Serum ghrelin levels and disturbances of the lipid profile in patients with acromegaly

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Abstract

It is unknown if altered ghrelin secretion might contribute to the development of metabolic complications in acromegaly.

The **AIM OF THE STUDY** was to: 1) assess if serum concentrations of total and acylated ghrelin in patients with acromegaly differ in the presence of various metabolic complications (hypercholesterolemia, hyperinsulinemia, hyperglicemia). 2) assess the correlations between concentrations of ghrelin and concentrations of GH, IGF-1, cholesterol, insulin and glucose in patients with acromegaly.

MATERIALS: 24 patients with previously diagnosed acromegaly (11 subjects with active and 13 subjects with inactive disease) and 12 healthy subjects. 23 subjects were treated in the past with neurosurgery, 3 subjects with radiotherapy. 7 patients were receiving octreotide LAR at the time of the study.

METHODS: In all studied subjects the concentrations of total ghrelin, acylated ghrelin, GH, IGF-1, insulin, glucose, total cholesterol, LDL cholesterol, HDL cholesterol, triglicerydes, were measured.

RESULTS: The concentrations of total and acylated ghrelin did not significantly differ between patients with active and inactive disease. The mean concentrations of total and acylated ghrelin were significantly higher in acromegalic patients who presented with hypercholesterolemia compared with patients with normocholesterolemia. In patients with hypercholesterolemia the ratio of acylated/total ghrelin was 16%. In patients with active acromegaly there was a statistically significant positive correlation between the concentration of total ghrelin and the concentration of total cholesterol and LDL cholesterol. There was also a positive correlation between the concentration of acylated ghrelin and LDL cholesterol (without statistical significance). In patients with inactive acromegaly there was a statistically significant positive correlation between the concentration of acylated ghrelin and the concentration of triglycerides and a positive correlation between total ghrelin and triglycerides, but statistically insignificant. There were no differences in ghrelin levels depending on the insulin and glucose concentrations.

CONCLUSIONS: Some metabolic complications of the disease might result not only from GH hypersecretion but also from altered ghrelin secretion.

ORIGINAL ARTICLE

Abbreviations

BMI – body mass index
GH – growth hormone
GHS-R – growth hormone secreatogogue receptor
GHSs – growth hormone secretagogues
IGF-1 – insulin-like growth factor 1
HDL – high density lipoproteins
LDL – low density lipoproteins
LAR – long acting release

INTRODUCTION

Ghrelin was originally identified as the endogenous ligand of the growth hormone (GH) secretagogue receptor (GHS-R) [Kojima *et al.* 2005; Kojima *et al.* 2001]. When administered exogenously, ghrelin has a strong potential to stimulate the secretion of GH from anterior pituitary. Thus, it was suspected to be a new integral element of hypothalamo-pituitary system influencing the function of somatotroph cells [Kojima *et al.* 1999]. Potentially, both circulating ghrelin produced in the stomach and ghrelin synthesized locally in the pituitary and hypothalamus, might play such role. However, according to available studies, it remains unclear whether endogenous ghrelin plays any role in the regulation of growth hormone secretion. There are studies which might confirm such hypothesis [Cummings *et al.* 2001; Tschop *et al.* 2001; Muller *et al.* 2002; Nagaya *et al.* 2001; Barkan *et al.* 2003] but also studies revealing no influence of ghrelin on GH release [Cummings *et al.* 2002; Dimaraki *et al.* 2006; Sun *et al.* 2003; Casanueva *et al.* 2005]. The question was raised whether the disturbances of ghrelin secretion might lead to the improper secretion of GH from the pituitary, but so far the role of endogenous ghrelin in the pathogenesis of acromegaly seems doubtful.

On the contrary, much information has been gained about non-endocrine and metabolic actions of ghrelin [Ghigo *et al.* 2005; Van der Lely *et al.* 2004; Horvath *et al.* 2002]. The best recognized functions of ghrelin are the metabolic effects and the regulation of energy balance. The peptide was shown to stimulate appetite, food intake, increase body weight, induce hyperglycaemia, inhibit insulin secretion and its actions, stimulate lipogenesis and inhibit lipolysis and increase the proliferation of adipocytes [Ghigo *et al.* 2005; Van der Lely *et al.* 2004; Horvath *et al.* 2002; Leite-Moreira *et al.* 2007]. There are studies which suggest the involvement of ghrelin in the pathogenesis of many diseases, including obesity, atherosclerosis, type 2 diabetes mellitus, metabolic syndrome, hypertension, cachexia, anorexia and polycystic ovary syndrome [Van der Lely *et al.* 2004; Leite-Moreira *et al.* 2007; Poykko *et al.* 2003a; Broglio *et al.* 2001; Choi *et al.* 2004; Poykko *et al.* 2006; Poykko *et al.* 2003b].

Acromegaly is known to be associated with various metabolic abnormalities, such as hyperinsulinaemia and insulin resistance, dyslipidaemia and disturbances of glucose metabolism [Colao *et al.* 2004]. It was well established that they result from the excess of GH, which counteracts the effects of insulin on glucose and lipid metabolism. It has not been demonstrated whether ghrelin, which regulates glucose metabolism and adipose tissue metabolism, might also contribute to the development of metabolic complications in acromegaly. Hypothesis might be raised that even though ghrelin is not connected with the disturbances of GH secretion and development of somatotroph tumor, it might play a role in the occurrence of metabolic complications of the disease. Modulation of ghrelin activity would thus be beneficial and represent a new therapeutic model in acromegaly and its complications.

AIM

The aim of the study was to assess: 1) whether serum concentrations of total and acylated ghrelin in patients with acromegaly differ depending on the activity of the disease and the presence of coexisting metabolic complications (hypercholesterolaemia, hyperinsulinaemia, hyperglicaemia); 2) whether there are any correlations between the concentrations of ghrelin and concentrations of GH, IGF-1, cholesterol, insulin and glucose in patients with acromegaly.

MATERIAL

The characteristics of examined subjects

The hormonal and biochemical measurements were performed in 24 patients with previously diagnosed acromegaly. There were 16 women and 8 men, at the age of 27–71 years (mean 52,5 years, SD 10,7). The disease had been recognized 7 weeks – 10 years prior to the study (mean 4,7 years, SD 3,0). In 23 subjects the transsphenoidal removal of the tumor had been performed (10 years – 3 months before the study was conducted) and 3 of them were subsequently treated with radiotherapy (3–10 years earlier). At the time of the study 7 patients were treated with a long-acting somatostatin analogue – octreotide LAR (Sandostatin LAR, Novartis Pharm., 30mg every 28 days) (the duration of the therapy – 3–39 months, mean 24 months, SD 10,7).

6 patients had been diagnosed with diabetes mellitus and were treated with oral medications (metformin, sulfonylurea drugs); none of them were treated with insulin. Furthermore, among all, 9 subjects with acromegaly were treated for dyslipidaemia (simvastatin, atorvastatin), 12 were receiving L-thyroxin because of euthyroid goiter or hypothyroidism (that developed due to the previous therapy of hyperthyroidism) and 5 were treated with bromocriptin because of coexisting hyperprolactinaemia.

The control group included 12 healthy subjects, 6 women and 6 men, at the age of 28–69 years (mean 35,4 years, SD 12,1). None of them presented with endocrine disorders, diabetes mellitus and one person from the control group was treated for hypertension.

The blood samples were collected from fasting subjects at 8 in the morning.

The consent of Local Bioethical Committee of University of Medical Sciences in Poznan was obtained for the study (No 202/05).

METHODS

In all studied subjects with acromegaly and all subjects from the control group the concentrations of total and acylated ghrelin, GH, IGF-1, insulin, glucose, total cholesterol, LDL cholesterol, HDL cholesterol and triglicerydes were measured. Moreover, in acromegalic patients oral glucose tolerance test (OGTT) was performed in order to assess the activity of the disease.

Hormonal and biochemical measurements were done with the use of the following methods: **total ghrelin** – RIA (Phoenix Pharmaceuticals); **acylated ghrelin** – ELISA (Sceti); **GH** and **IGF-1** – IRMA and RIA (Biosource Europe S.A.); **insulin** – electrochemiluminescence (ECL) (Roche Diagnostics); **total cholesterol** – enzymatic colorimetric method, **HDL cholesterol** – homogenic colorimetric enzymatic method, **triglycerides** – enzymatic colorimetric method with glycerophosphate oxidase and 4-aminophenazon. The concentration of **LDL cholesterol** was calculated on the basis of the concentrations of total and HDL cholesterol; **glucose** – oxidase method (Ebio Basic Analyser).

Statistical analysis

Statistical analysis was done with the use of Statistica 5.0. The results were presented as mean values ± standard deviation (SD). To compare the mean values of examined variables t-Student test for independent variables was used. The normality of distribution of examined variables was checked with Kolmogorov-Smirnov test and the homogeneity of variances in groups with Fisher-Snedecor test. In cases of no homogeneity of variances, t-Student test with Welch correction was used to compare the mean values of examined variables. Mann-Whitney test was used when there was no normal distribution of examined variables.

The analysis of correlations was performed with the use of Pearson linear correlation index. Spearman test was used in cases of the lack of the normal distribution of examined variables. Statistical significance was established as $\alpha \leq 0{,}05$.

RESULTS

1. Groups of acromegalic patients on the basis of the activity of the disease

Patients with acromegaly were divided into two groups depending on the activity of the disease, assessed on the basis of oral glucose tolerance test (GH concentration < or > 1μl/l):

Table 1. The characteristics of subjects and the mean values of hormonal and biochemical measurements in the group of patients with active acromegaly ($\bar{x}$– mean value; SD – standard deviation)

	$\bar{x}$ ± SD	Min - Max
Age (years)	50,4 ± 11,4	27-69
Height (cm)	169,5 ± 10,6	160-198
Weight (kg)	89,3 ± 19,5	74-145
BMI (kg/m2)	30,7 ± 2,6	26-37
Total ghrelin (pg/ml)	115,8 ± 94,2	37,9-365,3
Acylated ghrelin(pg/ml)	72,0 ± 92,3	23,9-329,8
GH (μg/l)	10,0 ± 9,8	2,9-29,5
IGF-1 (μg/l)	771,7 ± 391,3	409-1334
insulin (uU/ml)	14,7 ± 11,6	3,48-33,54
glucose (mg/dl)	104,0 ± 16,0	86-128
Total cholesterol (mg/dl)	201,9 ± 49,3	114-266
HDL cholesterol (mg/dl)	60,2 ± 9,5	38-72
LDL cholesterol (mg/dl)	113,9 ± 43,8	42,2-171,2
Triglycerides (mg/dl)	140,4 ± 76,5	51-314

a. Subjects with active acromegaly who did not meet the criteria of biochemical cure after administered therapy (the mean concentration of GH was 10 μl/l and the mean concentration of IGF-1 was elevated in relation to age and gender). There were 11 patients (7 women and 4 men); in 10 of them the neurosurgical removal had previously been conducted, one of them was further irradiated and another one had received 2 injections of Sandostatin LAR prior to the study. The characteristic of this group of patients is presented in Table 1.
b. Subjects with inactive disease, who met the criteria of biochemical cure of acromegaly (the mean concentration of GH was 0,7 μg/l and the concentration of IGF-1 was within normal values). There were 13 patients (9 women and 4 men). All of the subjects had previously been operated, 6 subjects were irradiated and 6 patients were treated with Sandostatin LAR at the time of the study (the duration of pharmacotherapy 3–39 months).The characteristics of this group of patients is presented in Table 2.

21 out of 24 examined subjects with acromegaly presented with BMI greater than 25. 7 patients with active acromegaly and 4 patients with inactive disease were obese (BMI > 30) and, respectively, 4 and 6 subjects were overweight (25 < BMI < 30).

The mean BMI in the control group was 22,5. The results of all hormonal measurements were normal. The characteristics of subjects from the control group is presented in Table 3. In 5 control subjects hypercholesterolaemia was diagnosed (previously unrecognized, untreated).

Table 2. The characteristics of subjects and the mean values of hormonal and biochemical measurements in the group of patients with inactive acromegaly ($\bar{x}$ – mean value; SD – standard deviation)

	$\bar{x}$ ± SD	Min - Max
Age (years)	54,4 ± 10,2	47-71
Height (cm)	167,1 ± 7,2	160-183
Weight (kg)	77,5 ± 12,2	63-97
BMI (kg/m2)	27,8 ± 4,2	22,0-35,9
Total ghrelin (pg/ml)	168,3 ± 118,3	42,3-353,5
Acylated ghrelin(pg/ml)	91,1 ± 75,1	6,7-236,4
GH (µg/l)	0,7 ± 0,5	0,1-1,0
IGF-1 (µg/l)	254,5 ± 124,9	72-408
insulin (uU/ml)	6,3 ± 2,8	2,56-10,9
glucose (mg/dl)	104,1 ± 25,4	80-182
Total cholesterol (mg/dl)	199,6 ± 34,8	158-288
HDL cholesterol (mg/dl)	63,6 ± 13,9	48-97
LDL cholesterol (mg/dl)	116,2 ± 30,6	72,2-196,3
Triglycerides (mg/dl)	99,7 ±42,0	51-170

Table 3. The characteristics of subjects from the control group ($\bar{x}$ – mean value; SD – standard deviation)

	$\bar{x}$ ± SD	Min - Maks
Age (years)	35,4 ± 12,1	24-63
Height (cm)	172,8 ± 4,5	165-180
Weight (kg)	68,6 ± 6,9	59-78
BMI (kg/m2)	22,9 ± 1,5	20,6-25,4
Total ghrelin (pg/ml)	120,5 ± 44,0	68,7-183,7
Acylated ghrelin(pg/ml)	95,4 ± 97,9	13,8-374,8
GH (µg/l)	0,8 ± 1,2	0,1-3,0
IGF-1 (µg/l)	281,9 ± 100,5	165,0-481,0
insulin (uU/ml)	9,2 ± 4,9	2,93-13
glucose (mg/dl)	88,0 ± 10,7	69,0-101,0
Total cholesterol (mg/dl)	215,5 ± 27,5	194,0-278,0
HDL cholesterol (mg/dl)	65,6 ± 16,2	49,0-97,0
LDL cholesterol (mg/dl)	129,7 ± 33,8	86,3-196,0
Triglycerides (mg/dl)	101,9 ± 37,8	62,0-174,0

2. Groups of acromegalic patients based on the concentration of cholesterol, insulin and glucose

Patients with acromegaly were also divided into subgroups on the basis of cholesterol, insulin and fasting glucose concentrations. 11 patients with acromegaly were found with increased concentration of total and LDL cholesterol (respectively >200mg/dl and >115mg/dl), remaining 13 subjects presented with normal cholesterol concentration. In 9 patients (6 with active and 3 with inactive acromegaly) increased concentration of fasting insulin were noticed; 15 subjects presented with normoinsulinaemia. Increased concentration of fasting glucose (>100mg/dl) were detected in 9 patients, previously diagnosed and treated for diabetes.

3. Statistical analyses

A. Ghrelin concentrations in patients with acromegaly in relation to the activity of the disease

The mean concentration of total ghrelin was not significantly different between subjects who met the criteria of cure and subjects with active acromegaly (168,3 ± 118,3 vs. 115,8 ± 94,2; p=0,17). There was no statistical difference in the concentration of acylated ghrelin between patients with active and inactive acromegaly (91,1 ± 75,1 vs. 72,0 ± 92,3; p=0,58) (Table 1 and Table 2).

The mean concentrations of total and acylated ghrelin in patients with active disease did not significantly differ from the concentrations in healthy subjects from control group (total ghrelin: 115,8 ± 94,2 vs. 120,5 ± 44,0; p=0,78; acylated ghrelin: 72,0 ± 92,3 vs. 95,4 ± 97,9; p=0,31) (Table 1 and Table 3).

B. Ghrelin concentrations in acromegalic patients in relation to coexisting metabolic disorders

The comparison of ghrelin concentrations in patients with hyper- and normocholesterolaemia

The comparison of ghrelin levels was done in two subgroups of patients with acromegaly: subjects with coexisting hyper-cholesterolaemia and subjects with normal cholesterol concentrations (Table 4).

The mean concentration of total ghrelin was significantly higher in patients with coexisting hypercholesterolaemia compared with patients with normal cholesterol concentrations (196,8 ± 111,5 vs. 99,7 ± 87,8; p=0,01) (Table 4, Fig.1.).

The mean concentration of acylated ghrelin was also significantly higher in patients with coexisting hypercholesterolaemia compared with patients with normal cholesterol concentrations (109,7 ± 93,2 vs. 44,1 ± 43,7; p=0,05) (Table 4, Fig.2.).

We demonstrated that the mean concentration of acylated ghrelin in patients with hypercholesterolaemia represented 16% of the mean concentration of total ghrelin.

The comparison of ghrelin concentrations in patients with hyper- and normoinsulinaemia and patients with hyper- and normoglicaemia

The mean concentrations of acylated and total ghrelin were compared in subgroups of acromegalic patients with hyperinsulinaemia and normal insulin concentrations and also in patients with fasting hyperglycaemia

Table 4. The concentration of total and acylated ghrelin in subgroups of acromegalic patients with normo- and hypercholesterolaemia (– mean value; SD – standard deviation)

	Patients with Hypercholesterolaemia		Patients with Normocholesterolaemia	
	$\bar{x} \pm SD$	Min - Maks	$\bar{x} \pm SD$	Min - Maks
Total ghrelin (pg/ml)	196,8 ± 111,5	60,6 - 365,3	99,7 ± 87,8	37,9 - 188,6
Acylated ghrelin (pg/ml)	109,7 ± 93,2	23,9 - 329,8	44,1 ± 43,7	7,4 - 159,5

Table 5. The concentrations of total and acylated ghrelin in subgroups of acromegalic patients with normo- and hyperinsulinaemia ($\bar{x}$ – mean value; SD – standard deviation)

	Patients with Hyperinsulinaemia		Patients with Normoinsulinaemia	
	$\bar{x} \pm SD$	Min - Maks	$\bar{x} \pm SD$	Min - Maks
Total ghrelin (pg/ml)	125,6 ± 85,2	41,5 - 316,5	158,3 ± 123,2	37,9 - 365,3
Acylated ghrelin (pg/ml)	54,8 ± 59,2	23,9 - 199,6	98,5 ± 92,6	6,7 - 329,8

Table 6. The concentrations of total and acylated ghrelin in subgroups of acromegalic patients with normo- and hyperglycaemia ($\bar{x}$ – mean value; SD – standard deviation)

	Patients with Hyperglycaemia		Patients with Normoglycaemia	
	$\bar{x} \pm SD$	Min - Maks	$\bar{x} \pm SD$	Min - Maks
Total ghrelin (pg/ml)	120,8 ± 79,0	55,3 - 316,5	160,4 ± 128,6	37,9 - 365,3
Acylated ghrelin (pg/ml)	71,0 ± 65,3	7,4 - 199,6	91,9 ± 95,6	6,7 - 329,8

vs. patients with normal fasting glucose concentrations (Table 5 and Table 6).

It was demonstrated that the mean concentrations of total and acylated ghrelin were not significantly different in patients with hyperinsulinaemia compared with subjects with normal insulin concentrations (total ghrelin: p=0,42; acylated ghrelin: p=0,22). No correlations were found between the concentrations of insulin and acylated and total ghrelin in any of examined subgroups.

The mean concentrations of total and acylated ghrelin did not significantly differ in patients with hyperglycaemia compared with subjects with normal glucose concentrations (total ghrelin: p=0,38; acylated ghrelin: p=0,54).

C. Correlations between the concentrations of ghrelin and concentrations of GH, IGF-1, cholesterol, insulin and glucose in groups of patients with active and inactive acromegaly

1. Correlations between the concentrations of ghrelin and GH and IGF-1

No statistically significant correlations between the concentrations of total or acylated ghrelin and the concentrations of GH or IGF-1 were found neither in patients with active or inactive acromegaly (p>0,05 for each analysis)

2. Correlation between the concentrations of ghrelin and parameters of the lipid profile

In patients with active acromegaly there was a statistically significant positive correlation between the concentration of total ghrelin and the concentrations of total cholesterol (p=0,03; r=0,63) (Fig.3) and LDL cholesterol (p=0,03; r=0,64) (Fig.4).

There was also a positive correlation between the concentrations of acylated ghrelin and the concentrations of LDL cholesterol, but with no statistical significance (p=0,07).

In the group of patients with inactive acromegaly there was a statistically significant positive correlation between the concentrations of acylated ghrelin and the concentrations of triglycerides (p=0,03, r=0,6) (Fig.5). A positive correlation between the concentrations of total ghrelin and the concentrations of triglycerides was also observed in this group of patients but there was no statistical significance (p=0,08).

The mean concentrations of total cholesterol, LDL and HDL cholesterol and triglycerides were not significantly different between the subgroups of patients with active and inactive acromegaly, and between subjects from the control group.

3. Correlations between the concentrations of ghrelin and concentrations of insulin, fasting glucose and BMI

There were no statistically significant correlations

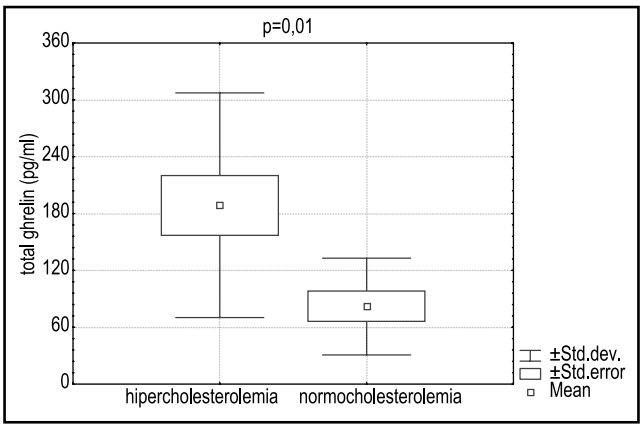

Fig.1. The comparison of mean concentration of total ghrelin in subgroups of acromegalic patients with hyper- and normocholesterolaemia

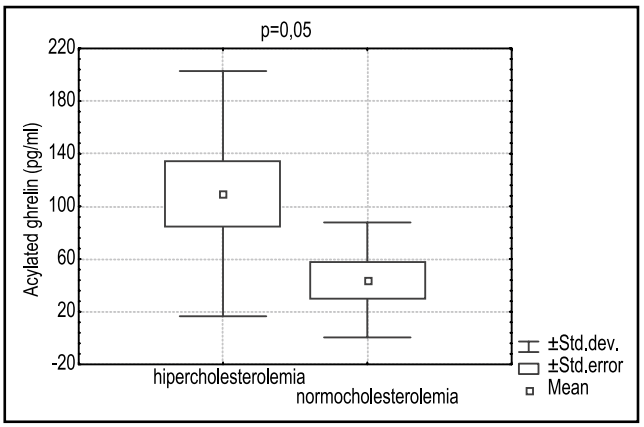

Fig.2. The comparison of mean concentration of acylated ghrelin in subgroups of acromegalic patients with hyper- and normocholesterolaemia

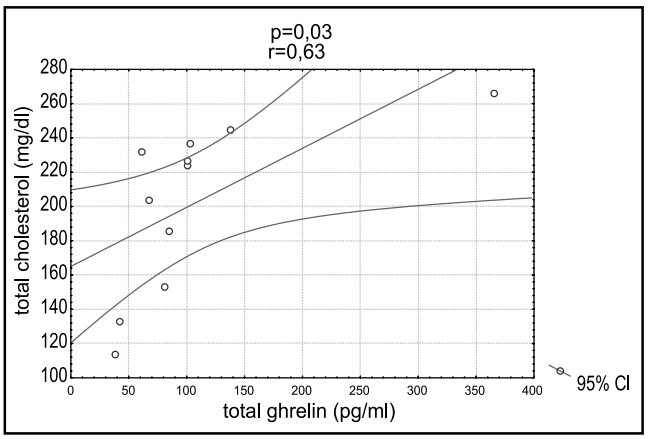

Fig.3. The correlation between the concentrations of total ghrelin and the concentration of total cholesterol in patients with active acromegaly.

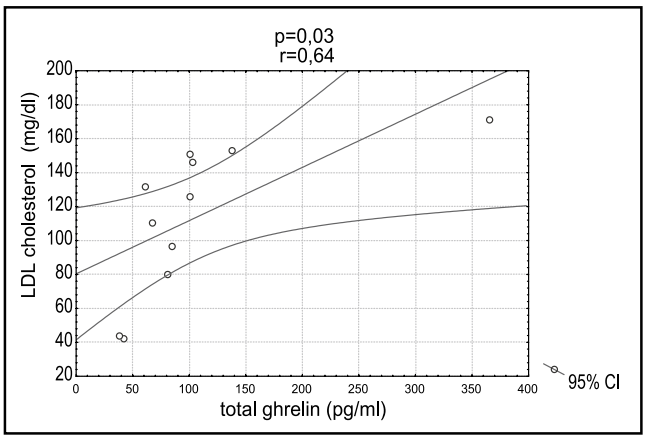

Fig.4. The correlation between the concentration of total ghrelin and the concentration of LDL cholesterol in subjects with active acromegaly.

between the concentrations of total and acylated ghrelin and the concentration of insulin in patients with active or inactive acromegaly (p>0,05 for all analyses). The mean concentration of insulin in subjects with active disease was significantly higher compared with subjects with inactive disease (p=0,04).

There were no statistically significant correlations between the concentrations of total and acylated ghrelin and the concentration of glucose in any examined groups of patients with acromegaly (p>0,05 for all analyses). The mean concentration of fasting glucose was not significantly different between groups (p=0,99).

There were no statistically significant correlations between the concentrations of total and acylated ghrelin and BMI in patients with active and inactive acromegaly (p>0,05 for all analyses). The mean BMI was not significantly different between groups (p=0,06).

DISCUSSION

The concentrations of ghrelin in patients with active and inactive acromegaly

It would be reasonable to indicate the role of ghrelin in the pathogenesis of acromegaly and presumably in the various clinical presentations of the disease and variable efficacy of the treatment. Moreover, it has not been demonstrated whether ghrelin, which regulates glucose and adipose tissue metabolism, might contribute to the development of metabolic complications of the disease. As known, acromegaly is connected with certain metabolic disturbances and it might be hypothesized that ghrelin would be the factor responsible for the occurrence of some of them. If so, the measurement of ghrelin concentrations would make it possible to monitor the activity of the disease, the efficacy of the treatment and the occurrence of metabolic complications.

In the presented study we demonstrated that there were no statistically significant differences in the mean concentrations of total and acylated ghrelin between

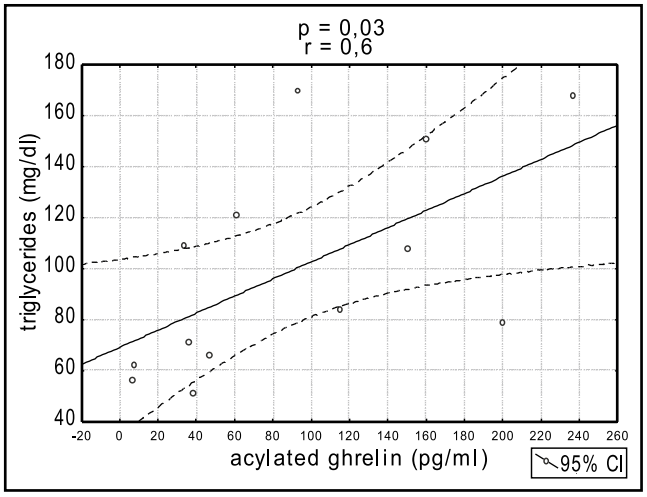

Fig.5. The correlation between the concentration of acylated ghrelin and the concentration of triglycerides in subjects with inactive acromegaly.

patients with active acromegaly and patients in whom the biochemical control of the disease was achieved. Moreover, the mean concentrations of total and acylated ghrelin in patients with active acromegaly were not significantly different from the concentrations in healthy subjects. There were no correlations between the concentrations of total/acylated ghrelin and GH/IGF-1 in any of two examined groups of acromegalic patients.

Based on the results obtained from the study, it might be suspected that there is no direct relationship between circulating ghrelin, produced in peripheral tissues, and the disturbances of GH secretion in acromegaly. In the accessible literature there are few studies analyzing the circulating ghrelin in subjects with acromegaly. However, most of them analyze the concentration of total ghrelin only [Barkan *et al.* 2003; Kozakowski *et al.* 2005; Freda *et al.* 2003; Norrelund *et al.* 2002; Capiello *et al.* 2002; Van der Toorn *et al.* 2002]. Some of them confirm the hypothesis of a negative feedback between ghrelin and GH/IGF-1 in acromegaly. Capiello *et al.* demonstrated that the concentrations of total ghrelin in patients with active acromegaly are lower compared with healthy individuals [Capiello *et al.* 2002]. Similar results were obtained by Freda *et al.* [Freda *et al.* 2003] and Kozakowski *et al.* [Kozakowski *et al.* 2005] who additionally revealed that the mean serum concentrations of ghrelin, lowered in subjects with active acromegaly, rose significantly after successful surgery of the somatotroph adenoma and this rise was accompanied with the fall of GH and IGF-1 levels. Presumably, elevated concentrations of GH and/or IGF-1 inhibit the production of ghrelin in the stomach and lead to the decrease of the peptide serum concentrations. On the contrary, our current report does not confirm such relationship. Moreover, the results of Kawamata *et al.* study [Kawamata *et al.* 2007] demonstrate that the concentrations of ghrelin in acromegalic patients did not change after the surgical removal of the tumor even if the concentrations of GH were normalized. The results of other studies also indicate the lack of a negative feedback between ghrelin and GH in acromegaly and demonstrate that the concentrations of ghrelin were not significantly different, compared with healthy individuals, despite elevated concentrations of GH [Barkan *et al.* 2003;Jarkovska *et al.* 2006; Norrelund *et al.* 2002]. Moreover, no correlations between serum concentrations of GH/IGF-1 and ghrelin were shown in any of accessible studies.

The role of endogenous ghrelin as the factor stimulating GH secretion from the pituitary and its role in the pathogenesis of acromegaly seem doubtful. Corbetta *et al.* [Corbetta *et al.* 2003] and Tsolakis *et al.* [Tsolakis *et al.* 2004] described patients with ghrelin-producing neuroendocrine tumors (ghrelinoma) who did not present with the clinical features of acromegaly and in whom the concentrations of GH and IGF-1 were found to be normal despite highly elevated serum ghrelin levels.

The discrepancies between the results of our current study and the results of other studies and the lack of the final conclusions considering the stomach-pituitary (ghrelin-GH) axis most probably result from much more complex regulation of ghrelin secretion. There are many factors that are known to influence ghrelin production and secretion and its serum concentration, like various hormones and drugs. Insulin, glucose, somatostatin, thyroid hormones are some of them [Ghigo *et al.* 2005; Van der Lely *et al.* 2004; Norrelund *et al.* 2002; Flanagan *et al.* 2003; Saad *et al.* 2002]. Thus, many other factors might have affected ghrelin levels in patients examined in our current study. Many patients examined in our study, both with active and inactive disease, presented with coexisting metabolic disorders (hyperinsulinaemia, hypercholesterolaemia, hyperglicaemia) that might have influenced circulating levels of ghrelin. Moreover, there were patients treated for hypertension and diabetes mellitus (oral medications) as well as patients receiving L-thyroxin, bromocriptin and hypolipemic drugs. Their influence on ghrelin levels is not yet known but there are data about changes of ghrelin concentrations after administration of certain other medications [Broglio *et al.* 2002; Thompson *et al.* 2004; Hosoda *et al.* 2004; Broglio *et al.* 2004].

Ghrelin secreted from the stomach seems not to be the factor influencing GH secretion from the pituitary. Another possibility is that endogenous ghrelin is a part of the ghrelin-GH axis but only in physiological conditions, and does not influence the secretion of GH from autonomous pituitary adenoma. The role of endogenous ghrelin in the regulation of GH secretion should not be however fully excluded as it remains possible that local dysregulation of ghrelin secretion in hypothalamo-pituitary system influences pituitary tumorigenesis and the release of GH from the cells of somatotroph adenoma in auto- and/or paracrine way.

Ghrelin and the disturbances of lipid profile in patients with acromegaly

Many researches have focused on the metabolic actions of ghrelin and indicate that these are the most remarkable actions of ghrelin [Ghigo *et al.* 2005; Leite-Moreira *et al.* 2007]. Much information has been collected about the effects that ghrelin exerts on lipid and glucose metabolism and the peptide has been considered one of the important factors influencing lipid metabolism [Leite-Moreira *et al.* 2007]. The changes of ghrelin levels have previously been shown to be associated with the risk of type 2 diabetes mellitus, atherosclerosis, insulin resistance and hypertension [Ghigo *et al.* 2005; Leite-Moreira *et al.* 2007; Poykko *et al.* 2003a; Broglio *et al.* 2001; Choi *et al.* 2004; Poykko *et al.* 2006; 2003b].

In our current report we demonstrated that the mean concentrations of total and acylated ghrelin were significantly higher in those patients with acromegaly who presented with hypercholesterolaemia compared with patients with normocholesterolaemia. Moreover, in patients with active acromegaly there was a statistically significant positive correlation between the mean concentration of total ghrelin and the mean concentrations of total cholesterol and LDL cholesterol.

Based on the obtained results, we might hypothesize that ghrelin is one of the factors determining the occurrence of metabolic complications of acromegaly and the changes of ghrelin levels might influence the pathological alterations of lipid profile in patients with the disease.

It seems uncertain whether such effect would be exerted by acylated or unacylated ghrelin. Acylated ghrelin has long been considered an active form of the peptide, but much information has been collected about the actions of unacylated ghrelin. It was demonstrated that both acylated and unacylated ghrelin have the ability to stimulate adipogenesis in adipocytes both *in vitro* and *in vivo*, to inhibit lipolysis and to stimulate the proliferation and differentiation of preadipocytes [Muccioli *et al.* 2004; Thompson *et al.* 2004]. Since acylated peptide is not the only active form of ghrelin, we might hypothesize that it's not only the changes of serum ghrelin concentration that might influence the occurrence of metabolic complications in acromegaly but also the changes of acylated/total ghrelin ratio. It has been demonstrated that in healthy individuals acylated ghrelin usually constitutes less than 10% of circulating total peptide [Van der Lely *et al.* 2004; Kawamata *et al.* 2007]. In our current report in those acromegalic patients who presented with hypercholesterolaemia, acylated ghrelin represented 16% of total ghrelin. It seems to be an interesting result. None of the available studies reports altered ratio of acylated/total ghrelin in subjects with acromegaly. Jarkovska *et al.* demonstrated that active ghrelin represented 2.7% of total ghrelin level in acromegalics and 2.8% in healthy controls, similarly to patients with GH-deficiency in whom active plasma ghrelin constituted 3.6% of total ghrelin level [Jarkovska *et al.* 2006]. Kawamata *et al.* demonstrated that the ratio acylated/total ghrelin in acromegalic patients was <10% [Kawamata *et al.* 2007], similarly to results obtained in healthy population [Hosoda *et al.* 2004].

It might be suggested that it's not only the change of serum ghrelin concentrations but also disproportion between its two forms that might affect the occurrence of hypercholesterolemia in acromegaly. Presumably, contradictory effects on the alterations of lipid profile are exerted by acylated and unacylated ghelin. Unacylated ghrelin might play a "protective" role, whereas increased proportions of acylated vs. unacylated ghrelin (increased acyl/total ghrelin ratio) would represent a constellation that promotes the disturbances of lipid profile and the occurrence of atherosclerotic changes. The study of Broglio *et al.* [Broglio *et al.* 2004] showing contradictory effects of both forms of ghrelin on insulin metabolism might be a confirmation of such hypothesis. Authors demonstrated that non-acylated ghrelin counteracts the effects of acylated peptide on glucose and insulin levels. It was shown that acylated peptide decreases serum concentrations of insulin and increases serum glucose levels, whereas unacylated peptide plays contradictory actions. Authors suggest that ghrelin might exert dualistic effects on glucose homeostasis depending on its state of acylation. Based on these observations it might be suspected that both forms of ghrelin might antagonize their actions also in relation to cholesterol levels. Thus, the changes of the proportion between acylated and unacylated ghrelin in different pathological conditions (e.g. acromegaly) would have various metabolic consequences, for example might initiate the development of hypercholesterolaemia or other alterations of lipid profile. In the view of above mentioned hypotheses it seems interesting to establish possible factors influencing the intensity of ghrelin acylation/desacylation. Moreover, the confirmation of those hypotheses might constitute an important element of the role of ghrelin in the occurrence of metabolic complications of acromegaly.

The hypothesis that acylated ghrelin would play a role in the development of atherosclerosis and the unacylated form of the peptide would exert antagonistic effects can be supported by the observations made for another GH-secretagogue. It has been reported that some growth hormone-releasing peptides (GHRPs) exert anti-atherosclerotic effects [Demers *et al.* 2004; Avallone *et al.* 2006]. Demers *et al.* demonstrated that hexarelin binds to CD36 receptor expressed in endothelium and monocytes/macrophages [Demers *et al.* 2004]. Receptor CD36 is involved in endocytosis of oxLDL (modified, more atherogenic form of LDL) by macrophages and mediates the accumulation of cholesterol in macrophages and the formation of atherogenic foam cells which are the element of atherosclerotic plaque [Gminski *et al.* 2003]. It was shown that the binding domain for hexarelin on CD36 overlaps with

that for oxLDL, thus anti-atherosclerotic effects of hexarelin might result from the competitive inhibition of CD36-mediated uptake of modified lipoproteins by macrophages [Demers *et al.* 2004]. It was reported that other synthetic GHSs might bind to CD36 with similar affinity as hexarelin. We might assume that if the same effects turned out to be true for unacylated ghrelin, it would bind to CD36 and exert anti-atherosclerotic effects, like hexarelin. Therefore, any changes of the proportion between acylated and unacylated peptide would influence the rate of atherosclerosis progression in patients with acromegaly. Such hypothesis seems interesting and needs further studies to be supported.

The results of the presented study demonstrated a significant positive correlation between the mean concentration of total ghrelin and the concentrations of total and LDL cholesterol in the group of subjects with active acromegaly. Similar correlations were not found in patients who did not achieve the biochemical criteria of cure. The mean concentrations of total and LDL cholesterol were similar in patients with active and inactive acromegaly as well as in healthy subjects. Only in patients with increased concentrations of GH and IGF-1 there was a correlation between ghrelin and cholesterol. Such results might indicate that ghrelin leads to the development of hypercholesterolaemia when GH is hypersecreted from the pituitary. It is again just a hypothesis which needs further studies to be confirmed.

It was reported that circulating ghrelin interacts with plasma lipoproteins [Beaumont *et al.* 2003; De Vriese *et al.* 2007; Purnell *et al.* 2003]. Beaumont *et al.* in 2003 demonstrated that ghrelin interacts with HDL-lipoproteins in humans. De Vriese *et al.* in 2007 demonstrated that incubation of ghrelin with LDL, HDL, and LPP subfractions led to the degradation to unacylated ghrelin and ghrelin desoctanoylation was the most significant in contact with LDL and LPP [45]. This suggests that enzymes responsible for ghrelin desoctanoylation are associated with lipoproteins and mainly with LDL. Thus, the interactions of ghrelin with serum lipoproteins might be responsible for the conversion of acylated to unacylated ghrelin, influence acylated/total ghrelin ratio and change the proportion between acylated and unacylated peptide.

The observation that ghrelin interacts with plasma LDL-lipoproteins might have other implications. It is known that lipoproteins contain proteins (apolipoproteins) which act as ligands for different receptors [Gminski *et al.* 2003]. It might be suggested that ghrelin, bound to LDL-lipoproteins, has a similar function. The receptor for unacylated peptide has not been yet identified. It seems probable that unacylated ghrelin acts through the receptor which "recognizes" it after binding to LDL-lipoproteins.

Moreover, it seems probable that interactions of ghrelin with LDL-lipoproteins might mediate the influence of ghrelin on lipid profile and the development of atherosclerosis. LDL cholesterol plays a major role in the development of atherosclerosis [Gminski *et al.* 2003]. Ghrelin might be the link between LDL cholesterol and atherosclerotic changes. It would explain a known fact of a faster progression of atherosclerosis in acromegaly. It is known that LDL-lipoproteins are modified (oxidation, glycation) before they become ligands for receptors in monocytes/macrophage and modified LDL-lipoproteins have a stronger atherogenic potential. Since ghrelin binds to serum LDL-lipoproteins, it might be suspected that ghrelin-modified LDL-lipoproteins become more atherogenic and are more easily scavenged by macrophages thereby predisposing to the atherogenic formation of foam cells.

It needs further studies to explain different correlations between ghrelin and lipid profile in groups of patients with active and inactive acromegaly. It also seems interesting to understand whether these correlations play any role in the development and progression of metabolic consequences of the disease and any alterations of the lipid profile in acromegalic patients. There are studies that confirm the role of ghrelin in the development of type 2 diabetes mellitus, hypertension, obesity and insulin resistance [Leite-Moreira *et al.* 2007; Poykko *et al.* 2003a; Broglio *et al.* 2001; Choi *et al.* 2004; Poykko *et al.* 2003b]. Some authors postulate that ghrelin plays an important role in the development of these disorders [Poykko *et al.* 2006]. It is also indicated that ghrelin gene polymorphism is connected with higher risk of a metabolic syndrome [Poykko *et al.* 2003a; Broglio *et al.* 2001; Choi *et al.* 2004; Poykko *et al.* 2006; Miraglia *et al.* 2004]. Moreover, the relationship between ghrelin and hypercholesterolaemia was also suggested [Choi *et al.* 2006]. Choi *et al.* reported the relationship between ghrelin gene polymorphism and increased concentrations of LDL cholesterol [48]. Poykko *et al.* showed a positive correlation between the concentration of ghrelin and the occurrence of atherosclerosis in carotid arteries [Poykko *et al.* 2006].

Ghrelin and insulin, glucose and BMI

In the presented report there were no significant differences in the mean concentrations of total or acylated ghrelin between patients with normal concentration of insulin and patients with hyperinsulinaemia. Moreover, the mean concentration of ghrelin in patients with active acromegaly was not decreased, compared with patients with inactive disease, despite elevated concentrations of insulin.

As known, insulin is the factor which inhibits the secretion of ghrelin [Ghigo *et al.* 2005; Van der Lely *et al.* 2004; Freda *et al.* 2003; Flanagan *et al.* 2003; Saad *et al.* 2002]. Moreover, both in healthy and obese subjects a negative correlation between the concentrations of insulin and ghrelin was found [Ghigo *et al.* 2005; Saad *et al.* 2002; Shiiya *et al.* 2002]. Freda *et al.* demonstrated that hyperinsulinaemia in patients with active acromegaly was accompanied with decreased levels of

ghrelin, which rose with the improvement of insulin sensitivity after the surgery [Freda *et al.* 2003]. Cappiello *et al.* revealed that in patients with acromegaly the more severe the insulin resistance was, the lower the concentrations of circulating ghrelin were [Cappiello *et al.* 2002]. No correlations were, however, found between ghrelin and insulin in acromegaly, unlike in healthy and obese subjects.

The lack of differences results most probably from the presence of other factors that determine serum concentration of ghrelin. As mentioned above, the groups of patients examined in our study were heterogenous regarding the activity of the disease (GH concentrations), other metabolic complications and aministered medications which might modify the secretion of ghrelin.

Another factor that might have influenced ghrelin levels is the concentration of fasting glucose. Patients examined in our study presented with normal or elevated fasting glucose levels. It is known that the changes of blood glucose concentrations might influence the levels of circulating ghrelin [Shiiya *et al.* 2002] and exogenous administration of glucose leads to a sudden drop of ghrelin concentration [Van der Lely *et al.* 2004 Cappiello *et al.* 2002]. The study of Cappiello *et al.* however demonstrated that ghrelin secretion did not change after the administration of glucose in patients with acromegaly, unlike in healthy and obese subjects [Cappiello *et al.* 2002]. In our current study there were no differences in the mean concentration of ghrelin between patients with normal and elevated levels of fasting glucose. Surely, the regulation of ghrelin secretion in our groups of acromeglic patients was much more complex and under the influence of other, above mentioned factors.

It has been demonstrated that in healthy subjects there is a negative correlation between ghrelin levels and BMI [Van der Lely *et al.* 2004; Norrelund *et al.* 2002]. Ghrelin secretion is decreased in obesity and rises together with weight loss [Cummings *et al.* 2001; Tschop *et al.* 2001]. In the presented study the BMI of patients both with active or inactive disease was increased but there was no correlation between ghrelin levels and BMI in any of these groups. Presumably, other factors influencing ghrelin secretion could have masked this relationship. Another possibility is that the changes of body composition in patients with acromegaly (decreased central body fat, increased soft tissue mass and total body water) are the reason for lack of relationship between ghrelin and BMI.

In summary, we did not find any relationship between circulatig ghrelin and GH in patients with acromegaly. The concentrations of ghrelin, both total and acylated, did not significantly differ between patients who achieved the biochemical criteria of cure and patients with active disease. Most probably, there is no direct relationship between circulating ghrelin, produced in peripheral tissues, and the disturbances of GH secretion or the degree of GH hypersecretion in acromegaly. We also demonstrated that the mean concentrations of ghrelin were significantly higher in patients with acromegaly and hypercholesterolaemia compared with patients with normal cholesterol concentrations. In the group of acromegalic patients with hypercholesterolemia the ratio of acylated/total ghrelin was 16%. Moreover, in patients with active disease there was a significant positive correlation between the concentrations of ghrelin and the concentrations of total cholesterol as well as LDL cholesterol. In patients with inactive acromegaly there was a statistically significant positive correlation between the concentration of acylated ghrelin and the concentration of triglycerides. Maybe there is a relationship between ghrelin which takes part in the regulation of lipid metabolism and the development of hyperlipidaemia in acromegaly. Presumably, some metabolic complications of the disease result not only from GH hypersecretion but also from altered ghrelin secretion.

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