

CLINICAL STUDY

Effects of GH replacement therapy in adults on serum levels of leptin and ghrelin: the role of lipolysis

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Abstract

Objective: The regulation and function of systemic ghrelin levels appear to be associated with food intake and energy balance rather than GH. Since GH, in turn, acutely induces lipolysis and insulin resistance in skeletal muscle, we aimed to study the isolated and combined effects of GH, free fatty acids (FFAs) and insulin sensitivity on circulating ghrelin levels in human subjects.

Design: Seven GH-deficient patients (aged 37 ± 4 years (mean \pm S.E.)) were studied on four occasions in a 2×2 factorial design with and without GH substitution and with and without administration of acipimox, which lowers FFA levels by inhibition of the hormone-sensitive lipase, in the basal state and during a hyperinsulinemic euglycemic clamp.

Results: Serum FFA levels decreased with acipimox administration irrespective of GH status. The GH-induced reduction in insulin sensitivity was countered by acipimox. Fasting ghrelin levels decreased insignificantly during GH administration alone, but were reduced by 33% during co-administration of GH and acipimox (Aci) (in ng/l): 860 ± 120 (-GH - Aci), 711 ± 130 (-GH + Aci), 806 ± 130 (+GH - Aci), 574 ± 129 (+GH + Aci), $P < 0.01$. The clamp was associated with a further, moderate lowering of ghrelin. GH and acipimox induced a reciprocal 25% increase in serum leptin levels ($\mu\text{g/l}$): 11.2 ± 4.4 (-GH - Aci), 11.7 ± 4.4 (-GH + Aci), 11.5 ± 4.4 (+GH - Aci), 13.9 ± 4.2 (+GH + Aci), $P = 0.005$.

Conclusion: Our data suggest that antilipolysis via suppression of the hormone-sensitive lipase in combination with GH administration is associated with significant and reciprocal changes in ghrelin and leptin.

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Introduction

The regulation and function of circulating ghrelin is complex and not fully understood. Since ghrelin, which is produced by the stomach, is the endogenous ligand of the growth hormone secretagogue (GHS) receptor, it was anticipated that the circulating levels of this peptide would be linked to the endogenous growth hormone (GH) secretion. However, the increased GH secretion observed during conditions such as exercise (1), fasting (2), and hypoglycemia (3), does not appear to be subserved by ghrelin. If anything, data obtained in acromegaly (4, 5) and following GH administration (6, 7) indicate that GH to a moderate extent suppresses systemic ghrelin levels. By contrast, the circadian pattern of ghrelin is closely associated with food intake with high preprandial levels, a postprandial suppression, and intermediary and more stable nocturnal concentrations (8). It has been suggested that ghrelin acts as a meal initiator,

which fits with experimental data in humans and in rodent models showing that ghrelin is orexigenic (9, 10). In addition, ghrelin levels are low in obesity and increase following weight loss (11, 12). Administration of insulin suppresses ghrelin levels irrespective of glycemia (13), and ghrelin is inversely correlated with endogenous insulin levels (8). Interestingly, insulin resistance *per se* also appears to be a negative determinant of ghrelin (14–17). GH induces lipolysis, insulin resistance and hyperinsulinemia (18), which may influence its impact on ghrelin secretion. In the present study, we have been able to factor out the effects of GH, insulin and insulin sensitivity on serum concentrations of endogenous ghrelin. Using a 2×2 factorial design we evaluated the impact of GH and acipimox, a potent inhibitor of lipolysis which antagonizes the insulin antagonistic effects of GH, on circulating ghrelin concentrations in the basal state and during a hyperinsulinemic euglycemic glucose clamp.

Subjects and methods

Seven adult GH-deficient patients (six men and one woman) were included in the study. Patient characteristics are summarized in Table 1. The diagnosis of GH deficiency was ultimately based on a GH response $< 3 \mu\text{g/l}$ after an arginine stimulation test and/or an insulin tolerance test. All pituitary replacement therapy, including GH treatment, was administered in an unchanged dosage for at least 6 months before the study.

Informed consent was obtained from all participants, and the local ethics committee approved the study.

All seven patients were studied four times in random order: after discontinuation of GH replacement for 2 days ($-GH - \text{Aci}$), after discontinuation of GH, but with administration of acipimox (Aci) ($-GH + \text{Aci}$), during continuation of GH replacement ($+GH - \text{Aci}$), and during continuation of GH replacement plus administration of acipimox ($+GH + \text{Aci}$). GH was administered as subcutaneous self-injections at 2200 h; for study conditions $-GH - \text{Aci}$ and $-GH + \text{Aci}$, the last two GH injections were discontinued. In study conditions $-GH + \text{Aci}$ and $+GH + \text{Aci}$, the patients received four doses of acipimox 250 mg, p.o., with two doses administered at 2000 and 2300 h the evening before and two doses administered at 0600 and 1000 h on the day of the metabolic study. The metabolic studies were performed between 0800 and 1400 h (0–360 min). Intravenous catheters were placed in an antecubital vein. The subjects were studied in the basal post absorptive state for 180 min (0800–1100 h). At 1100–1400 h a hyperinsulinemic, euglycemic clamp (insulin 0.6 mU/kg/min; Actrapid, Novo Nordisk, Gentofte, Denmark) was performed. During the insulin infusion plasma glucose was clamped at 5.0 mmol/l by adjusting the rate of infusion of 20% glucose according to plasma glucose measurements every 10 min. Insulin sensitivity was estimated by the level of glucose infusion rate (GIR) during the hyperinsulinemic, euglycemic clamp.

Serum ghrelin was measured in one run by a commercial radioimmunoassay (Phoenix Pharmaceuticals, Inc., Belmont, CA, USA) using ^{125}I -labeled bioactive ghrelin as a tracer and a rabbit polyclonal antibody vs octanoylated and des-octanoylated h-ghrelin (sensitivity: 8–20 pg/tube according to the

manufacturer's information. The assay recognizes the COOH-terminal of ghrelin and as such determines acylated as well as des-acylated ghrelin.

Serum leptin was measured in one run by a commercial ELISA kit (Linco Research, Inc., St Charles, MO, USA) (sensitivity was 0.5 ng/ml and the intra- and interassay coefficients of variation ranged from 2.6 to 4.6 and from 2.6 to 6.2% respectively according to the manufacturer's information).

Ghrelin and leptin were measured at $t = 0, 180$ and 360 min.

Plasma glucose was determined in duplicate immediately after the samples were withdrawn using a Beckman glucose analyzer (Beckman Instruments, Palo Alto, CA, USA). Free fatty acids (FFAs) were measured by a commercial kit (Wako Chemicals GmbH, Neuss, Germany).

GH was assayed by a time-resolved immunofluorometric assay (TR-IFMA) (DELFLIA, PerkinElmer Life Sciences and Analytical Sciences-Wallac, Turku, Finland). The intra- and interassay coefficients of variation of the GH assay were 1.7–2.4 and 1.9–3.0% respectively; the lower detection limit was 0.01 $\mu\text{g/l}$.

Insulin was measured by commercially available immunoassay (DAKO, Glostrup, Denmark).

All results are expressed as means \pm s.e. Statistical calculations were made by analysis of variance (ANOVA) for repeated measures. All non-normally distributed variables were log transformed to obtain normality. For time series (i.e. circulating hormones), the area under the curve (AUC) was calculated by the trapezoidal method, and comparisons were made by ANOVA. Where appropriate, *post hoc* comparisons of the different study days were made by means of a paired *t*-test. Correlations were evaluated by Pearson's test. All calculations were carried out using the computer program SPSS version 11.0 (SPSS, Chicago, IL, USA). $P < 0.05$ was considered statistically significant.

Results

Data on energy expenditure, substrate metabolism, and insulin sensitivity from this study have previously been published in a study focussing on the

Table 1 Characterization of subjects.

Patient	Sex	Age	BMI (kg/m ²)	Diagnosis	IGF-I ($\mu\text{g/l}$)	Insufficient pituitary axes
1	m	36	26.8	Juxtacellular glioma	350	GH, T
2	f	37	38	Clinically nonfunctioning pituitary adenoma	266	GH
3	m	33	38.5	Head trauma	287	GH, Gn
4	m	44	31.2	Craniopharyngeoma	94	GH, C, T, Gn, V
5	m	31	27.8	Idiopathic, childhood-onset	250	GH, T
6	m	22	28.1	Craniopharyngeoma	206	GH, T, Gn, V
7	m	58	26.6	Cushing disease	187	GH, C, T

m, male; f, female; BMI, body mass index; IGF-I levels at baseline during GH substitution; GH, growth hormone; T, thyrotropin; C, corticotropin; Gn, gonadotropin; V, vasopressin.

impact of ambient FFA levels on insulin sensitivity (19). In brief, basal levels of insulin, glucose and HbA_{1c} did not differ significantly between the four studies, although insulin levels tended to be lower during the study with acipimox alone (data not shown). Serum FFA levels during the basal period were decreased with acipimox administration irrespective of GH status: AUC_{FFA} (mmol/l × min): 82.5 ± 12.3 for the -GH - Aci group vs 18.0 ± 3.4 (-GH + Aci) vs 101.2 ± 10.7 (+GH - Aci) vs 26.4 ± 7.8 (+GH + Aci), $P < 0.01$. As expected, serum FFA levels decreased during the clamp, but the relative differences between the four studies prevailed (data not shown). The GH-induced reduction in insulin sensitivity was reversed by co-administration of acipimox: GIR (mg/kg/min): 4.0 ± 0.7 for the -GH - Aci group vs 5.0 ± 0.9 (-GH + Aci) vs 2.6 ± 0.6 (+GH - Aci) vs 4.0 ± 1.3 (+GH + Aci), $P < 0.01$. As expected, serum GH levels were significantly increased during GH administration: AUC_{GH} (µg/l × min): 32.3 ± 12.0 for the -GH - Aci group vs 84.8 ± 31.7 (-GH + Aci) vs 242.1 ± 31.4 (+GH - Aci) vs 237.0 ± 50.6 (+GH + Aci), $P < 0.001$.

Total circulating levels of ghrelin at baseline remained unchanged during GH administration alone (+GH - Aci) as compared with no treatment (-GH - Aci) and acipimox only (-GH + Aci). In contrast, during concomitant administration of GH and acipimox (+GH + Aci), total circulating levels of ghrelin were reduced by 33% (in ng/l: 860 ± 120 (-GH - Aci); 711 ± 130 (-GH + Aci); 806 ± 130 (+GH - Aci); and 574 ± 129 (+GH + Aci); $P < 0.01$) (Fig. 1). The suppressive effect of GH plus acipimox on ghrelin prevailed during the clamp period (in ng/l: 794 ± 200 (-GH - Aci); 570 ± 133 (-GH + Aci); 676 ± 141 (+GH - Aci); 478 ± 126 (+GH + Aci); $P < 0.02$) (Fig. 1). The AUC_{ghrelin} values were also suppressed by GH plus acipimox (in µg/l × min: 333.5 ± 61.8 (-GH - Aci); 254.8 ± 46.6 (-GH + Aci); 272.2 ± 41.5 (+GH - Aci); 219.0 ± 52.2 (+GH + Aci); $P < 0.02$), (Fig. 2). As expected, ghrelin levels declined during the clamp in all four studies, although the decline failed to reach statistical significance when GH was given alone (+GH - Aci) (Table 2).

Analysis for correlation between ghrelin and FFA, GH, insulin and GIR for each condition at the time points 0, 180 and 360 min failed to show significance. Linear regression between changes in serum ghrelin and insulin values before and after the clamp also failed to show significance.

Baseline leptin levels remained unchanged during GH administration alone, but were increased by 24% during concomitant administration of GH and acipimox (in µg/l: 11.2 ± 4.4 (-GH - Aci); 11.7 ± 4.4 (-GH + Aci); 11.5 ± 4.4 (+GH - Aci); 13.9 ± 4.2 (+GH + Aci); $P = 0.005$) (Fig. 3). After the clamp period no statistically significant differences in leptin levels remained.

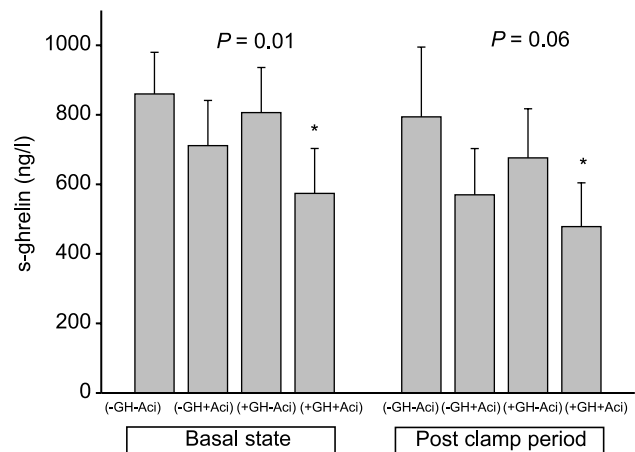


Figure 1 Total circulating serum (s) ghrelin levels (means ± s.e.) in the basal state and after the clamp period. Ghrelin decreased with concomitant GH and acipimox treatment (+GH + Aci) compared with all other conditions. P -value refers to overall ANOVA for repeated measurements. Asterisk refers to *post hoc* paired *t*-test comparing -GH - Aci with the other conditions; at $t = 0$ min, $P = 0.02$ and at $t = 360$ min, $P = 0.04$.

Discussion

The aim of this report was to investigate the isolated and combined effects of GH, FFA and insulin sensitivity on circulating ghrelin concentrations in human subjects. To this end, seven GH-deficient adult patients were studied on four occasions in a 2 × 2 factorial design with and without GH substitution and with and without administration of acipimox, which lowered FFA levels and increased insulin sensitivity. On each occasion ghrelin was measured in the basal state and during a hyperinsulinemic euglycemic glucose clamp. Our data demonstrate that the combined administration of GH and acipimox significantly suppresses ghrelin levels via apparently independent mechanisms.

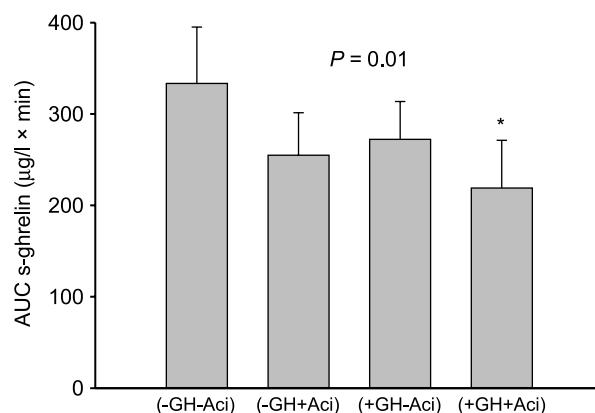


Figure 2 Area under the concentration curve (AUC; means ± s.e.) for total circulating serum (s) ghrelin levels from $t = 0$ to 360 min. P -value refers to overall ANOVA for repeated measurements. Asterisk refers to *post hoc* paired *t*-test comparing -GH - Aci with the other conditions; $P = 0.03$.

Table 2 The values for total circulating serum ghrelin at the time points 180 and 360 min for the four different treatment conditions.

Treatment group	S-ghrelin (ng/ml)	
	180 min	360 min
-GH - Aci	1025±186	794±200*
-GH + Aci	775±133	570±133*
+GH - Aci	771±101	676±141
+GH + Aci	690±166	478±126*

* $P < 0.05$, comparisons between time points 180 and 360 min.

Each study was performed during identical conditions as regards clock time and substrate background, which is important since ghrelin displays a distinct circadian variation in the fed (8) as well as in the fasting (2) state. Only the study with GH plus acipimox showed a significant change in ghrelin levels (Fig. 1). Since both GH and acipimox influenced FFA levels as well as insulin sensitivity it is difficult to factor out the precise ghrelin lowering mechanism of acipimox. But our data demonstrate that ghrelin levels are not significantly determined by isolated changes in either FFA levels or insulin sensitivity.

Intravenous infusion of lipids does not significantly impact on ghrelin levels (20), but a positive correlation between endogenous FFA levels and ghrelin has been reported (21).

As previously mentioned, the association between GH status and systemic ghrelin levels remains ambiguous, but the data so far seem to indicate a moderate suppressive effect of high GH levels on ghrelin (1, 4–7), whereas the increase in GH secretion during conditions such as exercise and hypoglycemia is not subserved by ghrelin.

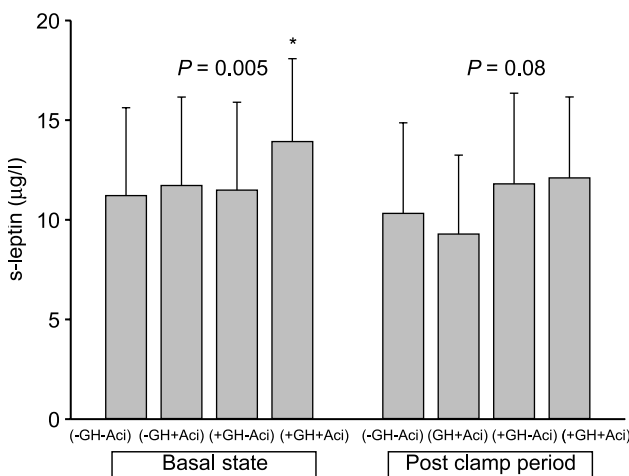


Figure 3 Serum leptin levels (s-leptin; means±S.E.) in the basal state and after the clamp period. Leptin increased in the group with concomitant GH and acipimox treatment (+GH + Aci) compared with other conditions in the basal state but not after the clamp period. P -values refer to overall ANOVA for repeated measurements. Asterisk refers to *post hoc* paired t -test comparing -GH - Aci to the other conditions; at $t = 0$ min, $P = 0.01$.

In most but not all (22) cross-sectional studies, reduced ghrelin levels are found in subjects with impaired insulin sensitivity (14–17), but insulin resistance has also been associated with a blunted postprandial suppression of ghrelin (3, 21). The specific role of insulin appears more evident since most studies including the present show that insulin administration lowers ghrelin levels irrespective of ambient glycemia. Because insulin levels during the basal period were not significantly different on the four study days, we could factor out insulin as a regulator of basal ghrelin levels in this trial. The causal relationship between ghrelin and insulin sensitivity is unclear and our data emphasize that short-term changes in insulin sensitivity *per se* do not significantly influence serum ghrelin levels. If insulin sensitivity is independently determining ghrelin levels we would, in the present study, expect +GH - Aci to significantly alter ghrelin levels, because insulin sensitivity was significantly decreased in that condition.

We therefore favor the hypothesis that antilipolysis *per se* as well as GH suppress ghrelin secretion. This observation fits with a recent study in rodents, where inactivation of fatty acid synthase decreased total circulating ghrelin levels and subsequently reduced both food intake and body weight (23). The most significant acute regulator of circulating (i.e. gut derived) ghrelin secretion seems to be food intake, where an increase is observed before each meal followed by a postprandial suppression (12). This has led to the hypothesis that ghrelin is a meal initiating signal, which is compatible with the orexigenic effects of ghrelin derived from experimental data in human as well as rodent models. In line with this view it makes teleological sense that suppression of lipolysis via inhibition of the hormone-sensitive lipase in adipose tissue would suppress ghrelin secretion, since mobilization of FFA is increased with fasting and blocked by food intake. The molecular mechanisms subserving the regulation of ghrelin secretion by the hormone-sensitive lipase (HSL) remains to be elucidated, but the available data suggest that it is not merely mediated by changes in circulating FFA levels. Our study also supports the notion that high GH levels may feedback-inhibit ghrelin secretion, but the effect appears to be moderate and of uncertain physiological significance.

We were surprised to observe that combined administration of GH and acipimox caused a $\approx 25\%$ increase in serum leptin levels, which was reciprocal to the changes in ghrelin. In rodents, leptin stimulates lipolysis and fat oxidation, which is assumed to be mediated by the HSL (24). It could thus be speculated that blockade of HSL-mediated lipolysis could lead to a feedback stimulation of leptin secretion. Long-term GH exposure in humans lowers leptin levels in parallel with a reduction in fat mass, whereas the short-term effects of GH on leptin are uncertain. Thus the reason why increased leptin levels were observed only when

acipimox and GH were co-administered is not readily explained.

In conclusion, our studies indicate that antilipolysis via suppression of the hormone-sensitive lipase in combination with GH administration is associated with significant and reciprocal changes in ghrelin and leptin. This novel observation adds to the evidence that the secretion and action of ghrelin predominantly depend on alterations in nutritional background.

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