

Short-Term Effects of Gastric Bypass Surgery on Circulating Ghrelin Levels

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Abstract

MORÍNIGO, ROSA, ROSER CASAMITJANA, VIOLETA MOIZÉ, ANTONIO M. LACY, SALVADORA DELGADO, RAMON GOMIS, AND JOSEP VIDAL. Short-term effects of gastric bypass surgery on circulating ghrelin levels. *Obes Res.* 2004;12:1108–1116.

Objective: To prospectively evaluate the short-term effects of Roux-en-Y gastric bypass (RYGBP) on ghrelin secretion and its relevance on food intake and body weight changes.

Research Methods and Procedures: Ghrelin response to a standardized test meal was evaluated in eight obese patients (BMI, 43.5 to 59.1 kg/m²) before and 6 weeks after RYGBP. Ghrelin response was compared with that of an age-matched group of six normal weight individuals (BMI, 19.6 to 24.9 kg/m²).

Results: Fasting serum ghrelin levels were lower in obese subjects compared with controls ($p < 0.05$). Meal ingestion significantly suppressed ghrelin concentration in controls ($p < 0.05$) and obese subjects ($p < 0.05$), albeit to a lesser degree in the latter group ($p < 0.05$). Despite a $10.3 \pm 1.5\%$ weight loss, fasting serum ghrelin levels were paradoxically further decreased in obese subjects 6 weeks after RYGBP ($p < 0.05$). Moreover, at this time-point, food intake did not elicit a significant ghrelin suppression. The changes in ghrelin secretion after RYGBP correlated with changes in insulin sensitivity ($p < 0.05$) and caloric intake ($p < 0.05$).

Discussion: This study showed that the adaptive response of ghrelin to body weight loss was already impaired 6 weeks after RYGBP. Our study provides circumstantial evidence for the potential role of ghrelin in the negative energy balance in RYGBP-operated patients.

Key words: ghrelin, gastric bypass, weight loss, energy intake, insulin sensitivity

Introduction

Roux-en-Y gastric bypass (RYGBP)¹ surgery is the most effective treatment to achieve and sustain major weight loss in patients with morbid obesity (1). However, the mechanisms by which RYGBP induces weight loss are not well understood (2). There is no question that the surgical creation of a small gastric pouch restricts food intake and thereby causes weight loss, because patients are forced to eat less. However, it would seem that simply restricting gastric capacity and inducing a very mild malabsorption by bypassing most of the stomach and the duodenum would not account for the massive weight loss and the changes in the eating pattern observed in patients undergoing RYGBP. Based on the current understanding of body weight regulation, weight loss resulting merely from mechanical restriction of food intake would predictably be associated with an amplification of hunger and eating behavior to compensate for the deficit in caloric intake (3). In sharp contrast, patients undergoing RYGBP present a sustained depressed appetite, despite profound negative energy balance (4). Therefore, it has been suggested that a disruption in the complex network of neural and hormonal signals involved in energy balance may help to explain the seemingly paradoxical eating behavior response in RYGPB subjects despite profound weight loss (2,5).

Ghrelin is a recently discovered 28 amino acid peptide that has been implicated in short- and long-term regulation of energy balance (6,7). Endocrine cells within the stomach fundus are the main source of this orexigenic peptide (7). Lesser amounts of the peptide have also been found in the intestinal tract, hypothalamus, pituitary gland, kidneys, and pancreas. As would be expected for a factor participating in

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¹ Nonstandard abbreviations: RYGBP, Roux-en-Y gastric bypass; HDL, high-density lipoprotein; LDL, low-density lipoprotein; HOMA-IR, homeostasis model assessment insulin resistance; AUC, area under the curve.

the control of food intake, ghrelin infusion is able to elicit food consumption (8,9), and plasma ghrelin levels increase preprandially and decrease after meal ingestion in humans and animals (10,11). Moreover, circulating ghrelin increases in response to weight loss associated with caloric restriction (12,13), anorexia nervosa (14), and cancer cachexia (15). Plasma ghrelin levels have been shown to be diminished in obese individuals (12,16,17) and to inversely correlate with BMI (12). Finally, the blockade of ghrelin signaling in the brain has been reported to decrease food intake, fat mass, and body weight in rodents (18).

A paradoxical decrease of circulating ghrelin levels has been hypothesized as one of the mechanisms underlying the reduced hunger and successful weight loss that follows RYGBP surgery (13). Cummings et al. (13) first reported that, despite a massive weight loss, plasma ghrelin levels were suppressed in patients who had undergone RYGBP. This is in sharp contrast with the significant increase in plasma ghrelin concentration observed in patients who had lost weight while following a dietary program. Unexpectedly, low circulating ghrelin levels after RYGBP have also been reported by Geloneze et al. (19) and Tritos et al. (20). Thus, these data suggest that the absence of the normal ghrelin adaptive response to weight loss could play a role in the decreased appetite in RYGBP patients. However, this hypothesis has been challenged in two recent prospective studies reporting elevated plasma ghrelin levels in patients who had undergone RYGBP (21,22).

It has been shown that the orexigenic effects of ghrelin are mediated, at least in part, through its action on the hypothalamic neurons of the arcuate nucleus (6), as well as by suppressing visceral afferent vagal nerve activity (2). However, the mechanisms regulating ghrelin secretion from the stomach remain unclear. Although it has been shown that ghrelin secretion is regulated by food intake, it is not well established to what extent this relates to contact of nutrients with the gastroenteric lumen and/or changes in nutrient or other metabolic factor plasma concentration. It has been suggested recently that the ghrelin-suppressing effect of oral glucose is associated with postgastric (pre- and/or postabsorptive) feedback mechanisms (23). Conflicting results have been reported on the effect of insulin on ghrelin secretion. An inverse correlation between fasting plasma ghrelin and insulin levels has been reported, suggesting that insulin may be a factor accounting, at least in part, for the lower plasma ghrelin concentration in obese subjects (16,21,22). However, whereas insulin infusion has been reported by some authors to induce a decrease in plasma ghrelin concentration, others have failed to observe such an effect when insulin was used at more physiological concentrations (24–27). The mediators underlying the changes in ghrelin secretion after RYGBP are largely unknown.

In this study, we aimed to further assess the possible role of ghrelin in the weight loss occurring after RYGBP. We first compared ghrelin secretion between normal weight subjects and obese patients about to undergo RYGBP, and subsequently, we prospectively evaluated the short-term effects of RYGBP on ghrelin secretion. Moreover, we examined the relationship between circulating ghrelin and the changes in body weight, caloric intake, and metabolic factors occurring in the same study period. Most studies on ghrelin secretion in RYGBP subjects have been performed after at least 6 months of follow-up. A prospective analysis of the ghrelin response to food intake in this group of patients has, to our knowledge, not been reported previously.

Research Methods and Procedures

Study Subjects

Eight obese white subjects (five women and three men; BMI, 43.5 to 59.1 kg/m²) about to undergo RYGBP were recruited from the Obesity Unit at the Hospital Clínic Universitari of Barcelona. Based on fasting plasma glucose, one was newly diagnosed with type 2 diabetes. As assessed from an oral glucose tolerance test before surgery, five patients had normal glucose tolerance, and two were categorized with impaired glucose tolerance. None was on any medication known to affect insulin sensitivity. Six healthy normal weight white volunteers (three women and three men; BMI, 19.6 to 24.9 kg/m²), matched for age with the obese group, were recruited as controls (43.8 ± 4.2 vs. 46.0 ± 3.1 years for normal weight vs. obese subjects, respectively). The body weight of all of the participants had been stable for at least the previous 4 weeks before entering the study. The study was approved by the Hospital Ethics Committee, and written informed consent was obtained from all participants.

Study Protocol

All obese subjects were evaluated within 8 weeks before RYGBP and at 6 weeks after the surgical procedure was performed. Subjects visited the Research Facility at 8:00 AM after fasting overnight. They were asked to avoid smoking from the night before and to refrain from strenuous exercise or alcohol in the 24 hours preceding the study. Wearing light clothing, subjects were weighed and measured, and a canula was inserted into a distal forearm for blood sample collection. Blood was withdrawn for measurement of plasma glucose, lipid parameters [total cholesterol, high-density lipoprotein (HDL)-cholesterol, triglycerides, and apolipoprotein B], insulin, leptin, adiponectin, and ghrelin in the fasting condition. Subjects consumed a 250-mL standard mixed liquid meal (Isosource Energy; Novartis, Basel, Switzerland) containing 398 kcal, with 50% calories as carbohydrates, 14.5% as protein, and 35% as fat. The liquid formula was well tolerated by all patients even after

RYGBP. Additional blood samples for ghrelin concentration assessment were collected at 10, 30, 60, and 120 minutes after meal ingestion. Serum samples were centrifuged immediately at -4°C . All samples were stored at -80°C until assayed. Samples from one individual obtained before and after RYGBP were assessed in the same assay.

Gastric Bypass

After failure of conservative therapy and complete evaluation by a multidisciplinary team, a standardized laparoscopic RYGBP was performed in the eight obese patients included in the study. A detailed description of the procedure can be found elsewhere (28). In brief, the surgical protocol applied to our study cohort consisted of the creation of a small gastric pouch ($<15\text{ mL}$) along the lesser curvature of the stomach, and the connection of a 100-cm Roux-en-Y limb as an enteroenterostomy to the jejunum 50 cm from the ligament of Treitz. The resultant gastric pouch-jejunal anastomosis was 1 to 1.5 cm in diameter. Vagal nerve integrity was preserved.

Biochemical and Hormonal Parameters

Plasma glucose was measured using a glucose oxidase method (Bayer Diagnostics, Munich, Germany). Total and HDL-cholesterol and triglycerides were measured using a commercial enzymatic colorimetric kit (Bayer Diagnostics). Low-density lipoprotein (LDL)-cholesterol was calculated according to Friedewald's equation (29). Apolipoprotein B was measured by a commercial immunoturbidimetric assay (Roche Diagnostics, Rotkreuz, Switzerland). Insulin levels were measured by monoclonal immunoradiometric assay (Medgenix Diagnostics, Fleunes, Belgium). The intra- and interassay coefficients of variation were $<5\%$ and $<7\%$, respectively. Data from insulin and glucose concentration were used to estimate insulin sensitivity by calculating the homeostasis model assessment insulin resistance index $\{\text{HOMA-IR} = [\text{insulin}] (\text{mU/L}) \times [\text{glucose}] (\text{mM})/22.5\}$ (30). Leptin and adiponectin concentrations were measured by a commercial radioimmunoassay (Linco Research, St. Charles, MO). For adiponectin measurement, samples were diluted 500 times before the assay. The intra- and interassay coefficients of variation for leptin and adiponectin measurement were $<8\%$ and 5% , respectively. Human plasma ghrelin was measured with a commercial radioimmunoassay (Ghrelin-Total RIA Kit; Linco Research). This assay uses ^{125}I -labeled ghrelin and a ghrelin antibody that is specific for total ghrelin and does not require the presence of the octonyl group on serine 3. The lowest level of ghrelin that can be detected by this assay is 100 pg/mL . The intra- and interassay coefficients of variation were $<10\%$ and $<15\%$, respectively. All blood work analysis was performed both before and 6 weeks after RYGBP.

Energy Intake

Subjects were asked to record in a structured food diary everything they consumed for 3 days. They received instructions from a dietitian (V.M.) on how to fill in the diary. With a computer program based on food tables (Dietsource v1.2; Novartis), a single dietitian (V.M.) used data on the food records to calculate intakes of total energy before and 6 weeks after RYGBP.

Statistical Analysis

Data are expressed as mean \pm SE. Values for the area under the curve (AUC) for glucose, insulin, and ghrelin secretion after a standard test liquid meal were calculated using the trapezoidal method. Because of the small sample size, two-tailed nonparametric unpaired (Mann-Whitney U) or paired (Wilcoxon rank) tests were used when appropriate. General linear model analysis with repeated measures was performed to evaluate the change in ghrelin from baseline after meal ingestion. Correlations were determined by univariate linear regression (Spearman's rank test). Statistical analysis was performed using SPSS 10.0 for PC (SPSS, Chicago, IL), with significance set at $p < 0.05$. Statistical power analysis was calculated using Power Analysis and Sample Size software (PASS 2002; NCSS Statistical Software, Keyville, UT). A sample size of eight achieves a $>90\%$ power to detect a difference between fasting plasma ghrelin levels before and after RYGBP with a significance level of 0.05.

Results

The preoperative average BMI of obese patients was $49.5 \pm 1.8\text{ kg/m}^2$ (range, 43.5 to 59.1 kg/m^2). Although patients were still markedly obese ($44.4 \pm 1.6\text{ kg/m}^2$; range, 39.2 to 51.8 kg/m^2), a significant reduction in BMI ($p < 0.05$) was already observed 6 weeks after surgery. At this time-point, BMI change was associated with a significant reduction in total cholesterol, LDL-cholesterol, triglycerides, insulin, and leptin (Table 1). Fasting plasma glucose concentration fell within the normal range in all patients, although as a group, it was not significantly different from that before surgery. A significant increase in plasma adiponectin was also observed. Plasma adiponectin ($p < 0.01$), but not plasma leptin, concentration changes 6 weeks after surgery were significantly correlated with changes in BMI in the same time period.

Before surgery, fasting plasma ghrelin levels were significantly lower in obese subjects compared with lean controls (980.9 ± 77.3 vs. $1221.8 \pm 65.01\text{ pg/mL}$ for obese vs. control, respectively, $p < 0.05$). General linear model analysis showed that plasma ghrelin levels were significantly suppressed after a standard test meal in both control ($p < 0.05$) and obese ($p < 0.05$) subjects (Figure 1). In the control group, plasma ghrelin concentration fell by $32.0 \pm$

Table 1. Patient characteristics before and 6 weeks after gastric bypass surgery

Variables	Preoperative	6 weeks
BMI (kg/m ²)	49.5 ± 1.8	44.4 ± 4.6*
Glucose tolerance status (n, NGT/IGT/DM)	2/5/1	—
Caloric intake (kcal/d)	1744.1 ± 176.0	699.4 ± 55.6
Glucose (mg/dL)	114.7 ± 15.4	92.0 ± 4.5
Cholesterol (mg/dL)	200.4 ± 13.0	165.3 ± 8.5*
HDL-cholesterol (mg/dL)	43.6 ± 2.3	38.3 ± 1.4
LDL-cholesterol (mg/dL)	126.8 ± 12.3	104.8 ± 7.6*
Triglycerides (mg/dL)	146.5 ± 16.8	110.1 ± 11.1*
Apolipoprotein B (mg/dL)	116.6 ± 10.7	99.5 ± 9.5
Insulin (mU/L)	26.6 ± 4.8	12.9 ± 1.6*
Leptin (mg/L)	35.0 ± 3.3	25.1 ± 4.0†
Adiponectin (mg/L)	13.7 ± 1.8	16.3 ± 2.5†

Data are expressed as mean ± SE.
 NGT, normal glucose tolerance; IGT, impaired glucose tolerance;
 DM, diabetes mellitus.
 * $p < 0.05$ and † $p < 0.01$ values are indicated for comparison between the two study time-points.

7.1% 60 minutes after the initiation of the meal, and at 120 minutes, plasma ghrelin levels were still 33.5 ± 7.5% lower compared with baseline. In obese subjects, ghrelin levels were maximally suppressed at 60 minutes after meal ingestion (18.5 ± 3.5%). However, as shown in Figure 2, the overall decline in plasma ghrelin levels after meal ingestion (represented as the AUC of ghrelin after a standard test meal) in obese subjects was only 39.3% of that observed in control subjects ($p < 0.05$).

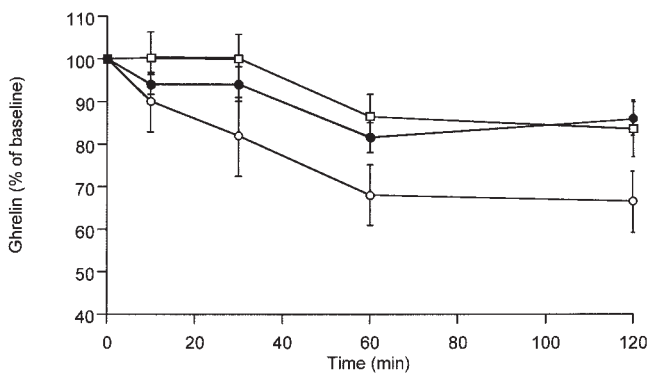


Figure 1: Mean ± SE ghrelin response in normal weight controls (○) and obese subjects before (●) or 6 weeks after (□) RYGBP.

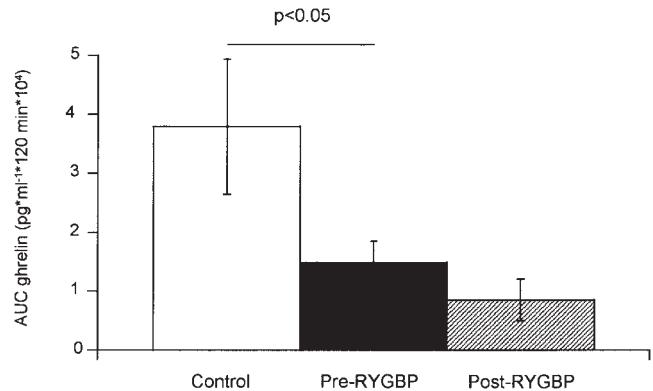


Figure 2: Mean ± SE AUC of ghrelin decline between 0 and 120 minutes after a standard liquid meal in normal weight controls (open bar) and obese subjects before (solid bar) or 6 weeks after (hatched bar) RYGBP.

Six weeks after surgery, the subjects had lost 10.3 ± 1.5% (range, 4.3% to 16.5%) of their initial body weight. Paradoxically, despite the weight loss, postoperative fasting plasma ghrelin levels were significantly lower than those observed before RYGB (844.1 ± 58.2 vs. 980.9 ± 77.3 pg/mL, $p < 0.05$). Despite the fact that the patients were actively losing weight, as shown in Figure 3, fasting ghrelin concentrations decreased in all but one patient. The fasting plasma ghrelin level 6 weeks after surgery was highly correlated to the presurgical value ($\rho = 0.905$, $p < 0.01$). General linear model analysis showed that 6 weeks after surgery, a standard test meal did not elicit a suppression of plasma ghrelin in patients who had undergone RYGBP (Figure 1). As a group, the mean ghrelin reduction in response to a standard test meal was 13.6 ± 5.2% and 16.5 ± 6.3% at 60 and 120 minutes, respectively (Figure 1). However, the individual responses were highly variable ($p < 0.001$; Figure 3). Such a high intersubject variability in the ghrelin response strongly weakened the statistical power of the comparison of the AUC of ghrelin suppression before and after RYGBP. This may account for the fact that the AUC of ghrelin decline after meal ingestion after RYGBP was not significantly different from that observed before surgery (Figure 2).

The insulin sensitivity, as assessed from the HOMA-IR index in the obese group before surgery, was significantly correlated neither with fasting plasma ghrelin nor with the AUC of ghrelin fall after meal ingestion. At this time-point, fasting plasma ghrelin was the only significant predictor of the postprandial fall ($\rho = 0.738$, $p < 0.05$). Interestingly, however, the improvement in insulin sensitivity observed after surgery was associated with a better suppressive effect of the standard test meal on plasma ghrelin concentration ($\rho = -0.786$, $p < 0.05$). Nevertheless, correlation analysis was negative when the association between the AUC for ghrelin suppression after a standard test meal and the

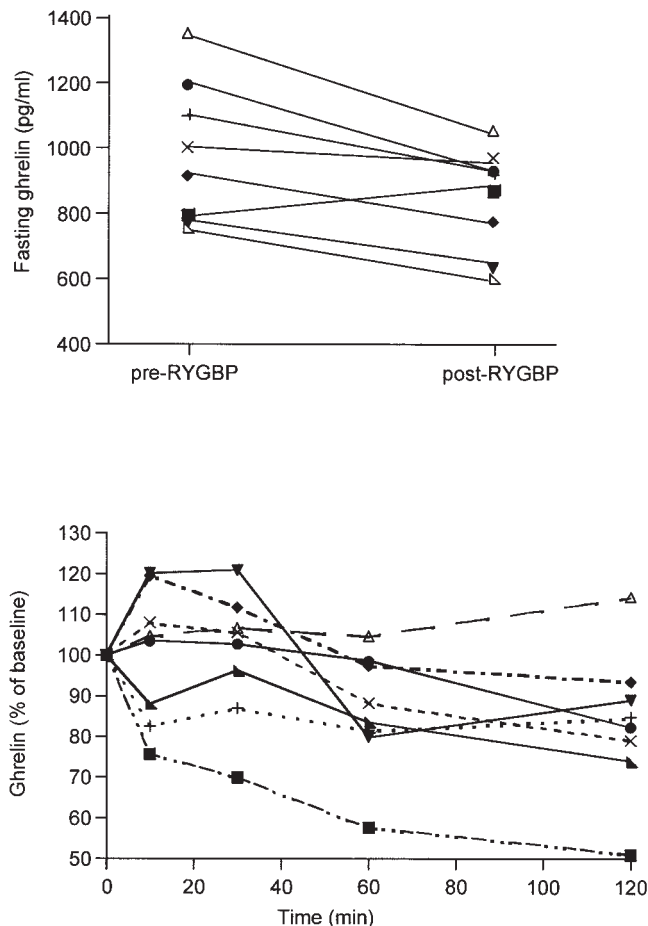


Figure 3: Individual fasting ghrelin levels in obese subjects before and 6 weeks after RYGBP (top). Individual ghrelin response to a standard liquid meal in obese subjects 6 weeks after RYGBP (bottom).

change in the adipose tissue-derived factors leptin and adiponectin was examined. These results were not affected by the exclusion from the analysis of the subject with newly diagnosed type 2 diabetes.

Finally, we aimed to evaluate whether changes in ghrelin secretion had an impact on the reduction in caloric intake and body weight loss associated with RYGBP. A negative correlation was found between postoperative AUC of ghrelin suppression and the daily caloric intake estimated from 3-day food records obtained 6 weeks after surgery ($\rho = -0.762, p < 0.05$). Furthermore, the percentage reduction in caloric intake after RYGBP was positively correlated with the changes observed in the ghrelin fall after meal ingestion ($\rho = 0.833, p < 0.05$; Figure 4). Thus, these data suggest that a better meal suppressive ghrelin effect is associated with a greater reduction in caloric intake in RYGBP subjects. However, unfortunately, neither preoperative fasting ghrelin nor preoperative AUC of ghrelin suppression predicted the percentage reduction in body weight

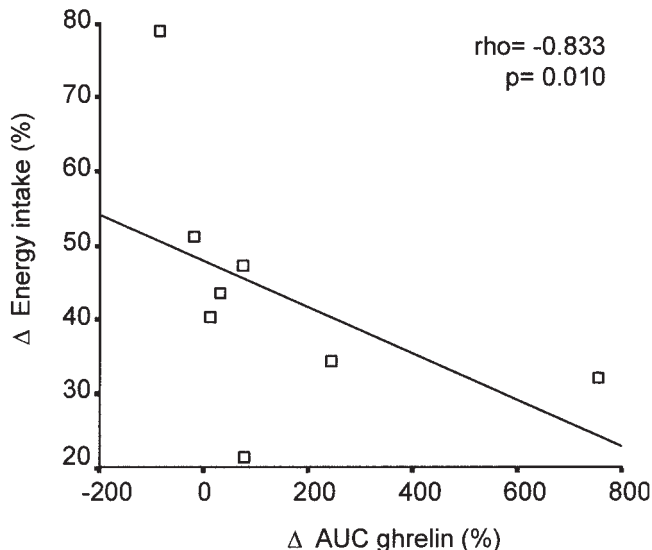


Figure 4: Inverse correlation between the changes in daily caloric intake and the changes in the AUC of ghrelin decline after a standard liquid meal in RYGBP operated patients.

that followed the surgical procedure ($p = 0.606$ and $p = 0.433$, respectively). Moreover, no significant correlation was observed between the percentage weight loss and the changes in ghrelin secretion parameters observed 6 weeks after surgery (Figure 5).

Discussion

This prospective study showed that RYGBP-induced weight loss was associated with a significant improvement in metabolic parameters and hormonal changes 6 weeks after the surgical procedure. Although the patients were still markedly obese at the end of the follow-up, a modest weight loss of 10% of the initial body weight was associated with a lowering of total cholesterol, LDL-cholesterol, triglycerides, and insulin resistance, as estimated by the HOMA model. Plasma lipid profile and insulin resistance have been shown to improve in obese patients after a similar percentage of body weight reduction, irrespective of the initial BMI (5,31). The seemingly paradoxical decrease in HDL-cholesterol in our study could be attributed to the short-term follow-up of our study. It has been shown that HDL-cholesterol changes after a hypocaloric diet are biphasic, showing a decrease in the short term and an increase in the long term (32). Circulating leptin concentration is known to reflect the amount of energy stored in fat and, therefore, correlate positively with indices of adiposity (33). The absence of a positive correlation between the amount of weight loss and the decrease in plasma leptin 6 weeks after surgery in our study is in agreement with the reported uncoupling of leptin from body fat mass after a 10-week caloric restriction in humans (34). It has been reported that

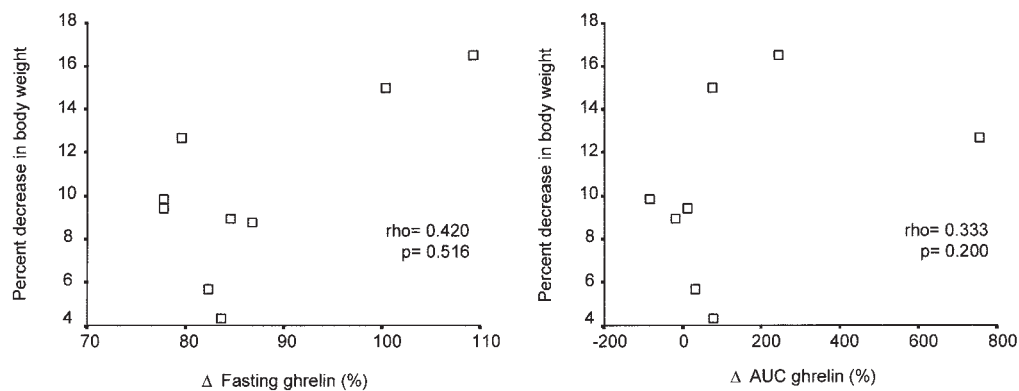


Figure 5: Absence of significant correlation between the changes in fasting plasma ghrelin (left) and the changes in the AUC of ghrelin decline after a standard liquid meal (right) and percentage decrease in body weight observed 6 weeks after RYGBP.

net energy balance, food composition, room temperature, exercise, and sleep pattern are short-term regulators of leptin concentration (35). Although little is known about the nutritional regulation of adiponectin, our study supports the contention that, in contrast to leptin, changes in body weight (21) rather than changes in caloric intake (35) have a substantial effect on the plasma concentration of this adipocyte cytokine.

Ghrelin is a recently discovered orexigenic peptide secreted primarily by the stomach and the proximal intestine (7). The main aim of our study was to prospectively evaluate the short-term RYGBP-associated changes in circulating ghrelin. We also compared ghrelin secretion between normal weight controls and obese subjects about to undergo RYGB. As reported by other authors, we observed that fasting plasma ghrelin levels were significantly lower in obese patients compared with normal weight controls (16,17). Moreover, fasting ghrelin levels were paradoxically further decreased 6 weeks after RYGBP surgery. Finally, 6 weeks after surgery, the suppressive effect of a standard liquid meal on plasma ghrelin was blunted. To our knowledge, this is the first report on the effects of RYGBP on circulating ghrelin levels 6 weeks after surgery.

In their seminal cross-sectional study, Cummings et al. (13) reported low fasting plasma ghrelin concentrations in obese patients 9 to 31 months after RYGBP compared with a group of matched obese controls. This finding was supported by the findings of the Geloneze et al. (19) longitudinal study, in which fasting plasma ghrelin levels were significantly lower at 1 year after surgery than those observed before RYGBP. Override inhibition has been postulated as the mechanism by which plasma ghrelin levels are paradoxically decreased in the fasting state after RYGBP (13). According to this hypothesis, a continuously empty stomach and duodenum would become depleted of ghrelin after prolonged fasting. Six weeks after RYGBP, patients in our cohort showed a decrease in fasting plasma ghrelin

levels. Compatible with a time-related phenomenon, post-surgical fasting ghrelin levels decreased in most patients and were highly correlated with the presurgical circulating hormone levels. According to the override inhibition hypothesis, we could speculate that a greater suppression of fasting ghrelin levels would have been observed with a longer follow-up. However, conflicting data with those of the Cummings et al. (13) and Geloneze et al. (19) studies have been reported in recent prospective studies in which plasma ghrelin concentration was assessed at ~6 months after surgery. Holdstock et al. (22) reported that the amount of weight loss in RYGBP patients was associated with a proportional increase in fasting ghrelin levels at both 6 and 12 months after surgery. Moreover, in the findings of Faraj et al. (21), ghrelin levels were inappropriately low 15 ± 6 months after surgery in subjects who had achieved a stable postoperative weight compared with baseline, but were higher than the preoperative values in patients who were still actively losing weight. In sharp contrast with these observations, data in our study showed that the disrupted normal adaptive response of ghrelin to weight loss occurring after RYGBP could already be observed at a time when patients were still in a profound negative energy balance. Despite the fact that all of the patients in our study were actively losing weight, fasting plasma ghrelin was paradoxically decreased in all but one patient. Moreover, data in our study showed that the paradoxical ghrelin decrease in fasting plasma ghrelin after RYGBP surgery occurred not only at massive weight losses similar to those reported by Cummings et al. (13) and Geloneze et al. (19), but also at weight losses comparable to those achieved by dietary restriction. The seemingly conflicting data between our study and those of Holdstock et al. (22) and Faraj et al. (21) are hard to reconcile. According to the override inhibition hypothesis, differences in the surgical technique from one center to another may affect fasting plasma ghrelin. A gastric pouch not completely excluding the gastric fundus or a short

biliopancreatic limb would allow for contact of nutrients with the main sites of ghrelin secretion. Thus, under these conditions, ghrelin secretion could still retain its dynamics after surgery for obesity, at least in part. Consistent with this prediction, it has been shown that gastric banding patients present increased postsurgical ghrelin levels despite significant weight loss (2). Gastric pouch size in our study was similar to that reported by Faraj et al. (21) and Holdstock et al. (22). Although biliopancreatic limb length in our study was longer than the reported 30-cm limb in the Holdstock et al. study (22), it was similar to that of 100 cm reported by Faraj et al (21). Therefore, it is unlikely that differences in these two anatomical parameters account for the discordant results. However, it does not preclude that other differences in the surgical technique could have played a role. Data from animal models have shown that vagal nerve integrity is an important determinant of ghrelin secretion dynamics (36). Unfortunately, vagal nerve handling in previous studies was not reported (13,19–22). Finally, there is no doubt that the comparison among the different studies is also hampered by differences in the length of follow-up. Whether the time course of fasting ghrelin concentration after RYGBP is best represented by a phasic pattern or by a progressive decline remains to be established.

Few studies have analyzed the suppressive effect of food intake on ghrelin secretion in obese patients and its changes after RYGBP. Supporting its potential role in energy homeostasis regulation, circulating ghrelin has been shown to increase preprandially and decrease postprandially in normal weight controls (10). Accordingly, we found that in control subjects, a 398-kcal liquid meal was able to elicit a 30% to 35% fall in plasma ghrelin levels that lasted for the 2-hour observation period. This is in agreement with the reported 20% to 25% fall in plasma ghrelin levels after lean controls were fed either a standard test meal or their habitual meal (24). Before surgery, obese patients in our cohort showed a significant, albeit impaired, acute ghrelin suppressive response to feeding. Our data are compatible with the preserved prandial pattern of ghrelin secretion observed in obese patients in the study by Cummings et al. (13), regardless of whether subjects had been evaluated before or after a dietary-induced 17% weight loss. However, our data contrast with the failure of food intake to suppress ghrelin production in obese subjects reported by English et al. (37). The energy intake in our study was lower than that in English et al. (37). We chose to restrict the administered volume, and consequently energy intake, to account for the reduction in postsurgical gastric volume. Because nutrient composition has been shown to play a role in ghrelin production (38,39), it is tempting to speculate that this may account, at least in part, for the different suppressive effect of food on circulating ghrelin in obese subjects in different studies. Six weeks after surgery, a standard liquid meal did not elicit a suppression of plasma ghrelin in patients who

had undergone RYGBP. However, it should be acknowledged that the individual suppressive response was highly variable, with some patients showing no suppression and some patients showing a response comparable with that observed in lean controls. Although, as reported by Cummings et al. (13), as a group, patients in our cohort failed to show the normal meal-related oscillation of plasma ghrelin, the high interindividual variability we observed is in sharp contrast with the reportedly homogeneous 24-hour, nearly flat ghrelin response reported in that study, in which patients were evaluated 9 to 31 months after surgery. However, the different length of follow-up and different experimental protocol may help to explain the differences between the two studies. Moreover, based on the demonstration that meal-related ghrelin suppression requires postgastric stimulation (23), it could be hypothesized that differences in gastric emptying and/or other intestinal factors responsible for the ability to generate such a signal may help to explain the differences among subjects in our cohort. Gastric emptying has been shown to differ among RYGBP patients (40) and to shorten over time after the operation (41). Nonetheless, further studies are needed to understand the potential relevance of gastric emptying on ghrelin secretion changes after RYGBP.

The mechanisms underlying the abnormal ghrelin levels in obese subjects and the disrupted adaptive changes in ghrelin after weight loss associated with RYGBP are largely unknown. Because leptin and insulin are established adiposity signals participating in the control of body weight (3), it has been suggested that they may participate in the control of ghrelin secretion. Leptin and ghrelin change reciprocally after changes in body weight (12,13,42). Therefore, it has been hypothesized that leptin negatively regulates ghrelin (43). However, as reported by others (12,42), we failed to observe a significant relationship between circulating ghrelin and leptin levels in both lean controls and obese subjects assessed either before or after RYGBP surgery. Considerable evidence favors a role for insulin in the short- and long-term regulation of ghrelin. Glucose clamp studies have shown that hyperinsulinemia is able to suppress ghrelin (26,27). Moreover, according to Tschöp et al. (16), insulinemia could explain as much as 36.5% of fasting plasma ghrelin variance in whites and Pima Indians. Therefore, it is conceivable that the hyperinsulinemia of obesity and the lower insulin levels in lean subjects could account, at least in part, for the variance in ghrelin concentration associated with different body mass. Consistent with this hypothesis, we observed that, 6 weeks after RYGBP surgery, the larger the improvement in insulin sensitivity, the larger the amelioration of the suppressive effect of food on ghrelin secretion. The exact pathways relating insulin and ghrelin secretion are not well established. In the short term, insulin could acutely exert a direct effect on ghrelin-secreting cells or could affect ghrelin secretion indirectly through regulation

of other humoral or neuronal factors (26). Because adiponectin is an adipose tissue–secreted product, mediating insulin sensitivity (35), we analyzed the relationship between ghrelin and adiponectin. The lack of an association between adiponectin and ghrelin levels suggests that this adipocyte cytokine is not a key mediator of insulin on circulating ghrelin. However, a larger number of patients would be needed to confirm this point.

Notwithstanding the different results among reports on ghrelin levels after RYGBP, a critical question is whether ghrelin suppression achieved by some operated subjects contributes to weight loss. The answer to this question could have potentially important implications for the surgical and medical approach to obesity treatment (44). To gain insight into this issue, we examined the relationship between plasma ghrelin concentration and changes in body weight and daily caloric intake. Interestingly, a significant correlation was found between the ability of food intake to elicit a fall in plasma ghrelin and the percentage reduction in caloric intake in operated patients. Ghrelin infusion has been shown to increase food intake in humans (8), and antagonism of ghrelin receptor has been found to reduce food intake in mice (18). Although food records may be of limited value in estimating caloric intake in obese patients (45), our observation could be viewed as circumstantial evidence supporting a role of ghrelin on energy intake. Unfortunately, neither the changes in fasting plasma ghrelin nor the changes in the magnitude of its suppression after a standard test meal were significantly associated with the extent of weight loss observed 6 weeks after RYGBP. However, it should be underscored that the small number of patients in our cohort severely weakened the strength of the analysis.

In summary, this study showed that the adaptive response of ghrelin to body weight loss was already impaired 6 weeks after RYGBP. The mechanisms underlying this phenomena remain largely unknown. Our data suggest that changes in insulin sensitivity could be a factor. Finally, our data provide circumstantial evidence for the potential role of ghrelin in the negative energy balance occurring in the first 6 weeks after RYGBP. However, further studies are needed to prove the relevance of plasma ghrelin changes in the weight loss observed shortly after RYGBP.

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