CLINICAL STUDY

Circulating ghrelin levels in basal conditions and during glucose tolerance test in acromegalic patients

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Abstract

Background: Ghrelin exerts a wide range of metabolic functions. In contrast to the body of information accumulated on the role of ghrelin on energy balance, the possible relevance of the peptide on GH secretion in physiological and pathological conditions has so far been poorly investigated.

Aim: The aim of the present study was to evaluate circulating ghrelin levels in acromegalic patients in basal conditions and in response to oral glucose tolerance test (OGTT).

Patients: Serum ghrelin, insulin and leptin levels were measured in 31 healthy normal weight subjects as controls, 25 patients with simple obesity and 17 non-diabetic acromegalic patients. Ghrelin and insulin response to OGTT was evaluated in six controls, four obese and six acromegalic patients.

Results: The acromegalic patients showed ghrelin levels lower than those observed in normal weight subjects (201 ± 20 vs 329 ± 32 pmol/l, $P < 0.05$) and similar to those found in obese subjects (165 ± 14 pmol/l, $P =$ not significant). Both obese and acromegalic patients had insulin levels significantly higher than controls, while high levels of leptin were detected only in obese subjects. Serum ghrelin levels showed a significant negative correlation with insulin, leptin and body mass index ($P < 0.05$) in normal and obese subjects. No correlation was observed in acromegalic patients, although those with severe insulin resistance showed the lowest ghrelin values (161 ± 20 pmol/l). In controls and obese subjects, ghrelin levels showed a significant decrease (25–40%) during OGTT, while no effect was detectable in acromegalic patients.

Conclusions: This study reports that patients with active acromegaly show low levels of circulating ghrelin that are not further reduced by OGTT, this pattern of secretion probably depending on both GH-induced insulin resistance and the putative GH/IGF-I negative feedback control on ghrelin secretion.

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Introduction

Ghrelin is an acylated peptide recently isolated from the rat stomach which exerts a wide range of metabolic functions (1–6). Although predominantly produced by the stomach, a certain amount of ghrelin has been found to derive from bowel, kidney, placenta, pituitary and hypothalamus (6). In particular, ghrelin has been identified in several regions of the hypothalamus, raising the possibility that growth hormone (GH) secretion may be under the control of ghrelin released from the arcuate nucleus (2, 3). In this view, ghrelin probably stimulates GH secretion by triggering multiple signals that include modulation of somatostatin and GH-releasing hormone pathways at the hypothalamic level, along with a direct stimulatory action at the pituitary level (3, 8, 9).

Although previously identified as a GH secretagogue hormone, some evidence indicates that ghrelin may be considered a novel ‘hunger hormone’ acting as the counterpart of the ‘satiety signals’, such as insulin and leptin. Indeed, ghrelin increases food intake in rodents and humans (10–12). Accordingly, ghrelin levels are influenced by acute and chronic changes in nutritional state and in these conditions a negative correlation with the levels of both insulin and leptin has been observed (13–16).

In contrast to the body of information recently accumulated on the role of ghrelin on energy balance, the possible relevance of this peptide on GH secretion in physiological and pathological conditions has so far been poorly investigated. In particular, while a recent study (17) reported that in patients with GH deficiency ghrelin levels were similar to those observed in healthy

subjects, no data on fasting ghrelin levels in patients with GH hypersecretion are at present available. Moreover, it is at present unknown whether stimuli, such as a standard meal or a glucose load which are effective in reducing circulating ghrelin levels in healthy normal and obese patients (18), also operate in the presence of high GH levels.

The aim of the present study was to evaluate circulating ghrelin levels in patients with active acromegaly in basal conditions and in response to an oral glucose tolerance test (OGTT), a diagnostic procedure widely used for the diagnosis of acromegaly and able to reduce ghrelin levels in healthy subjects (18).

Materials and methods

Subjects and testing

The study included 31 healthy subjects (6 men and 25 women, age 40 ± 4 years, body mass index (BMI) 21 ± 2 kg/m²), 25 patients with simple obesity (7 men and 18 women, age 45 ± 3 years, BMI 33 ± 2 kg/m²) and 17 non-diabetic patients with active acromegaly (9 men and 8 women, age 46 ± 3 years, BMI 28 ± 1 kg/m², percentage of body fat (BF%) 23 ± 1.3 , GH 8 ± 9 µg/l and insulin-like growth factor-I (IGF-I) 74 ± 26 nmol/l). The healthy subjects took no medication and had normal physical examination, electrocardiogram and blood tests. All obese patients (BMI ≥ 25 kg/m²) had no evidence of endocrine or gastrointestinal disease, cancer, renal or liver dysfunction or infection. Acromegalic patients had overt signs

and symptoms of the disease confirmed by the presence of elevated GH and high IGF-I levels for the age range, associated with modifications of the sellar morphology at magnetic resonance imaging or computed tomography scan, indicating the presence of a pituitary tumour (Table 1). At the time of the study no patient was being treated with somatostatin analogues or dopamine agonists. Blood was collected at 0800 h after an overnight fast. Six controls (six women, age 37 ± 3 years, BMI 20 ± 1 kg/m²), four obese (one man and three women, age 47 ± 4 years, BMI 37 ± 1 kg/m²) and six acromegalic patients (two men and four women, age 46 ± 5 years, BMI 26 ± 1 kg/m²) were given a 75 g/200 ml glucose solution orally (OGTT). Two healthy subjects were also given 200 ml water orally on a different day. Blood was collected at 0, 30, 60 and 120 min after glucose or water administration. All subjects gave written informed consent before participation. The protocol was approved by the local ethics committee.

Procedures

Insulin resistance (IR) was evaluated by the homeostatic model assessment (HOMA-IR), a computer-solved model used to predict the degree of IR starting from fasting plasma insulin (FI) and glucose concentrations (FG): $IR = FI \text{ (mU/l)} \times FG \text{ (mmol/l)} / 22.5$ (19, 20). Body composition was evaluated by whole body bioelectrical impedance analysis, using a portable impedance analyzer (RJL Systems, Detroit, MI, USA).

Table 1 Clinical and biochemical data of acromegalic male (M) and female (F) patients.

Patient	Sex/age	Body fat (%)	GH (µg/l)	IGF-I (nmol/l)	Insulin (mU/l)	HOMA-IR	Ghrelin (pmol/l)	Leptin (ng/ml)
1	F/52	ND	4.5	71	4.7	1.0	118	ND
2	F/26	24	10.0	60	4.5	1.0	271	0.5
3	F/37	17	2.8	49	5.4	1.1	267	ND
4	F/47	ND	12.3	98	8.4	1.5	329	ND
5	M/41	28	2.5	55	6.7	1.6	223	0.5
6	F/44	ND	7.0	54	8.8	1.9	270	18.5
7	F/59	ND	12.0	86	10	2.3	116	0.5
8	M/54	22	2.6	41	14.4	4.0	229	ND
9	M/50	26	8.0	125	18.4	4.2	140	7
10	M/60	ND	5.0	55	14.3	4.6	93	ND
11	F/69	ND	3.5	71	18.9	5.3	172	13.6
12	M/27	19	4.5	120	30.3	8.3	226	ND
13	M/36	ND	15.0	103	40	10.8	98	9.8
14	M/42	26	1.2	56	57	12.1	218	12.6
15	M/49	23	4.2	70	ND	ND	225	0.5
16	F/51	ND	2.8	45	ND	ND	347	ND
17	M/34	ND	40.0	96	ND	ND	70	12.2
Mean $\pm$ S.E.M.	46 ± 3	23 ± 1	8 ± 2	74 ± 6	17 ± 4	4.3 ± 1	200 ± 20	7 ± 2

ND = not done.

Normal ranges: BF%: (male) 25–34 years, 10–26; 35–44 years, 10–29; 45–54 years, 11–30; >55 years, 10–32; (female) 25–34 years, 17–32; 35–44 years, 18–33; 45–54 years, 17–34; >55 years, 22–43. GH: less than 2.5 µg/l. IGF-I: 20–30 years, 15–44 nmol/l; 30–40 years, 14–42; 40–50 years, 13–40; 50–60 years, 13–38; 60–70 years, 12–37. Insulin: 5–25 mU/l. Ghrelin: 75–675 pmol/l*. Leptin: 0.5–34 ng/ml*.

*Data evaluated in our 31 control subjects.

Assays

Blood samples were collected after an overnight fast, stored on ice, immediately separated by centrifugation at 3000 r.p.m. for 10 min at 4°C and stored at -80°C until assay. In agreement with previous reports (17), we did not observe difference between control samples assessed with or without extraction procedures (data not shown). Therefore, measurements were performed without an extraction procedure. Serum ghrelin was measured in one run with a commercially available radioimmunoassay (RIA) (Phoenix Pharmaceuticals Inc., Belmont, CA, USA) that uses a polyclonal antibody recognizing the C-terminal end of ghrelin, i.e. total ghrelin. Intra- and interassay coefficients of variation were 6% and 14% respectively. Serum GH levels were measured by IFMA (AutoDelfia kit; Wallac OY, Turku, Finland), IGF-I by RIA (Mediagnost, Tübingen, Germany), insulin by an immunoenzymetric one-step assay (Medgenics Diagnostics, Brussels, Belgium) and leptin by RIA (LINCO Research, Inc., St Louis, MO, USA) as previously reported (21, 22). Fasting glucose was measured with standard enzymatic methods.

Statistics

All results are expressed as means $\pm$ S.E.M. As the data were not normally distributed, a non-parametric test (Wilcoxon's rank sum test) was used for multiple comparisons among group means. Values of $P < 0.05$ were considered statistically significant.

Results

The normal subjects showed ghrelin levels ranging from 75 to 662 pmol/l without any difference by gender or age. As expected, in obese subjects, ghrelin levels were significantly lower than those found in controls (165 ± 14 vs 329 ± 32 pmol/l, $P < 0.05$) (Fig. 1). A similar reduction in ghrelin levels was also found in acromegalic patients (201 ± 20 vs 329 ± 32 pmol/l, $P < 0.05$) (Fig. 1).

Both obese subjects and acromegalic patients had insulin levels significantly higher than those observed in controls, while high levels of leptin were detected only in obese subjects. Acromegalic patients had leptin levels similar to controls (Fig. 1) as expected, owing to the normal BF% recorded in acromegalic patients ($n = 8$) (Table 1).

Serum ghrelin levels showed a significant negative correlation with insulin, leptin and BMI ($P < 0.05$) in normal and obese subjects (Fig. 2), while no correlation was observed in acromegalic patients. However, the subset of acromegalic patients with severe insulin resistance characterized by the highest values of HOMA-IR (Table 1) showed ghrelin values lower than those found in non-insulin-resistant acromegalic patients (161 ± 20 vs 246 ± 29 pmol/l, $P < 0.05$).

Finally, serum ghrelin levels did not correlate with GH and IGF-I levels ($r^2 = 0.2$ and $P = 0.06$ for GH; $r^2 = 0.1$ and $P =$ not significant for IGF-I) (Fig. 3).

In both controls and obese subjects, serum ghrelin levels significantly decreased during OGTT (Fig. 4) and did not change in response to administration of the same volume of water. By contrast, OGTT did not cause any reduction of ghrelin levels in acromegalic patients. As expected, all subjects showed a significant increase of serum insulin levels in response to OGTT

Figure 1 Serum levels (means $\pm$ S.E.M.) of ghrelin, insulin and leptin from 31 healthy subjects (controls), 25 patients with simple obesity (obese) and 17 patients with active acromegaly (acromegalics) after an overnight fast. * $P < 0.05$ compared with control levels.

Figure 2 Negative correlations between basal serum ghrelin, insulin and leptin levels in controls and obese subjects.

(Fig. 4) that was generally higher in acromegalic patients than in controls and obese subjects, while the reduction of serum GH levels below the cut-off value of $1 \mu\text{g/l}$ was only observed in controls and obese subjects (Fig. 4).

Discussion

This study provides the first evidence, as far as we are aware, that low ghrelin concentrations are present in acromegaly and confirms and extends previous observations indicating that circulating ghrelin levels are influenced by the nutritional state. Several data have been accumulating in the last 2 years indicating a strong influence of this peptide on appetite, fuel utilization, body weight and body composition in both humans and other animals (2, 6, 10–18). Indeed, circulating ghrelin levels are decreased in chronic or acute states of positive energy balance and increased by fasting or in cachectic states (13–16). Consistent with previous observations, in the present study serum ghrelin concentrations were found to be lower in subjects with

Figure 3 Absent correlations between basal serum ghrelin, IGF-I and GH levels in acromegalics.

simple obesity compared with normal weight control subjects and to negatively correlate with BMI and insulin (14, 18). Moreover, in agreement with a recent report (14), ghrelin and leptin levels negatively correlated, thus supporting the view that the 'hunger signal', ghrelin, may be the counterpart of the 'satiety signal' from insulin and leptin (6). Accordingly, it has been reported that *db/db* obese mice, an animal model characterized by a null mutation in the leptin receptor gene, have low levels of ghrelin mRNA in the gastric fundus (23).

The present study is, to the best of our knowledge, the first to describe that acromegalic patients have low circulating levels of ghrelin. However, since commercially available ghrelin RIA kits detect both the octanoylated and non-octanoylated ghrelin, we cannot conclude that changes in total ghrelin reflect changes in the biologically active peptide. Although similar to the values observed in obese subjects, the low levels of ghrelin found in acromegalic patients were not related to obesity. In fact, in agreement with the notion that acromegalic patients have a body

Figure 4 Effect of OGTT on serum ghrelin and insulin levels in six healthy subjects (controls), four patients with simple obesity (obese) and six patients with active acromegaly (acromegalics). * $P < 0.05$ compared with basal levels.

composition characterized by increased lean body mass and reduced fat mass compared with control subjects with the same BMI (24), most patients in the present series had a normal percentage of body fat mass. Moreover, in agreement with previous reports (25), acromegalic patients had normal levels of leptin which did not show any correlation with ghrelin.

The observations that impaired glucose tolerance is frequently present in acromegalic patients and that insulin and ghrelin are negatively correlated in normal and obese subjects suggest a possible role of insulin hypersecretion on ghrelin down-regulation in acromegaly. Although, in the present series, the correlation between ghrelin and insulin levels did not reach significance, the lowest ghrelin levels were found in

patients with the most severe insulin resistance, as indicated by high levels of fasting insulin and high values of HOMA-IR, an insulin resistance index that closely mirrors the euglycaemic clamp technique and predicts the development of type 2 diabetes mellitus (19, 20). This observation is consistent with the view that, in acromegaly, insulin resistance is not related to obesity, but is mainly due to hepatic and peripheral direct metabolic effects of the high GH levels (26).

Although the low levels of ghrelin observed in acromegalic patients may be, at least in part, attributed to insulin resistance, the putative negative feedback mechanism of GH and IGF-I on ghrelin secretion may also account for these findings. In fact, in transgenic mice over-expressing the GH gene, low levels of ghrelin have been reported, consistent with the existence of a negative control exerted by high GH levels on ghrelin secretion (27). Our data reporting the presence of low ghrelin levels in patients with GH hypersecretion support this hypothesis, although in the present series the correlation between ghrelin and GH levels did not reach significance. Accordingly, in GH-deficient patients, systemic ghrelin levels were reported to be similar to those found in controls and not modified by GH replacement therapy (17). However, the recent demonstration that changes in ghrelin levels followed by similar changes in serum GH concentrations occur in normal subjects only during prolonged fasting may account for the lack of a significant correlation between GH and ghrelin levels in this still limited series of acromegalic patients who were tested after a standard overnight fast (28).

Glucose loads lead to a rapid fall in serum ghrelin concentrations in both normal weight and obese subjects. A similar effect has been recently observed by Shiiya *et al.* (18) who also reported that the secretion of ghrelin was not affected by stomach wall extension, as oral or i.v. glucose administration equally reduced ghrelin levels. In the same study, type 2 diabetic obese patients showed a clear ghrelin reduction during a standard meal, consistent with the effect induced by OGTT in obese subjects reported here. Interestingly, ghrelin secretion was not affected by glucose load in acromegalic patients. Since the molecular signals triggered by glucose that regulate ghrelin secretion are unknown, it is difficult to hypothesize which alteration may be responsible for this defect. However, there is no evidence for a direct relationship between the lack of ghrelin reduction reported here and the impaired GH suppression by OGTT that is a hallmark of active acromegaly.

In conclusion, this study reports that patients with active acromegaly show low levels of circulating ghrelin that are not further reduced by OGTT. Further studies are needed to understand the role of GH-induced insulin resistance and the GH/IGF-I negative feedback mechanism in determining this pattern of ghrelin secretion in acromegaly.

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