

# The Effects of Gastric Surgery on Systemic Ghrelin Levels in the Morbidly Obese

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**Hypothesis:** Circulating ghrelin, produced primarily in the stomach, is a powerful orexigen. Ghrelin levels are elevated in states of hunger, but rapidly decline postprandially. Early alterations in ghrelin levels in morbidly obese patients undergoing weight reduction surgery may be attributed to gastric partitioning.

**Design and Patients:** Thirty-four patients underwent Roux-en-Y gastric bypass with a completely divided gastroplasty to create a 15-mL vertically oriented gastric pouch. Eight other patients underwent other gastric procedures that did not involve complete division of the stomach, including 4 vertical banded gastroplasties and 4 antireflux surgical procedures. Six additional patients undergoing antireflux surgery served as lean control subjects. Plasma samples were obtained before surgery and immediately after surgery. In a substudy, plasma was collected after Roux-en-Y limb formation and after dividing the stomach to identify any changes in plasma ghrelin levels.

**Setting:** Tertiary university medical center.

**Main Outcome Measures:** Ghrelin levels at different stages of surgical intervention.

**Results:** Mean  $\pm$  SEM preoperative and postoperative ghrelin levels in the gastric bypass group were  $355 \pm 20$  and  $246 \pm 13$  pg/mL, respectively ( $P < .001$ ). In the vertical banded gastroplasty group and in all patients undergoing antireflux surgery, ghrelin levels were not significantly changed.

**Conclusions:** Compared with morbidly obese humans, lean controls had significantly higher plasma ghrelin levels at baseline. A divided gastroplasty creating a small proximal gastric pouch results in significant early declines in circulating ghrelin levels that are not observed with other gastric procedures. This may explain, in part, the loss of hunger sensation and rapid weight loss observed following gastric bypass surgery.

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**I**N THE GASTROINTESTINAL TRACT, ghrelin is produced primarily in the gastric fundus and in far smaller quantities in the small intestine. It is the only known circulating appetite stimulant that triggers the arcuate nucleus of the hypothalamus, with the presumed role of regulating body weight.<sup>1</sup> Circulating ghrelin levels significantly increase before a meal and rapidly decline postprandially, implicating this hormone as a principal signal of hunger and meal initiation.<sup>2,3</sup> The administration of exogenous ghrelin stimulates feeding in humans and reduces host metabolic rate, fat catabolism, and body temperature, with resultant increase in body weight.<sup>4-6</sup> Indeed, weight loss such as that induced by dieting, cancer anorexia, or regular physical exercise is associated with high levels of circulating ghrelin, consistent with a compensatory

mechanism to restore body mass and fuel reserves.<sup>7-9</sup> In contrast, a low circulating level of ghrelin, indicative of the fed state, is found with weight gain and in severe obesity.<sup>10</sup>

Although the specific signals responsible for regulating gastric ghrelin secretion remain elusive, one cause for the rapid postprandial decline in ghrelin levels is postulated to be in response to nutrient passage through the cardia and fundus of an intact stomach and mediated in part by vagal parasympathetic signaling.<sup>11-13</sup>

Patients with morbid obesity have lower baseline ghrelin levels compared with lean counterparts. However, surgically induced weight loss with Roux-en-Y gastric bypass (RYGB) in morbidly obese patients appears to cause long-term, if not permanent, suppression of ghrelin secretion<sup>14,15</sup> that is not associated with other procedures such as ad-

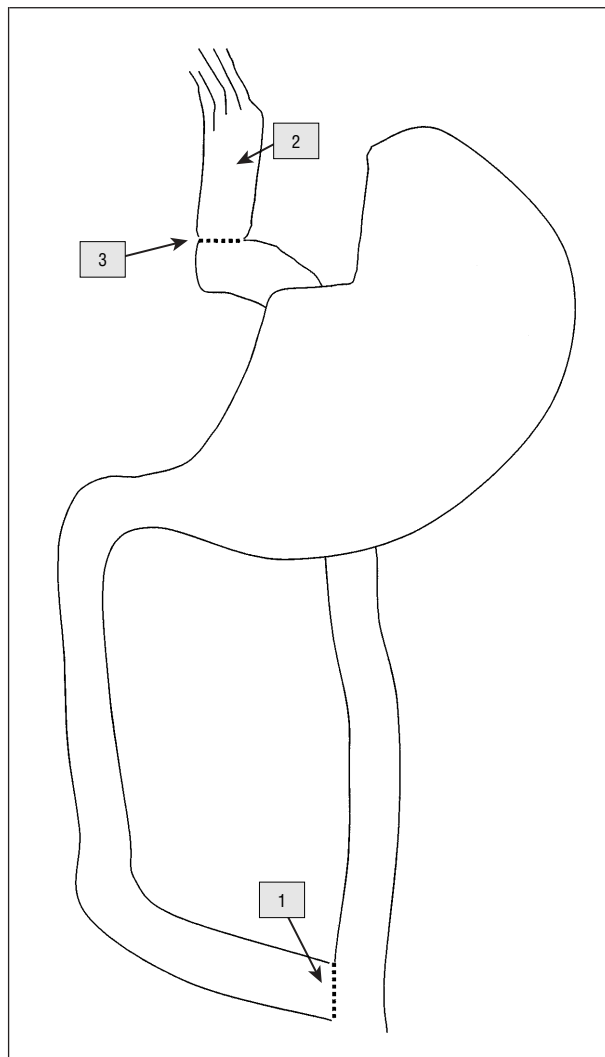
justable silastic banding.<sup>16</sup> In fact, patients who have had adjustable silastic bands exhibit rising ghrelin levels during follow-up of 12 months or longer.<sup>17</sup> It is postulated that the greater durability of an RYGB in maintaining long-term weight loss over any other procedure is due to the continued suppression of ghrelin levels following a RYGB with a divided vertical pouch. It has been proposed that suppression of ghrelin secretion from the gastric fundus is secondary to a permanent deprivation of nutrient stimulation to cells responsible for producing and releasing ghrelin. Supporting evidence is derived from human subjects who exhibit gradual declines in circulating ghrelin levels during an overnight fast.<sup>2,14</sup> In addition, rats subjected to long-term fasting had lower circulating ghrelin levels compared with rats that were fasted and refed.<sup>13</sup> These observations, however, have not been consistently shared. One group has reported no decline in ghrelin levels following RYGB, but rather an elevation in ghrelin levels in patients experiencing ongoing weight loss following surgery.<sup>18</sup> These seemingly opposing perspectives underscore the need to ascertain if the reduction in ghrelin levels following RYGB is indeed the result of stomach division. If bypass of the ghrelinogenic tissue in the gastric fundus with the creation of a vertical pouch proves to reduce circulating ghrelin, and thereby induce long-term weight loss, this information may affect how the restrictive portion of any weight loss operation should be performed. The information may also lend insight into the efficacy of other weight reduction procedures.

Gastrectomy studies<sup>19,20</sup> in rats demonstrated a reduced circulating ghrelin level 10 weeks after surgery that was proportional to the amount of fundus resected, suggesting that extragastric sources of ghrelin account for 20% of the hormone in circulation. Gastrectomy in humans has been associated with rapid (<30 minutes) declines in ghrelin levels.<sup>11</sup> The study reported herein describes the early effects of different gastric procedures on circulating ghrelin levels. This is the first report, to our knowledge, that compares the ghrelin-modulating effects of surgery specifically involving the gastric fundus in patients with similar body weight. We hypothesize that excluding the dominant portion of the stomach and the fundus by gastric division during RYGB results in early declines in circulating ghrelin levels.

## METHODS

### PATIENTS

Forty-two morbidly obese subjects (body mass index, calculated as weight in kilograms divided by the square of height in meters, >40) were studied prospectively according to a protocol approved by the institutional review board. Thirty-four of these patients underwent RYGB with a completely divided gastropasty to create a 15-mL vertically oriented gastric pouch (**Figure 1**). Eight patients underwent other gastric procedures that did not involve complete division of the stomach, including 4 vertical banded gastroplasties (**Figure 2**) and 4 antireflux surgical procedures (**Figure 3**). Six additional nonobese patients (body mass index, <25) undergoing antireflux surgery served as lean control subjects. All operations involved significant manipulations of the gas-



**Figure 1.** The 3 major steps of a Roux-en-Y gastric bypass operation with a resultant 15-mL vertical pouch: (1) dividing the jejunum and forming an enteroenterostomy to create a long (100-150 cm) Roux-en-Y limb; (2) completely dividing the stomach to create a vertical pouch that is 5 cm long and 1 to 1½ cm wide; and (3) connecting the Roux-en-Y limb to the vertical pouch.

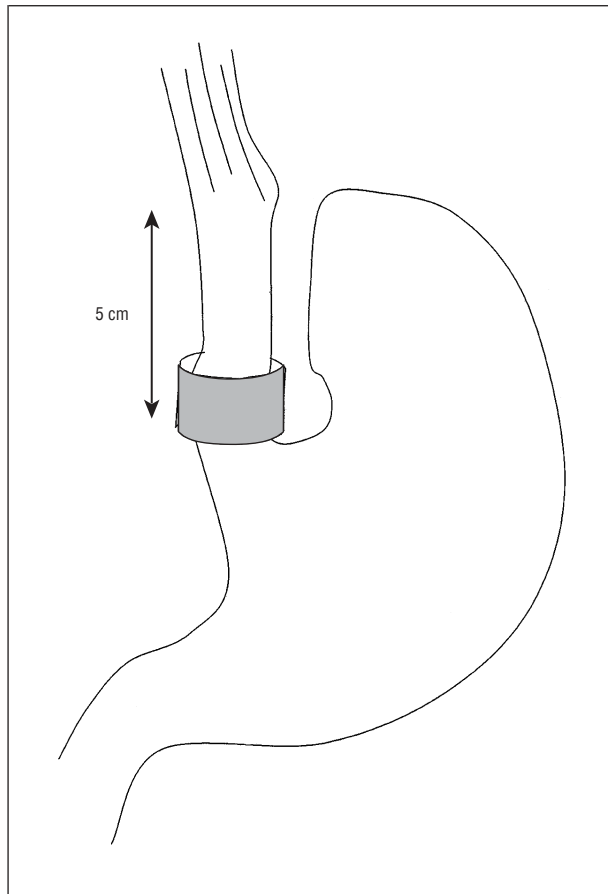
tric fundus, with only the RYGB resulting in a completely divided stomach.

### PLASMA COLLECTION

In all patients, 5 mL of venous blood was collected into cooled ethylenediaminetetraacetic acid tubes by venipuncture 30 minutes before and after surgery. Plasma was separated from whole blood in a centrifuge at 4°C and 3000g for 15 minutes. Plasma was immediately frozen in 1-mL aliquots at -80°C until the time of assay.

### PLASMA GHRELIN RADIOIMMUNOASSAY

Radioimmunoassay was performed using kits (Phoenix Pharmaceuticals, Belmont, Calif) using standardized methods. In brief, aliquots of plasma were thawed once, and samples were measured in duplicate. Rabbit antiserum against the peptide was used with radioiodinated (iodine I 125) tracer. The range of detection was 10 to 1280 pg/mL, with a lower limit of detection of 80 pg/mL. The interassay coefficient of variation is 8.7%.



**Figure 2.** The vertical banded gastroplasty only partially divides the stomach, with a restrictive pouch of similar dimensions as the Roux-en-Y gastric bypass. The outflow of the pouch is reinforced by a prosthetic band.

#### GASTRIC DIVISION AND GHRELIN LEVELS

To determine the stage of RYGB surgery that induces the greatest decline in circulating ghrelin, plasma was collected at 4 time points: before surgery, 10 minutes after transecting the jejunum to form the Roux-en-Y limb, 10 minutes after completely dividing the stomach to form the small vertical pouch, and after surgery.

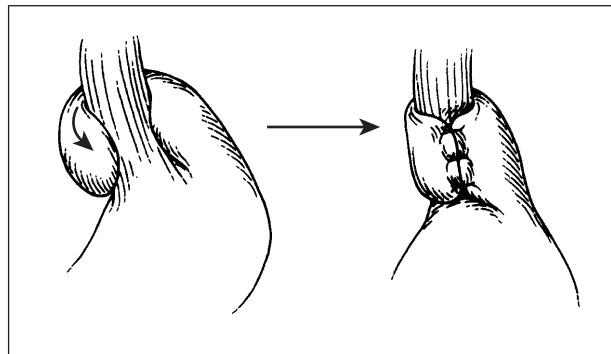
#### STATISTICAL ANALYSIS

Data were analyzed using analysis of variance with Newman-Keuls posttest and Wilcoxon matched-pairs test and reported as mean  $\pm$  SEM. Statistical significance was set at  $P < .05$ .

### RESULTS

#### PATIENT DEMOGRAPHICS AND BASELINE GHRELIN LEVELS

Age and body mass indexes are summarized in the **Table** for all study groups. The lean patients undergoing anti-reflux surgery had a body mass index of  $23.8 \pm 0.9$ . Consistent with available reports,<sup>10</sup> ghrelin levels at baseline were higher in lean patients than in morbidly obese patients (**Figure 4**).



**Figure 3.** The antireflux surgery or Nissen fundoplication performed for gastroesophageal reflux disease. The greater curvature is fully mobilized, and the fundus is wrapped around the esophagus to create an antireflux valve.

#### SURGICAL ALTERATION OF GHRELIN LEVELS

In all patients, there appeared to be a decline in ghrelin levels early after surgery involving the fundus. However, only RYGB surgery demonstrated a significant change between preoperative and postoperative ghrelin levels ( $355 \pm 20$  and  $246 \pm 13$  pg/mL, respectively;  $P < .001$ ), which was not observed with the vertical banded gastroplasty ( $319 \pm 32$  and  $294 \pm 38$  pg/mL, respectively;  $P = .6$ ). Antireflux surgery in lean and morbidly obese individuals yielded lower ghrelin levels after surgery, but this observation was not significant.

#### EFFECT OF GASTRIC DIVISION ON GHRELIN LEVELS

After observing a significant reduction in circulating ghrelin levels in patients undergoing RYGB, we proceeded to determine if this observation is the result of dividing the stomach. To this end, plasma ghrelin levels were measured before surgery, after transecting the jejunum to create the Roux-en-Y limb, after dividing the stomach to create the 15-mL pouch, and after surgery. The values were  $297 \pm 38$ ,  $254 \pm 24$ ,  $193 \pm 21$ , and  $175 \pm 18$  pg/mL, respectively. The most significant declines were measured following division of the stomach to form a small gastric pouch ( $297 \pm 38$  vs  $193 \pm 21$  pg/mL,  $P < .05$ ) (**Figure 5**).

#### COMMENT

This study demonstrates that complete division of the stomach, forming a small vertical pouch, contributes to the decline in circulating ghrelin levels. It further supports that the decline in ghrelin levels following RYGB surgery is not a gradual process, but occurs early following the procedure. This correlates well with the decline of hunger sensation in many RYGB patients early after surgery, although these patients are expected to be in negative energy balance.

The mechanism for the observed decline in ghrelin levels is not clear. Gastric bypass procedures that create a small vertical proximal pouch may partially denervate the gastric fundus, but the effect of this anatomic change is unknown. Vagal tone generally inhibits ghrelin release, but experimental blocking of cholinergic stimuli

### Age and Body Mass Index (BMI) for Patients Undergoing Various Gastric Fundus Procedures

Variable	RYGB (n = 34)	VBG (n = 4)	ARS (n = 4)	Lean ARS (n = 6)
Age, y	42.9 ± 1.9	37.0 ± 1.8	54.0 ± 6.7	44.8 ± 7.8
BMI*	47.0 ± 0.7†	43.7 ± 2.5	40.0 ± 2.0†	23.8 ± 0.9‡

Abbreviations: ARS, antireflux surgery; RYGB, Roux-en-y gastric bypass; and VBG, vertical banded gastroplasty.

\*BMI calculated as weight in kilograms divided by the square of height in meters.

† $P = .01$ , difference between RYGB and morbidly obese patients undergoing ARS.

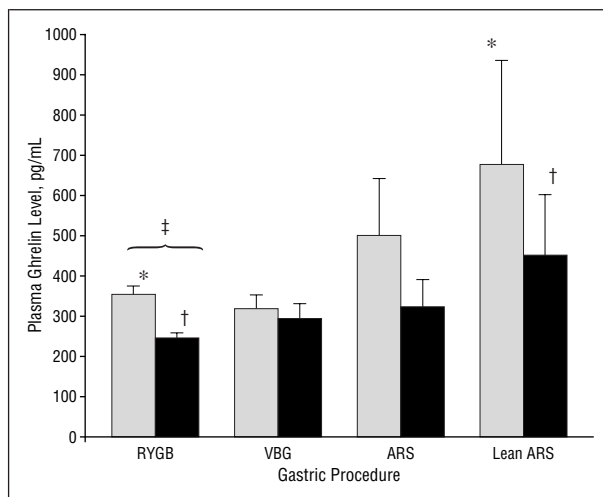
‡ $P < .001$ , difference between lean patients undergoing ARS and all patients with morbid obesity.

is associated with elevated ghrelin levels.<sup>21</sup> Moreover, during periods of fasting and associated low parasympathetic tone, ghrelin levels rise. Therefore, one would expect that partially denervating the fundus would result in an expected rise in ghrelin levels, which is not the case following gastric surgery. However, cholinergic input to the gastric fundus is not the sole innervation for the stomach, and other aspects of autonomic input may regulate ghrelin secretion. Furthermore, the levels of ghrelin never reach nil, indicating that other locations in the stomach and gastrointestinal tract may still have ghrelinogenic activity, albeit less robust.<sup>22</sup>

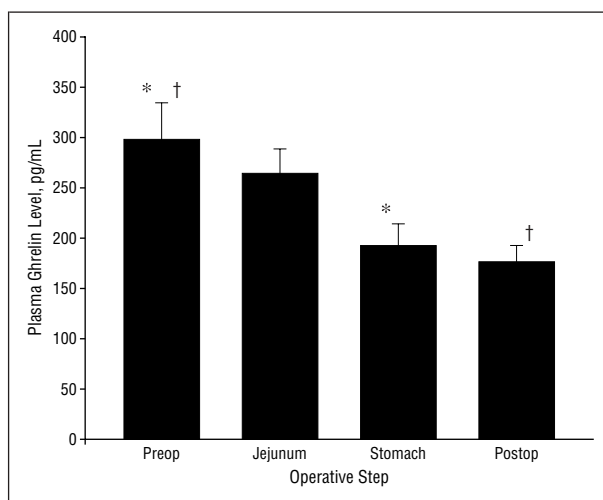
Another possibility for the observed reduction in ghrelin after RYGB may be due to paracrine effects exerted by endogenous gastrointestinal hormones such as somatostatin. Infusion of somatostatin analogues in humans suppresses ghrelin secretion.<sup>23,24</sup> However, the effect of weight reduction surgery on somatostatin activity has never been examined, to our knowledge, and the functional interactions between these 2 hormones, if any, are not established.

Although it is tempting to speculate that such early alterations in ghrelin levels may be involved in the resolution of insulin resistance commonly seen in the first 2 weeks following RYGB, data are inconclusive.<sup>25</sup> Administration of ghrelin in healthy volunteers appears to inhibit insulin production, with resultant hyperglycemia.<sup>26</sup> Human hepatoma cell lines exposed to ghrelin augment gluconeogenesis and compete against postreceptor insulin signaling.<sup>27</sup> In contrast, other investigators have shown that ghrelin administration in animals stimulates pancreatic insulin production.<sup>28,29</sup> Human obesity is associated with insulin resistance and low ghrelin levels.<sup>30-32</sup> Therefore, a lower ghrelin level alone as a result of RYGB surgery cannot directly account for the normalization of glucose metabolism in these patients.

Several animal studies<sup>1,12</sup> have demonstrated the important role of the gastric fundus in regulating ghrelin secretion. This is the first human study, to our knowledge, to demonstrate a reduction in circulating ghrelin early following division of the stomach during RYGB operation. We show that division of the stomach and exclusion of the gastric fundus play an important role in reducing circulating ghrelin levels from baseline levels. If ghrelin remains a principal stimulant of food intake



**Figure 4.** Preoperative and postoperative plasma ghrelin levels associated with each gastric fundus procedure. Gray bars indicate preoperative levels; black bars, postoperative values; double dagger,  $P < .001$ , difference between preoperative and postoperative levels following Roux-en-Y gastric bypass (RYGB); asterisk,  $P < .05$ , difference between lean antireflux surgery (ARS) patients and RYGB patients at baseline; dagger,  $P < .05$ , difference between lean ARS patients and RYGB patients after surgery; and VBG, vertical banded gastroplasty.



**Figure 5.** Circulating ghrelin levels in a gastric bypass operation (n=9). Measurements were obtained at 4 time points: before surgery (Preop), after dividing the jejunum to form the Roux-en-Y limb (Jejunum), after dividing the stomach to create the small pouch (Stomach), and after surgery (Postop). Asterisk indicates  $P < .05$ ; dagger,  $P < .01$ .

in morbid obesity, the results of this study would suggest that weight reduction procedures that do not sufficiently exclude gastric fundus tissue may not adequately lower ghrelin levels, reduce hunger, and induce optimal weight loss.

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Drs Lin and Gletsu contributed equally to this work.

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