Opioid Dysregulation After Biliopancreatic Diversion: Effect of Naloxone on Preprandial and Postprandial Growth Hormone (GH)-Releasing Hormone-Induced GH Release in Surgically Induced Weight Loss

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Previously, we have shown that in the opposite extremes of nutritional status (obesity and anorexia nervosa [AN]), the growth hormone (GH) response to GH-releasing hormone (GHRH) is not inhibited by the ingestion of a normal 800-kcal meal at noon. In obese subjects, GHRH-induced GH release is significantly increased (known as the "paradoxical response"). An opiate antagonist infusion (naloxone [NAL]) inhibited this postprandial meal-induced augmenting effect in obese subjects, suggesting opioid involvement in the paradoxical response. The paradoxical postprandial GH release persisted in obese subjects, who after biliopancreatic diversion (BPD) experienced a reduction in body weight, despite the elevation of fasting GH levels. We therefore tested a group of patients, before and after BPD, composed of 10 females, aged 23 to 54 years, who after surgery had experienced a significant reduction in body weight (mean body mass index [BMI], 25.78 ± 1.01 kg/mg *v* 44.68 ± 1.73 kg/mg). The subjects were studied 16 to 24 months after operation, in a phase of stabilized body weight. They underwent, in randomized order, the following tests: GHRH (1 μg/kg as an intravenous [IV] bolus) at 1:00 PM, in the fasting state; GHRH (1 μg/kg) at 1:00 PM, 45 minutes after a standard 800-kcal meal consumed between noon and 12:15 PM; and fasting state and postprandial GHRH (1 μg/kg) during NAL infusion (1.6 mg/h × 2.5 h, starting at noon).We found that NAL inhibited the paradoxical postprandial GH increase only in pre-BPD subjects (GH area under the concentration time curve [AUC] in μg/L/90 min)—*before meal*: after GHRH 237.54 ± 62.28, after NAL + GHRH 699.2 ± 271.57; *after meal*: after GHRH 575.46 ± 109.68, after NAL + GHRH 156.17 ± 24.96. On the other hand, NAL failed to have significant effects in post-BPD subjects (GH AUC in μg/L/90 min)—*before meal*: after GHRH 871.11 ± 256.38, after NAL + GHRH 449.19 ± 119.13; *after meal*: after GHRH 1,981.54 ± 319.92, after NAL + GHRH 1,665.91 ± 315.4. It could be hypothesized that the opioid system is radically modified by the surgical procedure, and that opioids are not the only mediators in the paradoxical response, which persists after BPD, despite the reversion of the hyposecretory GH state, which is a characteristic of obese subjects.

PREVIOUSLY, WE showed that in the opposite extremes of nutritional status, obesity and anorexia nervosa (AN), the growth hormone (GH) response to GH-releasing hormone (GHRH) is not inhibited by the ingestion of a standard meal consumed at lunch. In obese subjects, GHRH-induced GH release is significantly increased (known as the "paradoxical response").[1] An opiate antagonist infusion (naloxone [NAL]) completely blunted this postprandial meal-induced augmenting effect in obese subjects, suggesting an opioid involvement in the paradoxical response,[2] while in AN only a partial inhibition of the response was observed during NAL infusion.[3]

Surgically induced weight reduction in obese subjects offers the possibility to further explore this topic. We have previously reported that obese subjects who maintained decreased body weight after biliopancreatic diversion (BPD) showed a clear increase in fasting GH release, but a persistent paradoxical response, with a further augmenting effect after a meal.[4]

To explore the neuroendocrine mechanism underlying this phenomenon, we investigated the effect of a NAL infusion on the GH response to GHRH in a group of females, studied 16 to 24 months after BPD. NAL was infused at noon in a fasting state or after a meal; this time was chosen because our previous studies showed the most significant effects of food on GH secretion at midday among normal, obese, and AN subjects.[5] Moreover, we did not find a difference when comparing short fasting (between breakfast and lunch) and long fasting (from midnight to lunch).[3]

MATERIALS AND METHODS

Two groups of subjects were examined after providing informed consent. The first consisted of 10 morbidly obese patients who were scheduled to undergo therapeutic BPD[6] (pre-BPD subjects) after failing dietary therapy. The group consisted of 10 women aged 23 to 48 years, with a mean body mass index (BMI) of 44.68 ± 1.73 kg/m^2 (range, 33.70 to 52.03).

These subjects were also examined 16 to 24 months after having undergone a successful BPD (post-BPD subjects), when their body weight had returned partially or completely to normal (mean BMI at the time of testing, 25.78 ± 1.01 kg/m^2; range, 20.56 to 27.72). All patients were studied in a phase of stabilized body weight (±2% variation in body weight in the last 6 months before testing).

The control group consisted of 10 normal women aged 22 to 46 years, with a mean BMI of 23.46 ± 1.12 kg/m^2 (range, 21.21 to 24.66).

No subject was taking medications known to affect GH secretion, and none suffered from diabetes mellitus, thyroid disease, or any chronic disease. Preliminary oral glucose tolerance tests (100 g glucose) did not show glucose intolerance in any of the patients. The four tests were performed in all subjects, with an interval of at least 3 days, in randomized order. The tests were performed during the follicular phase of the menstrual cycle.

Before Meal Tests

GHRH test. After an overnight fast and having eaten a continental breakfast at 8 AM, a 150-mmol/L NaCl infusion was started at noon, and 1 hour later (time 0), a GHRH intravenous (IV) bolus (1 μg/kg

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body weight; range, 35 to 125 μg) was administered. Blood samples were collected at -60, -30, 0, 15, 30, 60, and 90 minutes.

NAL + GHRH test. On a separate day session, the same test was conducted during NAL infusion. NAL was administered at a rate of 1.6 mg/h × 2.5 h, starting at noon, 1 hour before GHRH administration. Blood samples were collected at -60, -30, 0, 15, 30, 60, and 90 minutes.

After Meal Tests

GHRH test. On a separate day, after an overnight fast and having had a continental breakfast at 8 AM, at noon the subjects consumed a 800-kcal meal, composed of approximately 55% carbohydrates, 32% lipids, and 13% proteins. At the same time, a 150-mmol/L NaCl infusion was also started, and 1 hour later (45 minutes after finishing the meal, time 0), a GHRH bolus (1 μg/kg body weight IV) was administered. Blood samples were collected at -60, -30, 0, 15, 30, 60, and 90 minutes.

NAL + GHRH test. On a separate day session, the latter test was repeated during NAL infusion. NAL was given at a rate of 1.6 mg/h × 2.5 hours, starting at noon. The women had a similar 800-kcal meal between noon and 12:15 PM and a GHRH bolus (1 μg/kg body weight IV) was administered at 1 PM (45 minutes after finishing the meal and 60 minutes after starting the NAL infusion, time 0). Blood samples were collected at -60, -30, 0, 15, 30, 60, and 90 minutes.

Post-BPD subjects underwent a second session of tests (both before and after a meal) 16 to 24 months after the surgical procedure.

GHRH 1-29 was provided by Serono (Milan, Italy), and naloxone chlorohydrate by Crinos (Como, Italy).

Blood samples were collected using lithium heparin as an anticoagulant and centrifuged within 2 hours of collection. The plasma was frozen at -20°C until assayed. Plasma GH levels were measured in duplicate by immunoradiometric assay (IRMA) with reagents purchased by Radim (Pomezia, Italy). The intra-assay variation coefficient ranged from 2.5% to 3.9%, and the interassay variation coefficient ranged from 5.8% to 8.5%. All samples from each individual subject were analyzed in duplicate and in the same assay. Normal plasma GH levels ranged from 0.25 to 5 μg/L.

Plasma insulin, free fatty acids (FFA), and insulin-like growth factor (IGF-1) were also measured at 8 AM, after an overnight fast, on the day of the first test. Plasma insulin was assayed by radioimmunoassay (RIA) using commercial kits by Abbott Diagnostics (Milan, Italy). Plasma IGF-1 was measured by RIA using commercial kits by Mediagnost (Tubingen, Germany); normal values range from 80 to 330 ng/mL. FFA levels were determined by enzymatic colorimetric methods (Boehringer Mannheim, Mannheim, Germany).

Statistics

All results are expressed as the mean ± SEM.

The distribution of the data was tested by the Kolmogorov-Smirnov test to verify whether the samples came from a specified distribution. We found that the data were not normally distributed.

Statistical analysis was performed using the Wilcoxon rank sum test, when comparing before and after meal studies within the same group of subjects (pre- or post-BPD and normal weight controls) and using the Mann-Whitney *U* test when comparing data from different groups, also weighted by Kruskal-Wallis multiple comparisons. Analysis was applied to GH peak and area under the curve (GH AUC) relative to zero, calculated by trapezoidal rule. The Friedman test was also applied when comparing different tests in the same group (preprandial and postprandial tests in obese subjects before and after surgery).

The level of statistical significance was set at $P < .05$. For statistical evaluation we used the software package Statistica (Statsoft, Tulsa, OK; release 5.0, 1996) for Windows 95.

RESULTS

Control Subjects

The results observed in normal women are shown in Fig 1 (single points) and Table 1 (peak and AUC). The GH response to GHRH was not significantly different when GHRH was administered before or after lunch. NAL infusion did not significantly modify the GH response to GHRH either before or after lunch (Table 1).

Pre-BPD Patients

The results observed in obese pre-BPD patients are shown in Fig 2 (single points) and Table 1 (peak and AUC). In obese pre-BPD fasting women, plasma GH levels were extremely low and were significantly lower than in controls (Table 1). NAL infusion, performed in the same women starting at noon, 1 hour before GHRH administration, did not induce significant variations in the GHRH-induced GH release (Table 1).

In the same group of subjects, when they were given lunch, GHRH-induced GH secretion was significantly increased in comparison to fasting (Table 1). When the test was performed during NAL infusion, both peak GH values and AUC were significantly lower than with GHRH alone (Table 1). Statistical analysis was confirmed by the Friedman multiple comparison test.

Post-BPD Patients

The results observed in post-BPD patients are shown in Fig 3 (single points) and Table 1 (peak and AUC). In post-BPD patients tested in fasting state, the GH response to GHRH was

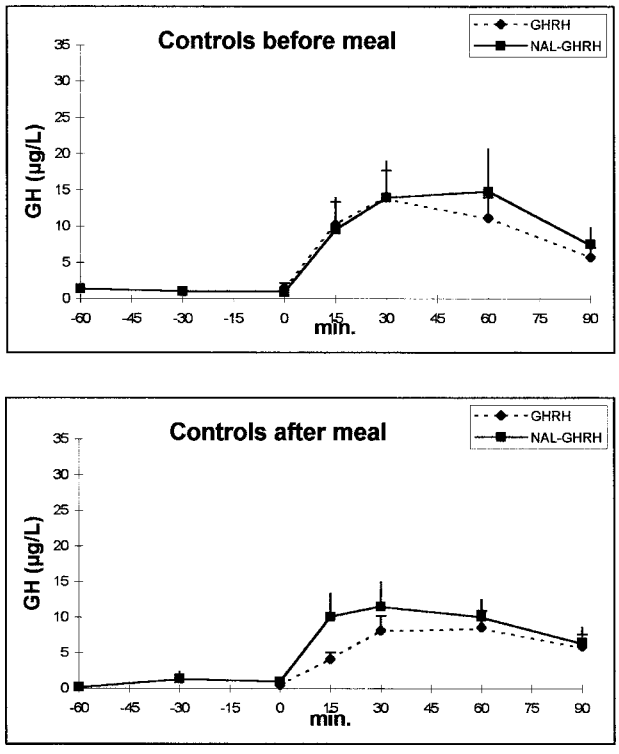

Fig 1. Mean (±SEM) GH response to GHRH, alone and during NAL infusion, in 10 controls, in fasting state (top) and after a standard meal (bottom).

Table 1. Mean ± SEM GH-AUC (μg/L/90 min.) and GH Peak Levels (μg/L) in Preprandial and Postprandial Tests, After GHRH Alone and During NAL Infusion, in All Subjects

	Before Meal		After Meal	
	GHRH	NAL-GHRH	GHRH	NAL-GHRH
Controls (n = 10)				
AUC	902.09 ± 214.28	791.42 ± 314.25	595.57 ± 146.49	448.03 ± 106.36
Peak	16.34 ± 3.83	15.10 ± 5.27	10.36 ± 2.26	14.30 ± 3.09
Pre-BPD (n = 10)				
AUC	237.54 ± 62.28*	699.2 ± 271.57	575.46 ± 109.68†	156.17 ± 24.96*‡
Peak	3.72 ± 0.99*	11.62 ± 3.44	11.51 ± 2.64†	4.43 ± 0.85*‡
Post-BPD (n = 10)				
AUC	871.11 ± 256.38§	449.19 ± 119.13‡	1,981.54 ± 319.92*†§	1,665.91 ± 315.4*§
Peak	14.77 ± 3.96§	9.19 ± 2.39	33.96 ± 5.27*†§	25.81 ± 4.61§

*$P < .05$ *v* controls.
†$P < .05$ *v* before meal.
‡$P < .05$ *v* GHRH alone.
§$P < .05$ *v* pre-BPD.

significantly greater than in obese pre-BPD patients (Table 1). NAL infusion induced a significant decrease in AUC, but not peak values (Table 1).

In the same group of subjects, when they were given lunch, GHRH induced a significant and greater GH release compared with the fasting state (Table 1). NAL infusion failed to induce significant GH variations in comparison to GHRH alone (Table 1). Statistical analysis was also confirmed by the Friedman multiple comparison test.

Table 2 shows the mean plasma levels of insulin, FFA, and IGF-1, as well as the glucose/insulin ratio (GIR), in the three groups of patients, together with their BMI. Pre-BPD patients exhibited higher insulin and FFA levels, and low GIR and IGF-1 levels, as previously described.[4] After BPD, insulin and FFA significantly decreased, GIR increased, and IGF-1 remained low and significantly different from control subjects.

DISCUSSION

Our data, confirming the persistence of a paradoxical postprandial GH response to GHRH in post-BPD patients, indicate

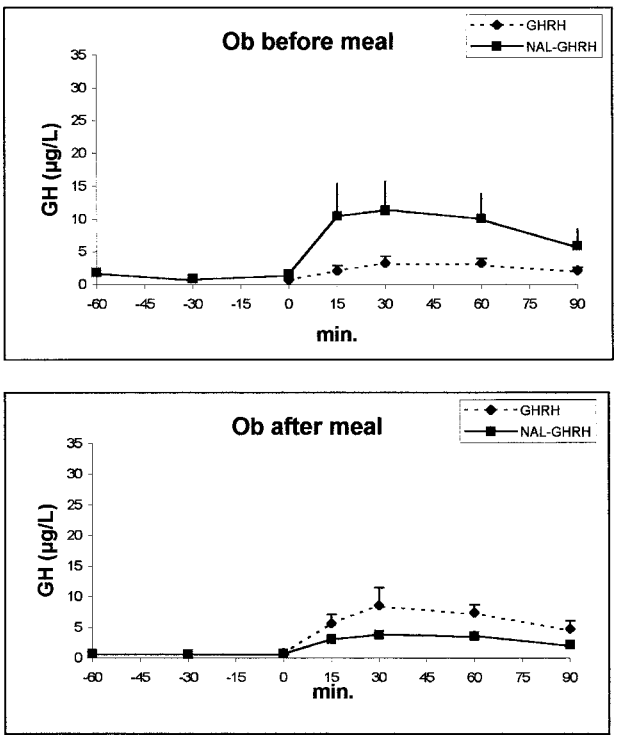

Fig 2. Mean (±SEM) GH response to GHRH, alone and during NAL infusion, in 10 obese patients, pre-BPD, in fasting state (top) and after a standard meal (bottom).

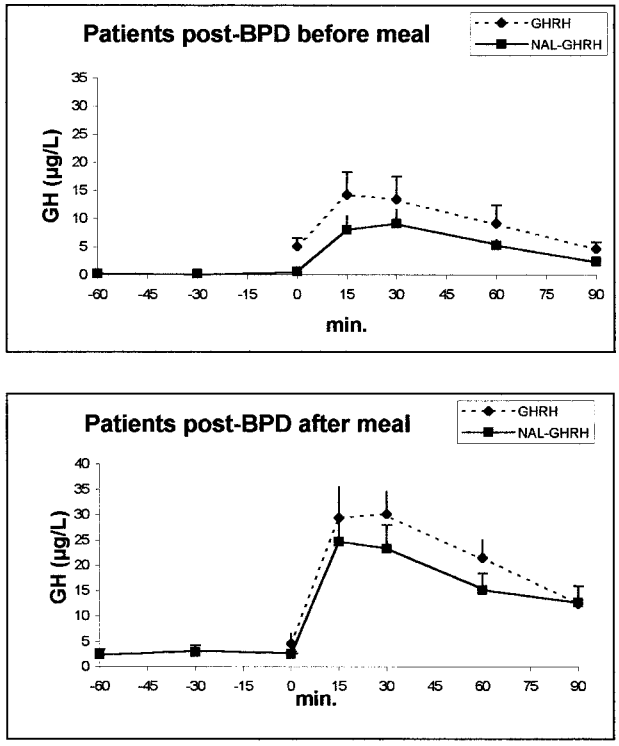

Fig 3. Mean (±SEM) GH response to GHRH, alone and during NAL infusion, in 10 subjects after BPD, in fasting state (top) and after a standard meal (bottom).

Table 2. Biochemical Data in All Subjects

	Insulin (pmol/L)	GIR	FFA (mg/L)	IGF-1 (μg/L)	BMI (kg/m^2)
Controls (n = 10)	47.14 ± 12.07	13.47 ± 3.81	129.3 ± 39.2	191.93 ± 21.39	23.46 ± 1.12
Pre-BPD (n = 10)	368.12 ± 29.84*	1.95 ± 0.19*	191.1 ± 23.9*	139.11 ± 7.75*	44.68 ± 1.73*
Post-BPD (n = 10)	110.31 ± 31.63†	7.12 ± 2.40†	116.9 ± 15.9†	129 ± 10.24*	25.78 ± 1.01†

*$P < .05$ *v* controls.
†$P < .05$ *v* pre-BPD.

that NAL is not capable of reversing this phenomenon, as it was demonstrated in simple obesity, and that therefore other factors than opioids are involved in the augmented in postprandial GHRH-induced GH release.

This observation reinforces the similarity of this model to AN. In fact, in a previous report, we demonstrated that NAL only partially blunted the postprandial GH response to GHRH. We concluded that the opioid control of GH in AN was different from that in normal subjects and obese patients, exhibiting a more definite opioid-dependence of postprandial GH secretion.[3] This hypothesis was also supported by different reports showing disturbances in opioid and GH regulation in AN patients.[7-10]

The surgical procedure of BPD induces modifications that can influence control pathway of GH secretion. Patients develop fat malabsorption (75% of ingested) and partial starch malabsorption while maintaining a normal absorption of monosaccharides and disaccharides (19% of ingested starch plus monosaccharides and disaccharides) and normal absorption of proteins.[11] The demonstrated metabolic and hormone variations include the following: (1) BPD reverts insulin resistance; (2) it increases diet-induced thermogenesis; (3) it induces modifications of gut hormones, such as gastrin, enteroglucagon, neurotensin, cholecystokinin.[12-15]

Different hypotheses could be formulated to explain the ineffectiveness of NAL in post-BPD subjects. First, beta-endorphins could be decreased after surgery. Opioid levels are elevated in obesity and are of peripheral origins. The modified anatomical connections due to BPD could modify also beta-endorphin release. Opioids could be also altered because of malnutrition: the reactivity of malnourished animals to psychoactive drugs acting through gamma-aminobutyric acid, catecholaminergic, serotoninergic, opioid, and cholinergic neurotransmitter systems is altered, as recently reviewed.[16] Moreover, different data indicate opioid and cholinergic dysfunction in AN.[3,9,17,18]

Second, insulin levels are markedly decreased after surgery. Experimental models in vivo indicate that an adequate amount of insulin is required to reveal inhibition by opioid-antagonists on GH secretion; studies performed using NAL in hyperandrogenic obese women suggest that the opiate antagonist treatment may act through the reduction of negative insulin feedback on GH secretion.[19] On the other hand, insulin may be low due to the decreased opioid levels: in fact, both physiologic and pharmacologic plasma levels of beta-endorphin are able to provoke marked islet hormone release in human obesity.[20] It has also been demonstrated that insulin decreases very early after surgery, with a pattern dissociated from the decrease of body mass.[21]

Third, the decrease in FFA after surgery[4] could be important in maintaining elevated GH levels, both preprandially and postprandially, and could overcome the inhibitory effect of NAL. In fact, it is known that FFA are important inhibitory factors in GH regulation.[22]

Finally, according to the hypothesis that NAL could inhibit GH secretion by inducing an increase in peripheral somatostatin release,[2] an altered somatostatin secretion, again related to anatomic postsurgical modification, can be invoked. Data from both animal models[23] and humans[24] indicate that endogenous opioids participate in the regulation of postprandial peripheral somatostatin release. Other data in acromegalic patients support the hypothesis that peripheral somatostatin can influence GH secretion.[25]

Whatever the case, our data surprisingly show the similarity of post-BPD patients and AN patients. GH secretion seems to be related rather to nutritional status than to body weight per se. Despite a normalized body weight induced by the surgical procedure, post-BPD subjects exhibit substantial GH secretion, but low IGF-1 levels,[4] as has been observed in malnourished patients.

In conclusion, BPD restores a normal GH secretion in fasting tests, according to previous studies, but does not normalize GH dynamics in postprandial studies. The paradoxical GH response after a meal seems to be opioid-independent. Our data support the hypothesis that this phenomenon is related to malnutrition rather than to persistent neuroendocrine abnormalities.

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