Pulsatile Growth Hormone Secretion in Normal-Weight and Obese Men: Differential Metabolic Regulation During Energy Restriction

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Metabolic changes such as obesity and fasting modulate pulsatile growth hormone (GH) release in man, but the underlying mechanisms are still elusive. We studied the temporal pattern of pulsatile GH release in five normal-weight men (mean ± SD: age, 29.8 ± 4.9 years; body mass index [BMI], 24.3 ± 1.8 kg/m^2) and five obese men (age, 27.8 ± 4.8 years; BMI, 38.9 ± 4.8 kg/m^2) during their regular energy consumption and the last 24 hours of a 96-hour fasting period. GH plasma levels were determined at 10-minute intervals and glucose level was measured every 20 minutes. GH pulse analysis was performed with three different algorithms. Insulin-like growth factor-I (IGF-I), IGF-II, IGF-binding proteins (IGFBP-1, -2, and -3), and IGF-binding capacity (IGF-BC) were evaluated in samples collected at 7:00 AM, 3:00 PM, and 11:00 PM. Twenty-four-hour mean GH was basally higher in normal subjects (1.1 ± 0.6 mU/L) than in overweight subjects (0.4 ± 0.2, $P < .01$ *v* normal). The significant fasting-induced GH increase in normal-weight men (to 5.6 ± 2.2 mU/L, $P < .05$ *v* basal) was inversely related to BMI ($r = -.86$, $P = .0006$). GH pulse amplitudes but not frequencies were different for both groups and were increased by fasting in normal subjects but not in obese subjects. Plasma glucose showed comparable mean levels basally, and fasting induced a more pronounced decrease of mean glucose in normal men (−1.9 ± 0.6 *v* −1.4 ± 0.3 mmol/L in obese) with a negative correlation between changes in mean GH and glucose ($r = -.70$, $P = .022$). IGF-I concentrations were similar in both groups and were unchanged with energy restriction, whereas IGF-II showed higher basal and fasting-associated values in obese subjects. The IGF-BC increased significantly during fasting in normal-weight men. In conclusion, nutritional regulation of GH release is associated with a modulation of GH pulse amplitudes, whereas GH pulse frequencies remain unchanged. These results suggest a stable pulse generation with amplitude modifications by direct substrate actions and/or metabolic influences of the somatostatinergic tone. Variations of free IGF-I and IGF-II are involved in the differential GH regulation of lean and obese subjects in periods of regular food intake. During short-term fasting, somatotrope GH release may also be influenced by changes in plasma glucose levels.

GROWTH HORMONE (GH) secretion is affected by numerous metabolic influences and has been found to be altered in obesity, but it is still controversial whether these endocrine alterations indicate sequelae of a long-term excessive energy consumption or represent neuroendocrine disturbances that may be potential pathogenetic factors for the development of obese states.[1-3]

An obesity-associated reduction of basal plasma levels and impairment of stimulated GH release were described in various clinical studies.[3-7] Since the secretory capacity of somatotropes was unaltered in obese subjects[8] and most of the disturbances of GH secretion were normalized with substantial weight loss,[9] the alterations probably indicate secondary changes due to a prolonged overnourishment. However, with regard to the lipolytic activity of GH and its potent effects on body fat distribution, the blunted release might perpetuate the obese state. The underlying mechanisms of obesity-induced hormonal changes are not fully elucidated.Since somatotrope GH release was found to be modulated by several peripheral factors, metabolic regulation via insulin-like growth factor-I (IGF-I),[10] free fatty acids (FFA),[11] and/or glucose[12,13] has been proposed as a possible explanation.[3,14,15]

To further evaluate the obesity-associated changes of pulsatile GH release, 24-hour temporal patterns were studied in normal-weight and obese young men during regular food intake and in the last day of a 96-hour fasting period. Additionally, we analyzed various metabolic factors that may be involved in the regulation of GH secretion during these metabolic states.

SUBJECTS AND METHODS

Study Design

Five young men with severe obesity (mean body mass index [BMI], 38.9 kg/m^2; range, 32.8 to 45.8) were compared with five age-matched normal-weight men (mean BMI, 24.3; range, 21.6 to 27.0) under two metabolic conditions in a crossover design. All volunteers had been carefully examined to exclude underlying diseases and gave written consent to the study design, which was approved by the local ethics committee. Subjects were admitted to our unit at noon, and a central venous catheter was inserted. At 6:00 PM, we started blood sampling at 10-minute intervals for 24 hours. Meals were eaten ad libitum during this basal metabolic period. After an interval of 3 to 6 weeks, the volunteers were admitted again and started fasting at 6:00 PM. All men remained on the ward for 4 days, where fasting states were controlled by measuring levels of ketone bodies in the urine daily. Blood was collected in the last 24 hours of energy restriction. During blood sampling, the lighting was dimmed between 11:00 PM and 6:00 AM and the subjects were allowed to sleep, which was monitored by continuous electroencephalography during the night.

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Measurements and Assays

We determined body composition by the bioimpedance method using the Akern-RJL-BIA 101/S (RJL-Systems, Detroit, MI). All hormones were analyzed in duplicate by specific assays. GH level was measured every 10 minutes with a sensitive immunoradiometric assay (hGH Coatria, Bio Mérieux, Marcy l'Etoile, France) with a detection limit of 0.02 mU/L. Intraassay and interassay variation coefficients were 2.5% and 7.1% for low values and 6.0% and 8.5% for high values, respectively. For statistical analysis, undetectable sample measures were assigned a value of 0.02 mU/L.

IGF-I, IGF-II, IGF-binding proteins ([IGFBP]-1, -2, and -3), and total IGF-binding capacity (IGF-BC) were analyzed in three blood samples (taken at 11:00 PM, 7:00 AM, and 3:00 PM, respectively). Serum IGF-I level was measured by an IGFBP-blocked radioimmunoassay (RIA) using a polyclonal antiserum[16] (kind gift of Drs B. Breier and P. Gluckman, University of Auckland, New Zealand) as described elsewhere.[17] This was a high-affinity antibody (half-maximal displacement at 50% B/B_0, 1.1 μg/L) with high specificity (cross-reactivity with IGF-II, <0.05%). Serum samples were diluted 1:150 in an acidic phosphate buffer to dissociate IGFs from IGFBPs. Upon reneutralization, the interference of IGFBPs in the RIA could be completely blocked by performing the assay in the presence of a large excess of IGF-II (25 ng per tube). Intraassay and interassay coefficients of variation were 3.1% and 8.1%, respectively. IGF-II was determined by a specific RIA using a polyclonal antiserum against the C-domain of hIGF-II.[18] Serum samples were extracted by acid-ethanol. Residual IGFBPs in the extract were blocked by adding an excess of IGF-I (25 ng per tube) to the assay mixture as described previously.[18] IGFBP-1,[19] IGFBP-2,[20] and IGFBP-3[21] levels were measured by specific RIAs as previously reported. The free IGF-BC was determined as follows: Duplicates of 200 μL serum were incubated with ^{125}I-IGF-II (30,000 cpm/100 μL phosphate-buffered saline, 0.25% bovine serum albumin) at room temperature for 2 hours. Unbound IGF-II tracer was then removed by incubation with excess IGF-II antiserum[18] (1:50 in 100 μL phosphate-buffered saline, 0.25% bovine serum albumin) for 1 hour and by sheep anti–rabbit IgG (IBL, Hamburg, Germany; 1:150 in 600 μL 4% PEG 6000) on ice for 40 minutes. After centrifugation (20 minutes at 4,000×; *g*), IGFBP-bound radioactivity in the supernatant was measured and referred to a normal human serum pool. One unit was defined as the IGF-II-BC of 1 mL of the serum pool.[19]

Glucose was analyzed every 20 minutes by the hexokinase/glucose-6-phosphate dehydrogenase method (Gluco-quant, Boehringer Diagnostica, Mannheim, Germany). Intraassay and interassay coefficients of variation were 1.3% and 1.6%, respectively.

GH Pulse Analysis

GH pulses were detected by three different approaches. As an heuristic method, we used the Cluster program[22]; in the present analysis, a 2 × 2 cluster configuration (two data points for a nadir and two for a peak) and a *t* statistic of 2.32/1.0 were applied. Alternatively, the Pulsar program developed by Merriam and Waechter[23] was used (G1 = 4.4, G2 = 2.6, G3 = 1.92, G4 = 1.46, and G5 = 1.13). Finally, we performed a deconvolution technique (Desade),[24] which is based on a one-compartment model. Although recent studies have demonstrated obesity-related variations in the metabolic rate of GH,[7] a single decay rate of 20 minutes for GH was used in this deconvolution analysis as an approximative estimation.[25]

Statistics

Data are presented as the mean ± SD and were calculated with the True Epistat software program (Epistat, Richardson, TX). The Wilcoxon sign-rank test (Table 2) and unpaired and paired *t* tests (Tables 1 and 3) were performed to determine differences between normal-weight and obese groups and between different metabolic states. We calculated Pearson's correlation coefficient for the association of different variables to GH changes and tested the significance of the correlation using the method of Bonferroni and Holm.

RESULTS

Clinical Data and Laboratory Values

Lean and obese men were comparable in age and body height, but were significantly different in BMI and body fat mass (Table 1). Body fat mass as measured by bioelectric impedance analysis showed a close correlation with BMI ($r = .92$ under the basal condition, $P < .001$).

Differences in FFA, cholesterol, and triglycerides between both groups were insignificant during the normal diet, but the decrease in triglyceride values was more pronounced in normal-weight men, who were becoming different from the obese with fasting (Table 1).

Pulsatile GH Secretion

During regular food intake, mean GH concentrations were higher in lean than in obese subjects, increasing significantly with energy restriction in the former group only (Table 1, Figs 1A and B). Fasting-induced changes in

Table 1. Comparison of Clinical Data and Laboratory Values of Five Normal-Weight and Five Obese Men During Regular Food Intake (basal) and in the Last 24 Hours of a 96-Hour Fasting Period

Parameter	Normal-Weight Men		Obese Men	
	Basal	Fasting	Basal	Fasting
Age (yr)	29.8 ± 4.9		27.8 ± 4.8	
Height (cm)	180 ± 5.0		184 ± 4.1	
BMI (kg/m²)	24.3 ± 1.8	23.7 ± 1.8	38.9 ± 4.8‡	38.0 ± 4.8‡
Fat mass (% of body mass)	14.5 ± 1.5	15.2 ± 3.3	38.2 ± 4.7‡	36.8 ± 6.3‡
Mean GH (mU/L)	1.08 ± 0.55	5.58 ± 2.15*	0.41 ± 0.20†	0.76 ± 0.47†
Mean glucose (mmol/L)	5.40 ± 0.41	3.55 ± 0.63*	5.56 ± 0.44	4.12 ± 0.58*
Insulin (mU/L)	8.92 ± 6.96	3.08 ± 1.93	13.92 ± 7.24	7.32 ± 4.95
FFA (mmol/L)	0.61 ± 0.35	0.94 ± 0.21	0.82 ± 0.29	0.92 ± 0.17
Cholesterol (mmol/L)	5.10 ± 1.24	4.83 ± 0.83	4.95 ± 0.26	4.33 ± 0.78
Triglycerides (mmol/L)	1.86 ± 1.25	0.85 ± 0.41	1.93 ± 0.48	1.61 ± 0.27‡

*$P < .001$ *v* basal.

†$P < .01$, ‡$P < .001$: *v* normal-weight men.

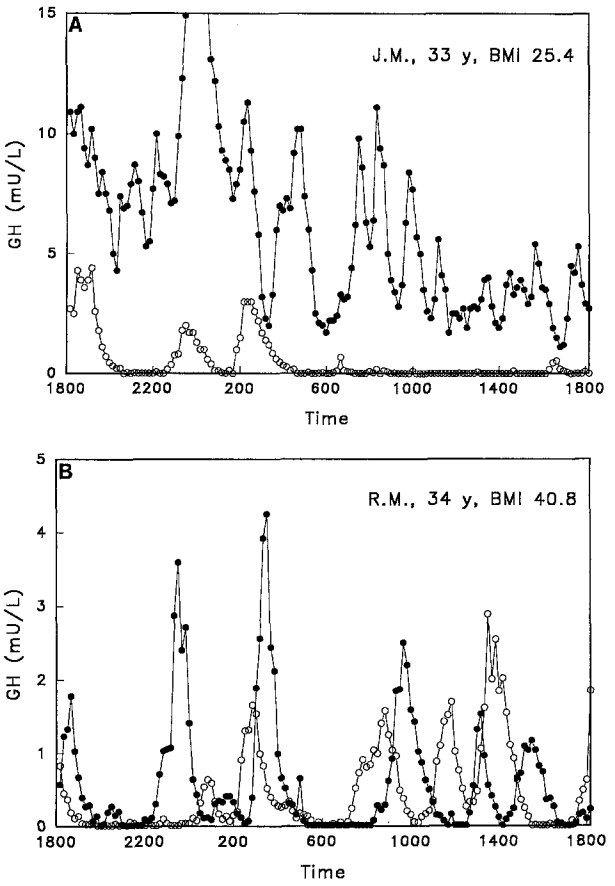

Fig 1. Typical temporal patterns of 24-hour pulsatile GH release in a lean man (A) and an obese man (B). (○) Basal metabolic conditions; (●) the last 24 hours of a 96-hour fasting period. Note the different scales for GH values in A and B.

mean GH plasma levels were inversely related to BMI ($r = -.86$, $P = .0006$). Determination of GH pulse frequency showed comparable results for both groups with all three detection algorithms; fasting had only small or no influences on pulse frequency (Table 2). However, lean men had higher GH pulse amplitudes than obese men during normal energy supply; GH amplitudes were further enhanced with fasting in the former group and were almost unchanged in the latter (Table 2). The numbers of GH pulses did not show any circadian variation (basal conditions: 0.5 ± 0.1 pulses per hour during sleep [11:00 PM to 6:00 AM] and 0.5 ± 0.2 pulses per hour during waking hours [6:00 AM to 11:00 PM]), but there was a sleep-associated increase of GH pulse amplitudes in the normal-weight group during basal energy consumption (5.5 ± 3.2 mU/L at night *v* 1.8 ± 1.4 during daytime, $P < .05$).

IGFs, IGFBPs, and IGF-BC

Under basal nutritional conditions and energy restriction, IGF-II but not IGF-I plasma concentrations were significantly higher in obese as compared with normal-weight men (Table 3). Four-day fasting had only insignificant effects on IGF-I levels, whereas IGF-II decreased in both groups. IGFBPs (-1, -2, and -3) showed comparable values during basal conditions. IGFBP-1 increased with energy restriction, with the increase being significant in lean men, whereas IGFBP-2 had a tendency to increase in normal men and to decrease in obese men (Table 3). We found a significant correlation between fasting-induced GH changes and IGFBP-1 ($r = .86$, $P = .0007$). IGF-BC was higher in lean men, increasing further during energy restriction in this group only (Table 3).

Plasma Glucose and Insulin Levels

Despite the large range of body weight, both groups had similar 24-hour mean concentrations of plasma glucose during regular food intake, with small interindividual variations (4.9 to 6.2 mmol/L, Table 1). Normal-weight men had a larger fasting-dependent decrease of mean glucose levels (−1.85 ± 0.58 mmol/L) than obese men (−1.44 ± 0.31 mmol/L, NS *v* normal). The increase of GH levels was closely related to the reduction of mean glucose concentrations ($r = .70$, $P = .022$; Fig 2).

Insulin levels measured at midnight showed large interindividual variations. Insulin plasma concentrations seemed to be higher in obese men and decreased during fasting in both groups, but the differences were insignificant (Table 1). Changes in insulin concentrations were not significantly correlated with the increase in GH ($r = -.40$, $P = .268$). However, a closer analysis of 24-hour insulin patterns has not been performed in this study.

Table 2. Comparison of GH Pulse Frequencies and Amplitudes in Five Normal-Weight and Five Obese Men During Normal Food Intake (basal) and the last 24 Hours of a 96-Hour Fasting Period

	Normal-Weight Men		Obese Men	
	Basal	Fasting	Basal	Fasting
GH pulse frequency (no. per 24 h)				
Pulsar	10.0 ± 2.0	14.6 ± 4.0	11.6 ± 5.7	13.6 ± 2.7
Cluster	11.4 ± 4.0	12.8 ± 1.6	10.6 ± 2.2	12.4 ± 1.5
Desade	11.8 ± 3.4	12.8 ± 1.8	15.4 ± 3.2	11.2 ± 3.7
GH pulse amplitude/peak height (mU/L)				
Pulsar	2.58 ± 1.47	5.88 ± 1.16*	1.14 ± 0.50	1.58 ± 0.63†
Cluster	2.44 ± 1.53	9.08 ± 2.80*	1.25 ± 0.72	1.75 ± 0.93†
Desade	4.60 ± 2.95	10.64 ± 3.05*	1.28 ± 0.67	2.18 ± 0.42‡

*$P < .01$ *v* basal.

†$P < .05$, ‡$P < .01$: *v* normal-weight men.

Table 3. Mean IGF-I, IGF-II, IGFBPs (-1, -2, and -3), and IGF-BC in Five Normal-Weight and Five Obese Men During Normal Food Intake (basal) and in the last 24 Hours of a 96-Hour Fasting Period

	Normal-Weight Men		Obese Men	
	Basal	Fasting	Basal	Fasting
IGF-I (μg/L)	173 ± 38	144 ± 54	146 ± 40	153 ± 53
IGF-II (μg/L)	623 ± 74	518 ± 92*	788 ± 125‡	727 ± 120‡
IGFBP-1 (μg/L)	14 ± 6	50 ± 18†	10 ± 2	19 ± 13§
IGFBP-2 (μg/L)	449 ± 153	505 ± 129	304 ± 138	275 ± 123§
IGFBP-3 (μg/L)	3,260 ± 399	2,962 ± 425	3,562 ± 312	3,167 ± 281*
IGF-BC (U/L)	520 ± 34	581 ± 43*	454 ± 32§	460 ±56‖

NOTE. Mean values are calculated from three samples collected at 7:00 AM, 3:00 PM, and 11:00 PM.

*$P < .05$, †$P < .001$: *v* basal.

‡$P < .05$, §$P < .01$, ‖$P < .01$: *v* normal-weight men.

DISCUSSION

Our study confirms previous data demonstrating reduced mean plasma GH levels in obesity.[3,5-7] Since GH secretion increases in lean but not in obese subjects with short-term fasting, the differences become even more pronounced during energy restriction.[11] Although the effects of obesity and short-term dietary manipulations on pulsatile GH release have recently been analyzed in a variety of clinical studies,[3,7,10,15,26] the present evaluation reveals different results. In our study, obesity-associated alterations of GH secretion, as well as fasting-induced changes, depended on a modulation of GH pulse amplitudes without any differences of pulse frequencies. In contrast, others proposed much lower numbers of GH pulses under basal conditions in obesity, suggesting a reduction of the pulse frequency in this metabolic state[5,7,10] and an increase of pulse numbers during fasting, as well.[15,26] Since recent studies using highly sensitive GH assays detected a significant amount of small micropulses in basal secretion periods of healthy adults[27,28] and adolescents,[29] the calculations based on less-sensitive detection methods might underestimate the number of micropulses becoming evident by amplitude enhancement during food restriction.

A negative feedback of IGF-I was claimed to be an important regulatory factor for GH during dietary manipulations[26,30] but previous studies generated controversial results.[13,15,26,31,32] A fasting-induced decrease of IGF-I levels found in normal subjects[10,13,31] was not seen in obesity.[13,32] However, in the present study, IGF-I plasma concentrations were similar in both groups and remained almost unchanged with short-term fasting. Dietary restriction induced an increase of IGFBP-1 and -2, which was less pronounced in the obese group. Additionally, IGF-BC was higher in lean than in overweight men, increasing further in the former group during fasting. Since variations of IGFBPs have been proposed to influence the biologic activity of IGF-I at the tissue level,[33] our results indicate changes of free IGF-I despite almost-constant values of total IGF-I. Hence, the fasting-associated increase of GH release might be related to a reduction of the biologically active fraction of IGF-I. Obese men also had higher IGF-II levels under a normal diet and a smaller fasting-induced reduction than normal-weight subjects. In view of recent experimental data demonstrating a synergistic central inhibitory action of IGF-I and IGF-II on GH secretion,[34] our data support a pivotal role of IGFs in the differential GH response under various nutritional conditions.

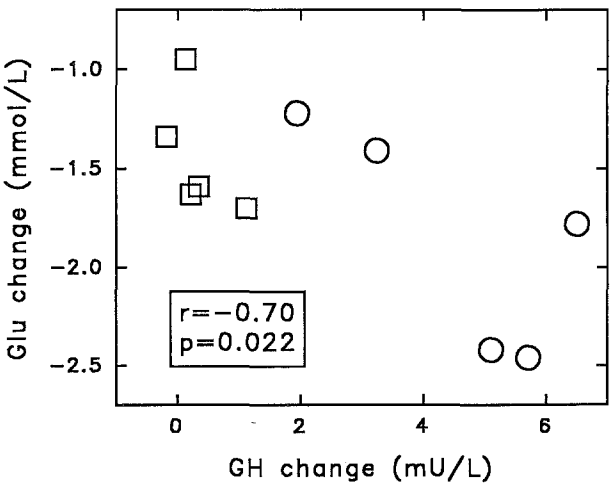

Fig 2. Relation of fasting-induced changes in 24-hour mean glucose and plasma GH concentrations in 5 lean (○) and 5 obese (□) men.

FFA have been found to inhibit GH secretion.[35,36] Since FFA concentrations were demonstrated to be higher in obese than in normal-weight men,[37,38] this difference may contribute to the obesity-associated GH suppression under basal nutritional conditions. However, during energy restriction, plasma GH levels increase in normal men despite the accompanying elevation of circulating FFA.[26,38] Therefore, a significant influence of FFA is unlikely to explain the hormonal changes during short-term fasting.

Although hypoglycemia is known to be a stimulus of GH release and glucose was proposed as an important factor associated with impaired neuroendocrine functions in obesity,[39] the significance of glucose in the metabolic regulation of GH has not been elucidated thus far. Plasma glucose levels were demonstrated to be higher in obese than in normal-weight subjects[32,40] and to decrease substantially in the latter group during fasting.[11,15] In the present study, 24-hour mean glucose levels were comparable during normal energy supply, tending to decrease more in normal-weight men. The negative correlation between the reduction of glucose and the increase of GH secretion indicates a

role for glucose in the short-term metabolic regulation of GH release and might account for the differential responses to energy restriction. However, this relation does not explain the blunted GH secretion in obese men during regular food intake.

Although insulin was shown to suppress GH release at the pituitary level,[41] hyperinsulinemia per se might not contribute to the reduced GH levels in obesity.[42] However, we could not establish significant correlations of insulin and GH in this study, and the influence of insulin on GH secretion during metabolic changes will remain elusive until detailed analyses have been performed.

Whereas the GH-releasing hormone–induced generation of GH pulses seems to remain stable during various metabolic states, nutritional regulation of GH secretion involves alterations of pulse amplitudes via direct pituitary actions and/or changes in the somatostatinergic tone.[4,43] Variations in glucose and the biologically active IGF-I and IGF-II seem to play the key role in the metabolic regulation of somatotrope activity.

The extent of changes during food restriction depends on body fat mass. Due to a larger reservoir of endogenous energy substrates, metabolic homeostasis seems more resistant to food depletion in chronically overnourished subjects than in normal ones. Therefore, the alterations of GH release appear to be less pronounced in obesity.

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