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Plasma Levels of Total and Active Ghrelin in Acromegaly and Growth Hormone Deficiency

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Summary

Ghrelin is an endogenous growth hormone (GH) secretagogue recently isolated from the stomach. Although it possesses a strong GH releasing activity *in vitro* and *in vivo*, its physiological significance in endogenous GH secretion remains unclear. The aim of this study was to characterize plasma ghrelin levels in acromegaly and growth hormone deficiency (GHD). We investigated plasma total and active ghrelin in 21 patients with acromegaly, 9 patients with GHD and 24 age-, sex- and BMI-matched controls. In all subjects, we further assessed the concentrations of leptin, soluble leptin receptor, insulin, IGF-I, free IGF-I and IGFBP-1, 2, 3 and 6. Patients with acromegaly and GHD as well as control subjects showed similar levels of total ghrelin (controls 2.004±0.18 ng/ml, acromegalics 1.755±0.16 ng/ml, p=0.31, GHD patients 1.704±0.17 ng/ml, p=0.35) and active ghrelin (controls 0.057±0.01 ng/ml, acromegalics 0.047±0.01 ng/ml, p=0.29, GHD patients 0.062±0.01 ng/ml, p=0.73). In acromegalic patients plasma total ghrelin values correlated negatively with IGF-I ($p<0.05$), in GHD patients active ghrelin correlated with IGF-I positively ($p<0.05$). In the control group, total ghrelin correlated positively with IGFBP-2 ($p<0.05$) and negatively with active ghrelin (p=0.05), BMI ($p<0.05$), WHR ($p<0.05$), insulin (p=0.01) and IGF-I (p=0.05). Plasma active ghrelin correlated positively with IGFBP-3 (p=0.005) but negatively with total ghrelin and free IGF-I (p=0.01). In conclusion, all groups of the tested subjects showed similar plasma levels of total and active ghrelin. In acromegaly and growth hormone deficiency plasma ghrelin does not seem to be significantly affected by changes in GH secretion.

Key words

Total ghrelin • Active ghrelin • Leptin • Acromegaly • Growth hormone deficiency

Introduction

Ghrelin, a 28-amino acid peptide with an *n*-octanoyl modification at serine 3, is a novel hormone isolated from the stomach as a specific endogenous ligand for the growth hormone secretagogue receptor (GHS-R) (Kojima *et al.* 1999). Ghrelin is predominantly produced by the stomach, although its production has been found in various other tissues including the bowel, kidney, placenta, hypothalamus and pituitary gland (Date *et al.* 2000, Wang *et al.* 2002, Jarkovská *et al.* 2005). Another molecular form of ghrelin, which is called des-

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acyl ghrelin, lacks the hydrophobic chain substitution at serine 3. Des-acyl ghrelin predominates in the systemic circulation in rats and humans (Hosoda *et al.* 2000, Yoshimoto *et al.* 2002) and is devoid of ghrelin endocrine actions.

Ghrelin is an orexigenic peptide participating in energy balance regulation. Ghrelin administration stimulates appetite and food intake and results in body weight gain (Wren *et al.* 2000). Plasma ghrelin levels are elevated in lean subjects (Shiiya *et al.* 2002) whereas obese subjects show decreased levels of ghrelin (Tschöp *et al.* 2001, Rosická *et al.* 2003). Circulating plasma ghrelin shows a diurnal pattern with preprandial increases and postprandial decreases (Shiiya *et al.* 2002) and the administration of glucose or insulin leads to the suppression of plasma ghrelin levels (Shiiya *et al.* 2002, Saad *et al.* 2002).

Exogenously administered ghrelin is a potent stimulator of pituitary GH release (Kojima *et al.* 1999, Arvat *et al.* 2000), but the physiology of ghrelin secretion and its role in endogenous GH secretion remains unclear. Controversial data on ghrelin concentrations in patients with either hypersecretion or deficiency of endogenous GH have been published. In acromegaly, ghrelin levels were found to be either normal (Barkan *et al.* 2003) or reduced (Cappiello *et al.* 2002, Freda *et al.* 2003). Similarly, patients with GHD were reported to have normal (Janssen *et al.* 2001, Malik *et al.* 2004) or decreased levels of ghrelin (Giavoli *et al.* 2004). Furthermore these studies did not determine the level of active ghrelin, which only possesses endocrinological activity, but total ghrelin, which is the sum of acylated (active) ghrelin and des-acyl ghrelin.

The aim of this study was to evaluate levels of total and active ghrelin and to correlate these values with hormonal, nutritional and anthropometric parameters in patients with active acromegaly and GHD.

Methods

We examined 21 patients with active acromegaly (8 men and 13 women), aged 57.6±3.0 years (mean ± S.E.M.), BMI 29.31±0.97 $kg.m^{-2}$. Eleven of them were newly diagnosed patients, 10 patients had undergone pituitary surgery. Active acromegaly was associated with increased serum levels of GH and IGF-I and a modification of sellar morphology at magnetic resonance imaging indicating the presence of a pituitary adenoma. No patients had been treated either with a somatostatin analogue, a dopaminergic agonist or radiotherapy.

The group of patients with GHD included 9 subjects (8 men and 1 woman), aged 39.6±6.7 years, BMI 28.98±2.46 $kg.m^{-2}$. All patients met criteria for severe GHD in adulthood. Eight patients had an adult-onset of GHD acquired after pituitary surgery for pituitary adenoma, one had childhood-onset idiopathic GHD. No patients had been treated with GH replacement therapy prior to our study. Other hormone deficiencies were adequately replaced.

As a control group we examined 24 healthy subjects (10 men and 14 women), aged 53.0±2.8 years, BMI 28.68±1.06 $kg.m^{-2}$.

In all subjects we assessed serum levels of total ghrelin, active ghrelin, leptin, soluble leptin receptor (SLR), insulin, GH (a mean of 3 consecutive samples taken in the morning at 1-hour intervals), insulin-like growth factor-I (IGF-I), free IGF-I, IGF binding proteins 1, 2, 3 and 6 (IGFBP-1, IGFBP-2, IGFBP-3 and IGFBP-6). The present study was conducted under written informed consent and approved by the Ethical Committee (First Faculty of Medicine, Charles University, Prague).

Blood samples were collected at 8:00 h after an overnight fast. On the same day all subjects underwent assessment of body composition (waist-hip ratio (WHR), BMI, percentage of body fat).

The human total plasma ghrelin levels were determined using the commercial Ghrelin (Total) RIA kit (Linco Research, USA), which measures both octanoylated ghrelin and des-acyl ghrelin. Plasma levels of active octanoylated ghrelin were determined with Ghrelin (Active) RIA kit (Linco Research, USA), which measures only acylated ghrelin.

The human serum leptin levels and soluble leptin receptor levels were detected using commercial ELISA kits (Bio Vendor, Czech Republic). GH and IGF-I serum levels were determined using commercial IRMA kits (Immunotech, Czech Republic), free IGF-I, IGFBP-1 and IGFBP-3 were measured by IRMA kits (DSL, USA), IGFBP-2 and IGFBP-6 were assessed by RIA kits (DSL, USA). Serum insulin levels were determined using RIA kits (CIS Bio International, France). The serum biochemical parameters were measured in the Institute of Clinical Biochemistry of the University Hospital, Prague by standard laboratory methods. The percentage of body fat was determined using Best's calliper.

The statistical analysis was performed by SigmaStat statistical analysis software (Jandel Scientific,

San Rafael, CA, USA). Statistical analysis of the differences between groups of subjects was performed using Student's t-test for unpaired data or by the Mann-Whitney non-parametric test when appropriate. Interdependence between variables within the separate groups was evaluated using the Pearson's or Spearman's correlation. All data are presented as means ± S.E.M. P<0.05 values were considered statistically significant.

Table 1. Clinical and laboratory characteristics of the compared groups of subjects.

	Patients with acromegaly	**Patients with GHD**	**Controls**
Age (years)	57.6±3.0	39.6±6.7	53.0±2.8
BMI (kg/m^2)	29.31±0.97	28.98±2.46	28.68±1.06
Body fat (%)	17.91±1.74	20.16±2.93	22.26±1.98
Total ghrelin (ng/ml)	1.755±0.16	1.704±0.17	2.004±0.18
Active ghrelin (ng/ml)	0.047±0.01	0.062±0.01	0.057±0.01
GH (mU/l)	55.02±15.58 [c]	0.54±0.12 [a]	4.03±1.31
IGF-I (µg/l)	1014.24±86.0 [e]	96.33±13.26 [b]	209.71±20.08
Free IGF-I (µg/l)	4.39±0.77 [e]	0.17±0.04 [d]	0.60±0.05
IGFBP-1 (µg/l)	14.28±2.34 [b]	28.90±8.79	27.63±3.78
IGFBP-2 (mg/l)	0.40±0.8	0.50±0.16	0.43±0.06
IGFBP-3 (mg/l)	6.74±0.29 [e]	3.66±0.38	4.30±0.19
IGFBP-6 (mg/l)	0.33±0.05	0.41±0.06	0.33±0.03
Leptin (ng/ml)	14.42±3.72	15.29±3.43	17.23±2.44
SLR (ng/ml)	19.29±1.31	18.74±2.96	20.75±1.53
Insulin (µU/ml)	45.39±7.37 [b]	38.92±21.59	21.62±2.29
Prealbumin (g/l)	0.32±0.01 [e]	0.23±0.02	0.25±0.01
Albumin (g/l)	43.11±0.67	43.09±1.40	42.58±0.45
Total protein (g/l)	71.72±0.80	70.99±1.25	73.20±0.78
Glucose (mmol/l)	6.04±0.18 [d]	4.93±0.34	5.19±0.13
WHR	0.88±0.02	0.87±0.05	0.88±0.02

Data are presented as mean ± SEM; BMI – body mass index; GH – growth hormone; GHD – growth hormone deficiency; IGF-I – insulin-like growth factor-I; free IGF-I – free insulin-like growth factor-I; IGFBP-1 – insulin-like growth factor binding protein-1; IGFBP-2 – insulin-like growth factor binding protein-2; IGFBP-3 – insulin-like growth factor binding protein-3; IGFBP-6 – insulin-like growth factor binding protein-6; SLR – soluble leptin receptor; WHR – waist-hip ratio. [a] p<0.05; [b] p<0.01; [c] p=0.001; [d] p<0.001; [e] p<0.0001 vs. controls.

Results

Characteristics of the studied groups of subjects are shown in Table 1. Patients with active acromegaly had higher levels of GH (p=0.001), IGF-I (p<0.0001), free IGF-I (p<0.0001), IGFBP-3 (p<0.0001), insulin (p<0.01), prealbumin (p<0.0001) and glucose (p<0.001), and lower level of IGFBP-1 (p<0.01) than the healthy controls. The two groups were sex-, age- and BMI-matched. Plasma levels of total and active ghrelin tended to be lower in acromegalics, but the difference did not reach statistical significance (total plasma ghrelin in acromegalics 1.755±0.16 ng/ml vs. in controls 2.004±0.18 ng/ml, p=0.31, active plasma ghrelin in acromegalics 0.047±0.01 ng/ml vs. in controls 0.057±0.01 ng/ml, p=0.29). Active ghrelin represented 2.7 % of total ghrelin level in acromegalics and 2.8 % in healthy controls. The analysis of gender differences showed, that acromegalic women had higher percentage of body fat (women 22.03 % vs. men 11.20 %, p<0.001) and lower WHR (women 0.81 vs. men 0.97, p<0.001) than acromegalic men. No gender difference was found in the levels of total and active ghrelin (data not shown).

Patients with GHD showed lower levels of GH (p<0.05), IGF-I (p<0.01) and free IGF-I (p<0.001) in comparison with the control group. Again we found similar levels of total and active ghrelin. Plasma level of total ghrelin tended to be lower in GHD patients, but the

Table 2. The interdependence between laboratory variables in the group of patients with acromegaly.

	Total ghrelin	**Active ghrelin**	**IGF-I**	**IGFBP-1**	**IGFBP-2**	**IGFBP-3**	**IGFBP-6**	**Leptin**	**SLR**	**Insulin**
Total ghrelin		NS	r = -0.46 p = 0.04	NS	NS	NS	NS	NS	NS	NS
Active ghrelin	NS		NS	NS	NS	NS	NS	NS	NS	NS
IGF-I	r = -0.46 p = 0.04	NS		r = -0.48 p = 0.03	r = -0.47 p = 0.03	r = 0.55 p = 0.01	r = -0.47 p = 0.03	NS	r = -0.45 p = 0.04	NS
IGFBP-1	NS	NS	r = -0.48 p = 0.03		r = 0.57 p < 0.01	NS	r = 0.55 p < 0.001	NS	r = 0.47 p = 0.03	NS
IGFBP-2	NS	NS	r = -0.47 p = 0.03	r = 0.57 p < 0.01		NS	r = 0.45 p = 0.04	NS	NS	NS
IGFBP-3	NS	NS	r = 0.55 p = 0.01	NS	NS		NS	NS	r = -0.52 p = 0.01	NS
IGFBP-6	NS	NS	r = -0.47 p = 0.03	r = 0.55 p < 0.001	r = 0.45 p = 0.04	NS		NS	NS	NS
Leptin	NS	NS	NS	NS	NS	NS	NS		r = -0.48 p = 0.03	r = 0.46 p = 0.04
SLR	NS	NS	r = -0.45 p = 0.04	NS	NS	r = -0.52 p = 0.01	NS	r = -0.48 p = 0.03		r = -0.65 p < 0.01
Insulin	NS	NS	NS	NS	NS	NS	NS	r = 0.46 p = 0.04	r = -0.65 p < 0.01	

IGF-I -insulin-like growth factor-I; IGFBP-1 -insulin-like growth factor binding protein-1; IGFBP-2 -insulin-like growth factor binding protein-2; IGFBP-3 -insulin-like growth factor binding protein-3; IGFBP-6 -insulin-like growth factor binding protein-6; SLR -soluble leptin receptor.

difference did not reach statistical significance (total plasma ghrelin in GHD patients 1.704±0.17 ng/ml vs. in controls 2.004±0.18 ng/ml, p=0.35, active plasma ghrelin in GHD patients 0.062±0.01 ng/ml vs. in controls 0.057±0.01 ng/ml, p=0.73). Because GHD patients and controls were comparable in BMI, but differed in age (GHD 39.6±6.7 years vs. controls 53.0±2.8 years, p=0.03), we generated a subgroup of age- and BMI-matched control subjects with the GHD patients (8 men, 1 woman, data not shown). The comparison of GHD subjects and a subgroup of matched controls gave the same results as mentioned above. Active plasma ghrelin in GHD represented 3.6 % of total ghrelin level.

We also analyzed gender differences in the control group. Healthy women had higher percentage of body fat (women 27.75 % vs. men 15.12 %, p<0.001), serum leptin (women 22.79 ng/ml vs. men 9.46 ng/ml, p<0.01) and IGFBP-3 (women 4.69 mg/l vs. men 3.75 mg/l, p=0.01), and lower IGFBP-6 (women 0.28 mg/l vs. men 0.42 mg/l, p=0.01) and WHR (women 0.82 vs. men 0.96, p=0.001). Again no gender difference was found in the levels of total and active ghrelin (data not shown).

In acromegalic patients plasma total ghrelin levels correlated negatively with IGF-I (p<0.05) and positively with prealbumin (r=0.56, p<0.05) and total protein (r=0.5, p<0.05), no correlation was found between active ghrelin and any studied variable. The interdependence between selected laboratory variables in acromegalics is shown in Table 2.

In GHD patients total plasma ghrelin showed no correlation with any other variable, active ghrelin positively correlated with IGF-I (p<0.05), prealbumin

Table 3. The interdependence between laboratory variables in the group of patients with growth hormone deficiency.

	Total ghrelin	**Active ghrelin**	**IGF-I**	**IGFBP-1**	**IGFBP-2**	**IGFBP-3**	**IGFBP-6**	**Leptin**	**SLR**	**Insulin**
Total ghrelin		NS	NS	NS	NS	NS	NS	NS	NS	NS
Active ghrelin	NS		r = 0.68 p = 0.04	NS	NS	NS	NS	NS	NS	NS
IGF-I	NS	r = 0.68 p = 0.04		NS	NS	NS	NS	NS	NS	NS
IGFBP-1	NS	NS	NS		NS	r = -0.81 p = 0.03	NS	NS	r = 0.81 p = 0.01	NS
IGFBP-2	NS	NS	NS	NS		r = -0.88 p < 0.01	NS	NS	NS	NS
IGFBP-3	NS	NS	NS	r = -0.81 p = 0.03	r = -0.88 p < 0.01		NS	NS	NS	NS
IGFBP-6	NS	NS	NS	NS	NS	NS		NS	NS	NS
Leptin	NS	NS	NS	NS	NS	NS	NS		NS	NS
SLR	NS	NS	NS	r = 0.81 p = 0.01	NS	NS	NS	NS		NS
Insulin	NS	NS	NS	NS	NS	NS	NS	NS	NS	

IGF-I -insulin-like growth factor-I; IGFBP-1 -insulin-like growth factor binding protein-1; IGFBP-2 -insulin-like growth factor binding protein-2; IGFBP-3 -insulin-like growth factor binding protein-3; IGFBP-6 -insulin-like growth factor binding protein-6; SLR -soluble leptin receptor.

(r=0.79, p<0.05) and glucose (r=0.72, p<0.05). The interdependence between laboratory variables is shown in Table 3. We found no correlations between plasma levels of total and active ghrelin and BMI, percentage of body fat, leptin or insulin in either group of patients.

In the control group, total ghrelin correlated positively with IGFBP-2 (r=0.47, p<0.05) and negatively with active ghrelin (r = –0.41, p=0.05), IGF-I (r = –0.41, p=0.05), BMI (r = –0.46, p<0.05), insulin (r = –0.50, p=0.01), and WHR (r = –0.50, p<0.05). Plasma active ghrelin positively correlated with IGFBP-3 (r = 0.55, p<0.01) and negatively correlated with total ghrelin and free IGF-I (r = –0.50, p=0.01).

Discussion

In this study we demonstrated, that plasma levels of total ghrelin were not significantly different in patients with acromegaly and GHD, when compared with healthy control subjects. This result is consistent with previously published observations, where ghrelin levels in acromegalics were reported to be similar to the controls (Barkan *et al.* 2003). Other authors detected lower ghrelin levels in acromegaly (Cappiello *et al.* 2002, Freda *et al.* 2003), but this difference is likely to depend on the control group chosen for comparison. In GHD the ghrelin levels were previously reported to be similar to the controls (Janssen *et al.* 2001, Malik *et al.* 2004). In one study however, the 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 our patients (Giavoli *et al.* 2004).

As far as we are aware, this study is the first to demonstrate, that levels of active ghrelin remain

unchanged in acromegaly and GHD. Plasma level of active ghrelin represents a small proportion of total ghrelin level (2.7 % in acromegalics, 3.6 % in GHD and 2.8 % in the controls).

Ghrelin was originally isolated as an endogenous secretagogue of GH. However, its physiological role in endogenous GH secretion and the existence of a putative negative feedback between ghrelin and GH remains unproven. On the one hand, peripheral administration of GH in rats leads to a suppression of pituitary ghrelin production (Kamegai *et al.* 2004), and in humans the changes in ghrelin levels follow similar changes in GH levels during prolonged fasting (Muller *et al.* 2002). On the other hand, ghrelin-null mice show identical size, growth rate, body composition and food intake as wild-type littermates (Sun *et al.* 2003), a suppression of GH does not modify ghrelin levels in humans (Barkan *et al.* 2003), and ghrelin levels in GHD are not modified by GH administration (Janssen *et al*. 2001).

In our study, total ghrelin correlated negatively with IGF-I in acromegalics and a similar negative correlation was observed between active ghrelin and IGF-I in healthy controls. This would support the idea of a negative feedback between ghrelin and the GH/IGF-I system. However, GHD subjects showed a positive correlation between active ghrelin and IGF-I and we did not confirm any correlation between ghrelin and GH in either group of subjects. Furthermore, our results of identical ghrelin levels in situations of normal production, excess as well as deficiency of endogenous GH production support the view that at least in acromegaly and GHD ghrelin levels in the systemic circulation are not an important regulator of GH secretion. At the same time, some degree of negative feedback exerted by ghrelin on GH/IGF-I secretion can not be excluded, mainly due to the complexity of ghrelin action and regulation. It is important to point out that this feedback is probably playing an important role at the local levels of the hypothalamus and the pituitary gland.

Previous studies reported negative correlations of ghrelin to leptin, BMI and insulin in healthy subjects (Tschöp *et al.* 2001), which we did not confirm in our patients, similarly to other observations (Cappiello *et al.* 2002, Giavoli *et al*. 2004). Certain studies also reported gender differences in plasma ghrelin levels with higher levels in women (Barkan *et al.* 2003). We did not confirm this finding in the group of acromegalics as well as in the control group, similarly to other studies in healthy subjects (Purnell *et al*. 2003). The reason for this discrepancy is not clear.

In conclusion, we demonstrated no significant differences in plasma levels of total and active ghrelin in patients with acromegaly, GHD and control subjects. Factors influencing plasma levels of total and active ghrelin in these conditions and the role of endogenous ghrelin in GH secretion remain uncertain and require further studies.

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