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PAPER

Effects of 36 hour fasting on GH/IGF-I axis and metabolic parameters in patients with simple obesity. Comparison with normal subjects and hypopituitary patients with severe GH deficiency

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OBJECTIVE: Reduction of growth hormone (GH) secretion in obesity probably reflects neuroendocrine and metabolic abnormalities. Even short-term fasting stimulates GH secretion and distinguishes normal from hypopituitary subjects with growth hormone deficiency (GHD). Marked weight loss improves GH secretion in obesity but the effect of fasting is controversial. We studied the effects of a 36 h fasting on the GH/IGF-I axis and metabolic parameters in obesity.
SUBJECTS: We studied nine obese patients (OB; three male and six female; age, 29.2 ± 4.8; range, 18 – 59 y; body mass index (BMI), $43.4 \pm 2.7\,kg/m^2$; WHR, 0.9 ± 0.1). Fifteen normal subjects (NS; eight male and seven female 28.9 ± 0.6, 25 – 35 y; $21.6 \pm 0.4\,kg/m^2$) and 10 adult hypopituitary patients with severe GH deficiency (GHD; seven male and three female; 37.6 ± 2.3, 29 – 50 y; $24.5 \pm 1.0\,kg/m^2$; GH peak $< 3\mu g/l$ after ITT and/or $< 9\mu g/l$ after GHRH + arginine) served as control groups.
STUDY DESIGN: We studied the effects of 36 h fasting on 8 h diurnal mean GH, insulin and glucose concentrations (mGHc, mINSc and mGLUc; assay every 30 min from 8.00 am to 4.00 pm) as well as on IGF-I, IGFBP-3, ALS, IGFBP-1, GHBP and free fatty acid (FFA) levels.
RESULTS: Before fasting, basal IGF-I and ALS levels in OB were similar to those in NS and both were higher ($P < 0.001$) than those in GHD. IGFBP-3 levels in OB were lower ($P < 0.01$) than in NS but higher ($P < 0.02$) than in GHD. GHBP levels in OB and GHD were similar and both were higher ($P < 0.01$) than in NS. Glucose levels were similar in all groups. FFA levels in OB were higher ($P < 0.01$) than in NS but similar to those in GHD. IGFBP-1 in OB were lower ($P < 0.05$) than in NS and GHD which, in turn, were similar. On the other hand, mINSc in OB was higher ($P < 0.01$) than that in NS and GHD which, in turn, were similar. The mGHc in OB was similar to that in NS but only the latter was higher ($P < 0.05$) than in GHD. The individual mGHc in the three groups overlapped. After fasting, IGF-I levels in GHD were unchanged while they decreased in OB ($P =$ NS) as well as in NS ($P < 0.01$). IGFBP-3 and ALS levels did not change. GHBP levels in OB and GHD were unchanged while they increased in NS ($P < 0.01$). Glucose and FFA levels were reduced and increased, respectively, in all groups ($P < 0.02$ and $P < 0.01$). IGFBP-1 increased while mINSc decreased in all groups ($P < 0.02$ and $P < 0.01$); in OB they persisted lower and higher ($P < 0.01$) respectively, than in NS and GHD. Fasting significantly increased mGHc in NS ($P < 0.001$) but not in OB as well as in GHD. Individual mGHc in OB showed persistent overlap with GHD.
CONCLUSIONS: Short-term fasting does not increase GH secretion in obesity and does not distinguish somatotroph function in obese from that in severe GHD adults. Short-term fasting in obesity has attenuated effects on insulin and IGFBP-1 secretion while it normally increases free fatty acids in spite of any change in GH secretion.
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Introduction

Growth hormone (GH) levels are reduced in obesity reflecting true reduction of 24 h GH production rate.[1] Obese patients also show reduction of the somatotroph response to provocative stimuli which is sometimes as impaired as in growth hormone deficient (GHD) patients.[2,3]

GH insufficiency in obesity could reflect neuroendocrine abnormalities including somatostatinergic hyperactivity[4] and/or, more likely, growth hormone releasing hormone (GHRH) hypoactivity.[5,6] Recent evidence favors the hypothesis for abnormalities in peripheral hormones and metabolic factors such as enhanced negative free insulin growth factor-I (IGF-I) feedback,[7] hyperinsulinism,[8] alterations in leptin activity[9] and elevated free fatty acids (FFA).[10,11]

Fasting strikingly stimulates somatotroph secretion in normal healthy humans;[12,13] reduction in the negative IGF-I feedback action as well as CNS-mediated mechanisms such as concomitant reduction in somatostatin activity and GHRH hyperactivity are likely to play major role.[14,15]

Somatotroph insufficiency in obesity is reversible after long-term diet-induced weight loss,[16] but the effect of fasting is still controversial. Short-term fasting has been reported to be able to enhance, but not restore, the GH response to GHRH as well as long-term diet does.[17] Moreover, the GH response to GHRH both alone and combined with arginine has been found to be unaffected by fasting in another study.[18]

We studied the effects of 36 h fasting on 8 h diurnal GH, insulin and glucose levels as well as on basal IGF-I, insulin-like growth factor binding protein-3 (IGFBP-3), acid labile subunit (ALS), IGFBP-1, growth hormone binding protein (GHBP) and FFA levels in obese patients. These results were compared with those in age-matched normal and hypopituitary subjects with severe GHD. In fact, fasting abolishes the overlap in mean GH concentrations between normal and GHD subjects which is generally observed in fed condition; this reflects the expected failure of hypopituitary patients to enhance GH secretion during fasting.[19]

Subjects and methods

Nine obese patients with visceral adiposity (OB; three male and six female; age 29.2 ± 4.8; range 18–59 y; body mass index (BMI), $43.4 \pm 2.7\,kg/m^2$; waist–hip ratio (WHR) 0.9 ± 0.1) were studied.

Fifteen normal subjects (NS; eight male and seven female; 28.9 ± 0.6, 25–35 y; $21.6 \pm 0.4\,kg/m^2$) and 10 adult hypopituitary patients with severe GHD (seven male and three female; 37.6 ± 2.3; 29–50 y; $24.5 \pm 1.0\,kg/m^2$; GH peak $< 3\,\mu g/l$ after insulin tolerance test (ITT) and/or $< 9\,\mu g/l$ after GHRH + arginine) had been already studied and served as control groups. All hypopituitary patients had acquired GHD; among them five had adult-onset (AO) and four had childhood-onset (CO) GHD. In all severe GHD in adulthood had been demonstrated by peak GH response below 3 μg/l after ITT and 9 μg/l after GHRH + arginine test. No patient had received recombinant human growth hormone (rhGH) for at least 3 months prior to testing while all patients with pituitary insufficiencies, other than GH, had been in optimised replacement therapy for at least 3 months with levo-tiroxine, cortisone acetate, gonadal steroids and DDAVP when appropriate.

The study protocol was approved by the local, independent Ethical Committee and all subjects gave their informed consent to participate in the study.

All subjects entered the department at 6.30 am the day of the test (day 1) after overnight fasting from 8 pm of the evening before. From 8 am to 4 pm blood samples were taken every 30 min from an antecubital vein of the forearm kept patent by slow infusion of isotonic saline for GH, insulin and glucose assays. IGF-I, IGFBP-3, ALS, GHBP, IGFBP-1 and FFA levels were also measured at 8 am. That day, all subjects had a 200 kcal breakfast (60% carbohydrate) at 8 am, a 800 kcal (50% carbohydrate, 30% lipid, 20% protein) lunch and dinner at 12 am and 8 pm, respectively. After dinner they started fasting. Being fasted, on day 3 (ie after 36 h fasting) all subjects underwent the same sampling protocol for hormonal assays.

Serum GH levels (μg/l) were measured in duplicate at each time point by the IRMA method (HGH-CTK, Sorin, Italy). The sensitivity of the method was 0.15 μg/l. The inter- and intra-assay coefficients of variation were 5.1–7.5% and 2.6–5.4%, respectively, at GH levels 2.9–42.4 and 2.8–41.2 μg/l, respectively.

Serum insulin levels (μU/ml) were measured in duplicate by RIA method (INSIK-5, Sorin, Saluggia, Italy). The sensitivity of the assay was 4.0 μU/ml. Inter- and intra-assay coefficients of variation were 5.9–6.3% and 3.5–8.6%, respectively.

Serum IGF-I levels (μg/l) were measured in duplicate by RIA method (Nichols Institute of Diagnostics, San Juan Capistrano, USA) after acid–ethanol extraction to avoid interference by binding proteins. The sensitivity of the method was 0.1 μg/l. The inter- and intra-assay coefficients of variation were 8.8–10.8% and 5.0–9.5%, respectively, at IGF-I levels 79.6–766.4 and 79.4–712.5 μg/1, respectively. In our laboratory the age-adjusted 3rd centile limits of normality for IGF-I levels were 108.5 μg/l between 20 and 30 y, 129.8 μg/l between 31 and 40 y and 72.3 μg/l between 41 and 50 y.

Serum IGFBP-3 levels (mg/l) were measured in duplicate by RIA method (Nichols Institute of Diagnostics, San Juan Capistrano, USA). The sensitivity of the assay was 0.25 mg/l. Inter- and intra-assay coefficients of variation were 5.3–6.3% and 3.4–8.0%, respectively.

Serum ALS levels (mU/ml) were measured in duplicate by sandwich immunometric assay using monoclonal antibodies directed against specific N- and C-terminal oligopeptides. To optimise immunorecognition, samples were pre-treated with 3 M urea and 0.05% SDS. A serum pool of healthy male volunteers was used for calibration and assigned 1 U/ml. The assay range is 500–5000 mU/ml, and

the intra- and inter-assay coefficients of variation were less than 9%.

Serum IGFBP-1 levels (μg/l) were measured in duplicate by IRMA method (Diagnostics Systems Laboratories, Webster, USA). The sensitivity of the assay was 0.33 μg/l. Inter- and intra-assay coefficient of variation were 3.5 – 6.0% and 2.7 – 5.2%, respectively.

Serum GHBP levels (pmol/l) were measured by a ligand immunofunctional assay (LIFA)[20] with a monoclonal anti-GHBP antibody 10B8.[21,22] Within-assay coefficient of variation was 3.4% at 1150 pmol/l, between-assay coefficient of variation was 10.9%.

Plasma glucose levels (mg/dl) were measured by glucooxidase colorimetric method (Menarini Diagnostics, Italy). Serum FFA levels (meq/l) were measured by enzimatic colorimetric method (Wako Chemicals, Richmond, USA). All samples from an individual subject were analysed together.

For the statistical analysis of the data we applied nonparametric statistics because the group sizes were very small and because in all groups the variables considered were not normally distributed. We chose the Kruskal – Wallis analysis of ranks for comparison in independent groups (the nonparametric equivalent to the parametric analysis of variance) and Mann – Whitney *U*-test as post-test analysis. To compare two variables in the same group we used Wilcoxon's matched pair test, the nonparametric alternative to the parametric *t*-test analysis.

Data in control groups (normal subjects and patients with GHD) have been partially published elsewhere.[19]

Results

Fed state (day 1)

Basal mean IGF-I and ALS levels in OB (216.3 ± 31.4 μg/l and 1004.4 ± 110.0 mU/ml) were similar to those in NS (249.5 ± 20.8 μg/l and 945.2 ± 68.9 mU/ml) and both were higher ($P < 0.001$) than those in GHD (45.9 ± 3.8 μg/l and 362.0 ± 91.6 mU/ml). IGFBP-3 levels in OB (2.8 ± 0.2 mg/l) were lower ($P < 0.01$) than in NS (4.2 ± 0.6 mg/l) but higher ($P < 0.02$) than in GHD (1.5 ± 0.4 mg/l). GHBP levels in OB and GHD (3630.8 ± 239.3 and 3305.3 ± 873.9 pmol/l) were similar and both were higher ($P < 0.01$) than in NS (1854.8 ± 390.1 pmol/l).

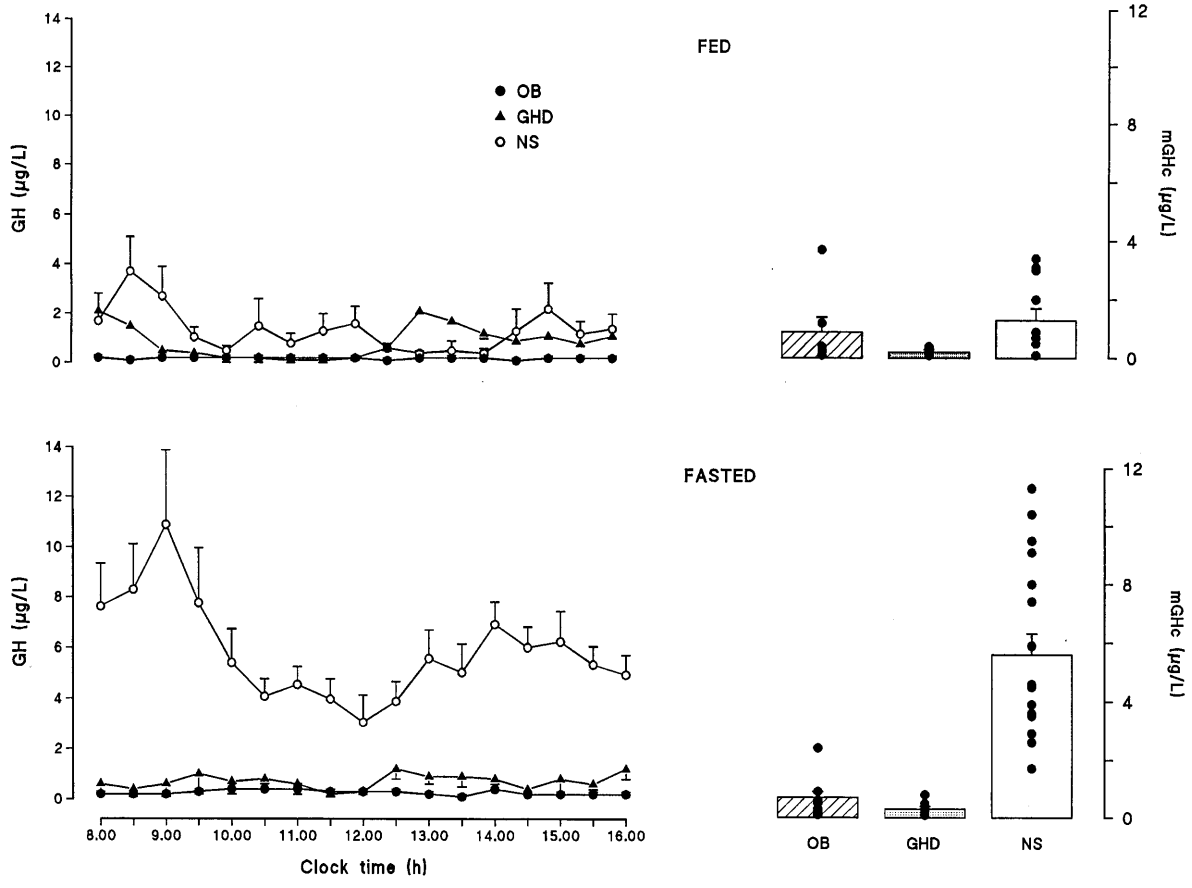

Figure 1 Mean (± s.e.) GH profiles (left panels) and mean and individual mean GH concentration (mGHc) (right panels) before and after fasting in patients with simple obesity or GHD and in normal subjects.

Table 1 Hormonal and biochemical details (mean ± s.e.) in obese, GHD patients and normal subjects before and after fasting

	IGF-I (μg/l)		*IGFBP-3 (mg/l)*		*ALS (mU/ml)*		*GHBP (pmol/l)*		*IGFBP-I (μg/l)*		*mGLUc (mg/dl)*		*mINSc (μU/ml)*		*FFA (meq/l)*	
	Before	*After*	*Before*	*After*	*Before*	*After*	*Before*	*After*	*Before*	*After*	*Before*	*After*	*Before*	*After*	*Before*	*After*
Obese patients	216.3 ± 31.4	189.8 ± 26.4	2.8 ± 0.2	3.5 ± 0.3	1004.4 ± 110.0	967.9 ± 108.4	3630.8 ± 239.3	3669.8 ± 312.5	9.0 ± 1.3	19.4 ± 5.0	89.8 ± 2.7	69.0 ± 2.7	41.4 ± 8.1	15.9 ± 2.8	0.6 ± 0.1	1.3 ± 0.1
GHD patients	45.9 ± 3.8	46.1 ± 4.2	1.5 ± 0.4	1.5 ± 0.3	362.0 ± 91.6	337.1 ± 80.7	3305.3 ± 873.9	3408.1 ± 1117.8	36.7 ± 9.7	129.5 ± 50.0	85.6 ± 4.8	67.5 ± 2.0	15.8 ± 2.6	5.9 ± 0.9	0.4 ± 0.1	0.9 ± 0.1
Normal subjects	249.5 ± 20.8	219.4 ± 20.4	4.2 ± 0.6	4.3 ± 0.2	945.2 ± 68.9	934.7 ± 58.5	1854.8 ± 390.1	1969.5 ± 436.4	27.4 ± 6.5	205.2 ± 39.1	82.2 ± 1.9	54.1 ± 1.9	18.6 ± 1.6	3.4 ± 0.6	0.2 ± 0.1	1.4 ± 0.1

Glucose levels were similar in all groups. FFA levels in OB (0.6 ± 0.1 meq/l) were higher ($P < 0.01$) than in NS (0.2 ± 0.1 meq/l), but similar to those in GHD (0.4 ± 0.1 meq/l). On the other hand, mean insulin concentration (mINSc) in OB (41.4 ± 8.1 μU/ml) was higher ($P < 0.01$) than that in NS and GHD (18.6 ± 1.6 and 15.8 ± 2.6 μU/ml) which, in turn, were similar. IGFBP-1 in OB (9.0 ± 1.3 μg/l) were lower ($P < 0.05$) than in NS and GHD (27.4 ± 6.5 and 36.7 ± 9.7 μg/l) which, in turn, were similar (Table 1).

The mGHc in OB before fasting was similar to that in NS (0.9 ± 0.5 μg/l and 1.3 ± 0.4 μg/l) and both were higher than in GHD (0.2 ± 0.01 μg/l), but the difference attained statistical significance only between GHD and NS ($P < 0.05$; Figure 1). Overlap among the individual mGHc of the three groups was present (ranges 0.1–3.7 *vs* 0.1–3.4 *vs* 0.1–0.4 μg/l).

Fasting (day 3)

Total IGF-I levels were unchanged in GHD (46.1 ± 4.2 μg/l) while they were decreased in OB (189.8 ± 26.4 μg/l, $P =$ NS) as well as in NS (219.4 ± 20.4 μg/l, $P < 0.01$). IGFBP-3 and ALS levels did not change significantly in OB (3.5 ± 0.3 mg/l and 967.9 ± 108.4 mU/ml) as well as in NS (4.3 ± 0.2 mg/l and 934.7 ± 58.5 mU/ml) and GHD (1.5 ± 0.3 mg/l and 337.1 ± 80.7 mU/ml). GHBP levels in OB (3669.8 ± 312.5 pmol/l) and GHD (3408.1 ± 1117.8 pmol/l) were unchanged, while they increased in NS (1969.5 ± 436.4 pmol/l, $P < 0.01$).

Glucose levels were reduced in all groups ($P < 0.02$). However, mGLUc in OB (69.0 ± 2.7 mg/dl) was similar to that in GHD (67.5 ± 2.0 mg/dl) but both were higher ($P < 0.001$) than in NS (54.1 ± 1.9 mg/dl). FFA levels increased in all groups ($P < 0.01$). However, FFA levels in OB and NS were similar (1.3 ± 0.1 and 1.4 ± 0.1 meq/l, respectively) and both were higher ($P < 0.05$) than in GHD (0.9 ± 0.1 meq/l). On the other hand, mINSc decreased in all groups ($P < 0.01$) but the decrease in OB (15.9 ± 2.8 μU/ml) was clearly lower than in NS and GHD (3.4 ± 0.6 and 5.9 ± 0.9 μU/ml). IGFBP-1 increased in all groups ($P < 0.02$) but levels in OB (19.4 ± 5.0 μg/l) persisted clearly lower ($P < 0.01$) than in NS and GHD (205.2 ± 39.1 and 129.5 ± 50.0 μg/l; Table 1).

Fasting markedly increased mGHc in NS (6.0 ± 0.6 μg/l, $P < 0.001$) while it did not modify that in OB (0.7 ± 0.2 μg/l) as well as in GHD (0.3 ± 0.1 μg/l). Individual mGHc in OB showed persistent overlap with GHD (ranges 0.1–2.4 μg/l and 0.1–0.8 μg/l, respectively). Fasting induced distinction between NS (range 1.2–11.3 μg/l) and GHD while a single OB had mGHc still within the NS range (Figure 1).

Discussion

The study shows that short-term fasting does not stimulate mean GH concentration in obesity and does not distinguish somatotroph secretion in obese from that in severe GHD adults. Short-term fasting in obesity has attenuated

inhibitory effect on insulin secretion and stimulatory effect on IGFBP-1 levels but normally increases FFA levels.

It is widely accepted that starvation enhances GH secretion in humans[12,13,23] as a result of increase in GH secretory burst frequency and amplitude without any change in estimated half-life.[12,13] Prolonged (more than 3 days) fasting and caloric restriction lead to peripheral GH resistance and reduced IGF-I synthesis and release.[24] This implies reduction in the negative IGF-I feedback action which plays a major role in the control of somatotroph function, as shown also by recent knock-out studies in mice and rhIGF-I studies in humans.[25–27]

Enhanced GH secretion has been recorded even after short-term fasting before significant reduction in total IGF-I levels;[13] however, we found slight decrease in total IGF-I levels in obese as well as in normal subjects, though statistical significance was reached in the latter group only.

CNS-mediated mechanisms including reduction in hypothalamic somatostatin release and hyperactivity of GHRH-secreting neurons[14] are likely to be involved in the prompt stimulatory effect of fasting on somatotroph secretion. It is not surprising that fasting did not stimulate spontaneous GH secretion in hypopituitary patients with severe GHD.[19] However, fasting abolished the overlap in mean GH concentrations between normal and GHD subjects which is generally observed in fed conditions;[19] this suggested as the opportunity to test the somatotroph responsiveness in obesity. In fact, GH secretion in obesity is restored by long term diet-induced weight loss[16] but the effect of fasting was still unclear.[17,18]

Our study shows that spontaneous diurnal GH secretion in obese patients is not modified at all by 36 h fasting and that this picture overlaps with that in hypopituitary patients with severe GHD. In fact, overlap between obese and GHD persisted even after fasting. The value of the study of spontaneous GH secretion for the diagnosis of adult GHD is therefore low even if it is performed after fasting.[28]

The lack of GH response to fasting in obesity could reflect impairment of neuroendocrine reaction to starvation. Hypothalamic somatostatin hyperactivity seems unlikely,[5] but there is evidence for reduced activity of GHRH-secreting neurons,[5] although short-term treatment with GHRH does not restore the GH response to GHRH itself.[15]

Abnormalities in peripheral hormones and metabolic factors could play a major role in making somatotroph cells and/or neuroendocrine mechanisms refractory to starvation.[10,29–32]

After fasting total IGF-I levels underwent a slight decrease in obese as well as in normal subjects who, however, showed striking increase in mGHc. Enhanced negative feedback action by free IGF-I has been hypothesised to explain GH hyposecretion in obesity. In agreement with insulin-dependent control of hepatic IGFBP-1 secretion,[33] in our fed obese patients we found low IGFBP-1 levels coupled with hyperinsulinsm. In agreement with the existence of peripheral insulin resistance, the inhibitory effect of fasting on insulin secretion in obese was lower than in normal and GHD subjects. This could explain why obese subjects showed attenuated fasting-induced IGFBP-1 increase which, in turn, could allow enhanced free IGF-I activity possibly blocking the somatotroph response to starvation. Moreover, hyperinsulinism *per se* could play a further inhibitory role on GH synthesis and release in obesity via action at both the pituitary and the hypothalamic level.[8,30,34]

Stimulatory role of leptin in the regulation of rat somatotroph function has been shown; in fact, fasting inhibits GH secretion and leptin restores it.[9,11] However, in humans fasting stimulates GH while inhibiting leptin levels[31] and the fasting-induced leptin decrease is more marked in normal than in obese subjects.[35] Thus, the lack of GH response to fasting in obesity is unlikely to reflect leptin-mediated mechanism. The chronic elevation of circulating FFA levels, which were further enhanced by fasting, could also have a key role in counteracting the GH response to starvation in obesity.[10,11,29] FFA has a strong inhibitory action on somatotroph secretion acting at either the pituitary or the hypothalamic level;[31,32,36] in fact, lipolysis inhibitors restore GH secretion in obesity.[10,11,29,37] In the absence of any significant increase in GH secretion obese patients in our study showed normal activation of lipolysis; this probably reflects the fasting-induced response of other contraregulatory hormones.[38]

In this context, modification in IGF-I bioactivity brought about by fasting-induced changes in the most important binding proteins could play a major metabolic role.[39,40]

In conclusion, short-term fasting does not stimulate mean GH concentration in obesity as well as in hypopituitary patients with severe GHD. This is impressive because a considerable pituitary GH releasable pool is available in obese subjects tested with appropriate stimuli such as GH secretagogs.[2,3] The marked insufficiency of spontaneous GH secretion and its response to starvation probably reflects alterations in the neuroendocrine and/or metabolic control of somatotroph function in obesity.

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