CLINICAL STUDY

Influence of pegvisomant on serum ghrelin and leptin levels in acromegalic patients

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Abstract

Introduction: Pegvisomant (peg) is a GH receptor antagonist. In *de novo* acromegalic patients with high GH levels, ghrelin and leptin levels are reduced, suggesting a direct GH-mediated effect. The aim of our study was to evaluate whether peg treatment in acromegalic patients may abolish the GH impact on ghrelin and leptin levels.

Methods: Ghrelin, leptin and endogenous GH were measured in ten peg-treated acromegalic patients (three females/seven males, 47 years (28–57)), ten patients with active (act) and ten patients with inactive disease (inact) as well as in ten gender-, age- and body mass index (BMI)-matched healthy volunteers (controls). Endogenous GH was measured using a special in-house assay without interference by peg; total ghrelin and leptin were determined using a commercial RIA and an immunofluorometric in-house assay respectively.

Results: Age and BMI did not differ significantly between groups. Endogenous GH was significantly higher in peg (6.3 µg/l (1.5–41)) and act (9.3 µg/l (1.7–70)) compared with controls (0.1 µg/l (0.1–3.1)) and inact (0.35 µg/l (0.1–2.0), $P < 0.001$). Ghrelin was significantly higher in peg (232 ng/l (96–351)) compared with act (102 ng/l (33–232), $P < 0.01$), whereas ghrelin was not significantly different between the other groups. Leptin was highest in controls (19 µg/l (4–57)) and lowest in act (6 µg/l (2–21)), but this difference did not reach significance.

Conclusion: Treatment with peg seems to disrupt the feedback loop of ghrelin and GH, leading to elevated ghrelin levels. Furthermore, peg therapy appears not to have a strong impact on leptin levels, as acromegalic patients with and without peg treatment showed similar leptin levels.

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Introduction

The GH receptor antagonist pegvisomant (peg) is a treatment option for acromegaly, if disease activity could be adequately controlled by other treatments such as surgery, somatostatin analogues or dopamine agonists (1, 2). It is a pegylated human GH analogue, which blocks the GH receptor (3). It normalizes insulin-like growth factor 1 (IGF1) levels in 80–90% of active (act) acromegalic patients (2, 4). During peg treatment, GH levels rise in a dose-dependent manner (2). As peg interferes with most of the conventional GH assays (5), new assays have been developed to measure endogenous GH without any cross-reactivity with the drug (6).

Some studies suggest that peg treatment improves insulin sensitivity in acromegaly (7–9), reduces free fatty acid concentrations (9) and suppresses lipid mobilization and oxidation (10). Intra-abdominal fat mass, a cardiovascular risk marker, seems to be increased during peg therapy (11). But other studies showed that fat metabolism is not altered by peg (8, 12).

Leptin and ghrelin are metabolic hormones influencing fat mass, food intake, body composition and energy expenditure (13, 14). Leptin levels are high in adiposity as the hormone is secreted by fat cells (13). Appetite is reduced due to high leptin levels by inhibition of hypothalamic neuropeptide Y (15). Conversely, ghrelin is a hunger hormone, whose levels are high during fasting and are reduced by food intake (16, 17). Ghrelin is produced in the gastrointestinal tract in the endocrine X/A-like cells of the gastric mucosa (18). It is also produced in the hypothalamus and stimulates GH secretion in the pituitary (19, 20). On the other hand, ghrelin can be reduced by GH. This could be shown in some (21, 22), but not all, studies (23) in act acromegaly where GH levels are elevated, as well as after GH substitution in GH deficiency (24) and in rats (25). After surgical cure, reduced ghrelin levels in acromegaly increase (26), whereas ghrelin levels are still low during somatostatin analogue therapy even if GH and IGF1 levels are normalized (27).

In act acromegalic patients, leptin levels are reduced (28), and disease control after surgery (29) or during somatostatin analogue treatment (30) has been shown to increase leptin levels. Furthermore, leptin and GH seem to directly interact (28, 31).

Only few studies have investigated the influence of peg on ghrelin (32) and leptin levels (33).

We performed this cross-sectional study to evaluate the influence of peg on ghrelin and leptin levels by comparing acromegalic patients treated with peg to healthy controls (controls), acromegalic patients treated with act disease and those patients treated with inactive (inact) disease after surgery. Our question was whether peg prevents the reducing influence of GH on ghrelin levels. Furthermore, we investigated whether the inhibition of lipolytic GH effects by peg leads to an increase in leptin levels.

Subjects and methods

Subjects

All patients with acromegaly currently treated with the GH receptor antagonist peg as monotherapy attending our outpatients' clinic were informed about this cross-sectional study. Eight patients agreed to participate (two females and six males). Furthermore, two patients of the Max Planck Institute of Psychiatry (Department of Endocrinology, Munich, Germany) were included in this study (one female and one male). After having obtained informed consent, altogether ten patients were included in the study. The investigation was reviewed and approved by the ethics committee of the Medical Faculty of the LMU Muenchen. Moreover, ten gender-, age- and body mass index (BMI)-matched healthy volunteers examined at the Charité – University Medicine in Berlin, ten acromegalic patients with act disease and ten acromegalic patients with inactive disease without any medical treatment examined at our clinic were referred to as control groups. Acromegalic patients with inactive disease activity are defined as patients with

an IGF1 level within the age- and gender-adjusted normal range (upper limit of normal = xULN) and GH nadir during an oral glucose tolerance test (OGTT) beyond 0.5 µg/l after pituitary surgery alone. The patients were not treated with any medication for acromegaly and had not received pituitary radiation. All patients on peg, all patients with inactive disease and two patients with act disease had transsphenoidal surgery. Six patients on peg, but none of the other acromegalic patients, had received additional conventional radiotherapy. All patients on peg had been on somatostatin analogue therapy before. None of the acromegalic patients with act or inactive disease were currently on any medication for acromegaly.

Median duration of peg treatment before study entry was 27 months (range 1–47 months) with a median dose of 15 mg (range 10–30 mg). Seven patients (70%) had xULN ≤ 1 and were therefore defined as in remission. Out of the three patients who were not in remission (xULN > 1), two patients had a xULN of IGF1 below two times ULN and one patient had a xULN of IGF1 above two times ULN.

Seven patients on peg, seven patients with act disease and five patients with inactive disease had partial pituitary insufficiency. For further details, see Table 1.

Patients with diabetes mellitus currently on insulin therapy were excluded from the study. One female patient on peg had diabetes mellitus type II (Dm II) and was treated with diet and metformin. One female patient with act disease had Dm II and was treated with pioglitazone and metformin; another female patient had yet untreated impaired glucose tolerance. None of the patients with inactive disease had Dm. See Tables 1 and 2 for more patients' characteristics.

Methods

Patients arrived between 0700 and 0900 h after overnight fasting for evaluation. First, baseline data including medical history, physical examination and basal blood samples for haematology, clinical chemistry

Table 1 Characteristics of all patients of the four groups. Data are given as median and range.

	Peg	Controls	Act	Inact	P value
Gender (F/M)	3/7	3/7	3/7	4/6	–
Operation (n)	10	–	2	10	
Pituitary insuff	7	–	8	5	
Corticotrope	6	–	2	2	
Gonadotrope	6	–	7	4	
Thyrotrope	3	–	0	1	
Complete	2	–	0	1	
Age (yrs)	47 (28–57)	46 (27–59)	48 (33–80)	49 (33–60)	NS
BMI (kg/m ²)	30 (25–36)	31 (26–35)	29 (26–36)	30 (25–35)	NS
Glucose (mg/dl)	87 (71–97)	90 (78–99)	102 (86–113)	95 (69–103)	NS
Insulin (µU/ml)	13 (7–25)	18 (15–29)	11 (6–42)	12 (3–24)	NS
xULN (IGF1)	0.8 (0.5–2.7)	0.5 (0.4–0.7)	3.6 (1.5–6.1)	0.5 (0.3–1.0)	<0.001

n, number of patients; yrs, years; F, female; M, male; pituitary insuff, pituitary insufficiency; BMI, body mass index; IGF1, insulin-like growth factor 1; NS, not significant.

Table 2 Characteristics of patients on pegvisomant treatment.

Patient	Sex	Age (years)	BMI (kg/m ²)	xULN (IGF1)	Radiotherapy (years)	peg duration (months)	peg dose (mg/day)	Dm
1	F	53	34	0.8	2000	41	10	Metformin
2	F	44	28	0.5	1998, 2002, 2004	34	20	No
3	F	55	31	0.8	No	47	30	No
4	M	44	28	0.8	2002	29	15	No
5	M	55	36	2.7	1976	1	20	No
6	M	50	29	0.7	2004	25	10	No
7	M	43	33	1.4	No	25	15	No
8	M	34	30	1.0	1996	18	15	No
9	M	28	25	1.6	No	12	15	No
10	M	57	30	0.7	No	41	10	No

F, female; M, male; BMI, body mass index; IGF1, insulin-like growth factor 1; peg, pegvisomant; Dm, diabetes mellitus II.

and endocrine parameters including IGF1 were taken. Afterwards, an OGTT was performed according to the World Health Organization criteria. Serum leptin, ghrelin, endogenous GH, glucose and insulin concentrations were collected at time points 0, 30, 60, 120 and 180 min following 75 g glucose (Dextro O.G.-T., Roche) administration. Blood samples were immediately chilled and centrifuged at 4 °C, and serum aliquots were frozen until assayed.

Laboratory values

A specific assay was designed to monitor endogenous GH secretion in patients treated with the GH analogue peg. This assay is free of interference with the drug peg and has been validated, and it is described in detail elsewhere (3, 6, 32). To ensure comparability, GH was measured for all patients in this special assay. The lower detection limit was 0.2 µg/l, and the upper end of the linear working range was 50 µg/l for 50 µl samples. Intra-assay variability was 4.1 and 3.9% at concentrations of 5.2 and 14.6 µg/l respectively. Inter-assay variability at the same concentrations was determined to be 7.3 and 9.2% respectively.

Immunoreactive total human serum ghrelin was measured by a commercially available RIA (Phoenix Pharmaceuticals, Mountain View, CA, USA). Serum ghrelin was measured by using ¹²⁵I-labelled bioactive ghrelin as a tracer and a polyclonal antibody that was raised in rabbits against the C-terminal end of human ghrelin. All samples from individual subjects were measured in the same assay. Intra- and inter-assay coefficients of variation (CV) were 5.3 and 13.6% respectively.

Serum leptin concentration was measured using an immunofluorometric in-house assay as described previously (34). The lower limit of quantification was 0.1 µg/l, and the linear working range was 0.2–40 µg/l. Samples measured above this range have been reassayed after dilution in zero standard. Intra-assay variability was 7.4, 4.3 and 5.6% at leptin concentrations of 0.8, 2.5 and 15.3 µg/l respectively. Inter-assay variability at the same concentrations was

8.3, 5.2 and 5.9% respectively. Spiking experiments revealed that this immunofluorometric assay (IFMA) for leptin measures total leptin independently from the formation of leptin/leptin receptor complexes.

IGF1 concentrations were measured using an automated chemiluminescent immunoassay (Immulite, Diagnostic Products Corporation, Los Angeles, CA, USA). Immulite IGF1 is a two-site, solid-phase, chemiluminescent enzyme immunoassay and is standardized according to the World Health Organization's 2nd IS 87/518 (35). IGF1 levels are given as the multiple of age- and gender-adjusted xULN.

Glucose levels were measured from whole venous blood by an automated glucose analyser (Care Eco solo I, Care Diagnostic, Voerde, Germany), and insulin levels were determined by the Adaltis Italia insulin RIA (S.p.A; Casalecchio di Reno, Italy) in the acromegalic patients. In the control group of healthy volunteers, capillary blood glucose concentrations were measured using the glucose oxidase method (Glucometer Biosen 5130, EKF-Diagnostic, Magdeburg, Germany), and insulin was measured by a solid phase two-site ELISA (Mercodia AB, Uppsala, Sweden). The lower detection limit was 1 mU/l. The intra- and inter-assay CV were 3.4 and 3% respectively.

Statistical analysis

Ten acromegalic patients on peg treatment (peg) were compared to ten gender-, age- and BMI-matched healthy controls (controls), ten acromegalic patients with act disease and ten acromegalic patients with inactive disease.

For data analysis, SPSS (version 16.0; SPSS Inc., Chicago, IL, USA) was used. Data were expressed as median and range due to non-normal distribution. For comparison between the groups, first Kruskal–Wallis test for unrelated measurements was used, followed by non-parametrical Mann–Whitney *U* test for unrelated measurements. For comparison within a group, first Friedman test for related measurements was used to evaluate possible significant differences. If differences were found to be significant, non-parametric Wilcoxon

signed-rank test for related measurements was used for further calculations. The area under the curve (AUC) was calculated by the trapezoidal rule. The nadir was defined as the lowest value during OGTT for all patients. An adequate suppression of endogenous GH by glucose was defined at a GH nadir $<0.5 \mu\text{g/l}$. A P value <0.05 was considered as the nominal level of significance.

Results

Age, BMI, fasting glucose and insulin levels were not significantly different between the four groups ($P=\text{NS}$; Table 1).

IGF1 levels given as xULN were significantly higher in act than in the other three groups (peg, $P<0.001$; controls, $P<0.001$; inact, $P<0.001$; Table 1, Fig. 1). Moreover, xULN of IGF1 was significantly higher in peg compared to controls ($P<0.001$) and inact ($P<0.05$), but in controls and in inact, they were not significantly different. Three patients of peg and all act had an IGF1 above the xULN >1 , whereas none of the controls and none of the inact had elevated IGF1 levels.

Baseline endogenous GH and the AUC_{180} of endogenous GH were significantly higher in peg and act compared with controls and inact ($P<0.001$, median AUC_{180} of eGH: peg, $873 \mu\text{g min/l}$ ($155\text{--}12\,660 \mu\text{g min/l}$); controls, $29 \mu\text{g min/l}$ ($18\text{--}318 \mu\text{g min/l}$); act, $2189 \mu\text{g min/l}$ ($233\text{--}15\,683 \mu\text{g min/l}$); inact, $55 \mu\text{g min/l}$ ($18\text{--}258 \mu\text{g min/l}$)). But they were not significantly different between peg and act or between controls and inact (Fig. 1). Endogenous GH was adequately suppressed during OGTT in all controls and in all inact, but not in peg or act.

Ghrelin levels significantly decreased during OGTT in acromegalic patients on peg ($P<0.01$), in controls ($P<0.005$), in act ($P<0.005$) and in inact acromegalic patients ($P<0.005$) with a minimum decrease at 120 min. The percentage decline was 27% (-7 to 46%) in peg, 17% ($2\text{--}34\%$) in controls, 13% ($5\text{--}35\%$) in act and 17% ($8\text{--}26\%$) in inact. The percentage decline was not significantly different between the four groups (Fig. 2).

Figure 1 Baseline endogenous GH and xULN of IGF1 levels for the four groups peg, controls, act and inact. * $P<0.001$. Values are given in boxplots showing median and range.

Figure 2 Baseline ghrelin levels (A) and the AUC_{180} of ghrelin (B) for the four investigated groups peg, controls, act and inact. Ghrelin levels during OGTT (C). + $P<0.01$, ** $P<0.005$. Values are given in median and range.

Ghrelin levels at baseline ($P<0.05$) and the AUC_{180} of ghrelin ($P<0.05$) were significantly different between the four groups. Ghrelin levels in act were lower than in healthy controls. In patients with disease control after surgery, ghrelin levels were higher. In peg-treated patients, ghrelin levels were the highest. This difference of high ghrelin levels in acromegalic patients on peg treatment compared with low ghrelin levels in act acromegalic patients reached significance ($P<0.01$ for baseline ghrelin and AUC_{180} of ghrelin). Ghrelin levels were not significantly different between the other groups (Fig. 2).

Leptin levels slightly but significantly decreased during OGTT in acromegalic patients on peg with a minimum decline at 60 min ($P<0.005$), in act ($P<0.05$) and in inact ($P<0.005$) acromegalic patients with a minimum decrease at 120 min, but not in the control group. Median percentage decrease was 18% ($3\text{--}41\%$) in peg, 5% (-19 to 25%) in controls, 18% (-1 to 33%) in act and 11% ($0\text{--}28\%$) in inact. The percentage decline was not significantly different between the four groups (Fig. 3).

Baseline leptin levels were the highest in controls and the lowest in act, but this difference did not reach statistical significance. But the AUC_{180} of leptin was

Figure 3 Baseline leptin levels (A) and AUC₁₈₀ of leptin (B) in peg, controls, act and inact. Leptin levels during OGTT (C). ** $P < 0.005$, ++ $P < 0.05$. Values are given in median and range.

significantly different between the groups ($P < 0.05$), which was caused by a significantly higher AUC₁₈₀ of leptin in controls compared to act ($P < 0.005$) and inact ($P < 0.05$) acromegalic patients (Fig. 3).

Discussion

We could show for the first time that ghrelin levels are high in acromegalic patients on peg therapy, whereas in acromegalic patients with act disease, ghrelin levels were significantly lower. Previous data have shown that high GH can reduce ghrelin levels in rats (25) as well as in acromegaly (21, 22, 36) and GH deficiency (24, 37), but our data revealed that treatment with the GH receptor antagonist peg can adequately prevent this effect. So it might be assumed that the direct reducing effect of endogenous GH on ghrelin could be mediated by the GH receptor. Still, it cannot be ruled out that peg itself increases ghrelin levels independently of GH and IGF1. In acromegalic patients with inact disease and normalized GH levels as well as in the healthy controls, ghrelin levels tended to be higher than in acromegalic patients with act disease and high GH levels, even if this difference did not reach significance. This missing statistical difference might be due to the rather small

investigated groups of only ten patients. Why ghrelin levels in these two groups are lower despite lower xULN levels compared with the peg group is not clear. It might be speculated that GH secretion and GH effects in the peg group are somewhat altered.

The physiological regulation of ghrelin by GH is not yet clarified, even as ghrelin is well known to be a stimulator of GH in the pituitary (19, 20). As far as we know, no data exist documenting GH or IGF1 receptors on ghrelin-secreting X/A cells. GH levels have been shown to rise during peg therapy (2). The reason why GH levels are lower in our peg-treated patients compared with the act patients in our cohort is probably because our patients on peg were all treated by surgery and many of them by radiotherapy before. It is still a contentious issue whether fat mass has a significant influence on ghrelin levels (38). If this was true, ghrelin levels would be high in act disease as fat mass is reduced and low in inact disease as fat mass is high. Our data confirms that the GH effect on ghrelin is stronger than the effect of fat mass.

One limitation of this study is that we measured only total ghrelin. Acylated ghrelin is the bioactive form of ghrelin. It interacts with the GH secretagogue receptor type 1a (GHSR1a) and stimulates GH secretion. The ratio between both ghrelin forms (acylated and desacylated ghrelin) is calculated to be about 1 according to Moller *et al.* (10) and Tong *et al.* (39). The lower ratio described in earlier studies by Kim *et al.* (40) and Lucidi *et al.* (41) might be due to different assay techniques and changed sampling procedures by known fast degradation of acyl ghrelin. The ratio between both ghrelin forms is closely maintained after food intake (41) or during oral glucose load (40). According to Kim *et al.* (40), these findings are independent of GH levels. In contrast, Moller *et al.* (10) found an elevated acyl/desacyl ratio during GH receptor blockade and a positive correlation between the acyl/desacyl ratio and glucose – a possible influence of elevated ghrelin levels on insulin sensitivity after GH receptor blockade was discussed by Moller *et al.* (10). Therefore, more experimental studies are necessary to clarify the effects of ghrelin and the role of ghrelin ratio as well as ghrelin acylation depending on GH levels.

Interestingly, three other studies performed on healthy males did not find an influence of peg on ghrelin levels (10, 32, 42). Gormsen *et al.* (42) measured ghrelin levels during a hyperinsulinaemic glucose clamp where they kept insulin and free fatty acid concentrations equal during the experiment to exclude influences of different insulin or free fatty acid levels on ghrelin concentrations. Then, they administered GH or peg (30 mg) which had no effect on ghrelin concentrations. Muller *et al.* (32) evaluated the influence of peg on the ghrelin rhythm during fasting. Fasting rapidly induced a diurnal ghrelin rhythm even when peg (80 mg) was administered before. However, GH levels rose higher during additional

peg treatment. Moller *et al.* (10) found a higher quotient of acyl/desacyl ghrelin without significant changes in acyl or desacyl ghrelin levels after GH receptor blockade by peg (15 mg) in ten healthy young men. The reason why peg did not affect total ghrelin levels in these studies might be due to the fact that it was performed on healthy males, and only a single peg administration was performed. In our study, the objectives were acromegalic patients on long-term peg therapy.

After glucose load, ghrelin levels decreased significantly in all four groups with a maximum decrease at 120 min. This decline was not significantly different between the four groups. Ghrelin levels are well known to decrease in response to glucose (16, 43). The physiological mechanism for that decline is still unknown. The amount of decrease in our groups was similar to that reported in act acromegalic patients before (19 and 26%) (26, 36), but somewhat smaller than described previously in healthy subjects (28% (44) and 34% (16)). In these studies, the ghrelin nadir was at 60 min earlier than in our study.

The next main conclusion of our study was that acromegalic patients on peg treatment had similar leptin levels compared with acromegalic patients with act and inact disease. Healthy controls tended to have higher leptin levels compared to the acromegalic patients. This is in accordance to previously published data of our group which also showed similar leptin levels in act, inact and inact acromegalic patients on somatostatin analogues (36). On the other hand, some studies revealed lower leptin levels in act disease (28, 29), which is thought to be due to lower fat mass caused by lipolytic GH (45). High levels of GH in acromegaly with act disease lead to a reduction in fat mass compared with the normal population (28, 45). Furthermore, leptin is positively correlated to fat mass (13). In contrast to our result that the healthy control group tended to have higher leptin levels than all acromegalic patients, Grottoli *et al.* reported similar leptin levels in act acromegalic patients and in normal woman (46). Our results indicate that treatment with the GH receptor antagonist peg does not have a strong impact on leptin levels, as acromegalic patient groups with and without peg therapy showed similar leptin levels.

In 16 acromegalic patients with act disease, treatment with peg led to a rise in leptin levels (33). One reason for these contradictory results may be that in the cited study, all 16 patients had a normalized IGF1 after peg treatment. In our patient group, only 70% had an IGF1 within the age- and gender-adjusted reference value. The duration of peg therapy might contribute to the leptin levels. However, our patient group was treated much longer (median 47 months) than the patient group of Parkinson's study (mean 7 months).

Leptin levels decreased slightly but significantly after glucose load in all acromegalic patients even during peg therapy. The percentage of decline was not significantly different between the three acromegalic groups.

Leptin levels did not decrease in the healthy control group. A similar decrease in leptin levels after glucose has been reported by our group in GH deficiency with and without GH substitution (11% in the substituted and 9% in the non-substituted group) (36) and in a larger group of acromegalic patients (median percentage decline in act disease 18%) (36). The reason why leptin decreases after glucose load in all acromegalic patients even on peg treatment but not in a healthy control group is unclear. But this finding confirms our previously stated hypothesis that leptin regulation is influenced by chronically high GH levels during act disease (36). A central dysregulation may be involved, which still persists after controlling disease activity. It has been speculated that leptin and GH directly interact (28, 31). Some of the GH cells in the pituitary contain leptin granules and have leptin receptors (31, 47). Moreover, homozygous mutations of the leptin receptor gene lead to decreased GH secretion (47).

Limitations of the study are small size of the study groups and their inhomogeneity caused by different treatment times with peg, previous treatment such as radiotherapy and surgery and including both genders. Pituitary radiation and pituitary surgery itself may change hypothalamic ghrelin and leptin sensitivity as the GH secretagogue receptor (GHSR) on which ghrelin binds is also located in the hypothalamus (48), and leptin granules and receptors were detected in GH cells of the pituitary (31, 47).

Declaration of interest

J Roemmler received travel grants from Pfizer. B Otto and A M Ararat have nothing to declare. M Bidlingmaier received lecture fees, travel grants and research grants from Pfizer. J Schopohl received lecture fees and travel grants from Pfizer.

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