Serum Leptin Is Increased in Growth Hormone–Deficient Adults: Relationship to Body Composition and Effects of Placebo-Controlled Growth Hormone Therapy for 1 Year

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The gene product from the *ob* gene, leptin, has recently been characterized in humans. The circulating level of leptin is related to body mass index (BMI) and more closely to estimates of total body fat, whereas visceral fat has been reported to be of minor importance. However, it is unknown if leptin is directly regulated by hormones that influence substrate metabolism and body composition. We studied leptin in adult growth hormone (GH)-deficient (GHD) patients substituted with GH treatment for 12 months in a parallel double-blind, placebo-controlled study. Twenty-seven GHD adults aged 44.9 ± 1.9 years underwent anthropometric measurements for determination of regional and total body fat (BMI, waist to hip ratio [WHR], computed tomographic [CT] scan, dual-energy x-ray absorptiometry [DEXA] scan, and bioimpedance analysis [BIA]) before and after 12 months of placebo-controlled GH substitution (2 IU/m^2) in a parallel design. The same measurements were performed in 42 healthy adults aged 39.1 ± 1.7 years. The logarithm of serum leptin levels correlated positively with abdominal subcutaneous fat and total body fat (BIA and DEXA) in untreated GHD patients and healthy subjects. Fasting insulin did not correlate with leptin levels in either of the groups. After 12 months of GH administration, the body composition of GHD patients was significantly changed with respect to a marked decrease in body fat. The relations of leptin to the estimates of body fat were maintained, and leptin was furthermore related to BMI and fasting insulin. In multiple linear regression analyses, additional estimates of visceral adiposity (intraabdominal fat and maximal anterior-posterior diameter determined by CT scan) were significant determinants of leptin in the healthy subjects. The increase in fasting insulin levels during GH substitution correlated negatively with the reduction in leptin levels ($r = -.823$, $P = .003$). At baseline, leptin levels were increased in the patients compared with controls in both sexes (women, 21.8 ± 3.3 *v* 11.3 ± 1.4 ng/mL, $P = .002$; men, 8.1 ± 1.2 *v* 4.7 ± 0.7 ng/mL, $P = .008$). Leptin levels were similar in GHD patients treated for 12 months compared with healthy controls for both women and men (women, 15.9 ± 2.3 and 11.3 ± 1.4 ng/mL, $P = .163$; men, 7.1 ± 2.8 and 4.7 ± 0.7 ng/mL, $P = .759$). In healthy adults and in GHD patients, leptin levels were significantly higher in women than in men (11.3 ± 1.4 *v* 4.7 ± 0.7 ng/mL, $P < .001$; 21.8 ± 3.3 *v* 8.1 ± 1.2 ng/mL, $P < .001$). Gender remained a significant determinant of leptin levels in several models of multiple linear regression analysis also including age, estradiol levels, insulin, and estimates of body fat. We conclude that leptin is increased but not differently regulated in GHD patients compared with normal subjects, and that leptin levels are closely related to estimates of body fat. This relationship is maintained during a decrease in body fat due to GH substitution.
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LEPTIN is thought to be a weight-regulating protein produced by the *ob* gene in adipocytes,[1-4] and receptors for leptin have been described in the choroid plexus in the brain,[5] but the mode of function has yet to be demonstrated in humans. In healthy adults and in insulin-dependent and non–insulin-dependent diabetic patients, leptin is positively correlated with the body mass index (BMI).[1,6-8] Leptin levels also correlate with more specific estimates of body fat.[1,9-11] So far, insulin, food intake, and body fat stores appear to increase leptin concentrations. In growth hormone (GH)-deficient (GHD) adults, body composition is markedly changed and becomes partly normalized during GH substitution.[12-20] It is unknown if a correlation between leptin and body fat is also attained in GHD patients with different body composition compared with normal subjects. Furthermore, the relation between GH secretory reserve and leptin is unknown. GH is a powerful stimulator of lipid oxidation and total energy expenditure[21-25] and also regulates body composition. Moreover, GH treatment is associated with an increase in circulating insulin, all of which makes it difficult to predict the impact of GH on leptin secretion. We therefore measured fasting serum leptin levels in GHD adults during 12 months of GH substitution therapy in a placebo-controlled, double-blind parallel design, and correlated the levels with various estimates of regional and total body fat. Furthermore, GH secretion, body composition, and leptin levels were determined in an age- and gender-matched group of healthy subjects.

SUBJECTS AND METHODS

Twenty-seven GHD adults and 42 healthy adults participated in the study, which was approved by the regional Ethics Committee and the National Board of Health. The participants provided informed consent.

The diagnosis of GHD was based on a maximum peak GH of less than 10 μg/L during an insulin tolerance test (blood glucose ≤ 2.0 mmol/L) performed within 12 months before inclusion in the study. When required, patients received additional replacement therapy with desmopressin, estradiol, hydrocortisone, L-thyroxine, and testosterone. The study in GHD patients was performed in a parallel randomized, double-blind, placebo-controlled trial. Norditropin/placebo (Novo Nordisk, Copenhagen, Denmark) was administered as daily subcutaneous injections at a dose of 2 IU/m^2 for 12 months. Anthropometric measurements were performed before and after 12 months of substitution therapy. The amount of intraabdominal (visceral) fat and abdominal subcutaneous fat and the maximal anterior-posterior abdominal diameter were evaluated by computed tomography (CT) with a Somatom Plus-S scanner (Siemens, Erlangen, Germany). The areas scanned were 10-mm cross-sectional slices at the midthigh and at the umbilical level using 120 kV and 330 mA. All scans were performed by the same technician and analyzed by the same radiologist. Percentage body fat was measured by two different methods: dual-energy x-ray absorptiometry (DEXA) using a QDR-2000 densitometer (Hologic, Waltham,

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Submitted October 2, 1996; accepted January 8, 1997.

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0026-0495/97/4607-0017$03.00/0

MA), and by measuring whole-body resistance (bioelectric impedance analysis [BIA]) using the BIA 101 (RJL Systems, Detroit, MI). Percentage body fat was calculated by the software supplied with the BIA 101. In addition, BMI and waist to hip ratio (WHR) were measured. In the group of healthy controls, 24-hour GH secretion was evaluated by deconvolution analysis as performed by Veldhuis et al.[26] Blood samples were collected every 20 minutes. Indirect calorimetry (Deltatrac; Datex Instrumentarium, Helsinki, Finland) was performed for 20 minutes in the basal state to assess energy expenditure. Further anthropometric measurements were performed as already described.

All analyses were performed in serum stored at −20°C. Leptin was determined by a commercial radioimmunoassay (RIA) (Linco, St Louis, MO). Insulin analyses were performed with a RIA as previously described.[27] Insulin-like growth factor-I (IGF-I) was determined by an in-house RIA.[28] GH binding protein (GHBP) was determined by a newly developed time-resolved fluoroimmunoassay.[29] Data on the body composition and characterization of the patients have been previously described.[30,31]

Statistics

Differences between groups were tested with Student's *t* test when data were normally distributed. Otherwise, the Mann-Whitney test was performed. For evaluating differences in leptin levels, the Student-Newman-Keuls method of multiple comparisons was performed when data were normally distributed; otherwise, Dunn's method was used. Simple linear and multiple linear regression analyses were used to relate variables. P values less than .05 were considered significant. In multiple linear regression analyses, a protected *P* value less than .05/(number of independent variables) was considered significant. Forward stepwise regression analysis was performed to evaluate the contributions of independent variables to the determination of leptin levels. A *P* value of .05 was used as a criterium for entry at each step. In simple, multiple linear, and forward stepwise regression analyses, the logarithmically transformed leptin concentrations were included to obtain normality. Results are expressed as the mean ± SEM unless otherwise stated.

RESULTS

Characteristics of the GHD patients and healthy controls are shown in Tables 1 and 2. Body fat estimated as BMI, WHR, intraabdominal fat, subcutaneous abdominal fat, and total body fat estimated by DEXA scan were significantly increased in GHD patients compared with normal subjects. Furthermore, IGF-I levels were decreased, whereas fasting insulin levels were similar in the two groups. After 12 months of GH substitution, body fat was significantly reduced in terms of intraabdominal fat, subcutaneous fat, and relative amounts of fat estimated by BIA and DEXA scans compared with values in the placebo group, whereas fasting insulin was increased (Table 1).

Serum leptin levels correlated positively with indices of adiposity in GHD patients at baseline: abdominal subcutaneous fat ($r = .721$, $P < .001$) and body fat by BIA ($r = .754$, $P < .001$) and DEXA ($r = .745$, $P < .001$). There was no correlation between leptin and BMI, WHR, or intraabdominal fat estimated by CT scan in the patient group ($r = .302$, $P = .148$; $r = .192$, $P = .358$; and $r = .363$, $P = .075$). Fasting insulin levels were not correlated with leptin ($r = .343$, $P = .094$). After 12 months of GH administration, the relations of leptin levels to the estimates of body fat were maintained in both the GH-treated group and the placebo group, and leptin was now related to BMI ($r = .672$, $P = .017$) and fasting insulin in the GH-treated group ($r = .776$, $P = .003$). Fasting insulin levels were significantly increased as compared with the placebo group, and the increase in insulin levels correlated inversely as with the reduction in leptin levels following 12 months of GH substitution ($r = -.823$, $P = .003$).

Table 1. Characteristics of the GHD Patients

Characteristic	Baseline	GH (12 mo)	Placebo (12 mo)
Sex (women/men)	9/18	4/9	5/9
Age (yr)	44.0 ± 1.8	—	—
BMI (kg/m²)	27.1 ± 0.7‡	27.5 ± 1.0	26.7 ± 0.95
IGF-I (μg/L)	86 ± 11.8‡	275 ± 29	89 ± 19‖
WHR	0.91 ± 0.01‡	0.92 ± 0.02	0.92 ± 0.01
Intraabdominal fat (cm²)	154.0 ± 11.3‡	120 ± 20	159 ± 15¶
Subcutaneous fat (cm²)	253 ± 18.3†	221 ± 37	239 ± 21‖
DEXA fat (%)	27.9 ± 1.4*	21.4 ± 2.5	27.9 ± 1.7¶
BIA fat (%)	26.1 ± 1.5	20.0 ± 2.7	28.0 ± 1.7¶
Insulin (mU/L)	10.5 ± 1.3	15.7 ± 2.6	9.9 ± 1.5§
Leptin (ng/mL)	21.8 ± 3.3†	15.9 ± 2.3	20.2 ± 3.6
	8.08 ± 1.2†	7.1 ± 2.8	7.98 ± 2.1

NOTE. Data are the mean ± SEM.

Significant difference between controls (see Table 2) and GHD patients at baseline: *$P < .05$, †$P \leq .01$, ‡$P \leq .001$.

Significant difference between 12-mo GH-treated group and placebo group: §$P < .05$, ‖$P \leq .01$, ¶$P \leq .001$.

In the control group, there was a positive correlation with the same estimates of body fat as in the group of patients: abdominal subcutaneous fat ($r = .594$, $P < .001$) and body fat by BIA ($r = .843$, $P < .001$) and DEXA ($r = .829$, $P < .001$). BMI, WHR, and intraabdominal fat were not correlated with leptin levels ($r = .278$, $P = .079$; $r = -.10$, $P = .536$; and $r = .149$, $P = .40$). Fasting insulin levels were not correlated with leptin ($r = .197$, $P = .218$). In normal subjects, leptin levels were not associated with any estimates of spontaneous GH secretion evaluated by deconvolution analysis of 24-hour GH secretion (mean 24-hour GH, production rate, mass of GH secreted, half-life, or basal secretion) or with arginine-stimulated GH secretion (data not shown). GHBP was also positively correlated with leptin levels in a linear regression analysis in the control subjects ($r = .426$, $P = .006$), but the significance disappeared in a multiple linear regression analysis including estimates of body fat.

At baseline, leptin levels were increased in GHD patients compared with controls for both sexes (women, 21.8 ± 3.3 *v* 11.3 ± 1.4 ng/mL, $P = .002$; men, 8.08 ± 1.2 *v* 4.66 ± 0.7 ng/mL, $P = .008$; Fig 1). After 12 months of GH substitution, leptin levels were no longer different from the levels in healthy controls in either sex (women, 15.9 ± 2.3 *v* 11.3 ± 1.4 ng/mL, $P = .163$; men, 7.1 ± 2.8 *v* 4.7 ± 0.7 ng/mL, $P = .759$), even though leptin levels did not significantly decrease in the GH-treated group compared with the placebo group (GHD: women *v* men, 2.02 ± 3.6 *v* 7.98 ± 2.1 μg/L, $P = .42$).

In healthy adults and GHD patients, leptin levels were significantly higher in women than in men (controls, 11.3 ± 1.4 *v* 4.7 ± 0.79 ng/mL, $P < .001$; GHD, 21.8 ± 3.3 *v* 8.1 ± 1.2 ng/mL, $P < .001$; Fig 1). In the group of normal controls, WHR, intraabdominal fat, maximal anterior-posterior diameter, and relative amount of body fat estimated by BIA and DEXA scan were different in women and men (Table 2). Nevertheless,

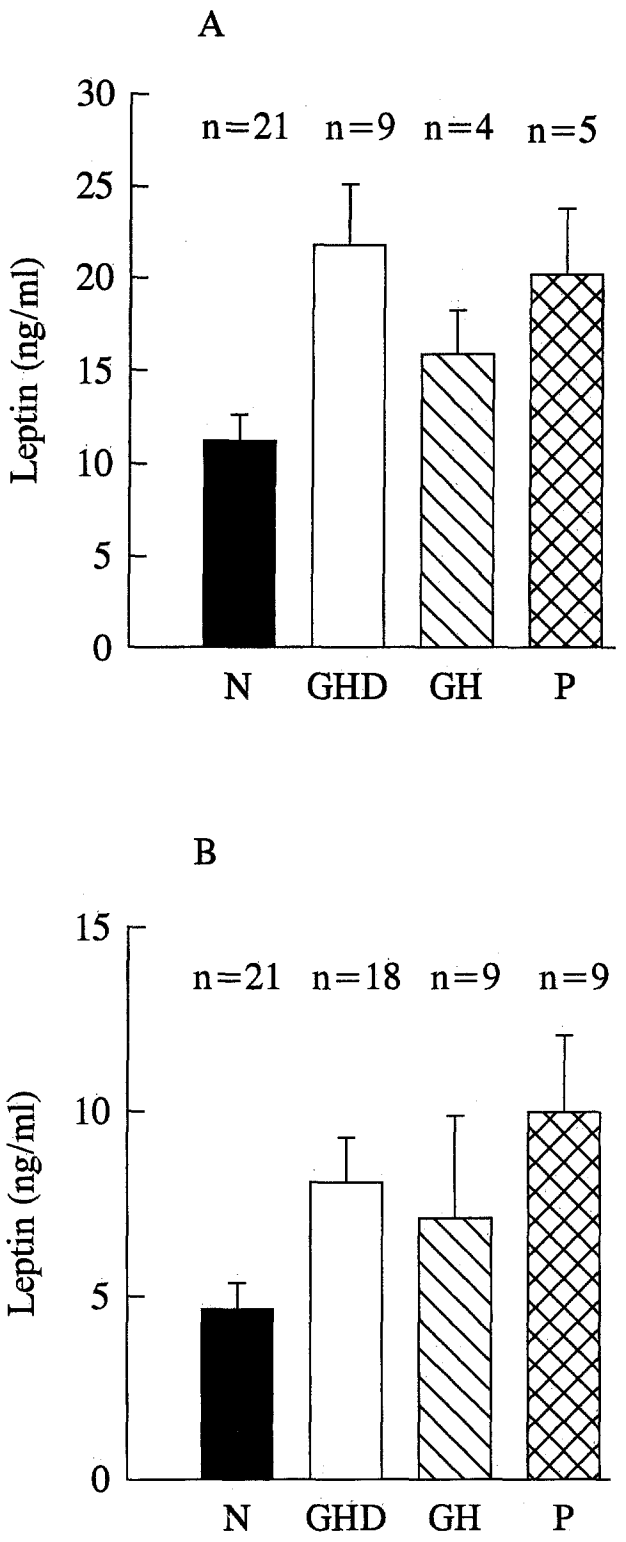

Fig 1. Serum leptin levels in (A) women and (B) men. N, healthy controls; GH, 12-month GH-substituted patients; P, placebo group. Leptin levels were increased in GHD patients at baseline compared with healthy subjects: A, $P = .002$; B, $P = .008$. Leptin levels were increased in women compared with men: N, $P < .001$; GHD, $P < .001$.

gender remained a significant independent determinant of leptin levels in most multiple linear regression models also including estimates of body fat, age, IGF-I, fasting insulin, and estradiol (data not shown). Furthermore, body fat estimated by BIA and DEXA scan, intraabdominal fat, and subcutaneous fat estimated by CT scan were independent significant determinants of serum leptin levels (data not shown), whereas IGF-I, fasting insulin, age, and estradiol did not contribute significantly to the prediction of leptin levels. Because of colinearity between different estimates of body fat, these variables were included in the multiple linear regression analyses one at a time. In the group of controls, estimates of GH secretory capacity were furthermore included in the analyses, but these estimates were not significant determinants of leptin levels. Analyzing the data from the controls plus the patients at baseline together and introducing a group variable showed an independent significance of the group variable in most models. In a forward stepwise regression analysis, it was demonstrated that 5.4% to 8.2% ($R^2 = .054$ to $.082$) of the variability in leptin levels could be explained by the group variable depending on the model applied. The majority of the variability was due to estimates of body fat, depending on the model (26.5% to 44.9%) (Table 3).

DISCUSSION

We have described serum leptin levels in GHD adults and in age- and gender-matched normal subjects. Furthermore, the effects of GH substitution in GHD patients were investigated. Leptin levels were increased in GHD patients and decreased to normal levels during 12 months of GH substitution. In both patients and healthy adults, leptin levels correlated with several indices of adiposity. The correlations with estimates of body fat were comparable in GHD patients and normal controls. Mul-

Table 2. Characteristics of the Normal Controls

Characteristic	Women (n = 21)	Men (n = 21)	All (n = 42)	P
Age (yr)	39.1 ± 2.4	39.1 ± 2.5	39.1 ± 17	.80
BMI (kg/m²)	23.1 ± 0.66	24.7 ± 0.73	23.9 ± 0.5	.093
IGF-I (μg/L)	153 ± 12	157 ± 9.6	156 ± 7.7	.685
WHR	0.81 ± 1.02	0.90 ± 0.02	0.85 ± 0.01	.001
Intraabdominal fat (cm²)	65.8 ± 10.4	121 ± 20	89.5 ± 11.2	.008
DEXA fat (%)	27.6 ± 1.45	19.3 ± 1.7	223.8 ± 1.3	<.001
BIA fat (%)	27.7 ± 0.9	19.1 ± 0.01	23.4 ± 1.0	<.001
Insulin (mU/L)	8.24 ± 0.74	12.7 ± 2.3	10.5 ± 1.25	.024
Leptin (ng/mL)	11.3 ± 1.4	4.66 ± 0.69	8.04 ± 0.94	<.001
Subcutaneous fat (cm²)	192 ± 18	167 ± 22	181 ± 14	.372
Max A:P (cm)	19.5 ± 0.58	22.1 ± 0.95	20.6 ± 0.56	.016
GHBP (nmol/L)	1.03 ± 0.08	1.01 ± 0.10	1.02 ± 0.06	.836
EE (mL/24 h)	1,422 ± 29	1,869 ± 53	1,646 ± 48	<.001
$\dot{V}o_2$max (mL/min/kg)	44.1 ± 2.8	45.6 ± 3.3	44.8 ± 2.09	.732
Estradiol (nmol/L)	0.19 ± 0.03	0.11 ± 0.00	0.15 ± 0.02	.077

NOTE. Data are the mean ± SEM. *P* values refer to significance levels of differences between the sexes.

Abbreviations: max A:P, maximum anterior-posterior abdominal diameter; $\dot{V}o_2$max, maixmum oxygen consumption; EE, energy expenditure.

Table 3. Forward Stepwise Regression Analyses With Five Independent Variables (dependent variable is leptin levels)

Independent Variable	Results
(1) Gender, (2) group, (3) IGF-I, (4) insulin, and (5a) fat (DEXA)	Step 1. fat (DEXA), R^2 = .449 Step 2. group, R^2 = .612 Step 3. gender, R^2 = .694
(5b) Subcutaneous fat	Step 1. subcutaneous fat, R^2 = .424 Step 2. gender, R^2 = .605 Step 3. group, R^2 = .659
(5c) Intraabdominal fat	Step 1. gender, R^2 = .258 Step 2. intraabdominal fat, R^2 = .542 Step 3. group, R^2 = .615
(5d) BMI	Step 1. gender, R^2 = .285 Step 2. BMI, R^2 = .550 Step 3. group, R^2 = .620

NOTE. Group is a dummy variable referring to GHD patients or control subjects. In each analysis, 5 variables are included; Estimates of body fat are included 1 at a time. $P < .001$.

tiple linear regression analysis showed that leptin levels depended on the group, ie, normal subjects versus patients, in addition to body fat and gender. Introduction of IGF-I levels did not eliminate the significance of the group variable. This relationship could suggest GH to be of some importance in the regulation of leptin levels. However, this is less probable, as we found no correlations between spontaneous and stimulated GH secretory capacity and leptin levels in normal subjects. The patients presumably differed further from the normal subjects due to chronic illness, additional pituitary lesions, and concurring medications. The decrease in leptin levels after GH substitution was probably caused by the decrease in body fat due to the lipolytic effect of GH.

The logarithm of serum leptin levels did not correlate with BMI in healthy adults or GHD patients at baseline. This finding is in contrast to previous reports.[6,11] The finding that BMI did not correlate with leptin levels might be partly due to the relatively small number of patients studied and to the fact that BMI is not a sensitive marker of adiposity in clinically non-obese subjects. It was observed in this study that leptin levels correlated better with more specific measures of adiposity (eg, body fat determined by DEXA scan and BIA, and subcutaneous abdominal fat and intraabdominal fat determined by CT scan). Leptin levels did not correlate with the amount of intraabdominal fat in a simple linear regression model, suggesting that abdominal/visceral fat is not of particular importance to leptin secretion, in accordance with previous reports.[11,32,33] It seems that the leptin level reflects the amount of total body fat and is not a marker of visceral fat stores, in contrast to the levels of fasting insulin and GHBP.[29,34] In most studies, GHBP correlates positively with BMI.[30,35,36] In the present study, leptin levels correlated only weakly with GHBP levels and GHBP did not remain significant in multiple linear regression, indicating that leptin is not influenced by GHBP. Since fasting insulin levels increase in adiposity, leptin levels would be expected to correlate with fasting insulin. However, we did not find any correlation in healthy subjects or GHD patients at baseline, suggesting that the two peptides are not coregulated in a simple manner, which is in contrast to previous reports.[10,33,37] In 12-month GH-treated GHD patients, fasting insulin increased significantly and correlated positively with leptin. The range of fasting insulin widened during GH substitution, which might have facilitated the demonstration of the positive relationship. Despite the increase in insulin levels after GH substitution, leptin levels decreased, as also illustrated by the negative correlation between the increase in insulin levels and the decrease in leptin levels. This could suggest that leptin reflects the amount of body fat and is not directly regulated by insulin. On the other hand, the observation of a negative correlation between the insulin increase and leptin reduction after GH therapy does not rule out that insulin per se may increase leptin secretion. Contrasting effects have been found concerning the acute influence of insulin on leptin levels in humans.[38-40] However, there is agreement that several hours of altered insulin levels are necessary to cause variations in leptin levels, suggesting that insulin regulates leptin levels indirectly through a trophic effect on adipocytes. Leptin levels were different in women and men both in the control group and in GHD patients. Gender remained a significant determinant of leptin levels in multiple linear regression analysis including estradiol and estimates of body fat, which is in contrast to previous findings in humans.[6,10,39] However, Rosenbaum et al[33] also found that gender remained significantly correlated with leptin levels when controlling for fat mass. In mice, gender has also been described as a determinant of leptin levels independently of body fat,[41] with female mice having higher leptin levels than male mice. In the control group, men had a significantly lower percentage of body fat (BIA and DEXA) but a significantly higher amount of intraabdominal fat than women, in accordance with previous studies.[42-44] Since leptin levels are increased in women compared with men, it emphasizes that total body fat rather than regional fat (intraabdominal fat) determines leptin levels.

In conclusion, serum leptin is increased in GHD patients and is related to estimates of body fat, as also seen in healthy adults. Furthermore, leptin decreases to normal levels in parallel with a significant decrease in body fat during substitution therapy with GH. We found no evidence of a direct effect of GH on the regulation of leptin levels.

ACKNOWLEDGMENT

The excellent technical assistance of Inga Bisgaard is gratefully appreciated. We thank Dr Kim Brixen, Aarhus Amtssygehus, for performing the DEXA scans, and technician Lisbeth Thingholm and Dr Anne Grethe Jurik, Aarhus Kommunehospital, for performing and analyzing the CT scans.

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