0021-972X/03/$15.00/0
Printed in U.S.A.

The Journal of Clinical Endocrinology & Metabolism 88(11):5193–5198

doi: 10.1210/jc.2003-030713

Effects of Growth Hormone (GH) on Ghrelin, Leptin, and Adiponectin in GH-Deficient Patients

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Ghrelin is a recently discovered gastric peptide that increases appetite, glucose oxidation, and lipogenesis and stimulates the secretion of GH. In contrast to ghrelin, GH promotes lipolysis, glucose production, and insulin secretion. Both ghrelin and GH are suppressed by intake of nutrients, especially glucose. The role of GH in the regulation of ghrelin has not yet been established.

We investigated the effect of GH on circulating levels of ghrelin in relation to its effects on glucose, insulin, body composition, and the adipocyte-derived peptides leptin and adiponectin. Thirty-six patients with adult-onset GH deficiency received recombinant human GH for 9 months in a placebo-controlled study. Body composition and fasting serum analytes were assessed at baseline and at the end of the study. The GH treatment was accompanied by increased serum levels of IGF-I, reduced body weight (−2%) and body fat (−27%), and increased serum concentrations of glucose (+10%) and insulin (+48%). Ghrelin levels decreased in 30 of 36 subjects by a mean of −29%, and leptin decreased by a mean of −24%. Adiponectin increased in the women only. The decreases in ghrelin and leptin correlated with changes in fat mass, fat-free mass, and IGF-I. The reductions in ghrelin were predicted independently of the changes in IGF-I and fat mass.

It is likely that the reductions in ghrelin and leptin reflect the metabolic effects of GH on lipid mobilization and glucose production. Possibly, a suppression of ghrelin promotes loss of body fat in GH-deficient patients receiving treatment. The observed correlation between the changes in ghrelin and IGF-I may suggest that the GH/IGF-I axis has a negative feedback on ghrelin secretion. (*J Clin Endocrinol Metab* 88: 5193–5198, 2003)

GHRELIN, THE ENDOGENOUS ligand specific for the GH secretagogue receptor, was originally purified from rat gastric mucosa (1). It is localized throughout the gastrointestinal mucosa with the highest content in the gastric fundus (2). Predominantly produced by the stomach in humans, lower amounts of ghrelin are also found in the bowel, kidney, and placenta as well as in the pituitary, hypothalamus (3), pancreas, liver (4), and testis (5). Ghrelin is the strongest releaser of GH known today (6–8). It has orexigenic effects (9) and increases food intake and adiposity in both animals and humans (10, 11). The circulating levels increase before meals (12) and are suppressed by the intake of nutrients, especially glucose (12–15).

Ghrelin promotes glucose oxidation and lipogenesis (11), whereas GH promotes glucose production and lipolysis (16, 17). The metabolic effects of GH are illustrated by the body fat mass typical of adults with GH deficiency (GHD) (18, 19) and by the tendency for hypoglycemia seen in children with GHD (20, 21). The role of ghrelin in GHD has not been clarified. A recent study found no effect of GH treatment on peripheral ghrelin levels in GHD adults (22), whereas another short-term study demonstrated lower ghrelin levels after exercise during GH replacement (23).

Leptin and adiponectin are peptides released from fat cells into the circulation. Leptin levels are known to correlate with adipose tissue mass (24). In patients with GHD, elevated serum levels of leptin have been found (25), reflecting the relative increase in total body fat. In obese subjects and in patients with type 2 diabetes, positive associations between leptin and insulin have been observed (26). In contrast to leptin, the circulating levels of adiponectin are low in obesity (27, 28) and increase after weight loss (29). The serum levels do not correlate with fat mass *per se* but rather with insulin sensitivity, and adiponectin is low in both insulin-resistant obese individuals (29, 30) and in patients with lipodystrophy and severe insulin resistance (31). To our knowledge, adiponectin has not previously been examined in patients with GHD.

The aim of the present study was to evaluate the effects of GH treatment on ghrelin and also on leptin and adiponectin in GHD patients in relation to the metabolic effects of GH affecting body composition and glucose homeostasis.

Subjects and Methods

Patients and study protocol

Thirty-six patients, 21 men (mean age, 44.7 ± 7.4 yr) and 15 women (mean age, 47.5 ± 6.6 yr) with GHD participated in a placebo-controlled trial. The mean duration of GHD was 10.4 yr (range, 1–33 yr). No patient had a GH response greater than 3 μg/liter during insulin-induced hypoglycemia (blood glucose ≤ 2.2 mmol/liter). All but two patients had complete pituitary insufficiency, and in all but two patients, hypopituitarism was acquired in adulthood. Eight of the women were on estrogen replacement therapy (oral, n = 4; transdermal, n = 4), and all men were receiving testosterone. Other hormone deficiencies were being adequately replaced with levothyroxine, adrenal steroids, and desmopressin (n = 9) for at least 6 months before enrollment. The patients were randomized to receive either GH or placebo for 9 months, and after 3 months of wash-out, the other treatment was given for an additional 9 months. The initial dose of recombinant human GH (Norditropin; Novo Nordisk A/S, Copenhagen, Denmark) was 0.5 U/m^2 body surface area. This dose was increased to a maximum of 2 U/m^2 and then reduced in patients who experienced side effects. The mean final dose was 2.4 U/m^2, with no difference between men and women (1.3 ± 1.7 U/m^2 and 1.2 ± 0.7 U/m^2, respectively). Serum samples were collected after an

Abbreviations: BMI, Body mass index; GHD, GH deficiency; HOMA, homeostasis model of assessment.

overnight fast and analyzed for glucose, insulin, and IGF-I. Measurements of body composition were performed before and after the treatment periods. Sera were stored at −70 C until analysis of ghrelin, leptin, and adiponectin. This study was approved by the ethical committee of the Uppsala University Hospital (Uppsala, Sweden).

Methods

Serum ghrelin levels were measured using a commercial RIA (Phoenix Pharmaceuticals, Inc, Belmont, CA). It uses ^{125}I-labeled bioactive ghrelin as a tracer and a polyclonal antibody raised in rabbits against the full-length, octanoylated human ghrelin. The intraassay and interassay coefficients of variance were 5.3% and 13.6%, respectively (22). Leptin was tested with a RIA kit (LINCO Research, Inc., St. Charles, MO) that used a rabbit antiserum against human leptin and ^{125}I-labeled human leptin. The sensitivity limit of the assay was 0.5 ng/ml, and the intra- and interassay coefficients of variation were 6.2% and 8.3%, respectively. Adiponectin serum levels were measured using the human adiponectin RIA (LINCO Research, Inc.), which uses ^{125}I-labeled murine adiponectin and a multispecies adiponectin rabbit antiserum. Serum IGF-I was measured with a RIA (Nichols Institute Diagnostics, San Juan Capistrano, CA) after extraction of binding proteins with acid ethanol. Fasting glucose was measured with routine clinical chemistry laboratory techniques at the Department of Clinical Chemistry, University of Uppsala. Serum insulin was measured by RIA (Pharmacia Insulin RIA, Pharmacia Uppsala, Sweden). Body composition was determined by dual-energy x-ray absorptiometry with the DPX-L equipment (Lunar Radiation Corp., Madison, WI). The homeostasis model of assessment (HOMA) index was calculated according to Matthews *et al.* (32).

Statistics

Serum values for ghrelin, leptin, IGF-I, and insulin were transformed into logarithms before analysis and are presented as geometric means (±SD). Values for weight, fat, fat-free mass, adiponectin, glucose, and HOMA index are presented as means (±SD). The unpaired two-tailed Student's *t* test was used for differences among groups, and the paired two-tailed Student's *t* test was used for statistical comparisons within the same group. Simple regression analyses were used to determine univariate relationships before and after GH treatment and between percentage changes. Forward stepwise regression was used to explore interrelations between variables.

Results

Effects of GH on body composition and weight

Treatment with GH for 9 months caused a decrease in total body fat and an increase in fat-free mass. The reduction in fat mass was larger in the men compared with the women ($P < 0.001$). A small decrease in body weight in the men was observed (Table 1). There was no change in body mass index (BMI).

Effects of GH on ghrelin, leptin, and adiponectin

At baseline, ghrelin levels were similar in men and women, whereas leptin was higher in the women, and adiponectin tended to be higher in the women (Table 1). Treatment with GH was accompanied by decreases in ghrelin and leptin in both men and women. Adiponectin increased in the women only (Figs. 1–3). There was no difference in serum levels of ghrelin, leptin, and adiponectin between women with and without estrogen replacement therapy either at baseline or after GH treatment.

Effects of GH on IGF-I, insulin, glucose, and HOMA index

IGF-I levels were higher in men both at baseline and after GH treatment (Table 1). Glucose, insulin, and HOMA index were similar in men and women at baseline. Glucose levels increased in the men, and IGF-I, insulin, and HOMA index increased in both men and women after GH treatment (Table 1).

Relation between ghrelin, adipocyte-derived peptides, body composition, and parameters of glucose metabolism before and after GH treatment and between percentage changes

The decrease in ghrelin correlated with the decreases in body fat (Fig. 4) and leptin levels ($r = 0.378$, $P < 0.05$) and inversely correlated with the increases in fat-free mass ($r = -0.382$, $P < 0.05$) and IGF-I (Fig. 5). Leptin levels were strongly related to total body fat at baseline ($r = 0.713$, $P < 0.001$) and after GH treatment ($r = 0.838$, $P < 0.001$) and inversely related to fat-free mass before ($r = -0.657$, $P < 0.001$) and after ($r = -0.482$, $P < 0.01$) treatment. The decreases in leptin and total body fat were positively correlated ($r = 0.416$, $P < 0.05$). Insulin and HOMA index correlated positively with leptin after GH therapy ($r = 0.413$, $P < 0.05$, and $r = 0.340$, $P < 0.05$, respectively). Adiponectin correlated inversely with insulin ($r = -0.376$, $P < 0.05$) and fat-free mass ($r = -0.338$, $P < 0.05$) at baseline. After GH treatment, the levels of adiponectin correlated with body fat ($r = 0.339$, $P < 0.05$).

TABLE 1. Measurements of body composition and regulatory peptides in 36 GHD patients (21 men and 15 women) before and after 9 months of GH treatment

Variables	Before GH therapy			After GH therapy		
	All	Men	Women	All	Men	Women
Weight (kg)	79.4 ± 14.0	85.3 ± 11.3	71.0 ± 13.5[b]	77.6 ± 12.7[d]	83.2 ± 11.2[d]	69.6 ± 10.3[c]
BMI (kg/m^2)	26.1 ± 3.1	26.6 ± 3.1	24.8 ± 3.4	25.8 ± 3.0	26.2 ± 3.0	25.1 ± 2.8
Fat (kg)	20.4 ± 7.5	17.4 ± 5.2	24.4 ± 8.6[b]	15.4 ± 8.0[f]	11.0 ± 5.3[f]	21.4 ± 7.2[c,d]
Fat-free mass (kg)	56.4 ± 13.2	65.2 ± 9.5	44.0 ± 5.2[c]	59.8 ± 14.0[f]	69.5 ± 9.2[f]	46.2 ± 5.8[c,f]
Ghrelin (pmol/liter)	64.3 (37.6–110)	62.9 (36.6–108)	66.3 (38.4–114)	48.8[f] (25.5–74.1)	40.9[f] (22.2–75.3)	47.4[f] (31.6–71.1)
Leptin (ng/ml)	9.4 (3.8–23.2)	5.2 (3.0–8.8)	21.5[c] (11.6–39.9)	6.6[f] (2.3–18.6)	3.3[f] (1.6–6.8)	17.1[c,d] (9.8–29.8)
Adiponectin (mg/liter)	23.4 ± 13.0	20.5 ± 12.5	27.4 ± 12.9	25.0 ± 14.5	17.3 ± 8.5	35.9 ± 14.4[c,d]
IGF-I (μg/liter)	73.3 (35.2–153)	106 (60.0–187)	43.7[c] (23.4–81.9)	327[f] (199–538)	405[f] (284–578)	242[b,f] (144–408)
Insulin (mU/liter)	6.6 (4.5–10.4)	6.5 (4.4–9.7)	6.8 (4.6–10.0)	9.0[f] (5.7–14.2)	8.4[c] (5.6–12.6)	10.0[c] (5.9–16.8)
Glucose (mmol)	4.1 ± 0.5	4.1 ± 0.4	4.2 ± 0.6	4.5 ± 0.7[f]	4.5 ± 0.5[c]	4.5 ± 0.9
HOMA index	1.33 ± 0.65	1.31 ± 0.66	1.35 ± 0.67	2.08 ± 1.25[f]	1.88 ± 0.94[d]	2.35 ± 1.57[c]

Values are given as means ± SD, geometric means ± SD, or means (range).
[a] $P < 0.05$; [b] $P < 0.01$; [c] $P < 0.001$ *vs.* men: [d] $P < 0.05$; [e] $P < 0.01$; [f] $P < 0.001$ *vs.* baseline.

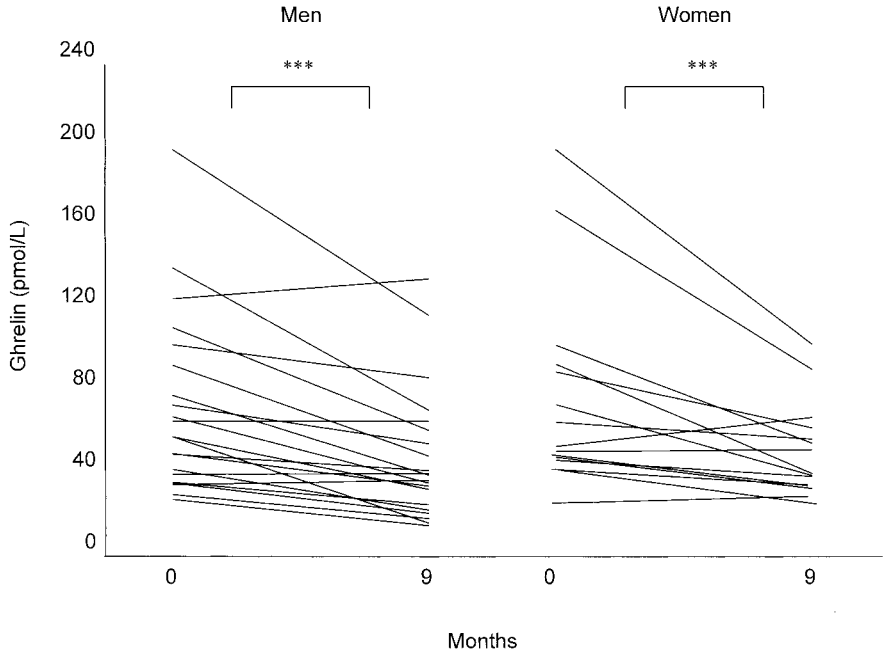

FIG. 1. Ghrelin levels (pmol/liter) before and after 9 months of GH therapy in 21 men and 15 women with GHD. ***, $P < 0.001$ *vs.* baseline.

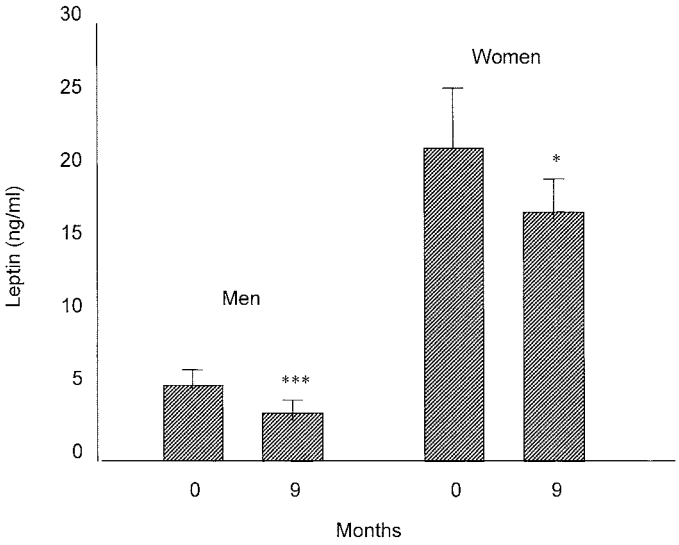

FIG. 2. Leptin levels (ng/ml ± SEM) before and after 9 months of GH therapy in 21 men and 15 women with GHD. *, $P < 0.05$; ***, $P < 0.001$ *vs.* baseline.

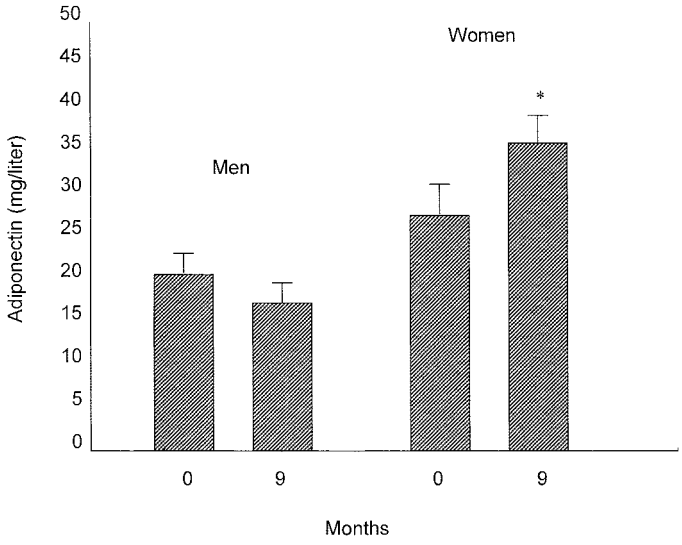

FIG. 3. Adiponectin levels (mg/liter ± SEM) before and after 9 months of GH therapy in 21 men and 15 women with GHD. *, $P < 0.05$ *vs.* baseline.

Forward regression analyses of changes in ghrelin

Changes in IGF-I and fat mass were significant predictors of the reduction in ghrelin ($F = 9.147$, $P < 0.01$, and $F = 6.660$, $P < 0.05$, respectively).

Discussion

In the present study of GHD patients, we observed a decrease in systemic ghrelin levels after long-term GH treatment. The reduction in ghrelin was related to changes in fat mass and IGF-I levels. Furthermore, a decrease in leptin levels was found, which strongly correlated with the reduction in fat mass. Because ghrelin stimulates appetite, it could be speculated that the fall in ghrelin in the GH-treated patients may reduce food intake and, thus, contribute to sustain effects on body composition during long-term treatment.

Ghrelin levels correlate negatively with BMI both in obese and normal subjects (15) and in patients with anorexia nervosa (13, 15). The levels of ghrelin increase after dietary and surgical weight loss (33, 34) and the increase correlates with the extent of weight loss (35). In the present study, however, a reduction in fat mass induced by GH treatment was accompanied by decreased ghrelin levels. This difference is likely to reflect the glucose-promoting and lipolytic activity of GH, which increases tissue substrate availability, in contrast to the negative energy balance during weight reduction. Possibly, the increase in insulin levels after GH treatment may also contribute to the fall in ghrelin. In the literature, there are several reports supporting an inverse relationship

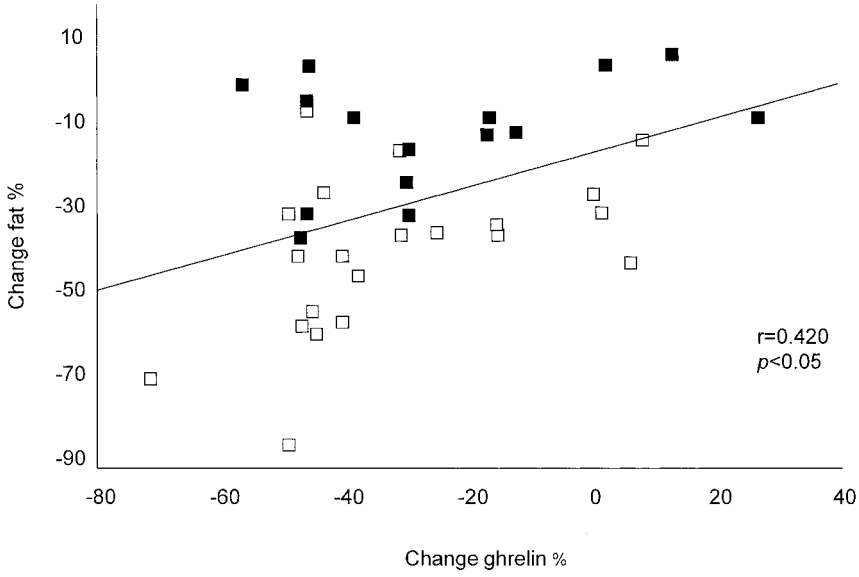

FIG. 4. Correlation between percentage changes in ghrelin levels and fat mass after 9 months of GH therapy in 21 men and 15 women with GHD. □, Men ($r = 0.485, P < 0.05$); ■, women ($r = 0.348, P =$ not significant).

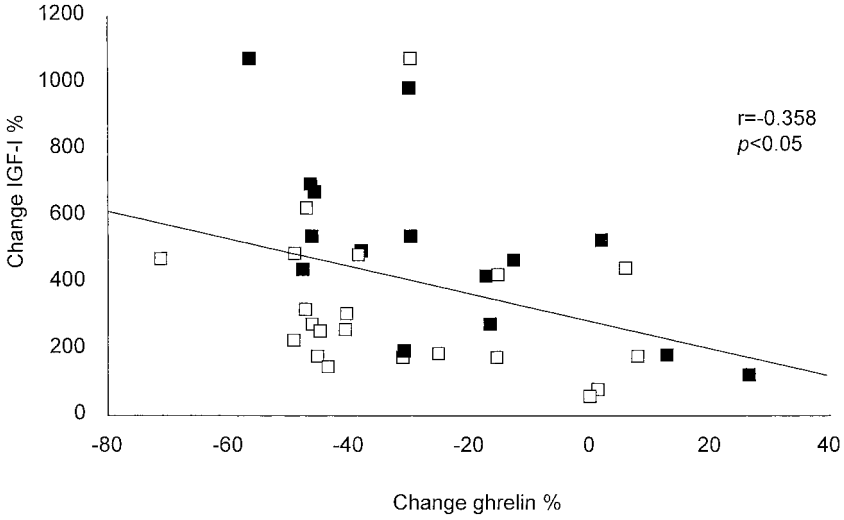

FIG. 5. Correlation between percentage changes in ghrelin and IGF-I levels after 9 months of GH therapy in 21 men and 15 women with GHD. □, Men ($r = -0.248, P =$ not significant); ■, women ($r = -0.647, P < 0.01$).

between ghrelin and insulin. For instance, low ghrelin levels have been found in obesity and in Pima Indians (36). Insulin infusion into humans resulted in a decrease in plasma ghrelin (37), whereas iv injections of ghrelin into healthy subjects reduced the insulin levels (38, 39). Recently, we have reported increased ghrelin concentrations after surgery weight loss, which strongly correlated with reductions in insulin levels (34).

One earlier report has investigated ghrelin levels after long-term GH treatment in GHD patients. In contrast to the present findings, no change in ghrelin levels was detected after 1 yr of treatment (22). Differences in the patient materials are likely to explain this discrepancy. The patients in the present study (n = 36) had severe GHD, with a stimulated GH response less than 3 μg/liter at an insulin tolerance test *vs.* less than 5 μg/liter at two separate tests (arginine and GHRH) in the patients (n = 24) of Janssen *et al.* (22). The latter patients were also more obese than our patients (BMI, 28.4 *vs.* 26.1, respectively) and had received, at the end of the study, a lower mean GH dose (1.52 IU *vs.* 2.4 IU, respectively). Furthermore, in the Janssen *et al.* study, changes in body fat were smaller, and glucose levels were not affected.

We observed a significant relationship between the changes in ghrelin and IGF-I both in univariate and in stepwise regression analyses that suggests a negative feedback effect on ghrelin secretion by IGF-I. This is supported by findings in a report of eight hypopituitary males with GHD in which ghrelin levels were examined during exercise, with and without GH therapy (23). Ghrelin was lower during GH replacement, suggesting that GH may inhibit systemic ghrelin release. This observation is further supported by a report of 17 patients with acromegaly in whom ghrelin levels were lower (201 ± 20 pmol/liter) than in normal subjects (329 ± 32 pmol/liter) and similar to those found in obese subjects (165 ± 14 pmol/liter). The levels did not correlate with insulin and BMI (as they did in normal and obese subjects), but those patients with acromegaly who had the most severe insulin resistance had the lowest ghrelin levels (40). Recently, a study in rats demonstrated that circulating levels of ghrelin were reduced by administration of GH in

normal rats and were 3-fold increased in hypophysectomized rats, suggesting a regulatory feedback loop involving the stomach and the pituitary in the regulation of gastric ghrelin secretion (41).

In the present study, leptin levels decreased in both men and women after GH replacement therapy. Leptin levels strongly correlated with fat mass, as did the changes in leptin with the changes in fat mass, in accordance with earlier studies of long-term GH treatment in GHD patients (42, 25). In the study by Kristensen *et al.* (25), after adjusting for the decrease in total adipose tissue, there was no change in the leptin levels during GH treatment, and leptin gene expression in abdominal tissue was unaffected by GH, which supports that the effect of GH on leptin is secondary to reductions in fat mass. Divergent results have been noted in leptin levels after short-term GH administration. In two studies of healthy young men, short-term GH exposure did not influence leptin levels (25, 43). In another study of healthy subjects, leptin levels increased significantly after a single dose and then decreased below baseline, suggesting that GH directly regulates leptin gene expression (44).

The production of adiponectin in adipocytes seems to be influenced by multiple factors (45), and the circulation concentrations appear to be regulated in a complex way. The serum levels are related to insulin sensitivity, and a recent study in Caucasians and Pima Indians indicated that the degree of hypoadiponectinemia was more closely related to the degree of insulin resistance than to the degree of adiposity (28). Another study in humans revealed lower adiponectin levels in obese compared with nonobese subjects and suggested a negative correlation to BMI (27). In line with these findings, increased adiponectin levels have been reported after surgical weight loss (29, 34). As a consequence of the increased insulin levels after GH therapy observed in the present study, one would expect adiponectin levels to decrease. On the other hand, the reduction in fat mass caused by the treatment could have an opposite effect. An increase in adiponectin was observed in the women only, although the effects of GH on insulin sensitivity and fat mass were no less in the men. Gender has an influence on adiponectin levels, with women having higher concentrations than men (27, 46), which has also been observed in the present study, and sex steroids are reported to affect the adiponectin levels. In mice, androgen treatment decreases plasma adiponectin, whereas ovariectomy has no effects (46). Because testosterone levels in women are not reduced by GH treatment (47), a change in sex steroid levels is not likely to explain the increase in adiponectin observed in the women. Further studies are needed to explain the difference in response between men and women.

In summary, we observed that 9 months of GH treatment in GHD patients led to a decrease in ghrelin levels, which was paralleled by changes in body fat and glucose homeostasis. Possibly, the fall in ghrelin levels contributed to the loss of fat mass seen during GH treatment. The close relation to changes in IGF-I suggests a negative feedback influence on ghrelin secretion by the GH/IGF-I axis.

Acknowledgments

We thank Margareta Ericson for her expert laboratory assistance.

Received April 22, 2003. Accepted July 21, 2003.

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This work was supported by grants from the Swedish Research Council and Uppsala University.

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