ORIGINAL ARTICLE

Effect of oral glucose on acylated and total Ghrelin secretion in acromegalic patients

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Abstract

OBJECTIVE: The pathophysiology of ghrelin secretion in acromegaly is unclear. Our aim was to study circulating fasting ghrelin levels and their response to oral glucose in acromegalic patients and normal control subjects.

Design and Patients: 9 acromegalic patients (4 male, 5 female; 59.4±3.6 years; 28.6±1.0 kg/m^2) and 9 age and BMI matched healthy control subjects (4 male, 5 female; 59.1±1.4 years; 26.5±0.8 kg/m^2) were included. We obtained blood samples for glucose, insulin, GH, total ghrelin and acylated ghrelin at times 0, 30, 60, 90 and 120 minutes after 75 g of oral glucose.

RESULTS: Fasting GH and IGF-I were statistically different between patients and controls: GH (μg/l): 6.7±1.4 vs. 0.8±0.4, p<0.01; IGF-I (ng/ml): 414±75 vs. 86±6, p<0.01. Fasting total ghrelin (pg/ml) were similar in the patient and in the control group, 916±132 vs. 844±169, p=ns. In both groups total ghrelin levels decreased during oral glucose, and nadir total ghrelin was lower than fasting ghrelin: patients: 916±132 vs. 747±95, p<0.05; controls: 844±169 vs. 625±90, p<0.05). The AUCs of total ghrelin (pg/ml•min) were not different between the two groups: 98953±13052 vs. 83773±13096, p=ns). Fasting acylated ghrelin (pg/ml) were similar in the patient and the control group 65±13 pg/ml vs.74±14 pg/ml, p=ns. In both groups acylated ghrelin levels decreased during oral glucose, and nadir acylated ghrelin levels were lower than basal acylated ghrelin levels: patients: 65±13 vs. 42±6, p<0.05; controls: 74±14 pg/ml vs. 37±4 pg/ml, p<0.05). The AUCs of acylated ghrelin (pg/ml•min) were not different between the two groups: patients: 6173±992 vs. controls 8648±2742, p=ns). In acromegalic patients there was a negative correlation between fasting, both total and acylated, ghrelin and both fasting and post oral glucose insulin levels.

CONCLUSIONS: These data suggest that circulating total and acylated ghrelin in acromegaly is regulated by insulin and not by GH hypersecretion.

INTRODUCTION

Ghrelin is a 28-amino-acid peptide, predominantly produced by the stomach, that shows a unique structure with an n-octanoyl ester at its third serine residue, which is essential for its potent stimulatory activity on somatotroph secretion (Kojima *et al.*, 1999; Howard *et al.*, 1996; Cordido *et al.*, 1993; Alvarez *et al.*, 2004). Besides stimulating GH secretion, ghrelin has other endocrine and nonendocrine actions (van der Lely *et al.*, 2004). Different studies suggest the importance of ghrelin in feeding and weight homeostasis (Tschop *et al.*, 2000; Nakazto *et al.*, 2001; Shuto *et al.*, 2002). The infusion of ghrelin led to short-term increases in hunger in human subjects (Wren *et al.*, 2001). Although obese patients with Prader-Willi syndrome, characterized by hyperphagia and obesity have elevated ghrelin levels (Cummings *et al.*, 2002), the concentrations of fasting ghrelin are increased in anorexia and cachexia but reduced in obesity (Cummings *et al.*, 2002; Tschop *et al.*, 2001; Shiiya *et al.*, 2001; Haqq *et al.*, 200; Muccioli *et al.*, 2002; Otto *et al.*, 2001) and plasma ghrelin levels are negatively correlated with body mass index, body fat mass and plasma leptin, insulin and glucose levels (Muccioli *et al.* , 2002; Ukkola, 2003, Asakawa *et al.*, 2003). Insulin resistance has been postulated to play a role in determining the lower fasting plasma ghrelin in the obese (McLaughlin *et al.*, 2004). Circulating plasma ghrelin increases before a meal and decreases following the consumption of nutrients and after an oral glucose tolerance test (OGTT) (Shiiya *et al.*, 2001; Cummings *et al.*, 2001; Kojima and Kangawa, 2005).

The best established action of exogenously administered ghrelin is its potent stimulation of pituitary GH secretion (Kojima *et al.*, 1999; Alvarez *et al.*, 2004; van der Lely, 2004 Kojima and Kangawa, 2005). However, the role of ghrelin in the regulation of endogenous GH secretion is not clear yet. Some, but not all, evidence indicates that circulating concentrations of GH and/or IGF-I could influence ghrelin secretion (Dall *et al.*, 2002; Janssen *et al.*, 2002; Malik *et al.*, 2004; Jung *et al.*, 2006; Giavoli *et al.*, 2004; Eden Engstrom *et al.*, 2003). Increasing evidence also suggests that insulin may be an important regulator of ghrelin secretion (Saad *et al.*, 2002; Purnell *et al.*, 2003 McLaughlin *et al.*, 2004) and, thus, hyperinsulinemia, a common metabolic abnormality in acromegaly, could be an important determinant of ghrelin secretion in acromegaly. It has also been shown that octreotide, which is known to suppress other gastrointestinal peptides, could suppress gastric ghrelin secretion in acromegaly (Freda *et al.*, 2003; Barkan *et al.*, 2003; Norrelund *et al.*, 2002; Wasko *et al.,* 2006).

Acromegaly is a rare disease with increased mortality (Hekimsoy *et al.*, 2005; Bolanowski *et al.*, 2006; Melmed, 2006). The pathophysiology of ghrelin secretion in acromegaly is unclear. Some data have suggested that ghrelin levels are lowered in patients with active acromegaly (Cappiello *et al.*, 2002; Freda *et al.*, 2003; Kozakowski *et al.*, 2005), while others have found normal levels (Van der Toorn *et al.*, 2002; Barkan *et al.*, 2003; Jarkovska *et al.*, 2006). Ghrelin secretion in acromegaly after an OGTT is also controversial. While Freda *et al.* (Freda *et al.*, 2003) have found that there is a decrease in ghrelin levels, Cappiello *et al.* (Cappiello *et al.*, 2002;) have found that there is no decrement of ghrelin levels after an OGTT. Due to this controversy and because changes of circulating ghrelin levels could be relevant to body composition changes or to altered GH secretion in acromegaly, further investigation of ghrelin physiology and the potential dysregulation of ghrelin secretion in acromegaly is needed.

Our aim was to study circulating fasting acylated and total ghrelin levels and their response to an oral glucose tolerance test in active acromegalic patients and normal control subjects matched for age, sex and BMI and their relation with glucose, insulin and GH.

PATIENTS AND METHODS

Patients

We included 9 patients with active acromegaly (4 male, 5 female), mean age 59.4±3.6 years, mean BMI of 28.6±1.0 kg/m^2 and mean percentage of body fat of 23.1±1.7%. 9 age, BMI and percentage body fat matched healthy subjects (4 male, 5 female), mean age 59.1±1.4 years, mean BMI of 26.5±0.8 kg/m^2 and mean percentage of body fat 23.8±2.1% were included as controls. Among the acromegalic patients there were two diabetics and two patients with glucose intolerance. Acromegaly was due to a pituitary macroadenoma in all the patients. All patients had previously undergone transsphenoidal surgery with confirmation of a GH-secreting tumor. After these procedures GH hypersecretion was still active, as defined by IGF-I levels above the normal range for age and sex and GH levels over 0.4 μg/L after a 75 g oral glucose tolerance test (OGTT). All the patients were under somatostatin analog treatment, which, in every case, was stopped at least three months before the study was performed. Patients with any degree of hypopituitarism were under appropriate and stable replacement hormone therapy. The two acromegalic patients that had been diagnosed of diabetes mellitus were being treated with dietetic therapy. Otherwise the patients received no other medications. All patients were ambulatory and none of them had active hepatic or renal disease. 9 age and BMI matched healthy or overweight subjects, selected from a pool of volunteers available to our unit, served as controls. They were receiving no treatments.

Study Procedure

Between 08:30 and 09:00 AM, after an overnight fast and while seated, a peripheral venous line was obtained. Fifteen minutes later 75 g of oral glucose were administered. We obtained blood samples for glucose, insulin, GH, total ghrelin and acylated ghrelin at baseline (fasting) and then at times 30, 60, 90 and 120 minutes. Basal levels of IGF-I were also measured. All blood samples were immediately centrifuged, separated and frozen at

–80 °C. Samples destined to determination of plasma ghrelin were specifically retrieved to chilled tubes containing aprotinin and EDTA-Na, and then immediately centrifuged at 4 °C, separated to aliquots and frozen at –80 °C. Body composition was evaluated by whole body bioelectrical impedance analysis (Biologica, Barcelona, Spain). The study protocol fulfilled the requirements of the ethical committee of our centre and written informed consent was obtained from all patients and controls.

Assays

Plasma glucose (mmol/l) was measured with an automatic glucose oxidase method (Roche Diagnostics, Mannheim, Germany). Insulin (mU/L) was measured with a solid-phase two-site chemiluminescent immunometric assay (Immulite 2000 Insulin, DPC, Los Angeles, CA, USA) and with intrassay coefficients of variation of 5.5%, 3.3% and 3.7% for low, medium and high plasma insulin levels respectively. Serum GH (μg/l) was measured by a solid-phase, two-site chemiluminescent enzyme immunometric assay (Immulite, EURO/DPC) with a sensitivity of 0.01 μg/l and with intrassay coefficients of variation of 5.3%, 6.0% and 6.5% for low, medium and high plasma GH levels respectively. IGF-I was determined by a chemiluminescence assay (Nichols Institute, San Clemente, CA, USA) and with intrassay coefficients of variation of 4.8%, 5.2% and 4.4% for low, medium and high plasma IGF-I levels 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%, and with intrassay coefficient of variation between 4.4–10%. Acylated ghrelin was measured by RIA, specific for acylated ghrelin (Linco Research Inc., St Charles, MO, USA), and with intrassay coefficient of variation between 6.7–9.5%.

All samples from a given subject were analysed in the same assay run. Hormone levels are presented as absolute values or as the mean nadir. The area under the secretory curve (AUC) was calculated by a trapezoidal method. Insulin resistance was calculated on the basis of fasting values of plasma glucose and insulin, according to the homeostasis model assessment (HOMA-IR) method (Matthews *et al.*, 1985) as follows: HOMA-IR=fasting insulin levels x fasting glucose levels/22.5, where basal insulin levels is in μUI/mL, and glucose is in mmol/L.

Statistical analysis

The results are presented as mean±SEM. All comparisons were based on univariate, nonparametric tests. Intragroup comparisons were based on Wilcoxon sign-rank test. Comparisons between patients and controls were based on Mann-Whitney U test. Numerical correlations were analyzed using the Spearman's correlation test. p-values ≤ 0.05 were considered to be significant. For graphic presentation we use mean values±SEM. The SPSS software 12.0 (Chicago, IL, USA) was used to produce statistical analysis.

Table 1. Clinical data and basal hormonal data (mean±SEM) of patients and controls.

	Patients	Controls	p-value
Male/Female	4/5	4/5	
Age (years)	59.4±3.6	59.1±1.4	NS
BMI (kg/m^2)	28.6±0.9	26.5±0.8	NS
Body fat (%)	23.1±1.7	23.8±2.1	NS
DM/GI	2/2	0/0	
GH (μg/l)	6.7±1.4	0.8±0.4	<0.01
IGF-I (ng/ml)	414±75	86±6	<0.01
Total ghrelin (pg/ml)	916±132	844±169	NS
Acylated ghrelin (pg/ml)	65±13	74±14	NS

DM=diabetes mellitus, GI=glucose intolerance.

RESULTS

Clinical data and baseline hormonal data of patients and controls are presented in Table 1.

Fasting serum levels

As expected, basal levels of GH and IGF-I were statistically different between patients and controls (GH 6.7±1.4 μg/l vs. 0.8±0.4 μg/l, p<0.01; IGF-I 414±75 ng/ml vs. 86±6 ng/ml, p<0.01). Although basal glucose and basal insulin were higher in the acromegalic than in the control group, the difference was not statistically significant (basal glucose 111±10.9 mg/dl vs. 94±1.4 mg/dl, p=ns; basal insulin 8.2±1.1 μUI/ml vs. 7.2±1.2 μUI/ml, p=ns; for patients and controls respectively). Basal total ghrelin levels were not statistically significantly different between patients and controls (916±132 pg/ml vs. 844±169 pg/ml, p=ns). Acylated ghrelin levels were not statistically significantly different between patients and controls (65±13 pg/ml vs.74±14 pg/ml, p=ns). Insulin resistance, as estimated by HOMA scores, was not statistically different between patients and controls (2.4±0.5 vs. 1.6±0.2, p=ns), although there was a tendency towards higher levels in the acromegalic group. In Figure 1 we show fasting serum levels of GH, IGF-I, total ghrelin and acylated ghrelin in acromegalic patients and controls.

Serum levels after oral glucose

During the OGTT patients and controls were statistically different in GH levels at all time points. The area under the curve (AUC) of GH was statistically significantly higher in patients than in controls (AUC of GH: 658±157 μg/l·min vs. 51±13 μg/l·min, for acromegalic and controls respectively, p<0.01).

Although glucose was higher in the acromegalic group than in the control group at all points of the OGTT, this difference was statistically significant only at 90´ (patients: 176±30 mg/dl vs. controls: 104±7 mg/dl; p<0.05). Insulin levels were not statistically different between the two groups at any point of the curve. Glucose mean peak and insulin mean peak were higher in the group of acromegalic patients, but again this difference was not significant (glucose mean peak 205±24 mg/dl vs. 150±9 mg/dl, p=ns; insulin mean peak 86±21 μUI/ml vs. 78±20 μUI/ml, p=ns). The AUC of glucose and the AUC of insulin were not different between patients and controls (AUC of glucose: 19922±2730 mg/dl·min vs. 14115±775 mg/dl·min, respectively, p=ns; AUC of insulin: 5864.0±1186 μUI/ml·min vs. 5918±1425 μUI/ml·min, respectively, p=ns).

In both groups total ghrelin levels decreased during the OGTT, and nadir total ghrelin levels were statistically lower than basal total ghrelin levels (patients: 916±132 pg/ml vs. 747±95 pg/ml, p<0.05; controls: 844±169 pg/ml vs. 625±90 pg/ml, p<0.05). Nadir total ghrelin levels were not different between the two groups (patients 747±95 pg/ml vs. controls 625±90 pg/ml, p=ns). Figure 2 shows serum total ghrelin levels in acromegalic and normal subjects during the OGTT. The mean reduction in total ghrelin levels during the OGTT was not different between the two groups (patients 169±50 pg/ml vs. controls 220±83 pg/ml; p=ns). In the control group total ghrelin levels at 120 min was statistically lower than basal total ghrelin levels (676±95 pg/ml vs. 844±169 pg/ml; p<0.05). In the group of patients total ghrelin level at time 120 min was not lower than basal total ghrelin level (948±173 pg/ml vs. 916±132 pg/ml; p= ns). The AUCs of total ghrelin were not different between the two groups (98953±13052 pg/ml·min vs. 83773±13096 pg/ml·min, p=ns).

In both groups acylated ghrelin levels decreased during the OGTT, and nadir acylated ghrelin levels were statistically lower than basal acylated ghrelin levels (patients: 65±13 pg/ml vs. 42±6 pg/ml, p<0.05; controls: 74±14 pg/ml vs. 37±4 pg/ml, p<0.05). Nadir acylated ghrelin levels were not different between the two groups (patients 42±6 pg/ml vs. controls 37±4 pg/ml, p=ns). Figure 3 shows serum acylated ghrelin levels in acromegalic and normal subjects during the OGTT. The AUCs of acylated ghrelin were not different between the two groups (patients: 6173±992 pg/ml·min vs. controls 8648±2742 pg/ml·min, p=ns).

Correlations

We analyzed if there was significant correlation between fasting total ghrelin levels and age, BMI, IGF-I,

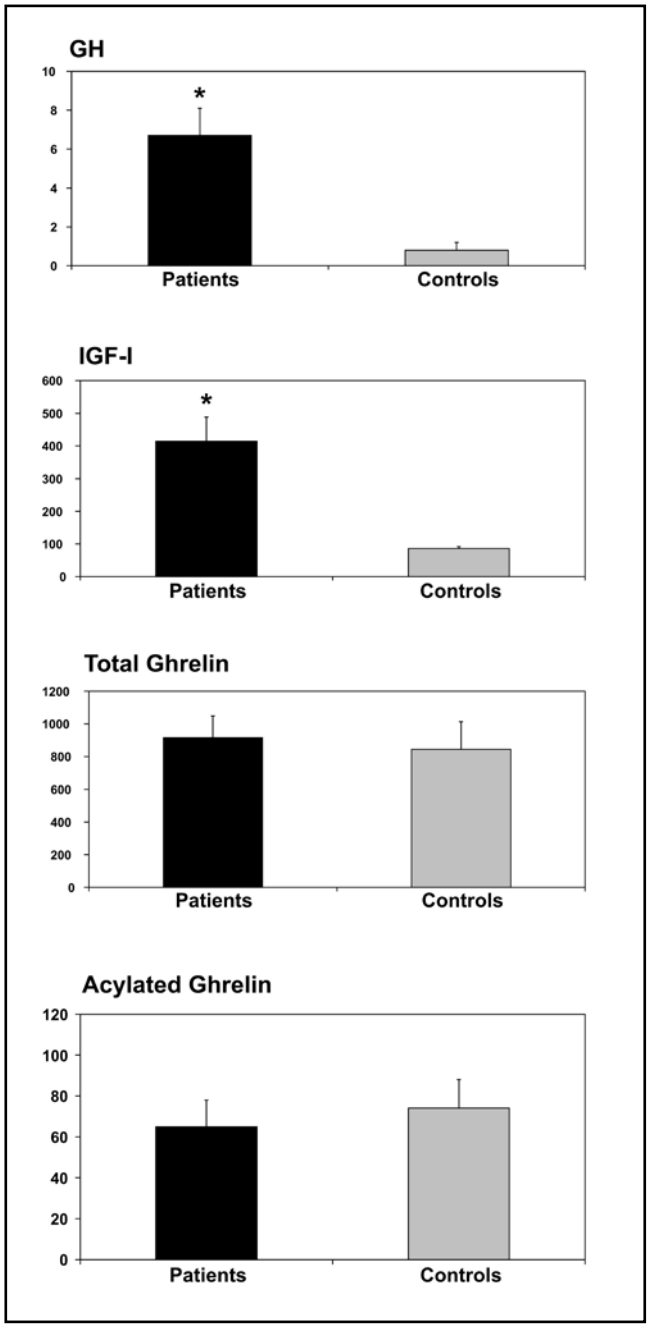

Figure 1. Fasting serum levels (mean±SEM) of GH (μg/l), IGF-I (ng/ml), total ghrelin (pg/ml) and acylated ghrelin (pg/ml) in acromegalic patients and controls. *p<0.01.

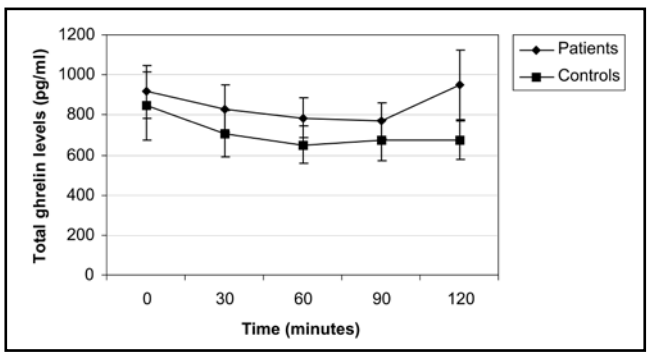

Figure 2. Mean±SEM serum ghrelin levels (pg/ml) in patients with active acromegaly and controls during the oral glucose tolerance test.

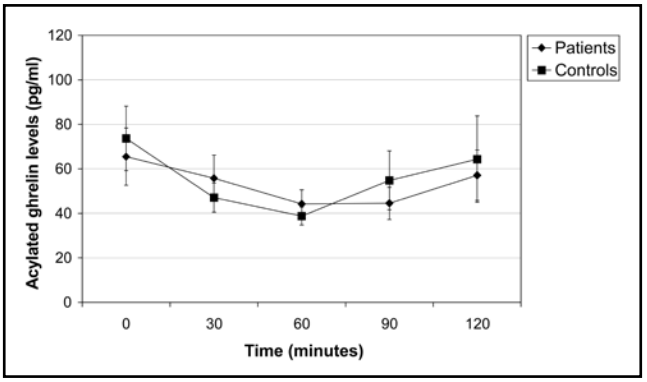

Figure 3. Mean ± SEM serum acylated ghrelin levels (pg/ml) in patients with active acromegaly and controls during the oral glucose tolerance test.

fasting glucose, glucose at any point during the OGTT, basal GH, GH at any point during the OGTT, fasting insulin, insulin at any point during the OGTT, insulin resistance as estimated by HOMA, glucose peak, insulin peak, glucose AUC and insulin AUC. In the acromegalic group fasting total ghrelin levels negatively correlated with basal insulin (r=–0.817; p<0.01) and AUC of insulin after OGTT (r=–0.683; p<0.05). Specifically fasting total ghrelin did not correlate with GH or IGF-I levels.

We analyzed if there was significant correlation between the degree of reduction of total ghrelin levels during the OGTT and age, BMI, IGF-I, fasting glucose, glucose at any point during the OGTT, basal GH, GH at any point during the OGTT, fasting insulin, insulin at any point during the OGTT, insulin resistance as estimated by HOMA, fasting total ghrelin levels, glucose peak, insulin peak, glucose AUC and insulin AUC. In the acromegalic group the degree of reduction in total ghrelin levels negatively correlated with basal insulin (r=–0.728; p<0.05), insulin at 60′ (r=–0.678; p<0.05) and positively correlated with basal total ghrelin levels (r=0.686; p<0.05). Specifically the degree of ghrelin suppression after oral glucose did not correlate with GH or IGF-I levels.

We analyzed if there was significant correlation between fasting acylated ghrelin levels and age, BMI, IGF-I, fasting glucose, glucose at any point during the OGTT, basal GH, GH at any point during the OGTT, fasting insulin, insulin at any point during the OGTT, insulin resistance as estimated by HOMA, glucose peak, insulin peak, glucose AUC and insulin AUC. In the acromegalic group fasting acylated ghrelin levels negatively correlated with basal insulin (r=–0.750; p<0.05) and AUC of insulin after OGTT (r=–0.672; p<0.05). Specifically fasting acylated ghrelin did not correlate with GH or IGF-I levels.

We analyzed if there was significant correlation between the degree of reduction of acylated ghrelin levels during the OGTT and age, BMI, IGF-I, fasting glucose, glucose at any point during the OGTT, basal GH, GH at any point during the OGTT, fasting insulin, insulin at any point during the OGTT, insulin resistance as estimated by HOMA, fasting total ghrelin levels, glucose peak, insulin peak, glucose AUC and insulin AUC. In the acromegalic group the degree of reduction in acylated ghrelin levels negatively correlated with basal insulin (r=–0.733; p<0.05), insulin at 60′ (r=–0.732; p<0.05) and positively correlated with basal acylated ghrelin levels (r=0.900; p<*0.01*). Specifically the degree of ghrelin suppression after oral glucose did not correlate with GH or IGF-I levels.

DISCUSSION

We found no significant differences in fasting and after oral glucose plasma levels of total or acylated ghrelin between patients with acromegaly and control subjects. Furthermore both total and acylated ghrelin levels were similarly suppressed in both groups after glucose ingestion. In acromegalic patients there was negative correlation between fasting, both total and acylated, ghelin and both fasting and post oral glucose insulin levels.

All endocrine systems are characterized by the existence of a negative feedback loop, and it would not be impossible to imagine one with GH adjusting its own secretion by inhibiting gastric ghrelin release. However, our data do not support this hypothesis, at least in acromegaly. Despite grossly elevated GH levels in patients with acromegaly, there were no differences in the plasma ghrelin levels, neither total nor acylated. These results are consistent with previously published observations, in which ghrelin levels were reported to be similar in acromegalic patients and controls (Van der Toorn *et al.*, 2002; Barkan *et al.*, 2003; Jarkovska *et al.*, 2006). Nevertheless other authors detected lower ghrelin levels in acromegaly (Cappiello *et al.*, 2002; Freda *et al.*, 2003). In agreement with our data, fasting acylated ghrelin has been recently studied in acromegalic patients and was found to be similar to controls, furthermore the suppression of acylated ghrelin levels were independent of the GH response after glucose (Kim *et al.*, 2006)

The discrepancies found in the results of the different studies could be due, at least in part, to the differences in the control groups. The studies that reported decreased ghrelin levels in acromegaly used, as controls, subjects that tended to be younger and with lower BMIs than the groups of patients (Freda *et al.*, 2003; Cappiello *et al.*, 2002). In the study of Capiello *et al.* (Cappiello *et al.*, 2002) when you compare the results of the acromegalic patients and a group of obese controls both groups have similar fasting ghrelin levels. An important confounding factor for circulating ghrelin levels is BMI (Cummings *et al.*, 2002; Tschop *et al.*, 2001; Shiiya *et al.*, 2001; Haqq *et al.*, 200). The differences in the BMIs of the acromegalic patients could explain, at least in part, the different ghrelin levels encountered in the different studies. Due to the altered body composition found in acromegaly, with increased lean body mass and decreased fat mass, we matched our control group for both BMI and percentage of body fat. Part of the difference could also be

explained by the different assays employed to measure ghrelin. If markedly increased GH levels are necessary to decrease significantly peripheral circulating ghrelin levels that are mainly secreted by gastrointestinal cells (Ariyasu *et al.*, 2001) then it could be expected a very active acromegaly to decrease ghrelin levels. An alternative explanation to our results could be that our patients had a less active disease than patients reported in other studies.This fact would also explain the normalization of the decreased ghrelin levels after the surgical treatment of acromegalic patients reported by Freda *et al.* (Freda *et al.*, 2003). The absence of markedly increased insulin levels in our acromegalic patients, at least when compared with our age and weight matched control group, is another putative explanation for the lack of difference in ghrelin levels between the two groups. The presence of similar insulin levels in acromegalic and control group is not straightforward, but could be due to the presence of a less active acromegaly, the partial deterioration of the beta cell function or the characteristics of the control group with a similar age and BMI. In any case the presence of normal insulin levels, with clearly increased GH and IGF-I levels in the group of patients, is an adequate clinical model to avoid the interference of increased insulin levels in the regulation of ghrelin in acromegaly. The correlation between fasting ghrelin and post oral glucose ghrelin response with fasting insulin and post oral glucose insulin response in the acromegalic patients suggest that insulin could be an important regulator of ghrelin secretion in those patients. There is evidence suggesting that insulin is an important regulator of circulating ghrelin levels (Saad *et al.*, 2002; Purnell *et al.*, 2003; McLaughlin *et al.*, 2004). A clear negative association between ghrelin and insulin secretion has been found in humans as well as in animals by the majority of authors (Cummings *et al.*, 2001; Tschop *et al.*, 2001; Saad *et al.*, 2002), although not by all (Caixas *et al.*, 2002; Schaller *et al.*, 2003). This negative association between ghrelin and insulin would reflect the inhibitory influence of insulin on ghrelin synthesis and secretion (Caixas *et al.*, 2002; Lucidi *et al.*, 2002). On the other hand, ghrelin is expressed within the endocrine pancreas (Date *et al.*, 2002; Volante *et al.*, 2002; Wierup *et al.*, 2002). In regard to the influence of ghrelin on insulin secretion in the human, most of the studies, although not all (Akamizu *et al.*, 2004; Alvarez-Castro *et al.*, 2006), suggest that acute ghrelin administration modulates glucose and insulin secretion in humans (Broglio *et al.*, 2001; Broglio *et al.*, 2003a; Broglio *et al.*, 2003b; Tassone *et al.*, 2003 ;Gauna *et al.*, 2004). In the animal model there also are studies suggesting the influence of ghrelin on insulin secretion, although the results are conflicting, some reporting a stimulatory (Date *et al.*, 2002; Lee *et al.*, 2002; 53Adeghate and Ponery, 2002) effect and other an inhibitory effect (Egido *et al.*, 2002; Reimer *et al.*, 2003). Both Freda *et al.* (Freda *et al.*, 2003;) and Capiello *et al.* (Cappiello *et al.*, 2002) found a significant correlation between insulin levels and HOMA score with plasma ghrelin, and a lack of correlation between GH and IGF-I levels with ghrelin in acromegalic patients. Our explanation for the different ghrelin levels found in the different acromegalic patients studied could be the different insulin levels found in those patients. The importance of insulin in the regulation of ghrelin in acromegalic patients is further emphasized by the similar decrement of ghrelin found in both normal and acromegalic patients after oral glucose administration (Freda *et al.*, 2003;).

In the other clinical model of altered GH secretion, GH deficiency (GHD), circulating ghrelin levels have also been studied. In agreement with our data, in GHD (GH deficiency) the ghrelin levels were previously reported to be similar to the controls (Janssen *et al.*, 2002; Malik *et al.*, 2004; Jung *et al.*, 2006). In one study, however, ghrelin levels were suppressed, which is in contrast to our observation, but again this could be explained by the different characteristics of body composition of subjects with GHD, who had a significantly higher body fat percentage than the control group (Giavoli *et al.*, 2004). It has also been found in GHD patients that after GH treatment ghrelin levels decreased. The decrease in ghrelin correlated with changes in fat mass and fat-free mass. It is likely that the reduction in ghrelin reflects the metabolic effects of GH on lipid mobilization and glucose production (Eden Engstrom *et al.*, 2003). In contrast other studies could not find a significant decrement in fasting ghrelin levels after GH administration to GHD patients (Vestergaard *et al.*, 2005).

In agreement with our data, in three different mouse models with altered GH secretion or action (Nass *et al.*, 2004), stomach ghrelin mRNA expression, as well as serum concentrations of ghrelin, did not change significantly in any of the three transgenic mouse groups compared to the respective control group. A lack of correlation between ghrelin and GH levels has also been found in different physiological situations that stimulate GH secretion in humans, such as exercise or fasting (Espelund *et al.*, 2005; Dall *et al.*, 2002), or with increased GH, such as renal failure (Perez-Fontan *et al.*, 2005).

In conclusion, we have found no significant differences in fasting and post-OGTT plasma levels of total or acylated ghrelin between patients with acromegaly and control subjects. Furthermore, total and acylated ghrelin levels were similarly suppressed in acromegalic patients after glucose ingestion. In acromegalic patients there was correlation between both total and acylated ghrelin and insulin. These data suggest that GH hypersecretion in acromegaly does not influence circulating ghrelin secretion, that is at least in part regulated by insulin.

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