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Nesfatin-1 hormone levels in morbidly obese patients after laparoscopic sleeve gastrectomy

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Abstract. – OBJECTIVE: To investigate changes in body mass index (BMI) and nesfatin-1 levels in patients with morbid obesity who had undergone laparoscopic sleeve gastrectomy (LSG).

PATIENTS AND METHODS: Blood samples were collected from, and the BMI calculated of 30 morbidly obese patients pre-surgery and at 3 and 6 months post-surgery. Nesfatin-1 hormone levels were measured using enzyme-linked immunosorbent assay (ELISA). Descriptive statistical analysis of the data was performed using Kruskal-Wallis variance analysis, one-way ANOVA, and the Bonferroni-Dunn test. The correlations between continuous variables not displaying normal distribution and those displaying normal distributions were analyzed using the Spearman correlation test and the Pearson correlation test, respectively.

RESULTS: The mean age of the 30 patients was 41.23 ± 10.37 years. The mean BMI values (kg/m^2) were 49.30 ± 7.92, 39,48 ± 7.32, and 34.39 ± 7.56 presurgery, three months post-surgery, and six months post-surgery, respectively ($p < 0.001$). Mean nesfatin-1 levels (ng/ml) were 22.80 ± 14.16, 60.23 ± 52.92, and 96.99 ± 40.20 presurgery, three months post-surgery, and six months post-surgery, respectively ($p < 0.001$). The postoperative months 3 and 6 BMI values were significantly lower than the preoperative BMI value and the postoperative month 6 BMI value was significantly lower than the postoperative month 3 BMI value ($p < 0.001$). The postoperative months 3 and 6 nesfatin-1 levels were significantly higher than the preoperative nesfatin-1 levels. A negative correlation was found between age and preoperative nesfatin-1 values ($p = 0.001$, r = -0.0557).

CONCLUSIONS: Observation of significant increases in nesfatin-1 hormone levels in morbidly obese patients who had undergone LSG indicate that nesfatin-1 has important anorexigenic effects post-surgery and may be an important component of future obesity treatments.

Key Words:
Anorexigenic, Body mass index, Gastric sleeve gastrectomy, Morbid obesity, Nesfatin-1.

Introduction

Obesity has become a widespread and important health concern throughout the world. An endocrine-metabolic disorder, obesity results in excessive fat deposition in the body causing pathology. As obesity has a significant impact on public health and is associated with significant mortality and morbidity, its treatment is mandatory[1]. While obesity has a complex genetic and environmental etiology, all the factors that play a role in its development mainly affect control mechanisms regulating energy intake and consumption in a manner that shifts energy equilibrium toward energy intake[2]. The emergent positive amount of energy that results when intake exceeds consumption increases the amount of adipose tissue.

The World Health Organization (WHO) defines body mass index (BMI) as an individual's body weight in kilograms divided by the square of the individual's height in meters (kg/m^2). WHO defines BMI values < 18.5 kg/m^2 as indicating underweight, between 18.5 and 24.9 kg/m^2 as normal weight, between 25.0 and 29.9 kg/m^2 as overweight, between 30.0 and 34.9 kg/m^2 as obese (class 1), between 35.0 and 39.9 kg/m^2 as obese (class 2), and ≥ 40.0 kg/m^2 as morbid obesity (class 3)[3]. Another accepted means of identifying obesity is measurement of adipose tissue. Women with adipose tissue constituting over 30% of total weight (normal range 20-25% of total weight) and men with adipose tissue constituting over 25% of total weight (normal range

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15-18%) are defined as obese. A third way to identify obesity is the determination of waist-hip ratio[4].

The hypothalamus is the primary organ responsible for maintaining the body weight/energy equilibrium. Many hormones affect the hypothalamus in a manner resulting in weight gain or loss. One of the most important hormones is nesfatin-1, a satiety molecule in the hypothalamus first identified in 2006 by Oh-I et al[5] nesfatin-1 has been found to suppress food intake by a mechanism independent from leptin but dependent on the melanocortin receptor. This hormone has been observed to be secreted in the brain by neurons present in energy-regulating areas as well as in peripheral tissues, such as adipose tissue, the stomach, the pancreas, the liver, and the testes[5,6]. Accepted as an anorectic and a multifunctional peptide, nesfatin-1can suppress appetite for 3 to 14 hours if injected subcutaneously, intraperitoneally, or intracerebroventricularly or by nasal spray. Nesfatin-1 has also been shown to regulate cardiac functions, decrease blood glucose levels, and induce behavior associated with fear and anxiety[7].

Bariatric surgery has become a very effective treatment option in the management of obesity. In the present study, changes in nesfatin-1 hormone levels were investigated in obese patients who had undergone laparoscopic sleeve gastrectomy (LSG).

Patients and Methods

A total of 30 morbidly obese (mean BMI = 49.30±7.92) patients who had undergone LSG between May 2014 and November 2014 after diagnosis of morbid obesity were included in the study. The BMI of the patients was calculated preoperatively and at postoperative months 3 and 6 using the standard (WHO) formula (kg/m^2). The study protocol was approved by the local ethics committee of the Antalya Training and Research Hospital and adhered to the tenets of the Declaration of Helsinki. Detailed written informed consent was obtained from each participant prior to inclusion in the study. The inclusion criteria were primary diagnosis of morbid obesity, primary treatment of LSG for morbid obesity, and age ≥ 18. The exclusion criteria were a previous bariatric surgery, a history of mental impairment, drug or alcohol addiction, recent major vascular event, and/or malignancy.

Surgical Method

The patient was placed on the operating table in the supine position. After the legs had been separated to each side, anti embolic stockings were fitted and a Foley catheter inserted. While the operating surgeon stood between the legs of the patient, a cameraman assistant stood on the right side of the patient and the assistant surgeon stood on the left side. A total of five trocars were positioned: a 10 mm trocar from the middle line 25 to 30 cm below the xiphoid and above the umbilicus, a 5 mm trocar from the left anterior axillary line, a 12 mm trocar from the left midclavicular line, a 10 mm trocar from the left midclavicular line, and a 5 mm trocar below the xiphoid process. After pneumoperitoneum had been achieved, the phrenoesophageal ligament was divided and left crus of the diaphragm was identified. The angle and proximal stomach were separated from upper part of spleen using a laparoscopic vessel sealing device (Covidien, Minneapolis, MN, USA). The short gastric vessels on the great curvature of the stomach were divided to allow entrance into the omental bursa. After this division had been advanced 4 cm proximal to the pylorus, a 32-French size bougie was passed through the mouth and placed along the small curvature of the stomach. Due to the thickness of the distal stomach, the stomach was divided toward the gastroesophageal junction using different types of staplers. After the stomach had been divided, a gastric tube providing the limiting intervention was formed. The entire staple line was covered with Tisseel (Tisseel; Baxter Healthcare, Deerfield, IL, United States) fibrin sealant to the distal stomach. The resected stomach was then removed from the 12 mm trocar hole. Finally, the entire stapler line was washed out with saline, the residual fluid was aspirated, and a drainage tube was positioned throughout the stapler line. To prevent fascial port side hernias, the fascia was closed using 2-0 vicryl. The skin was sutured using 3-0 prolene.

Measurement of Nesfatin-1

Venous blood samples (5 cc) were collected from the antecubital vein at 8 a.m. and 9 a.m. after a presurgery overnight fast and at 3 and 6 months post-surgery for measurement of nesfatin-1 levels by enzyme-linked immunosorbent assay (ELISA). Blood samples were transferred to a tube containing aprotinin and disodium eth-

ylenediamine tetraacetic (EDTA). After centrifugation at 300X, plasma was separated, dispensed into polypropylene tubes, and stored at -80^0 C until analysis. Nesfatin-1 levels were quantified using a commercially available ELISA kit (RayBiotech, Norcross, GA, USA; catalogue no. EIA-NES-1) according to the manufacturer's instructions. The minimum detectable concentration of nesfatin was 147 pg/mL. The inter- and intra-assay coefficients of variation were < 15% and < 10%, respectively. All samples were analyzed in duplicate, and the assay results were expressed in ng/mL.

Statistical Analysis

Descriptive statistical analysis of the data was performed using Kruskal-Wallis variance analysis, one-way ANOVA, and the Bonferroni-Dunn test. The results were expressed in terms of frequency, percentage, mean, standard deviation (SD), median, minimum (min), and maximum (max) values. As the sample size was smaller than 50, the Shapiro-Wilks test was performed to examine normal distribution. According to the time of each measurement, the Friedman test was performed if the measurements were not normally distributed and repeated measures ANOVA was performed if they were normally distributed. If the difference between the measurements was significant, paired comparison was performed using the Bonferroni-Dunn test for nonparametric tests or the Bonferroni test and Fisher's least significant difference test for parametric tests. The correlations between continuous variables not displaying normal distribution were analyzed using the Spearman correlation test and the correlations between variables displaying normal distribution were analyzed using the Pearson correlation test. The level of significance was defined as $p < 0.05$. All analyses were conducted using the SPSS 22.0 software package (SPSS Inc., Chicago, IL, USA).

Results

The clinical characteristics of the study cohort and statistical findings are summarized in Table I. The mean age of the 30 morbidly obese patients was 41.23 ± 10.37 years. The mean BMI values (kg/m^2) were 49.30 ± 7.92, 39,48 ± 7.32, and 34.39 ± 7.56 presurgery, three months post-surgery, and six months post-surgery, respectively ($p < 0.001$). Mean nesfatin-1 levels (ng/ml) were 22.80 ± 14.16, 60.23 ± 52.92, and 96.99 ± 40.20 presurgery, three months post-surgery, and six months post-surgery, respectively ($p < 0.001$). The results of the binary comparison of BMI and nesfatin-1 levels presurgery and at three and six months post-surgery are shown in Table II. As can be observed, the postoperative months 3 and 6 BMI values were found to have significantly decreased compared to the preoperative BMI value. Likewise, the postoperative month 6 BMI value was found to have significantly decreased compared to the postoperative month 3 BMI value ($p < 0.001$; Figure 1). The postoperative months 3 and 6 nesfatin-1 levels were found to have significantly increased compared to the preoperative nesfatin-1 levels (Figure 2). Sequential changes were observed in plasma nesfatin-1 levels and in BMI between preoperative and month 3 postoperative values (Figure 3A), in plasma nesfatin-1 levels and in BMI between preoperative and month 6 postoperative values (Figure 3B), and in plasma nesfatin-1 levels and in BMI between months 3 and 6 postoperative values (Figure 3C). A signifi-

Table I. Preoperative and postoperative months 3 and 6 BMI and nesfatin-1 levels.

	Mean	SD	Median	Minimum	Maximum	p
Preoperatıve BMI (kg/m^2)	49.30	7.92	46.95	39.07	68.03	< 0.001$^{\#}$
BMI 3 months post-surgery	39.48	7.32	37.38	31.45	57.50	
BMI 6 months post-surgery)	34.39	7.56	33.47	24.56	50.00	
Preoperative Nesfatin-1 level (ng/mL)	22.80	14.16	18.79	10.01	75.02	< 0.001$^{\#}$
Nesfatin-1 level 3 months post- surgery	60.23	52.92	30.94	8.33	172.58	
Nesfatin-1 level 6 months post-surgery	96.99	40.20	78.97	37.98	198.50	

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

Table II. Binary comparison of preoperative and postoperative months 3 and month 6 nesfatin-1 hormone levels and BMI.

	Comparison of preoperative and postoperative month 3 levels	Comparison of preoperative and postoperative month 6 levels	Comparison of postoperative months 3 and 6 levels
BMI[#]	< 0.001	< 0.001	< 0.001
Nesfatin 1[#]	< 0.001	< 0.001	0.158

BMI, body mass index; [#]Bonferroni-Dunn test.

cantly negative correlation was found between age and preoperative nesfatin-1 values ($p = 0.001$, $r = -0.557$; Figure 4).

Discussion

The increase in the excessive adipose tissue resulting in obesity is a risk factor for various diseases, including diabetes, hypertension, heart disease, and cancer, as well as early death. Endocrine, genetic, psychological, and cultural factors play discrete and combined roles in the disease etiology[8]. Despite various current pharmacological and surgical methods of treatment and the implementation of physical activity regimens, sufficient treatment success is not achieved by most obese patients. Nevertheless, there are hopeful expectations regarding the development of various surgical methods and pharmacological agents for obesity treatment and prevention of the disease.

In the early 1990s, a protein was identified in mouse and human cell lines termed nucleobindin or NEFA (DNA binding/EF-hand/acidic amino acid-rich region)[9]. Since then, two nucleobindins have been identified in rat studies, namely nucleobindin 1 (NUCB1 or CALNUC) and nucleobindin 2 (NUCB2 or NEFA). In 2006, Oh-I et al[5] defined nesfatin-1, a satiety hormone present in the hypothalamus derived from NUCB2 by prohormone convertase enzyme that contains 82 amino acids with a molecular weight of 9.7 kDa. Although the C-terminal fragment of NUCB2, a protein composed of 396 amino acids, does not play a role in nutrition regulation, its N-terminal plays a role in nutrition intake, and nesfatin-1 is a fragment derived from the N-terminal region[5,10]. Of the 396 amino acids, amino acids 1to82 form nesfatin-1, 85to163 form nesfatin-2, and 166to396 form nesfatin-3[5]. Of the three sub-segments composing nesfatin-1—N23 (1–23), M30 (24–53), and C29 (54–82)—M30 is the segment that affects nutrition intake[11,12].

Rat studies have shown that NUCB2/nesfatin-1 is present in the nuclei of paraventricular arcuate, supraoptic, and tractus solitarius of the hypothalamus, which plays a role in regulation of ap-

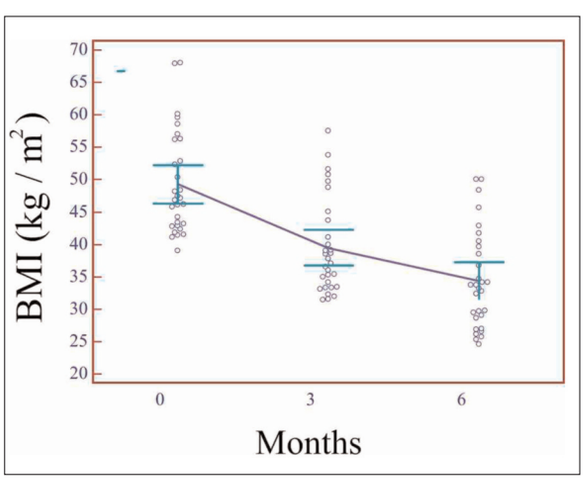

Figure 1. Preoperative, postoperative month 3, and postoperative month 6 BMI values.

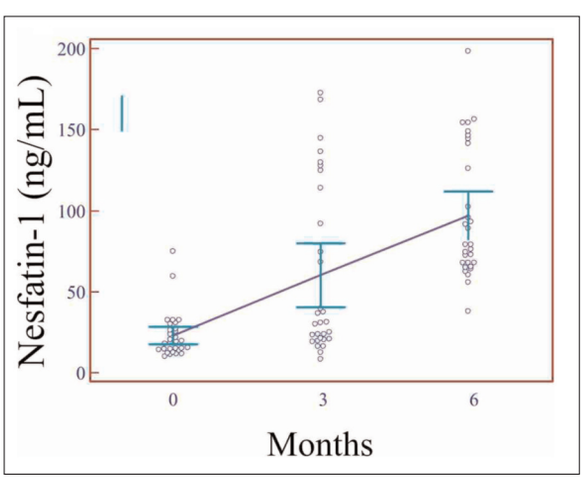

Figure 2. Preoperative, postoperative month 3, and postoperative month 6 plasma nesfatin-1 levels.

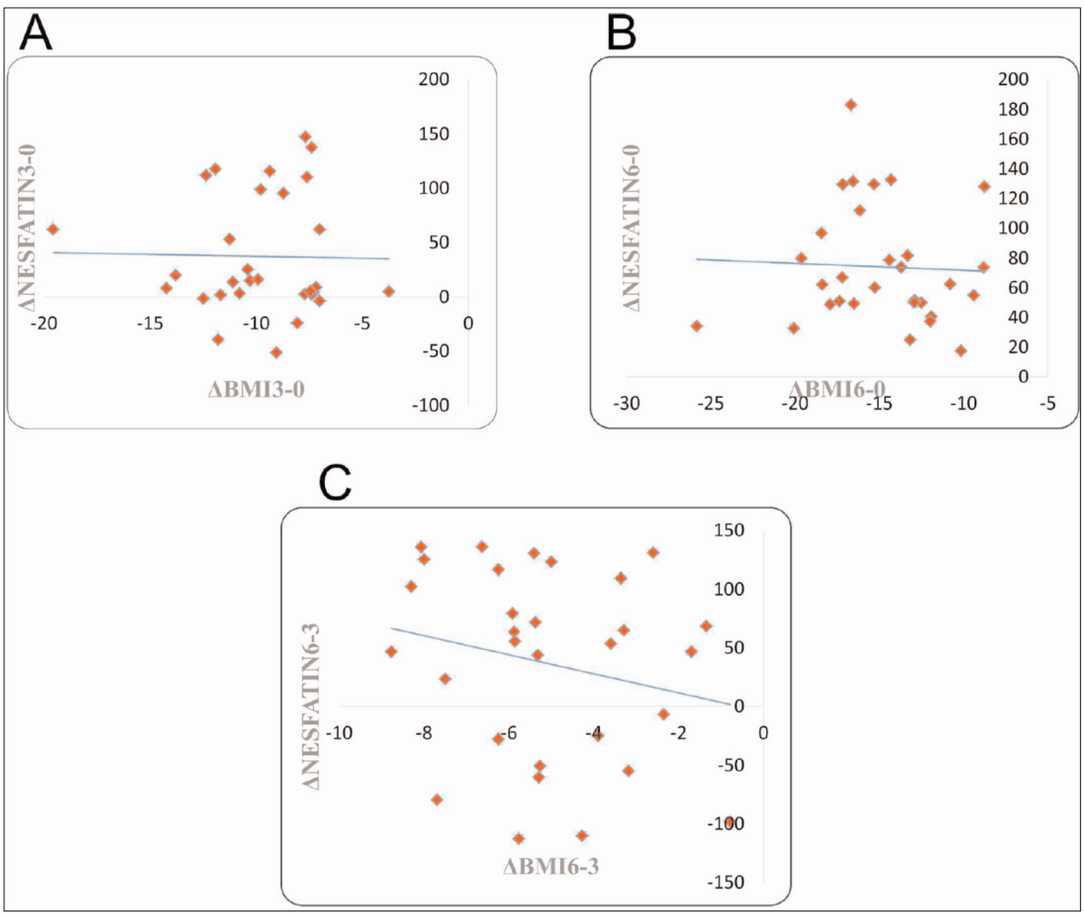

Figure 3. ***A,*** Sequential changes in plasma nesfatin-1 levels and BMI between preoperative (*baseline*) and postoperative month 3 values. ***B,*** Sequential changes in plasma nesfatin-1 and BMI between preoperative and postoperative month 6 month values. ***C,*** Sequential changes in plasma nesfatin-1 levels and BMI between postoperative month 3 and postoperative month 6-month values. Δ: change; ΔNESFATIN3-0: difference between postoperative month 3 and preoperative nesfatin-1 levels; ΔNESFATIN6-0: difference between postoperative month 6 and preoperative nesfatin-1 levels; ΔNESFATIN6-3: difference between postoperative month 6 and postoperative month 3 nesfatin-1 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 preoperative BMI.

petite and metabolism, as well as in the lateral hypothalamic area, dorsomedial hypothalamic nucleus, zona inserta, cellular bodies of the spinal cord, dorsal nucleus of the vagus, and the hypophyseal gland[5,13]. Nesfatin-1 is co-expressed with melanin-concentrating hormone (MCH) in neurons from the tuberal hypothalamic area, which is recruited during sleep states, especially paradoxical sleep[14]. The mRNA expression of nesfatin-1 is significantly decreased by fasting and significantly increased in the hypothalamus upon re-feeding[13]. While early studies[15,16] first detected mature nesfatin-1 in cerebrospinal fluid, later studies detected it immunohistochemically in peripheral tissues, such as the gastric oxyntic

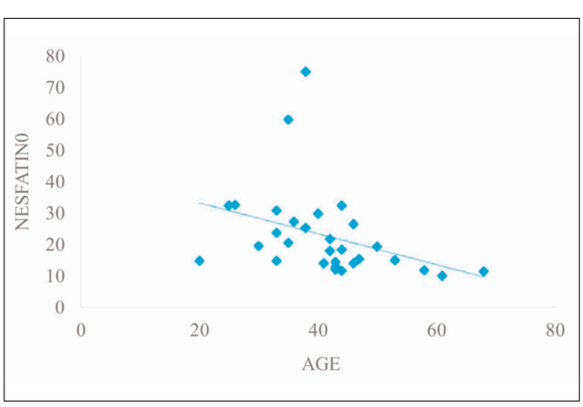

Figure 4. Negative correlation between preoperative nesfatin-1 level and age.

mucosa, adipose tissue, pancreatic endocrine Langerhans cells, testes, and pituitary gland. Stengel et al[15] also found that nesfatin-1 was co-expressed with ghrelin in X/A-like cells in rat gastric oxyntic mucosa. This finding accords with the hypothesis that hunger and gastric vagal stimulation stimulates ghrelin secretin from the stomach and was supported by the observation that vagal stimulation induces the anorexigenic effect of nesfatin-1[15,17].

The hypothalamus and adipose tissue determine the amount of nesfatin-1 produced, while the circulatory system, eating habits, and amount of adipose tissue play a role in its regulation[18]. In a study of Wistar rats[19], intracerebroventricular and intranasal administration of nesfatin-1 inhibited nutrient intake within six hours and decreased gastric emptying. A study described by Maejima et al[20] provided strong evidence for the involvement of an oxytocin pathway in nesfatin-1's inhibitory effect on food intake. Further evidence was provided by Yosten and Samson[21], who showed that an oxytocin antagonist injected intracerebroventricularly blocks the food-intake-suppressing effects of intracerebroventricular nesfatin-1 and melanocyte-stimulating hormone. These findings, together with the finding that intraperitoneal administration of nesfatin-1 inhibited nutrient intake for three hours and subcutaneous administration for 14 hours, has led to the hypothesis that subcutaneous nesfatin-1 administration will play an important role in the future in anti-obesity treatment[22,23].

Several studies[24-26] have demonstrated that nesfatin-1 can easily pass through the blood-brain barrier after peripheral administration, decreasing appetite. Pancreatıc β-cells have been found to insulin colocalize with nesfatin/NUCB2 in the islets of both mice and rats, indicating the possible involvement of nesfatin-1 in the regulation of insulin secretion from pancreatic β-cells[27]. Although several studies have indicated that nesfatin-1 levels are positively correlated with fasting blood glucose levels in healthy individuals, the mechanism underlying this correlation has not been definitively explicated. Despite this lack of understanding, Tsuchiya et al[26] found a significant negative correlation between plasma concentrations of nesfatin-1 and BMI, body fat percentage, and body fat weight in healthy nonobese Japanese males. Furthermore, they found that fasting concentrations of plasma nesfatin-1 were significantly lower in a group of high BMI subjects compared to nonobese subjects[26]. This negative correlation between nesfatin-1 and BMI suggests that nesfatin-1 plays a role in energy homeostasis and body weight, specifically that overweight or obesity could result from a deficiency of nesfatin-1 and thus that increasing the plasma nesfatin-1 concentration in the body could result in reduced body fat mass. Nevertheless, several studies have identified a positive correlation between nesfatin-1 levels and BMI[28].

Another important anorexigenic protein, leptin, has been found to be positively correlated with BMI, body fat percentage, and body fat weight. In accordance with the finding that elevated leptin levels repress appetite, obese individuals generally have been observed to have leptin resistance[29]. Moreover, intraperitoneal nesfatin-1 administration has been shown to decrease nutrient intake in mice with leptin resistance, such as genetically obese and genetically diabetic mice, fed a high-fat diet[11]. Several studies[30,31] have also observed that nesfatin-1 levels were high in the circulation in patients with generalized epilepsy. As a result, nesfatin-1 is currently being used as a new biomarker for epilepsy diagnosis and monitorization of treatment response[30]. As serum nesfatin-1 level has also been observed to be greatly decreased in patients with obstructive sleep apnea syndrome, it has been recommended as a new biomarker for sleep apnea diagnosis[31]. Dai et al[32] has also shown that fasting plasma nesfatin-1 levels are decreased in patients with myocardial infarction. In an investigation of changes in nesfatin-1 levels between obese patients and normal weight controls, Guo et al[33] found that acupuncture treatment decreased nesfatin-1 levels in the obese patients but not in the normal weight controls. In several studies in which nesfatin-1 levels in obese patients were high, this elevation was attributed to an increase in white adipose tissue mass, increasing the expression of NUCB2[34].

Laparoscopic sleeve gastrectomy (LSG), one of the foremost bariatric procedures, is believed to elicit weight loss by physically restricting gastric capacity via removal of 80% or more of the stomach, including the fundus and greater curvature. Due to reduced stomach size and removal of the highly distendable gastric fundus, ingestion of a specific volume of food will increase pressure much more quickly in the gastric sleeve compared with ingestion of the same volume in an intact stomach, thus triggering earlier satiety.

Unlike dieting, LSG is not associated with rebound hyperphagia, a factor critical in understanding the mechanism underlying LSG-induced weight loss. This lack of association with hyperphagia indicates that several physiological and hormonal changes, including satiety, are induced by the surgery that differs from the biological and behavioral changes associated with food restriction[35].

Bariatric surgery is also associated with marked decrease in appetite and food intake, which may directly or indirectly improve glycemic control and induce weight or fat reduction. Reduced occurrence of diabetes, heart disease, and cancer has also been associated with bariatric surgery[36]. Moreover, improvement in metabolic parameters, such as glucose tolerance, plasma lipid levels, and insulin secretion and sensitivity, all of which improve with weight loss, have also been reported with bariatric surgery. Bariatric surgery also alters circulating gastrointestinal peptides that affect food intake, influence the digestive process, and alter gastrointestinal propulsive motor function. However, it is unclear whether the changes in these peptides due to the anatomic rearrangement of the gastrointestinal tract with bariatric surgery are responsible for improved glucose homeostasis and weight loss[37,38]. Despite this uncertainty, it is likely that the intestinal adaptation to LSG contributes to the mechanisms underlying improvements in glucose homeostasis. Indeed, changes in gut hormones, including glucagon-like peptide, gastric inhibitory peptide, peptide YY, and amylin are often implicated as mechanisms underlying the weight-independent effects of LSG on glucose homeostasis[39]. Moreover, changes in endocrine and/or neural mechanisms and in vagal tone after LSG may result in altered gastric emptying.

The arcuate nucleus of the hypothalamus is a key component of central nervous system homeostatic circuitry, which plays an important role in appetite, glucose metabolism, and energy balance[40,41]. Given the importance of central melanocortin signaling and nesfatin 1 in regulating body weight, it has been hypothesized that the greater success of LSG compared with diet and exercise may be attributed to changes to this axis that reset the body's homeostatic system. Precise identification of the primary mechanism underlying weight loss after LSG should be investigated by research into changes in central melanocortin activity, hypothalamic changes, and nesfatin levels after LSG.

Conclusions

In our investigation of anorexigenic nesfatin-1 levels in patients with morbid obesity who had undergone LSG, we observed several significant changes in BMI and nesfatin-1 hormone levels that have important implications for the future of obesity treatment. Regarding BMI, we observed statistically significant decreases in BMI measurements at postoperative months 3 and 6 compared with preoperative values and statistically significant decreases in BMI measurements at postoperative month 6 compared with postoperative month 3. Regarding nesfatin-1 levels, we observed statistically significant increases at postoperative months 3 and 6 levels compared with preoperative levels, suggesting that a considerable amount of this hormone is secreted from sources other than the stomach, such as the hypothalamus, pancreas, liver, testes, and adipose tissue, and that increase in anorexigenic nesfatin-1 levels has a positive effect on post-LSG weight loss. New clinical research and observations that precisely identify the relationship between energy equilibrium-metabolism and nesfatin-1 will contribute significantly to the fight against obesity.

Ethical Approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the Institutional and/or National Research Committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

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