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Growth hormone, ghrelin and peptide YY secretion after oral glucose administration in healthy and obese women

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Abstract
The mechanism of the altered GH secretion in obesity is unclear. There is evidence that oral glucose (OG) administration initially decreases and subsequently stimulates GH secretion. Ghrelin is a peptide that displays strong growth hormone-releasing activity. Its physiological importance on GH regulation is unclear. Our aim was to study fasting GH concentrations and their response to OG administration in relation with ghrelin secretion in obese and healthy women, in order to elucidate the hypothetical participation of ghrelin on post-oral glucose GH secretion. 36 women were included in the study. After an overnight fast, 75 g of oral glucose was administered; glucose, insulin, ghrelin, and $PYY_{1–36}$ were obtained at baseline and during 300 min. The area under the curve between 0 and 300 min (AUC) of GH μ/l·min) was lower in obese patients than in controls; 262.5±57.5 vs. 534.9±95.6, p=0.01, for obese and controls respectively. GH peak (μg/l) was lower in obese patients than in controls; 3.7±0.7 vs. 7.1±1.0, p=0.012, for obese and controls, respectively. The AUC of total ghrelin (pg/ml·min) was lower in obese patients than in controls; 233 032±12 641 vs. 333 697±29 877, p=0.004, for the obese patients and controls respectively. $PYY_{1–36}$ was similar in obese and healthy women after OG. There were significant correlations between the different indices of post-oral glucose GH and ghrelin secretion. These data suggest that ghrelin is a physiological regulator of GH in the post-oral glucose state, and the decreased ghrelin secretion could be one of the mechanisms responsible for the altered GH secretion in obesity.

Key words
GH - oral glucose - women - obesity - ghrelin

Introduction

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 hormone-releasing activity mediated by the hypothalamus and pituitary GH secretagogue receptors [1–5]. 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 [6]. 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. Espelund et al. [7], Norrelund [8], and Natalucci et al. [9] did not find a correlation between ghrelin and GH under fasting conditions. Avram et al. [10] did not observe any relationship with GH under fed or fasting conditions. Dall et al. could not find a correlation between ghrelin and GH after exercise [11] . In situations with increased GH secretion such as acromegaly and renal failure [12, 13] there was no correlation between ghrelin and GH. Muller et al. [14] showed that fasting induced a diurnal rhythm for total ghrelin similar to that observed in GH, but the diurnal rhythm was not present under fed conditions. Koutkia et al. [15] found that there is a significant regularity in cosecretion between ghrelin and GH in the fasted state. Misra et al. [16], found that fasting ghrelin is an independent predictor of basal GH secretion and GH secretory burst frequency. Nass et al. [17] found that under normal conditions in subjects given regular meals, endogenous acylated ghrelin acts to increase the amplitude of GH pulses. These inconsistent findings may be due, at least in part, to differences in the study design, methodology and patients studied. None of the previous studies have examined the relation between ghrelin and GH secretion in obesity. Data exist, which suggest that other gastrointestinal hormones, like PYY, could participate in GH regulation [18].

In obesity there is a markedly decreased GH secretion. In both children and adults, the greater the body mass index (BMI), the lower the GH response to provocative stimuli, including the response to GH-Releasing Hormone (GHRH) [19]. The altered somatotroph function of obesity is not permanent; it can be reversed by a return to normal weight [20] or by short term calorie restriction [21]. The most striking example of secretory capacity appeared when obese subjects were treated with GHRH plus GH-Releasing Peptide-6 (GHRP-6) both at saturating doses, which resulted in a massive GH response for obese subjects [3]. This relative GH deficiency may contribute towards developing or maintaining the obese state [22], and GH treatment has been employed in obesity [23]. A meta-analysis of recombinant human GH as therapy for obesity in adults, suggests that GH therapy leads to a decrease in visceral adiposity and an increase in lean body mass, as well as beneficial changes in the lipid profile in obese adults, without inducing weight loss [23]. The mechanism of altered GH secretion in obesity is unclear. There is evidence that oral glucose (OG) administration affects GH secretion, initially decreasing GH secretion and subsequently stimulating GH secretion [24]. In human obesity, the oral glucose load loses its early inhibitory effect on GH but maintains its late stimulatory effect on somatotrope secretion. However, GH secretion after oral glucose was decreased in obese subjects when compared with the control group [25]. Circulating plasma ghrelin increases before a meal and decreases following the consumption of nutrients and after an oral glucose tolerance test [26–29]. Ghrelin secretion is altered in obesity [30, 31] and could be responsible for altered GH secretion in obesity.

Our aim was to study fasting GH concentrations and their response to OG administration in relation to ghrelin secretion in healthy and obese women, in order to elucidate the hypothetical participation of ghrelin on the late postprandial GH secretion.

Patients and Methods

Patients

We included a total of 36 women in the study (Table 1). 23 obese women, aged 39.8 ± 2.9 years, with a BMI of 38.8 ± 1.2 kg/m^2, were studied. As a control group, we studied 13 healthy women, selected from a pool of volunteers available at our unit, aged 34.4 ± 3.6 years and with a BMI of 22.3 ± 0.7 kg/m^2. Both groups were homogeneous and differ only in BMI. None of the obese patients or controls 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 informed 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 of obese women and healthy control women

	Control Woman	Obese Woman	P
Number	13	23	
Age (years)	34.4 ± 3.6	39.8 ± 2.9	NS
BMI (kg/m^2)	22.3 ± 0.7	38.8 ± 1.2	< 0.001
Mid-waist circumference (cm)	79.9 ± 1.5	109.7 ± 2.6	< 0.001
Total body fat (%)	31.4 ± 1.2	46.2 ± 0.9	< 0.001

Values are mean ± SEM. 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 was 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 beginning of the mestrual period. We obtained blood samples 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 [32].

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 μ g / 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 coefficients 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 inter-assay 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 2000 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 intra-assay and inter-assay coefficients of variation of 5.3% and 13.6%, respectively. Total ghrelin (pg/ml) was measured by a commercially available radioimmunoassay (RIA) kit (Linco Research Inc., St Charles, MO, USA), specific for total ghrelin, that uses ^{125}Ilabeled 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) kit (Linco, St Charles, MO, USA), with an intra-assay coefficient of variation of between 2.9 – 9.4% and an inter-assay 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 obese patients and controls. 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 patients and controls 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. For graphic presentation we use mean values ± SEM. SPSS 17.0 software (Chicago, IL, USA) was used to produce the statistical analysis.

Results

The basic characteristics of the healthy control group and patients are shown in Table 1.

Fasting serum levels

The fasting serum levels are presented in Table 2. Fasting glucose (mg/dl, mean ± SEM) levels were higher in obese women than in healthy control women; 98.4 ± 2.0 vs. 86.5 ± 3.0, p = 007, for the obese patients and controls respectively. Fasting insulin (μU/ml, mean ± SEM) levels were higher in obese women than in healthy control women; 17.7 ± 6.9 vs. 4.0 ± 0.8, p = 0.0001, for the obese patients and controls respectively. Fasting GH (μg/l, mean ± SEM) levels were similar in obese women and in healthy control women; 1.2 ± 0.4 vs. 1.6 ± 0.8, p = NS, for the obese patients and controls respectively. Fasting total ghrelin (pg/ml, mean ± SEM) levels were lower in obese women than in healthy control women; 898 ± 66 vs. 1 376 ± 163, p = 0.01, for the obese patients and controls respectively. Fasting IGF-I (ng/ml, mean ± SEM) levels were lower in obese women than in healthy control women; 107.0 ± 10.6 vs. 161.0 ± 11.2, p = 0.001, for the obese patients and controls respectively. Fasting leptin (ng/ml, mean ± SEM) levels were higher in obese women than in healthy control women, 65.5 ± 5.5 vs. 21.3 ± 2.6, p = 0.0001, for obese patients and controls

respectively. Fasting $PYY_{1–36}$ (pg/ml, mean ± SEM) levels were similar in our group of otherwise healthy obese women and control women; 135.4 ± 9.7 vs. 133.2 ± 13.3, p = NS, for the obese patients and controls respectively.

Table 2. Fasting and after oral glucose biochemical and hormonal data in obese women and healthy control women

	Control Woman	Obese Woman	p
Fasting glucose (mg/dl)	86.5 ± 3.0	98.4 ± 2.0	0.007
Fasting insulin (μUI/ml)	4.0 ± 0.8	17.7 ± 6.9	< 0.001
Fasting triglycerides (mg/dl)	72.5 ± 3.8	118.1 ± 6.4	0.006
Fasting GH (μg/l)	1.6 ± 0.8	1.2 ± 0.4	NS
Fasting IGF-I (ng/ml)	161.0 ± 11.2	107.0 ± 10.6	0.001
Fasting leptin (ng/ml)	21.3 ± 2.6	65.5 ± 5.5	< 0.001
Fasting total ghrelin (pg/ml)	1 376.1 ± 162.9	898.3 ± 65.7	0.01
Fasting $PYY_{1–36}$ (pg/ml)	133.2 ± 13.3	135.4 ± 9.7	NS
Peak glucose (mg/dl)	138.5 ± 7.4	170.4 ± 8.7	0.022
$AUC_{0–300}$ glucose (mg/dl min)	26 925.0 ± 1 245.7	31 469.3 ± 803.8	0.005
$AUC_{0–150}$ glucose (mg/dl min)	15 598.8 ± 886.4	19 342.2 ± 797.8	0.01
$AUC_{150–300}$ glucose (mg/dl min)	11 326.1 ± 440.8	12 127.2 ± 272.6	NS
Peak insulin (μUI/ml)	50.0 ± 6.1	115.7 ± 14.0	< 0.001
$AUC_{0–300}$ insulin (μUI/ml min)	5 705.3 ± 537.8	12 561.4 ± 1 029.6	< 0.001
$AUC_{0–150}$ insulin (μUI/ml min)	4 629.5 ± 497.5	9 778.0 ± 973.3	< 0.001
$AUC_{150–300}$ insulin (μUI/ml min)	1 075.8 ± 112.3	2 783.4 ± 240.5	< 0.001
Peak GH (μg/l)	8.6 ± 1.1	3.5 ± 0.8	< 0.001
$AUC_{0–300}$ GH (μg/l min)	652.1 ± 116.0	265.0 ± 64.1	0.001
$AUC_{0–150}$ GH (μg/l min)	142.0 ± 51.5	71.4 ± 23.1	NS
$AUC_{150–300}$ GH (μg/l min)	510.1 ± 89.0	193.6 ± 50.7	< 0.001
Nadir total ghrelin (pg/ml)	940.8 ± 93.8	667.5 ± 41.4	0.022
$AUC_{0–300}$ total ghrelin (pg/ml min)	362 687 ± 39 430	243 128 ± 14 867	0.01
$AUC_{0–150}$ total ghrelin (pg/ml min)	157 590 ± 15 772	113 673 ± 6 865	0.026
$AUC_{150–300}$ total ghrelin (pg/ml min)	205 097 ± 23 740	129 454 ± 8 230	0.005
Peak $PYY_{1–36}$ (pg/ml)	143.5 ± 16.1	162.0 ± 10.2	NS
$AUC_{0–300}$ $PYY_{1–36}$ (pg/ml min)	34 923.5 ± 3 441.3	36 937.8 ± 2 146.4	NS
$AUC_{0–150}$ $PYY_{1–36}$ (pg/ml min)	19 509.2 ± 2 071.8	20 237.6 ± 1 254.5	NS
$AUC_{150–300}$ $PYY_{1–36}$ (pg/ml min)	15 414.2 ± 1 432.9	16 700.2 ± 946.3	NS

Values are mean ± SEM. $AUC_{0–300}$: 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. Glucose was higher in obese women than in healthy control women after the 300-min OG (Fig. 1a). The AUC of glucose (mg/dl min, mean ± SEM) between 0 and 300 min was higher in obese patients than in controls; 31 169 ± 660 vs. 28 239 ± 1 176, p = 0.016, for the obese patients and controls respectively. Peak glucose (mean ± SEM) levels were higher in our group of otherwise healthy obese women and controls; 170.4 ± 8.7 vs. 138.5 ± 7.4, p = 0.022, for the obese patients and controls respectively. Insulin was higher in obese women than in healthy control women after the 300 min OG (Fig. 1b). The AUC of insulin (μU/ml min, mean ± SEM) between 0 and 300 min was higher in the obese patients than in controls; 12 616 ± 867 vs. 5 845 ± 605, p = 0.0001, for obese patients and controls respectively. Peak insulin (μU/ml, mean ± SEM) levels were higher in the obese patients than in the control subjects; 115.7 ± 14.0 vs. 50.0 ± 6.1, p = 0.0001, for the obese patients and controls respectively. GH was lower in the obese women than in the healthy control women after OG (Fig. 2a). The AUC of GH (μg/l min, mean ± SEM) between 0 and 300 min was lower in the obese patients than in the controls; 262.5 ± 57.5 vs. 534.9 ± 95.6, p = 0.01, for the obese patients and controls respectively. Peak GH (μg/l, mean ± SEM) levels were lower in obese patients than in control subjects, 3.7 ± 0.7 vs. 7.1 ± 1.0, p = 0.012, for obese patients and controls respectively.

Total ghrelin was lower in obese women than in healthy control women after OG (Fig. 2b). The AUC of total ghrelin (pg/ml min, mean ± SEM) between 0 and 300 min was lower in obese patients than in controls; 233 032 ± 12 641 vs. 333 697 ± 29 877, p = 0.004, for the obese patients and controls respectively. Nadir total ghrelin (pg/ml, mean ± SEM) levels were lower in obese women than in controls; 667.5 ± 41.4 vs. 940.8 ± 93.8, p = 0.022, for the obese patients and controls respectively. $PYY_{1–36}$ was similar in obese women and healthy control women after the 300 min OG (Fig. 3). The AUC of $PYY_{1–36}$ (pg/ml min, mean ± SEM) between 0 and 300 min was similar in obese women and healthy control women; 36 938 ± 2 146 vs. 34 923 ± 3 441, p = NS, for the obese patients and controls respectively. Peak $PYY_{1–36}$ (pg/ml, mean ± SEM) levels were similar in obese women and healthy control women; 162 ± 10 vs. 144 ± 16, p = NS, for the obese patients and controls respectively.

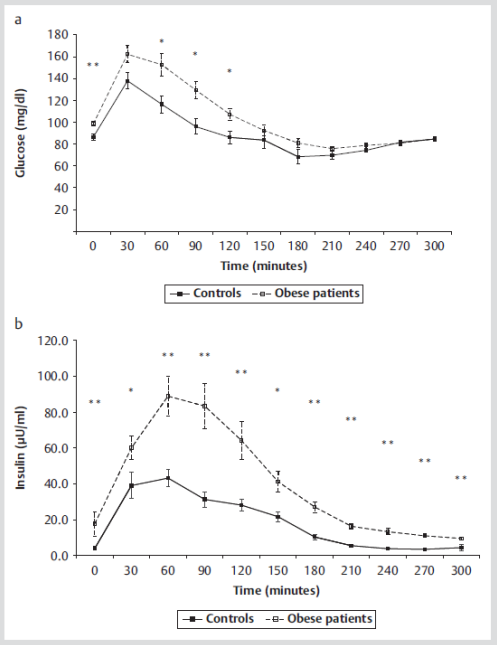

Fig. 1a: Mean ± SEM plasma glucose (mg/dl) in controls and obese women during the prolonged oral glucose tolerance test. *p<0.05 and **p<0.01 between controls and obese women at that time point.
b: Mean ± SEM plasma insulin levels (µU/ml) in controls and obese women during the prolonged oral glucose tolerance test. *p< 0.05 and **p<0.01 between controls and obese women at that time point.

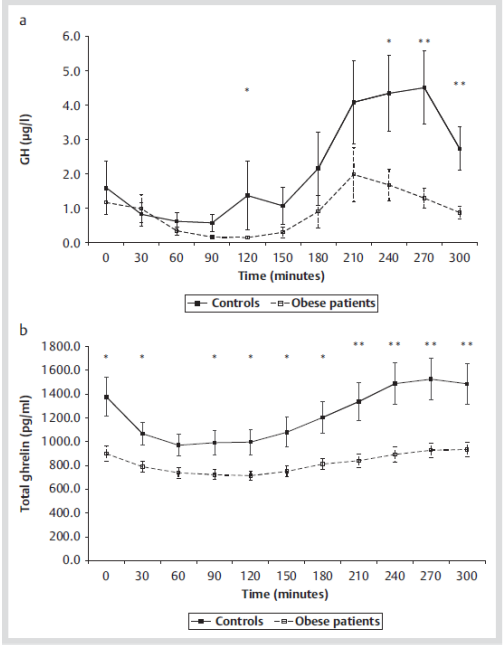

Fig. 2a: Mean ± SEM plasma GH levels (µg/l) in controls and obese women during the prolonged oral glucose tolerance test. *p<0.05 and **p< 0.01 between controls and obese women at that time point.
b: Mean ± SEM plasma total ghrelin levels (pg/ml) in controls and obese women during the prolongued oral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

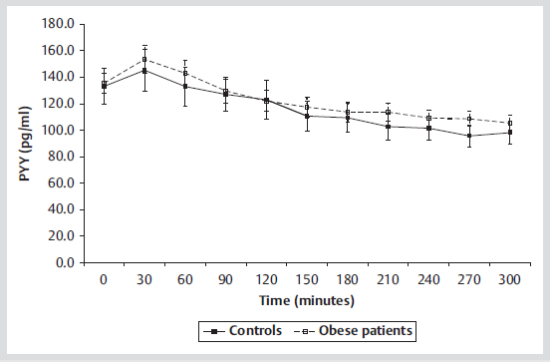

Fig. 3 Mean ± SEM plasma $PYY_{1–36}$ levels (pg/ml) in controls and obese women during the prolonged oral glucose tolerance test. The differences were nonstatistically significant between controls and obese women at any time point.

Correlations

We analyzed if there was any significant correlation between the GH secretion indices and ghrelin secretion indices in the entire group of obese women and healthy control women. There were signifi cant 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. These correlations persisted after adjusting for age, BMI and other obesity indices, glucose and insulin in a multiple regression analysis.

Table 3. Correlations between GH (Peak: µg/l and AUC: µg/l min) and ghrelin (Nadir: pg/ml and AUC: pg/ml min) secretion in the entire group of normal and obese women

	GH Peak		$AUC_{0-300}H$		$AUC_{150-300}GH$	
	r	p	r	p	r	p
Nadir total ghrelin	0.353	0.035	0.365	0.029	0.266	0.117
$AUC_{0–300}$ total ghrelin	0.419	0.011	0.425	0.010	0.330	0.049
$AUC_{0–150}$ total ghrelin	0.394	0.017	0.425	0.014	0.305	0.07
$AUC_{150–300}$ total ghrelin	0.442	0.007	0.447	0.006	0.373	0.025

$AUC_{0–300}$: 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 after OG in healthy and obese women there is a significant correlation between the AUC of ghrelin and the AUC of GH. After OG, initial GH secretion, between 0 – 150 min, is similarly suppressed in normal and obese patients, but the late response, between 150 – 300 min, is decreased in obese patients. These data show that ghrelin secretion after OG is probably an important regulator of late GH secretion, between 150 – 300 min, after OG in women and that the decreased GH secretion of obesity after an OG is probably due to the altered ghrelin secretion found in obese patients.

Ghrelin is a natural ligand for GHS-R and potently stimulates GH release when administered exogenously. Although ghrelin is more than a natural GHS, the best established action of exogenously administered ghrelin is its potent stimulation of pituitary GH secretion [1, 4, 28, 33]. The GH-releasing action of ghrelin takes place both at pituitary and hypothalamic level [6, 34]. Human studies with orally active long-acting GH secretagogues, which act through the GHS-R [35] have shown an increase in GH secretion. Animal studies by Zizzari et al. [36] using a GHS-R antagonist support the results that endogenous acylated ghrelin modifies circulating GH release during the fed state. Both sc and intracerebroventricular infusion in the rat with the GHS-R antagonist BIM-28163 lowered the GH pulse amplitude. In humans a GHS-R missense mutation, which impairs the constitutive activity of the GHS-R, is associated with short stature [37]. Consistent with these results, GHS-R-null mice have lower IGF-I levels when compared with wild-type animals [38]. These data implies that endogenous ghrelin plays a role in GH regulation. Several studies have investigated the relationship between ghrelin and GH secretion. Espelund et al. [7], Norrelund [8] and Natalucci et al. [9] did not find a correlation between ghrelin and GH under fasting conditions. Avram et al. [10] used an acylated ghrelin RIA, but did not observe any relationship with GH under fed or fasting conditions. Their differing results may be connected with protocol differences, and also their study population included both men and women. Dall et al. could not find a correlation between ghrelin and GH after exercise [11]. Our previous findings in pathophysiological situations with increased GH secretion such as acromegaly and renal failure [12, 13] could not find a correlation between ghrelin and GH. Muller et al. [14] showed that fasting induced a diurnal rhythm for total ghrelin similar to that observed in GH levels, but the diurnal rhythm was not present when subjects were tested under fed conditions. Koutkia et al. [15] used cross-approximate entropy analysis and found that there is a significant regularity in cosecretion between ghrelin and GH in the fasted state; however, this was only found during the night. Misra et al. [16], 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. [17] found a significant relationship between GH secretion peak amplitudes and mean circulating acylated ghrelin levels during the fed condition. In agreement with our results in healthy and obese women they conclude that under normal conditions in subjects given regular meals, endogenous ghrelin acts to increase the amplitude of GH pulses. On the other hand, the study by Zizzari et al. [36] using a GHS-R antagonist favors a direct modulatory role of circulating ghrelin on GH release as do studies with ghrelin mimetics [35]. 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 [39] . Our findings are in line with previous studies, which suggest that the inhibitory and stimulatory effects of glucose load on GH secretion are unlikely to be due to direct biphasic modulation of hypothalamic somatostatin [25] or GHRH release, as exogenous GHRH increases GH secretion after oral glucose administration [25]. As shown in Fig. 2 there is a relatively greater decline in GH in obese compared with healthy subjects, despite a smaller relative decline in ghrelin levels following glucose loading, that response could be due to the altered free fatty acids of obesity, as GH response to ghrelin is partially refractory to the inhibitory effect of free fatty acids [40] . We have previously shown that the decreased GH secretion of obesity is at least in part due to the increased free fatty acids present in that disease [41]. Based on our data, we cannot exclude the possibility that there is a negative feedback loop between GH and circulating ghrelin levels. However, the current literature on this subject is controversial [42], and our findings, together with those of other researchers, do not support the existence of any such negative feedback loop [13, 17, 43]. The relationship between ghrelin, which increases food intake, and GH found during the late fed state would be beneficial to man because the anabolic changes induced by GH require the presence of adequate nutrition [7, 17].

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 [44, 45]. Insulin rises after OG and has been suggested to decrease circulating ghrelin levels [46, 47]. Therefore, a possible role for insulin as a common regulator of circulating ghrelin and GH after OG cannot be excluded, even though the correlation between GH and ghrelin persisted after adjusting for insulin. 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 [18]. 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 [7 – 9, 11, 14 – 16], and there are concerns regarding the especificity of available acyl-ghrelin assays [48] 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 [49]. The primary cause of impaired GH secretion in obesity could be an altered hypothalamus, abnormal pituitary function, or a perturbation of the peripheral signals acting at either the pituitary or hypothalamic level. Different studies suggest that the pathophysiological mechanism responsible for GH hyposecretion in obesity is multifactorial, and there is probably a chronic state of somatostatin hypersecretion, increased FFA and decreased ghrelin [4, 41, 50, 51]. The findings of the present study where we have found that after OG in obese women there is a significant correlation between ghelin and GH secretion, suggest that the decreased ghrelin secretion in obesity is one of the mechanisms responsible for altered GH secretion in obesity. The decreased stimulation of GH release in obese subjects after OG may promote the retention of fat mass. However, studies have demonstrated that apparently all the defects in the GH IGF-I axis in obesity are reversible with diet-induced and surgical-induced large weight loss [20, 52 – 54]. The recovery of the GH IGF-I axis after weight loss suggests an acquired defect, rather than a preexisting disorder. However, the impaired GH IGF-I axis may intervene in the expansion and maintenance of fat mass, and contribute to perpetuation of the obese state. In conclusion, the data presented suggest that ghrelin is 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.

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