Ultradian ghrelin pulsatility is disrupted in morbidly obese subjects after weight loss induced by malabsorptive bariatric surgery\textsuperscript{1–3}

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ABSTRACT

Background: Suppression of ghrelin production after Roux-en-Y gastric bypass that suggested its contribution to appetite reduction has been reported.

Objective: Because biliopancreatic diversion (BPD) does not affect appetite, we compared ghrelin production and 24-h pulsatility between healthy control subjects and obese subjects before and after BPD.

Design: A computerized algorithm identified peak heights, clearance rate, and peak frequency of ghrelin over 24 h. Twenty-four-hour energy expenditure was measured in the calorimetric chamber, and energy intakes were computed. Insulin sensitivity was measured with a euglycemic-hyperinsulinemic clamp.

Results: Mean (±SD) 24-h plasma ghrelin concentrations were significantly ($P < 0.0001$) higher in control than in obese subjects (33.817 ± 22.09 and 164.47 ± 29.19 µg/L, respectively), but they increased to 204.64 ± 28.51 µg/L in the obese subjects after BPD ($P < 0.01$). The pulsatility index was 0.098 ± 0.016 and 0.041 ± 0.014 µg · L\textsuperscript{−1} · min\textsuperscript{−1} in control and obese subjects, respectively ($P < 0.01$), and decreased to 0.025 ± 0.007 µg · L\textsuperscript{−1} · min\textsuperscript{−1} after BPD ($P < 0.05$). Energy intakes before and after BFP did not differ significantly. Although metabolizable energy after BPD was 40% of the energy intake, that (per kg fat-free mass) after BPD did not different significantly from that before BPD.

Conclusions: Weight loss induced by malabsorptive bariatric surgery is associated with greater ghrelin concentrations, which, however, remain consistently lower than those in control subjects, whereas ghrelin pulsatility is subverted. Higher ghrelin concentrations may contribute to the high calorie intakes observed in post-BPD subjects. The lack of normal pulsatility may explain the new impulse of these subjects to eat very frequently. Am J Clin Nutr 2006;83:1017–24.

KEY WORDS Biliopancreatic diversion, morbid obesity, ghrelin, pulsatility, appetite, insulin sensitivity, energy intake

INTRODUCTION

Ghrelin, a gut hormone with a rapid, short-lived orexigenic effect in rodents (1–4), is a 28-amino acid acylated peptide that has a molecular weight of 3300. It is primarily secreted by cells in the oxyntic glands of the stomach and duodenum (5).

Cummings et al (6) showed that the circulating concentrations of ghrelin rise preprandially and fall postprandially in healthy subjects, which suggests that this hormone plays a role in meal initiation in humans. Twenty-four–hour profiles of circulating ghrelin in patients with simple obesity and anorexia nervosa consistently showed significantly lower and higher concentrations, respectively, than in healthy, normal-weight subjects (7). On the basis of those reported data, we hypothesized that the nutritional state is a determinant of plasma ghrelin concentrations in humans and that ghrelin secretion is up-regulated under conditions of negative energy balance and down-regulated under conditions of positive energy balance.

Plasma ghrelin does not decline in obese subjects after a test meal as it does in lean subjects. This lack of suppression after a meal in obese subjects could lead to greater food consumption and suggests that ghrelin may be involved in the pathophysiology of obesity (8).

Cummings et al (9) showed that 24-h plasma ghrelin concentrations increase in response to diet-induced weight loss, whereas they remain low after Roux-en-Y gastric bypass (RYGBP), which raises the possibility that this bariatric surgical procedure reduces weight at least in part by suppressing ghrelin production—and thus the appetite. In contrast, Holdstock et al (10) found that ghrelin decreases in obese subjects who underwent

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RYGBP were strictly related to the subsequent reduction in body mass index (BMI; in kg/m²), which suggests that RYGBP surgery per se has no effect, independent of weight loss, on ghrelin concentrations. Nevertheless, the role of ghrelin in the regulation of body weight in humans remains unclear, and, moreover, no published studies have reported the rhythmic oscillations within 24 h, or the ultradian rhythm, of plasma ghrelin in healthy and obese persons. Therefore, we investigated the pulsatility of ghrelin in obese subjects before and after weight loss induced by a malabsorptive bariatric procedure—the biliopancreatic diversion (BPD)—which led to a marked increase in appetite, and compared the data with those from a group of healthy, normal-weight control subjects. Bariatric surgery currently represents the most effective way to obtain a controlled weight reduction in morbidly obese subjects (11).

Thus, blood samples for ghrelin measurement were collected from severely obese women every 60 min for 24-h periods immediately before and again 14 mo after BPD, when the subjects’ weight had been stable for ≥2 mo. Insulin-mediated glucose disposal was measured by using a euglycemic-hyperinsulinemic clamp.

SUBJECTS AND METHODS

Subjects

The study groups consisted of 6 severely obese (BMI: >40) women, 35 ± 5 y old, who were studied during the follicular phase of their menstrual cycle on 2 separate occasions—before and 14 after BPD. None had impaired glucose tolerance, diabetes mellitus, or any other endocrine or nonendocrine disease. At the time of the 2 studies, all subjects were following an ad libitum diet.

Six healthy, normal-weight women, aged 34 ± 6 y, recruited from the medical staff of the Catholic University School of Medicine volunteered for this study. All subjects had a normal physical examination and no history of gastrointestinal or endocrine disorders. The subjects were studied on 2 separate days, one for the assessment of hormone rhythm during a standardized 24-h period and one for the measurement of insulin sensitivity by using the glucose clamp method.

All subjects gave written informed consent. The study protocol was approved by the Institutional Review Board of the Catholic University School of Medicine.

Study protocol

24-h Nitrogen and lipid outputs

Twenty-four-hour urine and stool collections were carried out on the day that was spent in the calorimetric chamber. Urinary nitrogen was analyzed with the use of a blood urea nitrogen (BUN) analyzer (Beckman Instruments, Fullerton, CA). Stool aliquots were homogenized and analyzed for nitrogen, carbohydrate, and lipid content by using a Fenyr analyzer (PerCon Prüfgeräte, Hamburg, Germany). The SD of repeated (n = 3) measurements of 24-h fat loss in BPD patients was found to be < 0.5 g/d.

Food intake

Energy intake and composition were assessed by using weighed food records kept over three 3-d periods that included 2 weekends and that were evenly spaced over the 6 mo preceding the study. Each subject was asked to record everything she ate or drank for a total of 9 d. During each recording period, the dietitian had 2 individual meetings with the subject, the first to provide instructions and the second to check the food records. All of the subjects were followed by the same dietitian. The diet during the day spent in the calorimetric chamber reflected each subject’s habitual diet according to their food records. To calculate the energy balance, we used the data for the food intake recorded during the stay in the calorimetric chamber.

The nutrient content of every food item was calculated by using computerized tables from the FOOD PROCESSOR II food and diet analysis software system (version 7.4; ESHA Research, Salem, OR, modified according to the food tables of the Istituto Nazionale di Nutrizione, Rome, Italy). The energy content of the food was computed as follows: 4.3 kcal/g for protein, 4.2 g for starch (or starch equivalent), and 9.3 kcal/g for fat (12).

Metabolizable energy evaluation

BPD patients were treated with oral antibiotics for 1 wk before the study to exclude intestinal bacterial overgrowth, as assessed by using the breath-hydrogen test, which could increase carbon dioxide uptake production (13). The metabolizable energy intake was defined as the gross energy intake minus fecal and urinary losses. The 9-d food records were used to calculate energy intake. With those data, the diet in the calorimetric chamber was prepared to reflect the diet usually consumed by the subjects. To calculate the energy intake on the day that was spent in the calorimetric chamber, the food given and the food returned were weighed. In the same way, 24-h urine and stool collections were carried out and the values found were used to calculate the metabolized energy intake.

Biliopancreatic diversion

BPD, an essentially malabsorptive surgical procedure (14), consisted of an ≈60% distal gastric resection and stapled closure of the duodenal stump. The residual volume of the stomach was 300 mL. The small bowel was transected 2.5 m from the ileocecal valve, and its distal end was anastomosed to the remaining stomach. The proximal end of the ileum, which includes the remaining small bowel carrying the biliopancreatic juice and is excluded from food transit, was anastomosed to the bowel in an end-to-side fashion, 50 cm proximal to the ileocecal valve. The total remaining length of absorbing bowel was 250 cm, of which the final 50 cm, the so-called common channel, represents the site where ingested food and biliopancreatic juices mix.

Body composition

Body weight was measured to the nearest 0.1 kg with the use of a beam scale, and height to the nearest 0.5 cm with the use of a stadiometer (both: Holtain, Crosswell, United Kingdom). Total body water (TBW) was measured by using 0.19-Bq tritiated water in 5 mL saline solution, which was administered as an intravenous bolus injection. Blood samples were drawn immediately before and 3 h after the injection. Radioactivity was measured in duplicate on 0.5 mL plasma by using a β-scintillation counter (Model 1600TR; Canberra-Packard, Meriden, CT). Corrections were made (5%) for nonaqueous hydrogen exchange; water density at body temperature was assumed to be 0.99371 kg/L. TBW (in kg) was computed as ³H₂O dilution
space (in L) × 0.95 × 0.99371. The within-subject CV for this method is 1.5%. Fat-free mass (FFM; in kg) was obtained by dividing the TBW by 0.732 (15).

**Euglycemic-hyperinsulinemic clamp procedure**

Peripheral insulin sensitivity was evaluated by using the euglycemic-hyperinsulinemic clamp procedure (16). After one cannula was inserted in a dorsal hand vein for sampling arterialized venous blood and another was inserted in the antecubital fossa of the contralateral arm for infusions, the subjects rested in the supine position for ≥1 h. They were placed with one hand warmed in a heated air box set at 60 °C to obtain arterialized blood samples. Whole-body insulin-mediated glucose disposal (M value) in μmol kg\(^{-1}\) FFM/min was measured during a primed constant infusion of insulin at the rate of 6 pmol · min\(^{-1}\) · kg\(^{-1}\). The fasting plasma glucose concentration was maintained throughout the insulin infusion by means of a variable glucose infusion and blood glucose measurements every 5 min. Whole-body peripheral glucose utilization was calculated during the last 40 min of the steady state insulin infusion.

**24-h Studies in the calorimetric chamber**

The subjects spent 24 h (beginning at 0800) in the respiratory chamber at the Metabolism Unit of the Catholic University School of Medicine (Rome, Italy). The characteristics of the device were described previously (17).

During the study day, the pre-BPD and post-BPD obese subjects were assigned a diet with an energy content of 12 200 kJ consisting of 42% carbohydrate, 47% fat, and 11% protein, which reflected the diet habitually consumed by the subjects. Control subjects received 8650 kJ, which accorded with their reported average daily energy intake. This amount was divided as follows: 20% at breakfast, 40% at lunch, 10% as an afternoon snack, and 30% at dinner. The 4 meals served in the chamber were prepared by a dietitian who used common foods such as meat, fish, vegetables, bread, and fruit. The food given and the food returned were weighed to the nearest 1.0 g on precision scales (KS-01; Rowenta, Berlin, Germany). The nutrient content of all food items was calculated as detailed above.

At 1600, the subjects performed a physical exercise session on the motorized treadmill, walking up a 10% gradient for 30 min at a constant speed of 3 km/h. Hourly blood samples were drawn from a central venous catheter to which was attached a long plastic tube, which reached outside the chamber, for the hormone measurement.

**Analytic methods**

Plasma samples were frozen at −70 °C for later measurement of ghrelin. For measurement of active ghrelin, a standardized radioimmunoassay kit (Linco Research Inc, St Charles, MO) with a highly specific antibody directed against the octanoyl-modified portion of the ghrelin molecule was used. The lowest concentration of active ghrelin that can be detected by this assay is 1 fmol/mL when a 50-μL sample is used. Intraassay and interassay CVs were 4.1% and 4.6%, respectively. Plasma glucose was measured by using the glucose oxidase method (Beckman). Plasma insulin was assayed by using a microparticle enzyme immunoassay (MEIA; Abbott, Pasadena, CA) with a sensitivity of 1 μU/mL and an intraassay CV of 6.6%. All samples for each subject were assayed at the same time each hour.

**Diurnal variability analysis**

Fourier analysis (18) was applied to the 24-h hormonal time series to study fluctuations on selected time scales. Serum concentrations of ghrelin for each subject were first low-pass filtered over a frequency range of 0 to 0.1 cycles/h to extract the low-frequency components. These low-pass-filtered time series were used to study the long-term variations. Each filtered dataset was rescaled so that the 24-h average was set to equal 100% and so that data at each time point were defined as a percentage of the 24-h average.

**Pulsatility analysis**

We used PULSEFIT, a computerized algorithm (19, 20), to identify the pulses in the 24-h time series. PULSEFIT uses the strict mathematical definition of a pulse—an instantaneous rise followed by an exponential decline—to model circulating hormone measurements.

The optimal peak heights and clearance rate are measured by using least-squares means. The optimal peak location is measured by stepwise regression, and the optimal peak number (ie, peak frequency) is measured by minimizing the predictive error with the use of the generalized cross-validation index. The program returns a pulsatility index, which measures the “pulsiness” of a series. Specifically, the pulsatility index is defined as the SD of the positively constrained, optimal, discrete deconvolution of the logarithm of the circulating hormone measurements.

The PULSEFIT program requires data input at least every 10 min, and experimental data are collected every hour. However, because, in a curve, natural cubic splines pass through a sequence of control points, we used an interpolating function that passed precisely through the experimental values. A cubic spline is a spline constructed of piecewise third-order polynomials that pass through a set of m control points. The second derivative of each polynomial is commonly set to zero at the endpoints, because that setting provides a boundary condition that completes the system of m – 2 equations. This procedure produces a so-called “natural” cubic spline and leads to a simple tridiagonal system, which can be solved easily to give the coefficients of the polynomials. Therefore, before using the PULSEFIT program, the experimental hormone concentration time series (24 points: 1 point/h) was fitted by cubic splines to obtain an estimate of circulating hormone concentrations every 10 min.

The fitting procedure was obtained by using the “csaps” function of MATLAB software (version 5.0; Mathworks, Natick, MA) in which values equaling csaps (ie, x, y, p, and xx) return the values at xx of the cubic-smoothing spline for the given data (x and y) and depending on the smoothing parameter p from 0 to 1. For p = 0, this is the least-squares straight-line fit to the data, and, at the other extreme—ie, for p = 1—this is the “natural” or variational cubic spline interpolant. In this study, p was assumed to be 0.995.

**Statistical analysis**

Data are reported as means ± SDs, unless otherwise specified. We performed Wilcoxon’s signed rank test to compare data from the same subjects before and after BPD and made intergroup comparisons by using the Mann-Whitney U test. Bonferroni correction for multiple comparisons was performed. An approximately linear plot of the residuals signifies that the data are reasonably Gaussian. Data analyses were performed with SPSS.
TABLE 1
Anthropometric characteristics of the study subjects

<table>
<thead>
<tr>
<th></th>
<th>Control subjects (n = 6)</th>
<th>Obese subjects (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BMI (kg/m²)</strong></td>
<td>23.02 ± 1.40</td>
<td>28.78 ± 1.76(^2,3)</td>
</tr>
<tr>
<td><strong>FFM (kg)</strong></td>
<td>52.88 ± 5.80</td>
<td>60.03 ± 6.50(^2,3)</td>
</tr>
<tr>
<td><strong>FM (kg)</strong></td>
<td>10.07 ± 1.79</td>
<td>20.97 ± 3.27(^2,3)</td>
</tr>
</tbody>
</table>

\(^1\) All values are \(\bar{x} ± SD\). BPD, biliopancreatic diversion; FFM, fat-free mass; FM, fat mass.
\(^2\) Significantly different from control subjects, \(P < 0.01\) (Mann-Whitney U test with Bonferroni correction).
\(^3\) Significantly different from before BPD, \(P < 0.0001\) (Wilcoxon’s signed rank test with Bonferroni correction).

RESULTS

Body composition

The anthropometric characteristics of the subjects are reported in Table 1. The average preoperative BMI was 58.98 ± 10.12. BMI was dramatically reduced after surgery, to 28.78 ± 1.76. Also after BPD surgery, fat mass decreased from 75.42 ± 15.19 to 20.97 ± 3.27 kg (\(P < 0.0001\)), and FFM decreased, although to a lesser degree (from 95.59 ± 15.19 to 60.03 ± 6.50 kg; \(P < 0.001\)).

Energy balance

The energy intake of the obese subjects did not change significantly after BPD. However, as shown in Table 2, the metabolizable energy became only 40% of the energy intake after the operation, mainly as a consequence of the fecal fat loss. The fecal loss of carbohydrate accounted for 10.78 ± 2.17% of the carbohydrate intake, that of lipids was 80.7 ± 5.1%, and that of proteins was 17.3 ± 4.9%. However, the metabolizable energy normalized by kg\(_{FFM}\) was not statistically different before and after BPD.

24-h Energy expenditure and insulin sensitivity

The 24-h energy expenditure was 6768.9 ± 722.0 kJ in control subjects and 11 300.6 ± 1457.6 and 7278.2 ± 773.6 kJ before and after BPD, respectively, in obese subjects (\(P < 0.0001\) for comparisons of control and obese subjects and of pre-BPD and post-BPD obese subjects). However, when the EE was normalized by using the FFM, no significant difference between groups was detected (125.1 ± 7.8, 125.0 ± 7.6, and 121.3 ± 3.4 kJ/kg\(_{FFM}\) in control, pre-BPD obese subjects, and post-BPD obese subjects, respectively).

Whole-body insulin-mediated glucose disposal doubled after BPD (57.6 ± 3.4 compared with 27.5 ± 33. \(\mu\)mol · kg\(_{FFM}\)\(^{-1}·\text{min}^{-1}\); \(P < 0.0001\)) and became similar to that of control subjects (55.6 ± 7.2 \(\mu\)mol · kg\(_{FFM}\)\(^{-1}·\text{min}^{-1}\)). The plasma insulin concentration at the euglycemic-hyperinsulinemic clamp steady state did not differ significantly between the 3 groups (544.7 ± 44.9, 550.5 ± 52.9, and 552.7 ± 51.9 pmol in control subjects and pre-BPD and post-BPD obese subjects, respectively).

Correlation

The correlation between fasting plasma ghrelin concentrations and BMI was stronger before BPD (fasting ghrelin, –0.168; BMI, –85.417; \(R^2 = 0.67, P < 0.001\)) than after BPD (fasting ghrelin, –22.175; BMI, 853.9; \(R^2 = 0.59, P < 0.05\)). Circulating ghrelin concentrations also were correlated with BMI in control subjects (fasting ghrelin, –80.198; BMI, 2248.8; \(R^2 = 0.68, P < 0.001\)).

Plasma ghrelin concentrations were significantly correlated with whole-body insulin-mediated glucose disposal (\(M\) values) in all of the groups studied (ghrelin, 4.817; \(M\) value, –4.8882; \(R^2 = 0.76, P < 0.001\) before BPD; ghrelin, 3.4538; \(M\) value, 12.852; \(R^2 = 0.69, P < 0.001\) after BPD; ghrelin, 15.497; \(M\) value, –444.4; \(R^2 = 0.72, P < 0.001\) in control subjects).

TABLE 2
Energy intake and metabolizable energy in obese subjects before and after biliopancreatic diversion (BPD)

<table>
<thead>
<tr>
<th></th>
<th>Before BPD (n = 6)</th>
<th>After BPD (n = 6)</th>
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</thead>
<tbody>
<tr>
<td><strong>kJ (% of energy intake)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Energy intake</td>
<td>12 126.0 ± 510.1</td>
<td>12 203.8 ± 1160.6</td>
</tr>
<tr>
<td>Carbohydrate intake</td>
<td>5462.9 ± 228.6</td>
<td>4968.5 ± 878.3</td>
</tr>
<tr>
<td>Fat intake</td>
<td>5733.3 ± 490.1</td>
<td>5700.1 ± 502.6</td>
</tr>
<tr>
<td>Protein intake</td>
<td>1275.7 ± 165.1</td>
<td>1535.3 ± 249.2</td>
</tr>
<tr>
<td>Metabolizable energy</td>
<td>7352.1 ± 738.3</td>
<td>3.4538 ± 12.852</td>
</tr>
<tr>
<td>Metabolizable carbohydrate</td>
<td>4968.5 ± 878.3</td>
<td>3.4538 ± 12.852</td>
</tr>
<tr>
<td>Metabolizable fat</td>
<td>1653.6 ± 504.4</td>
<td>1266.4 ± 202.1</td>
</tr>
<tr>
<td>Metabolizable proteins</td>
<td>135.8 ± 18.8</td>
<td>120.9 ± 18.5</td>
</tr>
</tbody>
</table>

\(^1\) All values are \(\bar{x} ± SD\). Intralgroup comparison (obese subjects before BPD compared with obese subjects after BPD) was performed by using Bonferroni-corrected Wilcoxon’s signed rank test.
\(^2\) Significantly different from before BPD, \(P < 0.0001\) (Mann-Whitney U test).
\(^3\) Significantly different from after BPD, \(P < 0.0001\) (Mann-Whitney U test).
\(^4\) Expressed per kg fat-free mass.
Ultradian variability (pulsatility) analysis

The single, interpolated plasma ghrelin concentration time course for each control subject is shown in Figure 1. The hourly experimental points of plasma ghrelin concentration together with the fitted curves for each obese subject before and after BPD are shown in Figure 2. A clear rhythm in the ghrelin circulating concentrations can be observed in control subjects; that rhythm is less, but still present, in pre-BPD obese subjects, whereas it is disrupted in post-BPD obese subjects. The individual variation in ghrelin timing was not consistent in the subject groups. There is no information from this research or in the literature on the reproducibility of ultradian ghrelin curves.

Ghrelin

The area under the curve for plasma ghrelin was higher in the control subjects (4035.75 ± 545.03 μg·L⁻¹·h⁻¹; P < 0.001) than in the other 2 groups; it significantly (P < 0.05) increased in the obese subjects after BPD, from 1083.05 ± 268.64 to 1728.52 ± 3490.72 μg·L⁻¹·h⁻¹. The mean plasma ghrelin concentration was significantly (P < 0.0001) higher in control subjects than in pre-BPD obese subjects (338.17 ± 22.09 and 164.47 ± 29.19 μg/L, respectively), and it increased significantly (P < 0.01), to 204.64 ± 28.51 μg/L, after BPD.

The pulsatility index was 0.098 ± 0.016 μg·L⁻¹·min⁻¹ in control subjects and 0.041 ± 0.014 μg·L⁻¹·min⁻¹ in obese subjects before BPD (P < 0.01), and it decreased to 0.025 ± 0.007 μg·L⁻¹·min⁻¹ in obese subjects after BPD (P < 0.05). The ghrelin clearance rate did not differ significantly between the 3 groups of subjects: it was 0.0027 ± 0.0012 and 0.0015 ± 0.0006 min⁻¹ in control and obese subjects, respectively, before BPD and 0.0015 ± 0.0012 min⁻¹ in obese subjects after BPD.

DISCUSSION

This is the first report comparing the ultradian rhythm of ghrelin in normal-weight persons with that in morbidly obese subjects before and after weight-reduction surgery. Cummings et al (6) first reported the presence of a pattern in plasma ghrelin concentrations—ie, a rise shortly before and a drop shortly after each meal—which indicates that this hormone plays a role in the initiation of eating. In another report (9), that same group described changes in plasma ghrelin profiles in morbidly obese subjects after dieting and after restrictive bariatric surgery. However, in both cases, the pulsatility of the ghrelin profiles was not analyzed.

The major findings of our study are that the amplitude and the frequency of ghrelin pulses are significantly higher in healthy control subjects than in morbidly obese subjects; the control subjects had a rapid rise in plasma ghrelin concentrations very early in the morning, which did not occur in the obese subjects. The pre-meal drop in ghrelin pulses in control subjects were
absent in obese subjects, which confirms the observation of English et al (8) that obese persons do not have the postprandial decline in plasma ghrelin seen in lean persons.

Some controversy exists in the literature regarding the time relation of circulating ghrelin concentrations with meals. The rise and fall in circulating ghrelin concentrations have been described as food-related (6). However, it is interesting that an analogous pattern of 24-h ghrelin secretion was recently shown in fasting subjects. In other words, periodical increases and decreases in plasma ghrelin concentrations also occur in the absence of food consumption (21).

Furthermore, Cummings et al (22) showed that the duration of the drop in ghrelin concentrations after a meal varies during the day: it is shorter after the first meal and progressively longer after the later meals. Those authors concluded that, whereas the response to the first meal is rapid, a subsequent preprandial increase occurs over a longer period (320–425 min). And, in fact, we found in the current study that the duration of the ghrelin trough increased progressively from breakfast to dinner, when it reached ≈4 h.

Plasma ghrelin concentrations were consistently higher in post-BPD than in pre-BPD obese subjects, but they still were significantly lower than in the control subjects. It is interesting that the rhythm of ghrelin pulses, either in connection to meals or to the nocturnal rise, is completely lost after the weight loss resulting from the bariatric surgery.

Our data differ from the post-RYGBP findings of Cummings et al (9) with respect to the changes in plasma ghrelin concentrations, but not with respect to the shape of plasma ghrelin profiles. In fact, whereas they found a large reduction in ghrelin concentrations after surgery, we observed a significant increase, although the hormonal pulsatility was disrupted.

Many reasons may account for these different results. The first of these reasons is the different types of bariatric operation performed. In RYGBP, gastric capacity is reduced to a few milliliters (23) because of the exclusion of the main part of the stomach from contact with food, but, in BPD, the remaining gastric volume is ≈300 mL. The stomach is the main source of ghrelin in humans and rats (24–27), although the hypothalamus, pituitary, duodenum, jejunum, and lung also contribute to ghrelin secretion (25). Therefore, the presence in post-BPD patients of a large portion of the stomach that is in contact with nutrients, compared with RYGBP, may explain the different observed action of the plasma ghrelin concentration. Fasting plasma ghrelin concentrations have also been reported to significantly increase after BPD surgery (28). In the current study, postoperative ghrelin concentrations were consistently higher throughout the day than preoperative.

**FIGURE 2.** The time courses of plasma ghrelin concentrations in each morbidly obese subject before and after biliopancreatic diversion surgery. Each graph represents the experimental values (●) with the best-fitting line. Sleeping hours are from 2300 to 0700. The arrows indicate the times of breakfast, lunch, snack, and dinner.
concentrations had been, and the post-BPD patients reported a return to normal appetite 2–3 mo after the operation. Moreover, the lack of a normal rhythm in plasma ghrelin pulses may explain the peculiar change in eating behavior reported by post-BPD patients, who, 3 mo after the surgery, experienced an unrestrainable impulse to eat. Adami et al (29), in fact, reported that their post-BPD subjects would have to be considered to have abandoned any concern about food, weight, and diet and to have become truly free eaters.

Furthermore, the BMI achieved in the post-BPD subjects in the current study (range: 58.98 ± 10.12 to 28.78 ± 1.76) was remarkably lower than that reported by Cummings et al (9) afterRYGBP (range: 68.0 ± 7.8 to 43.5 ± 6.0), whereas the ghrelin values given by those authors are referred to as total ghrelin rather than as active ghrelin, which we used; this difference makes it difficult to compare data.

It is of unquestionable interest that ghrelin plasma concentrations are increased after weight loss in post-BPD subjects, although ghrelin’s ultradian rhythm is disrupted. This observation suggests that weight loss—as shown by the significant inverse correlation between ghrelin concentrations and BMI observed in control subjects and post-BPD obese subjects—is essential to measuring the elevation of circulating ghrelin, despite the curtailment of an important site of ghrelin source, and it indicates that the gastric fundus plays a more relevant role in ghrelin production than does the gastric antrum, at least in humans. However, the loss of regular pulsatility of this hormone may suggest that it is the antrum where the ghrelin pacemaker might be located. In this regard, the data in the literature are highly contradictory. Whereas Tomasetto et al (30) found the maximal expression of ghrelin in the stomach fundus and corpus of dogs and no expression in the antrum or in the duodenum, jejunum, ileum, colon, or liver, Moesgaard et al (31) observed that ghrelin was highly expressed in both the corpus and gastric antrum of C57BL/6J mice.

Another explanation for the increase in ghrelin concentrations after weight loss may be the drastic reduction in circulating insulin, as observed in the current series. In fact, it has been noted that ghrelin was suppressed during induced hypoglycemia or hyperglycemia—and thus by the induced hyperinsulinemia—which suggests a role for insulin in the regulation of ghrelin production (31). However, this hypothesis is not in agreement with hypotheses of other authors, who stated that the meal-related suppression of ghrelin appears not to be directly regulated by glucose or insulin (32).

However, because insulin sensitivity reverses to normal after BPD, as previously reported (33) and as confirmed in the current study, it is conceivable that the reversion of insulin resistance may stimulate ghrelin secretion, as suggested by Ikezaki et al (34) in a study of overweight children and adolescents. Those authors hypothesized that the down-regulation of ghrelin secretion may be a consequence of higher insulin resistance associated with visceral fat accumulation and elevated concentrations of plasminogen activator inhibitor 1. Accordingly, we found a significant negative correlation between 24-h averaged ghrelin plasma concentrations and the $M$ value. McLaughlin et al (35) recently found that, in a multivariate analysis, both insulin resistance and hyperinsulinemia independently predicted low ghrelin concentrations, which suggests that insulin resistance or related metabolic abnormalities may participate in the regulation of body weight in humans.

In conclusion, weight loss induced by BPD surgery is associated with higher concentrations of circulating ghrelin; however, the concentrations remain consistently lower than those observed in lean control subjects, but ghrelin pulsatility is subverted in post-BPD studies. Therefore, we speculate that the increase in ghrelin concentrations may contribute to the high calorie intakes observed in post-BPD subjects, and the lack of ghrelin pulsatility may explain the impulse of these persons to eat very frequently.

GM was responsible for the design of the study and for writing the manuscript. EVM, AI, and MM were responsible for the experimental studies in the calorimetric chamber and data collection. LG performed the mathematical and statistical analyses. RB, GN, MC, and MC contributed to the design of the study, sample analysis, and interpretation of results. All authors contributed to the writing and revision of the manuscript. None of the authors had a personal or financial conflict of interest.

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