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Comparison of Weight Loss, Ghrelin, and Leptin Hormones After Ligation of Left Gastric Artery and Sleeve Gastrectomy in a Rat Model

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Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
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Background: Ligation of the left gastric artery (LLGA), which supplies the fundus of the stomach, may reduce the appetite hormone ghrelin, resulting in weight control. The aim of this study was to compare LLGA and sleeve gastrectomy (SG) in terms of postoperative outcomes in a rat model.

Material/Methods: Fifteen male Wistar albino rats, weighing >350 grams (range 350–525 grams), were enrolled in LLGA (N=5), SG (N=5), and control (N=5) groups. Blood samples were drawn preoperatively and also during the first and fourth week postoperatively to assay ghrelin and leptin hormone levels. Body weight was measured in each group.

Results: The maximum reduction in ghrelin level (41.5%) was found in the LLGA group. Considerable% total weight loss (TWL) (mean 24.1%) was observed in the SG group, and slight%TWL was noted in the control and LLGA groups (means of 0.1% and 2.1%, respectively). There was no significant difference in mean percent weight change between the LLGA and the SG groups (*p*=0.08). Blood sample analysis revealed no statistically significant changes in ghrelin or leptin levels between the groups (*p*=0.9 and *p*=0.3, respectively).

Conclusions: We present evidence that LLGA causes the same reduction in ghrelin hormone levels as SG at 4 weeks after surgery in a rat model. However, LLGA did not cause the same%TWL as SG. The mechanism of weight loss in SG is most likely due to restriction and to the effects of the procedure, rather than due to neurohormonal changes.

MeSH Keywords: **Bariatric Surgery • Ghrelin • Models, Animal**

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Background

The number of overweight and obese individuals has grown over the past several decades. Currently, obesity is a global health problem [1]. Bariatric surgical procedures, such as sleeve gastrectomy (SG) and Roux-en-Y gastric bypass (RYGB), are aggressive and effective treatment options for morbidly obese patients that enable weight loss and resolve or improve several obesity-related co-morbid conditions [2,3]. SG is a longitudinal gastrectomy and entails resection of the entire fundus, greater curvature and partial antrum of the stomach [4]. This procedure provides a reduction in gastric volume, decreases food intake, and causes a reduction in the level of the orexigenic hormone ghrelin, all of which lead to weight loss [5]. Potential complications after SG include leakage, bleeding, stricture, and mortality, all of which have a low reported incidence [6,7].

Ghrelin hormone, which has potent orexigenic and adipogenic properties, is produced by cells that are located mainly in the upper body and also in the greater curvature of the stomach, and induces hunger, increases gastrointestinal system motility, and suppresses insulin production [8–11]. Leptin is a hormone that is mainly produced by adipocytes; it promotes energy expenditure and reduces hepatic gluconeogenesis and insulin resistance [12]. Plasma levels of leptin increase during and after eating [13]. Studies have shown that ghrelin and leptin levels decrease after bariatric surgery [14]. The vascular supply to the gastric fundus is predominantly from the LGA. Currently, less invasive and effective obesity treatment options are available, including percutaneous, catheter-directed, transarterial embolization of the left gastric artery (LGA), which has been shown to modulate body weight in animal models [15]. The present study aimed to compare the ligation of the left gastric artery (LLGA) and SG in terms of postoperative outcomes, such as weight loss and ghrelin and leptin hormone levels, in a rat model.

Material and Methods

This study was performed in the Experimental Animals Research Laboratory and the research protocol was approved by the Local Animal Ethics Committee. All steps were in accordance with the regulations governing the care and use of laboratory animals as set forth in the Declaration of Helsinki.

Fifteen healthy male Wistar albino rats, aged 12-15 months and weighing >350 grams (range of 350-525 grams), were maintained in standard metabolic cages under controlled temperature (21–23°C), humidity, and light (12 h light/12 h dark) with standard pellet feed manufactured specifically for animals, and tap water. The rats were divided into 3 groups: SG (n=5, 418±69.3 g), LLGA (n=5, 432.4±61.4 g), and sham operation (n=5, 423.6±35.1 g). All the types of surgery were performed by both surgeons on the same day. Feeding was stopped for all rats 12 h before surgery and restarted ad libitum 4 h after surgery. All groups were fed shredded chow during the first 3 days after surgery and then resumed eating regular pellet feed. All blood samples were taken in the morning after overnight fasting from the external jugular vein under deep anesthesia with ketamine/xylazine. Blood samples were collected preoperatively and during the first and fourth week after surgery to assess ghrelin and leptin hormone levels. The sample tubes were centrifuged at 3000 rpm for 10 min at 4°C. Serum plasma was separated and then transferred to a fresh Eppendorf tube and stored at –80°C. The plasma levels of ghrelin and leptin were assayed using ELISA kits. Body weight was measured in each group at weekly intervals through week 4. At the end of the fourth week, all rats were sacrificed.

Surgical procedures

After a 12-h overnight fast, the rats were anesthetized with an intraperitoneal ketamine/xylazine solution. All surgical procedures were performed under sterile conditions. For the SG group, a standard 14F green nasogastric tube was used, and a 3-cm midline incision was made. After ligation of the greater omentum and the gastrosplenic ligament, a vascular clamp was placed along the antrum to the fundus. The greater curvature of the stomach was removed 5 mm above the level of the pylorus using scissors. Gastric capacity decreased in volume by 80–90%. The divided stomach was closed with 5.0 polyglycolic acid suture (Maxon, Surgical US, USA) to create the gastric sleeve (Figure 1A). The muscle layers and skin were closed with 3.0 polyglycolic acid sutures. For the LLGA group, a 3-cm midline incision was made; the celiac plexus and LGA were found, and then the LGA was ligated with 5.0 polyglycolic acid suture (Figure 1B). For the sham operation, a 3-cm midline incision was made, and the stomach was only manipulated. Closure was the same for all operated groups. After surgery, all animals were given 2 ml of subcutaneous sterile saline to prevent dehydration and then were returned to their cages.

Statistical analysis

Statistical analyses were performed using SPSS (PC version) 14.0 software. All data are expressed as the mean ±SD, with a confidence interval of 95%. A value of $p<0.05$ was considered to be statistically significant. One-way analysis of variance (ANOVA) was used for inter-group comparisons.

Results

All procedures were performed successfully and there were no mortalities. There were no significant differences in baseline

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Figure 1. A rat stomach after LLGA (**A**) and SG (**B**).

Table 1. Body weight and ghrelin and leptin levels at baseline. Values are the mean ±SD; no significant differences (P>0.05) were detected among the 3 groups.

Characteristics	SG group	LLGA group	Sham-operated group
Body weight (gr)	418±69.3	432.4±61.4	423.6±35.1
Ghrelin (pg/ml)	65.72±54.2	67.32±81.7	45.6±32.7
Leptin (pg/ml)	449.1±149.1	387.6±272.2	187.7±106.2

ghrelin or leptin levels or body weight among the 3 groups (Table 1).

The mean preoperative weights of the LLGA, SG and sham-operated groups were 432.4±61.4 g, 418±69.3 g, and 423.6±35.1 g, respectively. Four weeks after the operations, the LLGA, SG, and sham-operated groups had average weights of 432±62.6 g, 317.6±38.4 g, and 414±39.6 g, respectively (Figure 2). Significant total weight loss (%TWL) was observed in the SG group (mean 21%), whereas the LLGA and sham-operated groups showed small decreases in weight (mean 0.1% and 2.1%, respectively), but there were no statistically significant differences in mean weight before and after the operations in either group (*p*=0.08).

In the SG group, the average ghrelin level decreased from 65.7 pg/ml to 54.6 pg/ml. The plasma ghrelin level increased in the sham-operated group from 45.6 pg/ml to 50.4 pg/ml. Before and after the operations, mean plasma ghrelin levels did not differ significantly among the groups (*p*=0.9). However, the maximum reduction in ghrelin levels (41.5%) was found in the LLGA group (decreasing from 67.3 pg/ml to 39.3 pg/ml at the end of the experiment) (Figure 3).

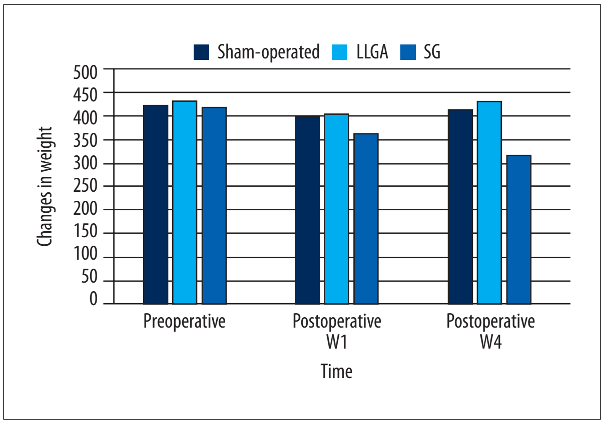

Figure 2. The mean change in weight among the groups. A steady decline is seen in the SG group.

Fasting leptin levels increased in each group, but a minimal rise was observed in the SG group (the preoperative and 4 weeks postoperative levels were 449.1 pg/ml and 583.8 pg/ml, respectively). The highest increase in the leptin level was found in the sham-operated group (187.7–710,1 pg/ml). The average leptin values of the LLGA group preoperatively and postoperatively were 387.7 pg/ml and 734.1 pg/ml, respectively. Leptin levels did not differ significantly between the groups (*p*=0.3). The differences in leptin levels are shown in Figure 4.

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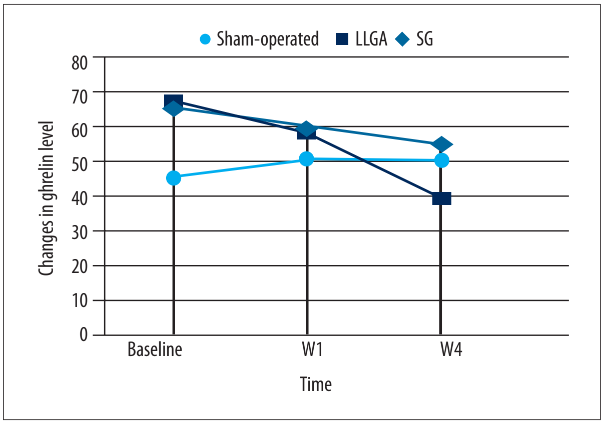

Figure 3. Changes in ghrelin levels from baseline over 4 weeks in the 3 groups. The decrease in ghrelin was not significantly different between the LLGA and SG groups (p>0.05).

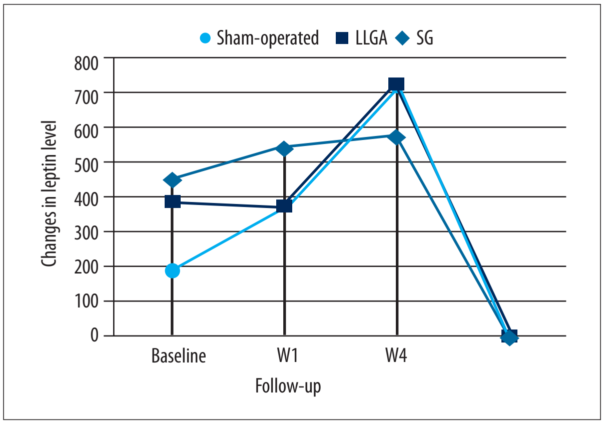

Figure 4. Changes in leptin levels from baseline over 4 weeks in the 3 groups. The minimum rise in the leptin level was in the SG group, but there was no significant difference among the 3 groups (p=0.3).

Discussion

The success of bariatric surgery is not completely related to malabsorption or restriction of the stomach volume, because the neuroendocrine effects are very important for total weight loss and the improvement or resolution of related co-morbidities. Some of the neuroendocrine signals that arise from the gastrointestinal tract are regulated by body weight and control energy expenditure and fat metabolism [9]. Ghrelin, which is a multifunctional hormone, is the only known appetite-stimulant hormone, and it causes positive energy balance and decreases energy expenditure [16–19]. Many studies have shown that individuals who have undergone bariatric surgery experience a significant reduction in ghrelin levels and that this effect is related to the resection of portions of the gastric fundus [20]. Decreased levels of ghrelin contribute to weight loss due to its role in regulating food intake. However, according to the results of this study, the maximum decrease in ghrelin was in the LLGA group, although there was not significant %TWL in this group. Additionally, there was considerable %TWL in the SG group, despite the fact that ghrelin levels decreased less than in the LLGA group. Based on these parameters, weight loss after bariatric surgery depends not only on a decrease in ghrelin, but also on an important mechanism that is not correlated with a decrease in ghrelin. Yehoshua et al. showed that the increased intragastric pressure in the narrow lumen and lessened distensibility of the stomach may contribute to weight loss after SG [21]. In a study by Goitein, 20 morbidly obese patients underwent SG, and total plasma ghrelin levels were monitored before and 3 months after surgery. These patients showed both a significant reduction in total plasma ghrelin levels and %TWL 3 months after surgery; however, the authors stated that there was no correlation between decreased ghrelin levels and body weight [5]. Some of the expected results, such as a decline in plasma ghrelin and a decline in body weight in the SG group, were observed in our study; however, there were no significant differences among the groups (p=0.918 and p=0.08, respectively). The emergence of these results may be affected by the short follow-up period. The study, in which Wran et al. used rat model, showed that weight gain and increased adiposity occur after continuous systemic ghrelin administration, and their results provided evidence that ghrelin is important in long-term control of food intake and body weight [22].

Umemura et al. correlated the levels of adipokines in the serum and omentum-derived adipocytes and visceral adipose tissue between non-obese patients who underwent elective abdominal surgery and SG patients. They found that mean serum leptin levels were significantly higher and that mean adiponectin levels were significantly lower in the SG group than in the non-obese group [23]. Most studies show that leptin levels are decreased after bariatric procedures in humans [24]. In our study, there was no significant difference in leptin levels among the groups.

Researchers are investigating novel treatment options to affect endocrine hormones, such as bariatric embolization, in the management of obesity, which may be an effective and less invasive alternative. Arepally et al. described the first gastric artery chemical embolization, showing reductions in ghrelin levels in an animal model and suggested the use of this less invasive technique to treat obesity [15]. In a study by Bawudun et al., after they performed left gastric artery embolization (LGAE) in a canine model, they found that plasma ghrelin values were significantly lower than at baseline and that significant weight loss also occurred [25]. Currently, there are many image-guided interventional studies regarding embolization of the left gastric artery and its effectiveness with respect to total weight loss and decreased ghrelin levels. In this study,

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we aimed to compare the effects and outcomes of SG, which is known as an effective technique, and LLGA, an alternative treatment option for obesity.

Bariatric embolization is not used routinely in humans for the treatment of obesity, but some retrospective studies on the relation between left gastric artery embolization and %TWL have been published in the literature. Gunn and Oklu compared the weights of patients who underwent LGA embolization (N=19) and embolization of other arteries (N=28) for upper gastrointestinal bleeding. In this retrospective study, they found that the LGA embolization group lost a significant portion of their initial body weight (7.3%) 3 months after embolization (p=0.006). According to these data, they proposed that LGA embolization may modulate body weight and may therefore be a therapeutic option for obesity treatment [26].

Limitations

The analytic power of the current study was limited by the low number of experimental animals. Future studies with multivariate analysis and large animal groups would further our understanding of the differences between bariatric embolization and surgery. To the best of our knowledge, the vascular anatomical variations that provide blood supply to the stomach cannot at present be predicted. To evaluate the results of similar studies, the metabolic differences between humans and experimental animals should also be considered.

Conclusions

The results of this study demonstrate that LLGA can reduce the plasma levels of ghrelin and overall body weight, but SG resulted in the most effective %TWL. Contrary to common perceptions, ghrelin hormone reduction may not be very effective for %TWL after bariatric surgery. These data suggest that the mechanism of weight loss after SG most likely involves restriction and its effects rather than neurohormonal changes. Prospective, randomized studies based on a larger number of animals and a longer follow-up period are needed to safely and effectively perform LLGA or LGAE for the treatment of obese patients in the future.

Acknowledgement

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Conflict of interest

The authors declare that they have no conflicts of interest.

Ethics approval

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted.

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