Sexual dimorphism on growth hormone secretion after oral glucose administration

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Abstract
Sexual dimorphism of GH secretion is unclear in humans. There is evidence that oral glucose (OG) administration initially decreases and subsequently stimulates GH secretion. Our aim was to study fasting GH concentrations and their response to OG administration in obese and healthy women and men, in order to elucidate the mechanism of sexual dimorphism of GH secretion and the possible contribution of ghrelin. We selected 33 women and 11 men as obese and healthy subjects. After an overnight fast, 75 g of oral glucose were administered; glucose, insulin, ghrelin, and PYY_{1-36} were obtained at baseline and during 300 min. Fasting GH (μg/l) was higher in women than men; 1.3±0.3 vs. 0.2±0.1, p=0.009, for women and men, respectively. The area under the curve between 0 and 150 min (AUC) of GH (μg/l · min) was higher in women than men; 98.2±25.9 vs. 41.5±28.6, p=0.002, for women and men, respectively. The AUC of total ghrelin (pg/ml · min, mean±SEM) between 0 and 150 min was borderline and significantly higher in women than men; 128 562.3±8 335.9 vs. 98 839.1±7 668.6, p=0.069, for women and men, respectively. Several initial time points were higher in women than men. Glucose, insulin, and PYY_{1-36} were similar in women and men after OG. There were significant correlations between indices of post-oral glucose GH and ghrelin secretion. Fasting and initial GH secretion is higher in women than men, in contrast to peak and late GH secretion, which is similar in both cases. Sexual dimorphism in the regulation of GH secretion probably involves ghrelin.

Key words
GH – ghrelin; Oral glucose; Sexual dimorphism

Introduction

In the animal model there is a sexually dimorphic pattern of GH secretion [1]. Male rats display a discrete high amplitude, pulsatile pattern of GH secretion, with a peak periodicity of 3 h, whereas pulsatile secretion in the female is at low amplitude, with irregular periodicity [1]. In humans, women have more uniform GH pulses throughout the day and men have a large nocturnal GH pulse and relatively low GH output over the rest of the day [2]. After IGF-I administration, pulsatile- and GHRH-induced GH secretion is less potently suppressed in women than men [2]. In nonprimate species, mean circulating GH concentration is greater in males than in females, whereas in primate species, mean circulating GH concentrations are greater in females than in males [1]. In studies done with the infusion of a GHRH antagonist in humans, trough and basal GH significantly decreased in women but not in men, although mean GH, pulse amplitude, and GH response to GHRH decreased in both sexes [3]. When studied by deconvolution analysis, basal GH secretion, pulsatile GH secretion, and total GH secretion were higher in women than in men, although the number of pulses was similar [4].

Ghrelin is a 28 amino acid peptide, predominantly produced by the stomach, which has a unique structure with an *n*-octanoyl ester at its third serine residue, which is essential for its potent stimulatory activity on somatotroph secretion. It displays strong growth hormonereleasing activity mediated by the hypothalamus and pituitary GH secretagogue receptors [5–7]. The GH-releasing action of ghrelin takes place both directly on pituitary cells and through modulation of GHRH from the hypothalamus; some functional anti-somatostatin action has also been shown [8]. Its physiological importance in GH regulation is unclear. Studies to determine the effects of endogenous ghrelin on the control of GH secretion have yielded conflicting results. In situations with increased GH secretion such as renal failure [9] there was no correlation between ghrelin and GH. Misra et al. [10] found that fasting ghrelin is an independent predictor of basal GH secretion and GH secretory burst frequency. Nass et al. [11] found that under normal conditions in subjects given regular meals, endogenous acylated ghrelin acts to increase the amplitude of GH pulses. We have recently found that ghrelin could be a physiological regulator of GH in the postprandial state, and that the decreased ghrelin secretion in obesity could be one of the mechanisms responsible for the altered GH secretion in obesity [12]. Circulating ghrelin have been found increased in women when compared with men in some [13, 14] but not all studies [15]. However, the hypothalamic pituitary mechanisms controlling the sexual dimorphism and the importance of ghrelin are unclear. Data exist, which suggest that other gastrointestinal hormones, like PYY, could participate in GH regulation [16]. There is evidence that oral glucose (OG) administration affects GH secretion, initially decreasing GH secretion and subsequently stimulating GH secretion [17]. Circulating plasma ghrelin increases before a meal and decreases following the consumption of nutrients and after an oral glucose tolerance test [18–20].

Our aim was to study fasting GH concentrations and their response to OG administration in obese and healthy women and men, in order to elucidate the mecanism of sexual dimorphism of GH secretion and the possible contribution of ghrelin and PYY.

Patients and Methods

Patients

We selected a total of 44 healthy (12 women, 4 men) or obese subjects (21 women, 7 men) (Table 1). 33 women, aged 36.0 ± 1.8 years, with a BMI of 32.8 ± 1.6 kg/m^2 , and 11 men aged 30.9 ± 2.9 years and with a BMI of 32.8 ± 2.5 kg/m^2 , were studied. We paired by age and BMI women and men, 3 women were studied for each men. Both groups were homogeneous and differ only on sex. None of the subjects had diabetes mellitus or other medical problems nor were they taking any drugs. The subjects had been eating a weight-maintaining diet for several weeks prior to the study. We specifically instructed the patients that they should maintain their usual eating and exercise habits during the previous 2 weeks of the study. All the studies have been conducted in accordance with the Declaration of Helsinki. The study protocol was approved by our center's ethical committee, and written informed consent was obtained from all patients and controls.

Table 1. Basic characteristics (mean ± SEM) of women and men.

	Women	Men	p
Age (years)	36.0 ± 1.8	31.5 ± 2.7	0.271
BMI (kg/m^2)	32.8 ± 1.6	32.4 ± 2.3	0.936
Mid-waist circumference (cm)	98.9 ± 3.1	104.8 ± 4.0	0.391
Total body fat (%)	40.8 ± 1.5	27.4 ± 2.4	< 0.001
Total body fat (kg)	36.7 ± 2.9	26.9 ± 4.1	0.111

BMI: body mass index

Study procedure

Between 08:30 and 09:00 AM, after an overnight fast and while seated, a peripheral venous line was obtained. 15 min later 75 g of oral glucose were administered. All studies were performed during the follicular phase of the menstrual cycle. In order to study the patients during the follicular phase of the menstrual cycle, all studies were done during the first 10 days from the begining of the mestrual period. Blood samples were obtained for glucose, insulin, GH, ghrelin and PYY$_{1-36}$ at baseline (fasting) and then at 30, 60, 90, 120, 150, 180, 210, 240, 270, and 300 min. Basal levels of leptin and IGF-I were also measured. All blood samples were immediately centrifuged, separated and frozen at − 80°C. Samples destined to be used for the determination of plasma ghrelin were specifically retrieved in chilled tubes containing aprotinin and EDTA-Na, and then immediately centrifuged at 4°C, separated to aliquots, and frozen at − 80°C. Mid-waist circumference was measured as the midpoint between the iliac crest and the lowest rib, with the patient in the upright position. Measurement of the hip circumference was performed at the widest point, also with the subject in an upright position. Total body fat was calculated through bioelectrical impedance analysis (BIA), as previously described [21].

Assays and other methods

Serum samples were collected and stored at − 80°C. Serum GH (μg/l) was measured by a solid-phase, 2-site chemiluminescent enzyme immunometric assay (Immulite, EURO/DPC) with a sensitivity of 0.01 μg/l and with intra-assay coefficients of variation of 5.3, 6.0, and 6.5 % for low, medium, and high plasma GH levels, respectively; and with inter-assay coeffi cients of variation of 6.5, 5.5, and 6.6 % for low, medium, and high plasma GH levels, respectively. IGF-I (ng/ml) was

determined by a chemiluminescence assay (Nichols Institute, San Clemente, CA, USA) and with intra-assay coefficients of variation of 4.8, 5.2, and 4.4 % for low, medium, and high plasma IGF-I levels, respectively; and with interassay coefficients of variation of 7.7, 7.4, and 4.7 % for low, medium, and high plasma IGF-I levels, respectively. Insulin (µU/ml) was measured with a solid-phase 2-site chemiluminescent immunometric assay (Immulite 2 000 Insulin, DPC, Los Angeles, CA, USA) and with intra-assay coefficients of variation of 5.5, 3.3, and 3.7 % for low, medium, and high plasma insulin levels, respectively; and with inter-assay coefficients of variation of 7.3, 4.1, and 5.3 % for low, medium, and high plasma insulin levels, respectively. Leptin (ng/ml) was measured by radioimmunoassay (Mediagnost, Tübingen, Germany) and with intraasay and interassay coefficients of variation of 5.3 % and 13.6 %, respectively. Total ghrelin (pg/ml) was measured by a commercially available radioimmunoassay (RIA) (Linco Research Inc., St Charles, MO, USA), specific for total ghrelin, that uses 125 I-labeled ghrelin tracer and rabbit antighrelin serum with a specificity of 100 %, with an intra-assay coefficient of variation between 3.3–10 %, and an inter-assay coefficient of variation between 14.7–17.8. Plasma glucose (mg/dl) was measured with an automatic glucose oxidase method (Roche Diagnostics, Mannheim, Germany). All samples from a given subject were analyzed in the same assay run. PYY 1–36 (pg/ml) was measured by a commercially available radioimmunoassay (RIA) (Linco, St Charles, MO, USA), with an intra-assay coefficient of variation of between 2.9–9.4 % and an interassay coefficient of variation between 5.5–8.5 %; the lower detection limit was 10 pg/ml.

Statistical analysis

The results are presented as mean values ± standard error of the mean (SEM). Fasting and post-oral glucose biochemical and hormonal data were compared between the women and men. The area under the secretory curve (AUC) was used to summarize serum values at 30, 60, 90, 120, 150, 180, 210, 240, 270, and 300 min after oral glucose. AUC was calculated with the trapezoidal rule (0–300 min), and separately for the 0–150 min and 150–300 min intervals. All comparisons were based on univariate, nonparametric tests. Comparisons between the womwn and men were based on the Mann-Whitney U test. Numerical correlations were analyzed using Spearman's correlation coefficient. p-Values ≤ 0.05 were considered to be significant, and all tests were considered as being 2-sided. Mean values ±SEM was used for graphic presentation. SPSS 17.0 software (Chicago, IL, USA) was used to produce the statistical analysis.

Results

The basic characteristics of both groups are shown in Table 1.

Fasting serum levels

The fasting serum levels are presented in Table 2. Fasting GH (µg/l, mean ± SEM) levels was higher in women than men; 1.3 ± 0.4 vs. 0.2 ± 0.1, p = 0.009, for women and men, respectively. Fasting leptin (ng/ml, mean ± SEM) levels was higher in women than men; 50.4 ± 5.5 vs. 17.2 ± 4.9, p = 0.001, for women and men, respectively.

Table 2. Fasting and after oral glucose biochemical and hormonal data (mean ± SEM) in women and men.

	Women	Men	p
Fasting glucose (mg/dl)	93.9 ± 2.1	99.3 ± 2.1	0.088
Fasting insulin (µU/ml)	13.1 ± 4.9	12.3 ± 2.4	0.169
Fasting GH (µg/l)	1.3 ± 0.4	0.2 ± 0.1	0.009
Fasting IGF-1 (ng/ml)	126.4 ± 9.5	133.9 ± 16.7	0.593
Fasting leptin (ng/ml)	50.4 ± 5.5	17.2 ± 4.9	0.001
Fasting total ghrelin (pg/ml)	1072.1 ± 87.2	812.2 ± 67.9	0.161
Fasting PYY_{1-36} (pg/ml)	132.4 ± 6.9	174.6 ± 26.8	0.187
Peak glucose (mg/dl)	158.4 ± 7.1	173.3 ± 10.8	0.237
AUC_{0-300} glucose (mg/dl min)	29 747.7 ± 833.8	31 232.7 ± 1255.6	0.669
AUC_{0-150} glucose (mg/dl min)	17 993.2 ± 728.3	18 804.5 ± 1103.2	0.630
$AUC_{150-300}$ glucose (mg/dl min)	117 545 ± 256.5	12 428.2 ± 265.3	0.105
Peak insulin (µU/ml)	94.6 ± 11.3	103.6 ± 15.2	0.348
AUC_{0-300} insulin (µU/ml min)	10 282.1 ± 937.9 10	919.6 ± 1471.5	0.348
AUC_{0-150} insulin (µU/ml min)	8 054.9 ± 817.3	9 004.2 ± 1321.7	0.362
$AUC_{150-300}$ insulin (µU/ml min)	2 227.1 ± 226.0	1915.4 ± 251.5	0.689
Peak GH (µg/l)	5.6 ± 0.8	4.8 ± 1.4	0.437
AUC_{0-300} GH (µg/l min)	424.0 ± 70.2	309.2 ± 101.1	0.348
AUC_{0-150} GH (µg/l min)	98.2 ± 25.9	41.5 ± 28.6	0,002
$AUC_{150-300}$ GH (µg/l min)	325.8 ± 55.2	267.7 ± 90.0	0.504
Ghrelina total nadir (pg/ml)	757.2 ± 49.5	590.2 ± 52.1	0.124
AUC_{0-300} total ghrelin (pg/ml min)	28 3991.8 ± 20469.6	221 095.9 ± 16975.8	0.105
AUC_{0-150} total ghrelin (pg/ml min)	128 562.3 ± 8335.9	98 839.1 ± 7668.6	0.069
$AUC_{150-300}$ total ghrelin (pg/ml min)	155 429.5 ± 12273.8	122 256.8 ± 9574.6	0.226
Peak PYY_{1-36} (pg/ml)	155.1 ± 9.2	194.4 ± 26.2	0.178
AUC_{0-300} PYY_{1-36} (pg/ml min)	35 360.0 ± 1873.9	44 616.8 ± 5411.8	0.187
AUC_{0-150} PYY_{1-36} (pg/ml min)	19 507.3 ± 1114.8 24	425.4 ± 2987.2	0.153
$AUC_{150-300}$ PYY_{1-36} (pg/ml min)	15 852.7 ± 807.7	20 191.3 ± 2552.9	0.169

AUC_{0-300} : area under the secretory curve between time 0–300 min; AUC_{0-150} : area under the secretory curve between time 0–150 min;
$AUC_{150-300}$: area under the secretory curve between time 150–300 min

Serum levels after oral glucose

The post-oral glucose serum levels are presented in Table 2 . The AUC of glucose (mg/dl min, mean ± SEM) between 0 and 300 min was similar in women and men; 29 747.7 ± 833.8 vs. 31 232.7 ± 1 255.6, p = NS, for women and men, respectively (Fig. 1a). The AUC of insulin (µU/ml min, mean ± SEM) between 0 and 300 min was similar in women and men; 10 282.1 ± 937.9 vs. 10 919.6 ± 1 471.5, p = NS, for women and men, respectively (Fig. 1b). The AUC of GH (µg/l min, mean ± SEM) between 0 and 300 min (Fig. 2) was similar in women and men; 424.0 ± 70.2 vs. 309.2 ± 101.1, p = NS, for women and men, respectively. The AUC of GH (µg/l · min, mean ± SEM) between 0 and 150 min was higher in women than men; 98.2 ± 25.9 vs. 41.5 ± 28.6, p = 0.002, for women and men, respectively. The AUC of total ghrelin (pg/ml · min, mean ± SEM) between 0 and 300 min (Fig. 3) was similar in women and men; 283 991.8 ± 20 469.6 vs. 221 095.9 ± 16 975.8, p = 0.105, for women and men, respectively. Several initial time points were higher in women than men (Fig. 3). The AUC of total ghrelin (pg/ml · min, mean ± SEM) between 0 and 150 min was borderline significantly higher in women than men; 128 562.3 ± 8 335.9 vs. 98 839.1 ± 7 668.6, p = 0.069, for women and men, respectively. The AUC of PYY 1–36 (pg/ml min, mean ± SEM) between 0 and 300 min was similar in women and men; 35 360.0 ± 1 873.9 vs. 44 616.8 ± 5 411.8, p = NS, for women and men, respectively.
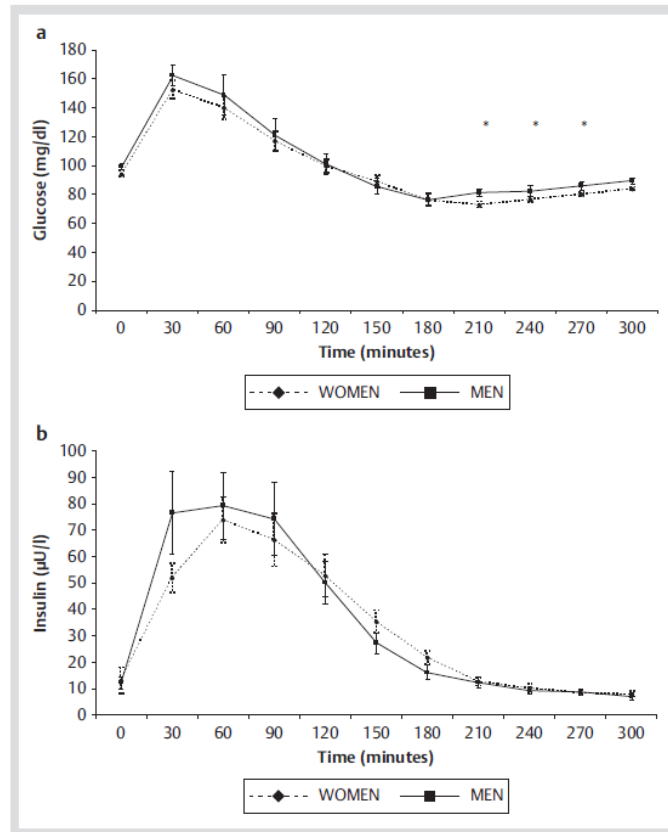
Fig. 1 a Mean ± SEM plasma glucose (mg/dl) in women and men during the prolongued oral glucose tolerance test. *$p < 0.05$ between women and men at that time point. **b** Mean ± SEM plasma insulin levels (μU/ml) in women and men during the prolongued oral glucose tolerance test. The differences were non statistically significant between women and men at any time point.
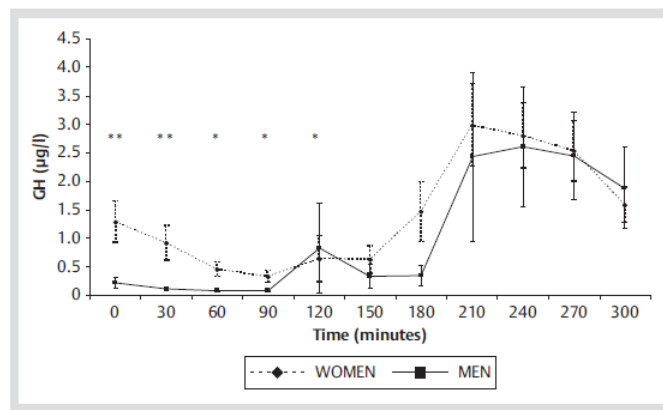

Fig. 2 Mean ± SEM plasma GH levels (μg/l) in women and men during the prolongued oral glucose tolerance test. *$p < 0.05$ and **$p < 0.01$ between women and men at that time point.
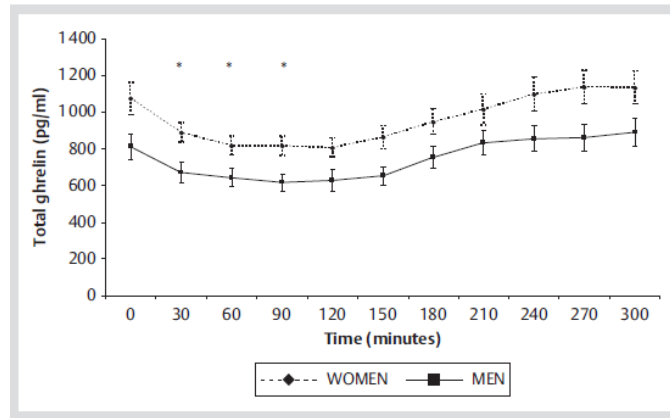
Fig. 3 Mean ± SEM plasma total ghrelin levels (pg/ml) in women and men during the prolongued oral glucose tolerance test. $*p < 0.05$ between women and men at that time point.

Correlations

We analyzed if there was any significant correlation between the GH secretion indices and ghrelin secretion indices in the entire group of women and men. There were significant correlations between the different indices of GH secretion and ghrelin secretion (Table 3), suggesting that higher ghrelin values are associated with higher GH secretion values.

Table 3 Correlations between GH (fasting and peak: µg/l and AUC: µg/l min) and ghrelin (nadir: pg/ml and AUC: pg/ml min) secretion in the entire group of healthy and obese women and men.

	Fasting GH		Peak GH		AUCGH $_{0-300}$		AUCGH $_{0-150}$		AUCGH $_{150-300}$	
	r	p	r	p	r	p	r	p	r	p
Fasting total ghrelin	0.387	0.006	0.250	0.083	0.285	0.047	0.391	0.005	0.219	0.131
Nadir total ghrelin	0.362	0.011	0.121	0.409	0.149	0.308	0.306	0.032	0.076	0.602
AUC total ghrelin $_{0-300}$	0.358	0.011	0.190	0.192	0.221	0.126	0.334	0.019	0.157	0.280
AUC total ghrelin $_{0-150}$	0.399	0.005	0.174	0.232	0.207	0.153	0.361	0.011	0.134	0.360
AUC total ghrelin $_{150-300}$	0.308	0.031	0.197	0.175	0.231	0.110	0.276	0.055	0.185	0.203

AUC $_{0-300}$: area under the secretory curve between time 0–300 min; AUC $_{0-150}$: area under the secretory curve between time 0–150 min; AUC $_{150-300}$: area under the secretory curve between time 150–300 min

Discussion

We have found that GH secretion after OG in healthy and obese is different in women than men. Fasting and initial GH secretion is higher in women than men, in contrast peak and late between 150–300 min, after OG GH secretion is similar. The AUC of ghrelin between 0 and 150 min was borderline and significantly higher in women than men. There were significant correlations between the different indices of GH secretion and ghrelin secretion, suggesting that higher ghrelin values are associated with higher GH secretion values. These data suggest that sexual dimorphism in the neuroendocrine regulation of GH secretion probaly involves ghrelin.

Ghrelin is a natural ligand for GHS-R and potently stimulates GH release when administered exogenously. Although ghrelin is more than a natural GHS [22 – 24], the best established action of exogenously administered ghrelin is its potent stimulation of pituitary GH secretion [5, 6, 19]. The GH-releasing action of ghrelin takes place both at pituitary and hypothalamic level [8, 25]. Human studies with orally active long-acting GH secretagogues which act through the GHS-R [26] have shown an increase in GH secretion. In humans a GHS-R missense mutation, which impairs the constitutive activity of the GHS-R, is associated with short stature [27]. These data implies that endogenous ghrelin plays a role in GH regulation. Several studies have investigated the relationship between ghrelin and GH secretion. Misra et al. [10], using deconvolution analysis for GH and total ghrelin in healthy adolescents and adolescents with anorexia, found that fasting ghrelin is an independent predictor of basal GH secretion and GH secretory burst frequency. Blood samples were measured overnight for 12 h (20:00–08:00 h) every 30 min. In studies carried out on 8 healthy young men, Nass et al. [11] found a significant relationship between GH secretion peak amplitudes and mean circulating acylated ghrelin levels during the fed condition. On the other hand, the study by Zizzari et al. using a GHS-R antagonist favors a direct modulatory role of circulating ghrelin on GH release as do studies with ghrelin mimetics [26]. Recent studies in mice without the Ghrelin *O* -acyltransferase (GOAT) gene have found that an essential function of ghrelin in mice is elevation of GH levels during severe calorie restriction, thereby preserving blood glucose and preventing death [28].

We have found the most important correlations between ghrelin and fasting GH and the AUC of GH between 0 and 150 min, suggesting that ghrelin could regulate mainly fasting and early GH secretion after OG. Our results are in agreement with those of Veldhuis et al., who found that, basal GH secretion, pulsatile GH secretion, and total GH secretion was higher in women than in men, although the number of pulses was similar [4]. These results are in accordance with the increased circulating ghrelin levels found in women when compared with men [13, 14]. Our findings are in line with our report that ghrelin could be a physiological regulator of GH in the postprandial state, and that the decreased ghrelin secretion in obesity could be one of the mechanisms responsible for the altered GH secretion in obesity [12]. The present results do not exclude the possibility that other factors could contribute to the sexual dimorphism on GH secretion, such as GHRH [3, 29]. Studies with the infusion of a GHRH antagonist in humans, found trough and basal GH significantly decreased in women but not in men, although mean GH, pulse amplitude and GH response to GHRH decreased in both sexes [3]. Based on our data, we cannot exclude the possibility that there is a negative feedback loop between GH and circulating ghrelin levels. However, our findings, together with those of other researchers, do not support the existence of any such negative feedback loop [11, 30, 31]. The relationship between ghrelin, which increases food intake, and GH would be beneficial to man because the anabolic changes induced by GH require the presence of adequate nutrition [11].

The concentration of leptin in plasma is proportional to the percent of body fat in different clinical situations [32, 33]. This is likely the main reason for the higher concentration of leptin in the post-absorptive state observed in women [34]. However, for any given degree of obesity, observed leptin levels are higher in women than in men, suggesting a state of leptin resistance in the former [34 – 36]. Most studies have suggested that leptin either has no effect or can decrease GH secretion [21, 37 – 40]. Treatment of acromegalic patients with a GH receptor antagonist

seems to disrupt the feedback loop of ghrelin and GH, leading to elevated ghrelin levels, but does not modify leptin levels [41]. In addition, treatment with recombinant growth hormone in patients with growth hormone deficiency decreased fat mass and leptin levels [42]. In our study, we have found that fasting and initial GH secretion and fasting leptin levels were higher in women than in men. These data suggest that leptin does not participate in the sexual dimorphism of GH secretion after OG.

Because our study was not interventional and the analysis is based on correlation, we cannot exclude the existence of one common or several separate factors that control both GH release and circulating ghrelin levels simultaneously. For example, insulin inhibits GH secretion and has a direct transcriptional inhibitory regulation of the GH gene [43, 44]. Insulin rises after OG and has been suggested to decrease circulating ghrelin levels [45]. Therefore, a possible role for insulin as a common regulator of circulating ghrelin and GH after OG cannot be excluded. During mid-puberty, at a time when GH levels are the highest, PYY is at a nadir, and log nadir GH correlated inversely with log PYY [16]. These associations remained significant even after controlling for BMI, suggesting that PYY could have a role in the nutritional regulation of GH secretion. This is why we also measured PYY, and could not find any correlation between GH and PYY secretion. Another concern regarding our study is that we did not measure acylated ghrelin. Although acylated ghrelin has proved to be the biologically active form in the control of GH secretion, most of the leading studies on the correlation between GH and ghrelin secretion have focused on the estimation of total ghrelin [10]; and there are concerns regarding the especificity of available acyl-ghrelin assays [46] and more important the stability of plasma acylated ghrelin once collected. Recent studies have evaluated the different methods for the stabilization of acylghrelin in human blood collections, in order to improve its stability [47].

In conclusion, these data show that fasting and initial GH secretion after OG is higher in women than men and suggest that sexual dimorphism in the regulation of GH secretion probably involves ghrelin.

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