

Original Article

Relationship between body composition, growth hormone and ghrelin secretion in morbidly obese subjects before and after surgically induced weight loss

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ABSTRACT: Objective: The aim of the present study was to evaluate the relationship between body composition, ghrelin and growth hormone (GH) following a GHRH/arginine test in severely obese patients before and after surgically induced weight loss.

Materials and methods: Fourteen morbidly obese women (age 36 ± 4 years, BMI 47.8 ± 3.2 kg/m²) were studied at baseline and 18 months after biliopancreatic diversion (BPD) with a stable body weight (± 2 kg over a 6-month period) and with a BMI of 31.5 ± 2.5 kg/m². Body composition was measured by anthropometry and dual-energy X-ray absorptiometry. A GHRH/arginine test was performed before and 18 months after BPD to evaluate ghrelin and GH interactions. Active ghrelin and GH were measured by a RIA and chemiluminescence assay, respectively.

Results: Fasting serum GH levels and GH area under the curve (AUC) significantly increased from 0.25 ± 0.05 ng/mL to 1.02 ± 0.08 ng/mL ($p<0.05$) and from 548 ± 99 to 1940 ± 586 ng/mL x 120 min after BPD ($p<0.05$), respectively. Although no significant changes in fasting ghrelin levels were observed (573 ± 78 before BPD vs 574 ± 33 after BPD), ghrelin AUC significantly increased from -3228 ± 1990 to -1128 ± 874 pg/mL x 120 min after BPD ($p<0.05$).

Conclusion: Our data demonstrate that the GHRH/arginine test is a stimulus for GH secretion in morbidly obese patients before and after surgically induced weight loss and that this response could influence ghrelin levels. (Nutritional Therapy & Metabolism 2007; 25: 73-8)

KEY WORDS: Biliopancreatic diversion, Ghrelin, GHRH/arginine test, Growth hormone, Morbid obesity

INTRODUCTION

A great deal of attention has been recently devoted to the relationship between ghrelin and growth hormone secretion in massive obesity, as defined by a body mass index (BMI) exceeding 40 kg/m² (1, 2). Biliopancreatic diversion (BPD) is a surgical procedure that combines moderate volume restriction and severe lipid malabsorption, thus allowing massive weight loss with patients maintaining a stable body weight essentially due to reduction of fat mass despite a large energy intake (3).

Among the substances able to determine body weight maintenance, ghrelin, the endogenous ligand of the GH secretagogue receptor (GHS-R) type 1a, is secreted predominantly by the gastric enteroendocrine cellular system and has been recently identified as one of

the most relevant hormones that regulate appetite and energy expenditure in both experimental animals and humans (4, 5).

Circulating ghrelin levels in humans increase in response to caloric deprivation, as happens in anorexia nervosa (6) and cancer cachexia (7); the levels decrease after refeeding and in obesity after total gastrectomy (8-10). In addition, ghrelin secretion is inhibited by both intravenous and oral glucose load and in a hyperinsulinemic condition (11, 12).

On the other hand, GH controls several complex physiological processes including growth and energy substrate metabolism through the growth hormone-insulin-like growth factor (GH-IGF) axis. GH secretion is finely regulated by several factors including stress, exercise, nutrition and sleep, and by a negative feedback

loop involving GH itself and IGF-1. An influence of ghrelin on GH secretion has also been reported (13).

A marked reduction of both GH and ghrelin secretion was reported in obesity (14, 15). GH negatively correlates with BMI, and its half-life, amplitude and pulsatility are reduced in obesity; a progressive increase in GH secretion is observed as body weight decreases (16). Furthermore, a reduced GH response to growth hormone-releasing hormone (GHRH) administration has been reported in morbidly obese patients (17) and this has been attributed to central factors such as impairment of endogenous GHRH tone or increased somatostatin release as well as peripheral factors such as increased bioavailability of IGF-1 and its possible increased negative feedback action on somatotroph cells (18).

A feedback inhibition of GH on ghrelin secretion following a GHRH/arginine test in healthy subjects has been postulated by several reports, with high GH levels determining the lowering of ghrelin secretion (19, 20). The GHRH/arginine test is a potent reproducible and reliable test to explore somatotroph function, showing excellent sensitivity and specificity that is not affected by gender and aging (21). The aim of the present study was to measure GH and ghrelin secretion after a GHRH/arginine test in morbidly obese women before and after BPD, and to evaluate the relationship between these 2 hormones and body composition changes.

PATIENTS AND METHODS

Subjects

The study involved 14 morbidly obese women aged 36 ± 14 years, with a mean BMI of 47.8 ± 3.2 kg/m², who, 18 months after having undergone biliopancreatic diversion, were restudied in a phase of stabilized body weight (± 2 kg variation in 4-6 months), when they had reached a BMI of 31.5 ± 2.5 kg/m². Exclusion criteria at enrolment were presence of endocrine, hepatic and renal disease. None of the patients were taking drugs able to influence data collection. All subjects were studied in the follicular phase of the menstrual cycle, and none were using oral contraceptives.

Baseline GH and ghrelin levels were determined in blood samples drawn at 8:00 a.m. after an overnight fast, and immediately thereafter 1 µg/kg sermorelin acetate (Geref, Industria Farmaceutica Serono S.p.A., Rome, Italy) was given as an intravenous bolus. L-arginine (0.5 g/kg [maximum dose = 30 g]) was administered intravenously over 30 minutes. Subsequent blood samples to measure GH and ghrelin levels were drawn at +15, +30, +45, +60, +90 and +120 minutes.

The study protocol was approved by the ethics committee of the Catholic University of Rome and all participating subjects signed an informed consent form.

Body composition assessment

Body weight was measured to the nearest 0.1 kg using a beam scale with the subjects wearing light clothes and no shoes; height was measured in the same conditions using a wall-mounted stadiometer (Holatin, Crosswell, Wales, UK). BMI was computed as the ratio between body weight (kg) and height (m²). Body composition, i.e., fat mass and fat-free mass, was assessed by dual-energy X-ray absorptiometry (DXA), using a whole body densitometer (Lunar DPX-L, Madison, WI, USA; software version 3.65).

Biliopancreatic diversion

This malabsorptive surgical procedure consists of a ~60 cm distal horizontal gastric resection with stapled closure of the duodenal stump. The residual volume of the stomach is 250-500 mL, depending on individual patient's characteristics. The remnant stomach is anastomosed to the distal 250 cm of the small intestine (alimentary limb). The excluded small intestine (including the duodenum, the jejunum and part of the proximal ileum) carries the bile and pancreatic secretions (biliary limb), and is connected to the alimentary canal 50 cm proximal to the ileocecal valve. This common limb is the only segment where bile and nutrients mix (Fig. 1). Fat and starches are absorbed in the short common limb, whereas the alimentary limb (usually 200-250 cm in length) allows absorption of the noncaloric essential nutrients (22).

Analytical assays

Fasting plasma samples were collected in tubes in an ice bath and frozen immediately at -80°C . Plasma glucose was measured by the glucose oxidase method (Beckman, Fullerton, CA, USA). Insulin was assayed by RIA using kits from Abbott Diagnostics (Milan, Italy). GH and IGF-1 were measured by a sensitive chemiluminescence assay (Nichols Advantage, San Clemente, CA, USA). Ghrelin was measured using a Ghrelin (Active) radioimmunoassay kit (LINCO Research Inc, St. Charles, MO, USA). The intra-assay and interassay coefficients of variation for the high control were 2.65% and 16.2%, respectively; the intra-assay and interassay coefficients of variation for the low control were 1.15% and 15.1%, respectively. The sensitivity of the ghrelin assay was 2.98 pg/mL.

Statistical analysis

Data are given as means \pm SEM. The distribution of the data was assessed by the Kolmogorov-Smirnov test to verify whether the samples came from a specified distribution. The significance of differences between the GHRH/arginine test before and after BPD was assessed by the nonparametric Wilcoxon signed-rank test or by a paired *t*-test. A 2-tailed *p* value <0.05 was considered statistically significant.

RESULTS

Table I reports the main anthropometric and biochemical parameters of the patients examined. After BPD there was a significant decrease in BMI (from 47.8 ± 3.2 kg/m² to 31.5 ± 2.5 kg/m² [$p < 0.0001$]), as well as body fat mass ($43.4 \pm 4.8\%$ vs $32.9 \pm 3.8\%$; $p < 0.0001$).

The GH and ghrelin responses to the GHRH/arginine test before and after BPD are shown in Figures 2 and 3, respectively. GH peaked 60 minutes after the stimulatory test before and after BPD (8.42 ± 1.64 vs 28.9 ± 9.8 ng/mL, $p < 0.0001$) and the shape of the curve was similar. GH and ghrelin plasma concentrations were also expressed as AUC after the GHRH/arginine test, calculated by the trapezoidal rule and expressed as ng/mL \times 120 min for GH and pg/mL \times 120 min for ghrelin. Fasting serum GH levels increased significantly from 0.25 ± 0.05 ng/mL to 1.02 ± 0.08 ng/mL ($p < 0.05$). GH AUC increased significantly from 548 ± 99 to 1940 ± 586 ng/mL \times 120 min after BPD ($p < 0.05$).

No significant changes in ghrelin fasting levels were observed (570 ± 72 pg/mL before BPD vs 602 ± 44 pg/mL after BPD). Ghrelin AUC significantly increased from -3228 ± 1990 to -1128 ± 874 pg/mL \times 120 min ($p < 0.05$).

An inverse relationship was found between body fat percentage and GH level ($r = 0.88$; $p < 0.0001$) and ghrelin concentration ($r = 0.75$; $p < 0.001$), while no correlation was found between body composition parameters and hormonal changes after GHRH stimulation in our series.

Plasma insulin concentration decreased from 302 ± 34 before BPD to 165 ± 22 pmol/L after BPD ($p < 0.01$). No significant changes in fasting serum IGF-1 concentration were found before and after BPD (138 ± 18 vs 152 ± 25 ng/mL, respectively).

DISCUSSION

In the last decades, a great deal of attention has been devoted to the investigation of the mechanisms involved in body weight regulation in humans; several substances

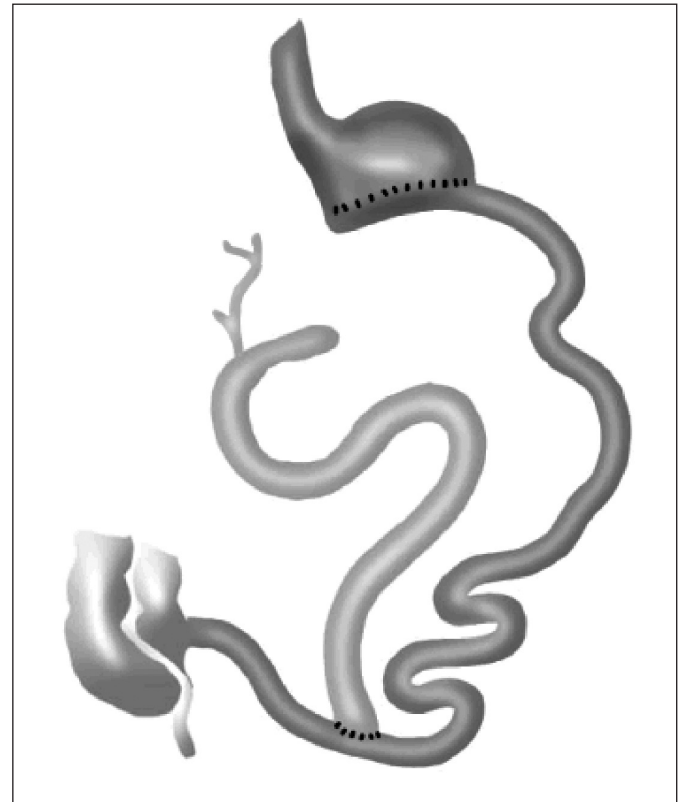


Fig. 1 - Description of ad hoc biliopancreatic diversion.

TABLE I - ANTHROPOMETRIC AND BIOCHEMICAL CHARACTERISTICS OF THE SUBJECTS EXAMINED

	Before BPD (N=14)	After BPD (N=14)
Body weight (kg)	125.2 \pm 10.4	82.4 \pm 6.2*
BMI (kg/m ²)	47.8 \pm 3.2	31.5 \pm 2.5*
Fat mass (%)	43.4 \pm 4.8	32.9 \pm 3.8
Fat mass (kg)	54.3 \pm 4.8	27.1 \pm 2.5
IGF-1 (fasting levels, ng·mL ⁻¹)	138 \pm 18	152 \pm 25
GH (fasting levels, ng·mL ⁻¹)	0.25 \pm 0.05	1.02 \pm 0.08*
Ghrelin (fasting levels, pg·mL ⁻¹)	570 \pm 72	602 \pm 44
GH AUC (ng·mL ⁻¹ ·120min)	548 \pm 99	1940 \pm 586*
Ghrelin AUC (pg·mL ⁻¹ ·120min)	-3228 \pm 1990	-1128 \pm 874*

BPD, biliopancreatic diversion; BMI, body mass index; GH, growth hormone; AUC, area under the curve; * $p < 0.05$

and hormones, acting both centrally and peripherally, have been identified (1, 2).

In this context, ghrelin is considered part of a new physiological system with 2 principal actions: (a) stimulation of food intake through upregulation of the hypothalamic orexigenic hormones neuropeptide Y (NPY)

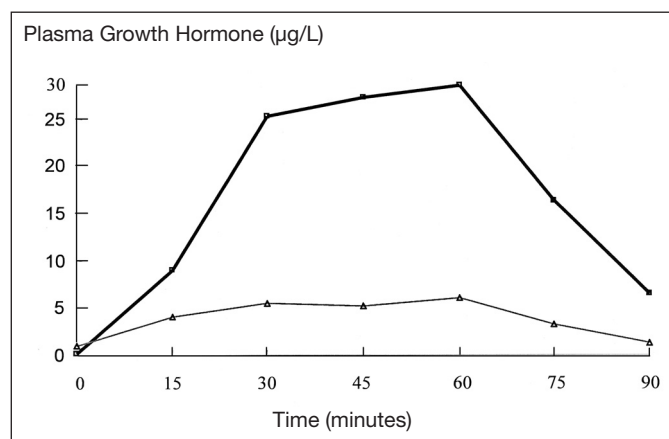


Fig. 2 -GH AUC after GHRH/arginine test in pre- and post-surgical obese women (* $p < 0.05$).

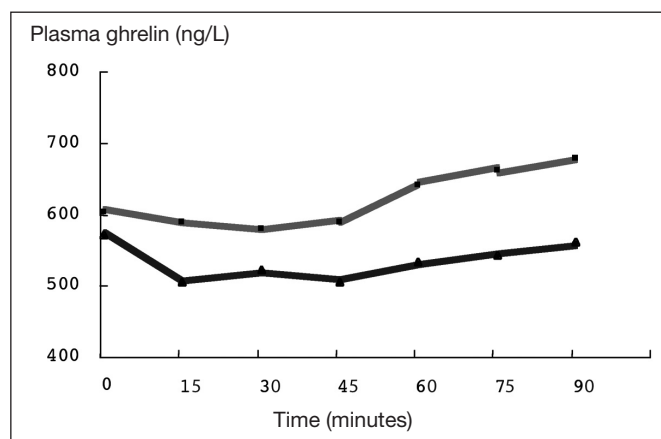


Fig. 3 - Ghrelin AUC after GHRH/arginine test in pre- and post-surgical obese women (* $p < 0.05$).

and orexin as well as inhibition of pro-opiomelanocortin (POMC)/ α -melanocyte-stimulating hormone (α MSH); (b) GH secretion from the pituitary (4, 5). In addition, more rapid gastric emptying was reported in obese than in lean subjects; whereas in control subjects the gastric kinetics seem to be regulated by ghrelin, this mechanism seems to be lost in obese individuals, who show accelerated gastric emptying (21). On the other hand, GHRH-dependent regulation of pituitary ghrelin gene expression and peptide content has been shown in healthy subjects (19, 20).

In this study, body composition and the effect of the GHRH/arginine stimulation test on ghrelin secretion were investigated in obese women before and after surgically induced weight loss. Bariatric surgery, such as BPD, induces massive and stable weight loss, essentially due to lipid malabsorption (16); thus, post-BPD patients represent an intriguing model for investigating the mechanisms that regulate energy metabolism and body weight homeostasis.

In several reports, a negative feedback of GH on ghrelin has been reported, with an enhanced stimulus of GHRH plus arginine on ghrelin secretion compared with the effect of GHRH alone, because arginine inhibits somatostatin secretion, which has an inhibitory effect on ghrelin production (19, 23).

Recently, a decreased ghrelin concentration after short-term fasting (4-day complete fasting) associated with an increase in GH concentration was found in healthy women (24), and GH administration over 5 days was able to reduce ghrelin level in young women on an *ad libitum* diet.

In our series, we found that during the GHRH/arginine test in both morbidly obese and post-obese women

whose BMI was still over 30 kg/m², the maximal increase in GH as well as the maximal decrease in ghrelin appeared at the same time, with an early GH response (after 15 minutes). Before surgery, the maximal ghrelin increase occurred 30 minutes after the GH peak, while after BPD it was delayed by 45 minutes. Furthermore, after weight loss, the effect of the GHRH/arginine test on the feedback involving GH and ghrelin was enhanced.

In agreement with other studies (25, 26), we found that fasting circulating ghrelin levels did not significantly increase after bariatric surgery, while other investigations performed 12 months after BPD reported an increase in fasting total ghrelin, present in serum at a 2.5-fold higher concentration than the active form (3, 27).

In our series, total ghrelin secretion as shown by its AUC significantly increased after BPD ($p < 0.05$), probably as a consequence of the increased stimulus in ghrelin expression and secretion caused by the GHRH/arginine test (28).

No correlation was found between body composition parameters and hormonal changes after GHRH stimulation in our series, while an inverse relationship between body fat percentage and GH level and ghrelin concentration was found. Since weight loss after BPD is essentially due to fat mass reduction, our data suggest that this does not represent a key factor in the response to the GHRH/arginine test stimulus after bariatric surgery.

Interestingly, a recent study reported that BPD with duodenal switch was associated with markedly suppressed ghrelin levels, possibly contributing to the weight-reducing effect of this operation (29).

In conclusion, our data support the hypothesis that the reciprocal influence between GH and ghrelin is not

modified by the partial gastrectomy changes resulting from the BPD procedure (30) or by the massive surgically induced weight loss and that the GHRH/arginine test is a stimulus for GH secretion in our obese female patients before and after bariatric surgery.

Further studies on a larger sample are needed to elucidate the correlation between body composition changes, GH and ghrelin concentration in severely obese patients who underwent surgical weight loss procedures.

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Received: January 31, 2007

Accepted: June 18, 2007