIN3-0: difference between postoperative month 3 and preoperative Ghrelin levels; ΔGHRELIN6-0: difference between postoperative month 6 and preoperative Ghrelin levels; ΔGHRELIN6-3: difference between postoperative month 6 and postoperative month 3 Ghrelin levels; ΔBMI3-0: difference between postoperative month 3 and preoperative BMI; ΔBMI6-0: difference between postoperative month 6 and preoperative BMI; ΔBMI6-3: difference between postoperative month 6 and postoperatıve month 3 BMI.

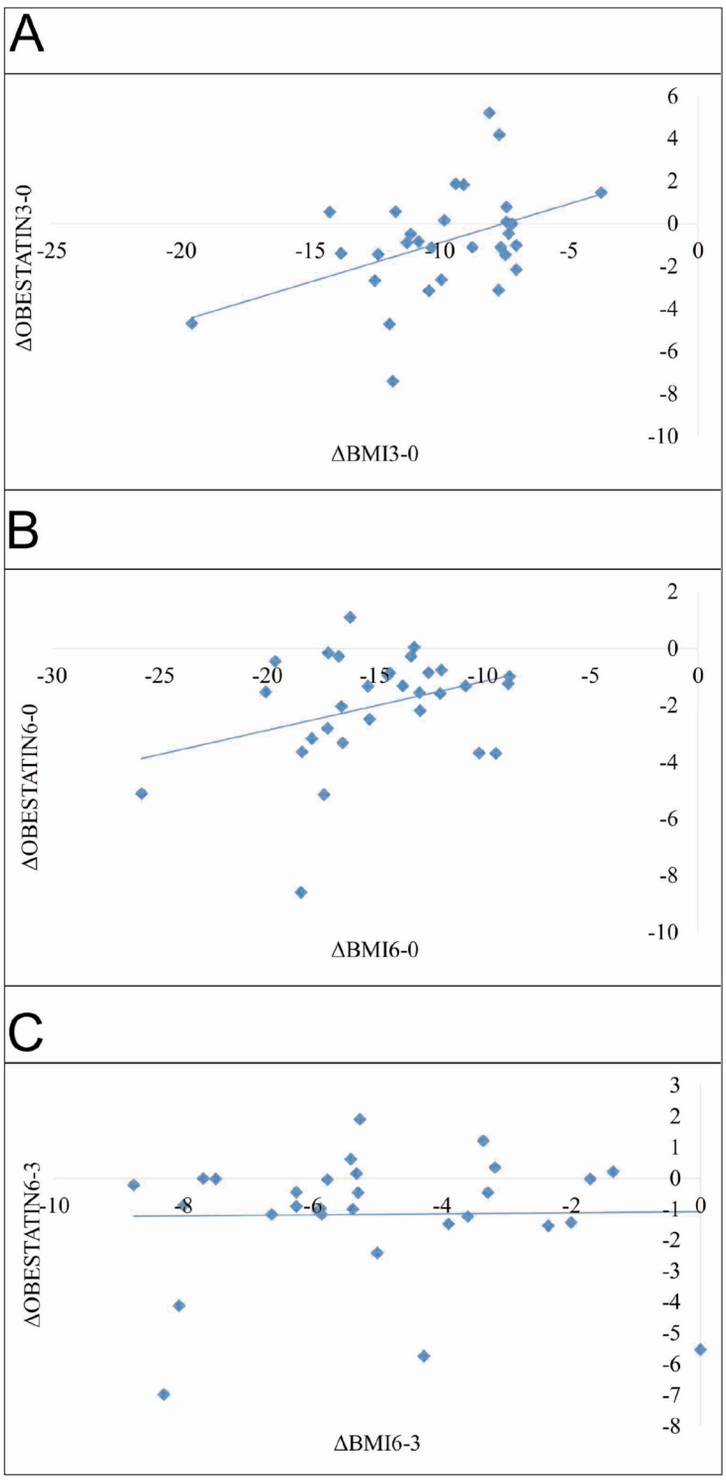

Figure 5. ***A,*** Sequential changes in plasma Obestatin levels and BMI between preoperative (*baseline*) and postoperative month 3 values. ***B,*** Sequential changes in plasma Obestatin and BMI between preoperative and postoperative month 6 month values. ***C,*** Sequential changes in plasma Obestatin levels and BMI between postoperative month 3 and postoperative month 6 month values. Δ: change; ΔOBESTATIN3-0: difference between postoperative month 3 and preoperative Obestatin levels; ΔOBESTATIN6-0: difference between postoperative month 6 and preoperative Obestatin levels; ΔOBESTATIN6-3: difference between postoperative month 6 and postoperative month 3 Obestatin levels; ΔBMI3-0: difference between postoperative month 3 and preoperative BMI; ΔBMI6-0: difference between postoperative month 6 and preoperative BMI; ΔBMI6-3: difference between postoperative month 6 and postoperatıve month3 BMI.

Results

The mean age of 30 morbidly obese patients was 41.23 ± 10.37 years. BMI (kg/m^2) was 49.30 ± 7.92 preoperatively, 39.48 ± 7.32 at postoperative month 3, and 34.39 ± 7.56 at postoperative month 6. BMI statistically significantly decreased at postoperative months 3 and 6 compared to preoperative values, and at postoperative month 3 compared to month 6 values ($p < 0.001$). Ghrelin (ng/ml) was 17.62 ± 7.86 preoperatively, 18.87 ± 4.56 at postoperative month 3, and 26.50 ± 7.38 at postoperative month 6. Ghrelin values were higher at postoperative month 6 compared to the preoperative and postoperative month3 values ($p < 0.001$). Obestatin (pg/ml) was 3.63 ± 2.05 preoperatively, 2.79 ± 2.10 at postoperative month 3 and 1.63 ± 0.75 at postoperative month 6. Obestatin values were lower at postoperative month 6 compared to the preoperative and postoperative month3 values ($p < 0.001$) (Table I). Insulin and glucose values were statistically significantly lower at postoperative months 3 and 6 compared to preoperative values, whereas there was no difference between months 3 and 6. HOMA-IR score was significantly lower at postoperative month 3 compared to preoperative values ($p < 0.001$) (Table II).

Discussion

Obesity is a disease characterized by increased adipose tissue in the body, which develops due to several genetic, endocrine, and environmental factors[12]. Today, many researchers investigate how different surgical methods affect obesity and metabolic syndromes. Bariatric surgery reduces food intake functionally, but it may also cause hormonal changes and altered eating behaviors. Both ghrelin and obestatin are gastrointestinal peptides originating from a precursor hormone called preproghrelin[13]. Ghrelin is a fatty acid containing eight carbons, which undergoes a posttranslational modification caused by ghrelin-O-acyl-transferase (GOAT). The growth hormone secretagogue receptor is activated by and its impact on growth hormone release and food take are mediated by ghrelin acylation[14]. Ghrelin also has physiological effects on adipogenesis, gastrointestinal motility, glucose homeostasis, pancreatic and cardiovascular functions, cell proliferation, bone metabolism, and reproduction. It is involved in brain functions, memory and sleep regulation[15,16]. It has been demonstrated that intracerebroventricular injections induce growth hormone secretion in rodents[17]. The administration of ghrelin has been recently shown to trigger food intake by activating 5'-AMP-activated protein kinase (AMPK) in the hypothalamus[18].

In addition to acyl ghrelin, functional or nonfunctional molecules such as desacyl-ghrelin and obestatin are also encoded by the gene preproghrelin. Desacyl-ghrelin, the inactive form of ghrelin, has no endocrine effect on food intake, but it has been shown to have an impact on cell proliferation and adipogenesis. Acyl ghrelin, the active form, has an effect on food intake and growth hormone release through growth hormone secretagogue receptor[19]. Previous studies demonstrated that ghrelin transmits hunger signals to the central nervous system through vagal afferent and efferent fibers[20].

Obestatin is a hormone derived from the C-terminal portion of the precursor preproghrelin, which opposes the effect of ghrelin and produces a central feeling of satiety. It delays gastric emptying by inducing the vagal afferent fibers.

Table I. Preoperative and postoperative 3 and 6 months of BMI, ghrelin, and obestatin hormone levels.

	Mean	SD	p
Preoperatıve BMI (kg/m^2)	49.30	7.92	< 0.001$^{\#}$
BMI (3 months after surgery)	39.48	7.32	
BMI (6 months after surgery)	34.39	7.56	
Preoperatıve ghrelin level (ng/ml)	17.62	7.86	< 0.001$^{\#}$
Ghrelin level (3 months after surgery)	18.87	4.56	
Ghrelin level (6 months after surgery)	26.50	7.38	
Preoperative obestatin level (pg/ml)	3.63	2.05	< 0.001$^{\#}$
Obestatin level (3 months after surgery)	2.79	2.10	
Obestatin level (6 months after surgery)	1.63	.75	

BMI, body mass index; $^{\#}$Kruskal-Wallis variance analysis, $p < 0.05$.

Table II. Preoperative and postoperative 3 and 6 months of insulin, glucose and HOMA index levels.

	Mean	SD	p
Preoperative insulin level (mIU/mL)	17.32	19.00	< 0.001[#]
Insulin level (3 months after surgery	8.20	5.26	
Insulin level (6 months after surgery	8.09	7.13	
Preoperative glucose level (mg/dl)	109.70	42.75	< 0.001[#]
Glucose level (3 months after surgery)	73.77	13.98	
Glucose level (6 months after surgery)	76.37	12.83	
Preoperative HOMA Index	4.69	7.92	0.002[#]
HOMA (3 months after surgery)	1.53	1.22	
HOMA (6 months after surgery)	1.55	1.50	

[#]Kruskal-Wallis variance analysis, $p < 0.05$; HOMA.

Obestatin has been also reported to be involved in cell survival, the release of the pancreatic hormone, sleep regulation, memory, and anxiety[21,22]. The anorectic effect of obestatin was not observed in some studies, whereas it was found to block some of the ghrelin effects when applied together with ghrelin in rodents. The pharmacological interaction of two hormones at the hypothalamic level modulates food intake and growth hormone secretion. It was first believed that ghrelin and obestatin are present in the rat and mouse plasma at equal concentrations[23], whereas the subsequent studies using ultra-sensitive assay methods reported that obestatin has a lower concentration. The levels of circulating ghrelin are pre-prandially higher and post-prandially suppressed. Nutrition has a differential effect both on acyl and desacyl-ghrelin. After a 36-hour fast, acyl ghrelin levels drop down to the postprandial levels and desacyl-ghrelin levels rise almost to the peak (pre-prandial) levels; however, no change was observed in the total ghrelin levels[24]. Many studies demonstrated that obesity is associated with low total ghrelin levels. This, in fact, is the mechanism developed as adaptive to excess calorie. The absent is the desacyl form, and acyl ghrelin may be high, low or remain unchanged. Ghrelin levels were shown to be considerably lower compared to healthy and normal individuals, and to elevate in levels after weight loss[25]. Among obesity syndromes, Prader-Willi syndrome is characterized by hyperphagic behavior and associated with hyperghrelinemia. In other obesity syndromes, it is known that only acyl ghrelin is elevated. Today, the ghrelin/obestatin ratio is considered as an important marker for evaluating nutritional status[26].

Even minor changes in obestatin levels play a major role in energy homeostasis and fat accumulation[27]. Known as ghrelin-associated peptide, obestatin was found at low levels in obese male and female individuals in the study by Guo et al[28]. Recently, interaction with the glucagon-like peptide 1 receptor has been suggested, in line with obestatin-positive effects on glucose and lipid metabolism. In addition, obestatin displays a variety of cellular effects, by regulating metabolic cell functions, increasing cell survival and proliferation, and inhibiting apoptosis and inflammation in different cell types[29]. Anorexia nervosa patients were found to have high plasma levels of ghrelin[30]. It was shown that the plasma levels of both ghrelin and obestatin increased significantly and simultaneously upon weight loss after 6 months of lap-band surgery in patients who were morbidly obese[31]. Vicennati et al[32] reported higher obestatin and lower ghrelin levels in obese females compared to the control group and a negative correlation between ghrelin/ obestatin ratio and BMI. Although the effect of obestatin on the obesity development mechanism is still unknown, studies focus on the balance between ghrelin and obestatin and insulin resistance. Zou et al[33] showed increased ghrelin, obestatin, and ghrelin/obestatin ratio with weight loss in obese children.

Different bariatric surgical methods exhibit different effects on weight loss and hormone levels. Ghrelin and obestatin hormones were high, low, or unchanged in patients after bariatric surgery in different studies. Hormonal functions are very important for maintenance after weight loss. No correlation has been found between weight loss and appetite hormone levels in obese patients. Hormone levels are jointly affected by surgical technique, postoperative follow-up duration, weight loss amount, and body energy homeostasis. Zhou et al[34] showed in an obese rat mod-

el that ghrelin increased after month 3 of LSG and in the early postoperative period in the gastric bypass group, while obestatin increased at various degrees. The postoperative elevation in ghrelin is believed to occur with increased hormone production specifically from extra-gastric sources producing ghrelin as a compensatory mechanism secondary to weight loss[35].

Although the gastric fundus producing ghrelin is removed in LSG, the bioactive form acyl ghrelin decreases by only 20-30%. Thus, the main source of the circulating acyl-ghrelin is likely to be the duodenum, although the gastric fundus contains most of the cells producing ghrelin. It is believed that the ghrelin production by the duodenum increases as compensatory to the increased gastric emptying into the duodenum and the occurring negative energy homeostasis[36,37]. Ghrelin secretion is mainly regulated by metabolic signals and, in turn, the modulatory action of ghrelin on the control of food intake and energy metabolism seems to be among its most important biological actions[38]. Hady et al[39] demonstrated that ghrelin decreases after LSG in obese patients, but the ghrelin level starts to increase after month 6 in a compensatory way. They established a statistically significant improvement in the postoperative levels of insulin, glucose, and HOMA-IR scores compared to the preoperative levels. LSG provides the control of long-term weight loss in obese patients, as well as improves many comorbidities caused by obesity, such as insulin resistance[40,41]. LSG may alter circulating gastrointestinal peptides, orexigenic and anorexigenic hormon levels[36,42].

Conclusions

In the present study, the patients lost weight effectively after LSG. BMI, insulin, and glucose levels significantly decreased compared to the preoperative values. Ghrelin levels were higher at postoperative month 6, whereas obestatin levels were lower compared to the preoperative levels. The change in the ghrelin and obestatin levels was mild at postoperative month 6, while it was more evident at month 6. Ghrelin released by the duodenum and other sources increased as a compensatory response to the negative energy homeostasis developed after LSG, while obestatin, which is coded by the same gene and uses the same protein precursor, decreased. Today, bariatric surgery does not only exhibit a restrictive effect on food intake in obesity treatment, but it also has important effects on the plasma levels of ghrelin and obestatin hormones. New studies will provide significant contributions to bariatric techniques and standards and obesity treatment.

Ethics Approval

The present study performed all procedures on humans according to the ethics principles of the institutional and/or national committee of research and the Declaration of Helsinki (1964) as amended or corresponding ethical principles.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

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