Changes in body composition and leptin levels during growth hormone (GH) treatment in short children with various GH secretory capacities

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Abstract

Objective: The aim of this study was to follow changes in body composition, estimated by dual-energy X-ray absorptiometry (DXA), in relation to changes in leptin during the first year of GH therapy in order to test the hypothesis that leptin is a metabolic signal involved in the regulation of GH secretion in children.

Design and Methods: In total, 33 prepubertal children were investigated. Their mean (s.d.) chronological age at the start of GH treatment was 11.5 (1.6) years, and their mean height was -2.33 (0.38) s.d. scores (SDS). GH was administered subcutaneously at a daily dose of 0.1 ($n = 26$) or 0.2 ($n = 7$) IU/kg body weight. Ten children were in the Swedish National Registry for children with GH deficiency, and twenty-three children were involved in trials of GH treatment for idiopathic short stature. Spontaneous 24-h GH secretion was studied in 32 of the children. In the 24-h GH profiles, the maximum level of GH was determined and the secretion rate estimated by deconvolution analysis (GH_t). Serum leptin levels were measured at the start of GH treatment and after 10 and 30 days and 3, 6 and 12 months of treatment. Body composition measurements, by DXA, were performed at baseline and 12 months after the onset of GH treatment.

Results: After 12 months of GH treatment, mean height increased from -2.33 to -1.73 SDS and total body fat decreased significantly by 3.0 (3.3)%. Serum leptin levels were decreased significantly at all time points studied compared with baseline. There was a significant correlation between the change in total body fat and the change in serum leptin levels during the 12 months of GH treatment, whereas the leptin concentration per unit fat mass did not change. In a multiple stepwise linear regression analysis with 12 month change in leptin levels as the dependent variable, the percentage change in fat over 12 months, the baseline fat mass (%) of body mass and GH_t accounted for 24.0%, 11.5% and 12.2% of the variability respectively.

Conclusions: There are significant correlations between changes in leptin and fat and endogenous GH secretion in short children with various GH secretory capacities. Leptin may be the messenger by which the adipose tissue affects hypothalamic regulation of GH secretion.

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Introduction

Growth hormone (GH) secretion is known to be low in obesity (1, 2). Even in individuals of normal stature, some of the variation in GH secretion is related to body composition (3). Abdenur *et al.* reported an inverse correlation between GH secretion and indices of adiposity in children with idiopathic short stature (ISS) (4). However, the reason why healthy children with a relatively large fat mass require less GH to maintain normal growth than children with less fat is not clear.

A logical candidate for conveying information from adipose tissue to the hypothalamus is the hormone

leptin, which is secreted by adipose tissue (5, 6) and regulates both appetite and energy expenditure at the hypothalamic level; leptin receptors are present in different hypothalamic nuclei (7). The circulating concentration of leptin is closely correlated with body fat mass in both humans and mice (8), obese humans having increased leptin concentrations. Furthermore, leptin levels decline in humans who lose weight (9). Several studies have reported that leptin administration increases plasma levels of luteinizing hormone, follicle-stimulating hormone, testosterone, and thyroxine in fasted mice, suggesting that leptin can play a role in the regulation of anterior pituitary hormone secretion (10–12).

The aims of the present study were to determine the relationship between changes in body composition and changes in leptin levels during the first year of GH therapy in children of various GH secretory capacities, and to investigate how GH treatment affects leptin levels and relationship to fat mass.

Subjects and methods

Subjects

In total, 33 children (5 girls and 28 boys) were investigated at the Children's Hospital (Göteborg, Sweden). All children were prepubertal during the study period. Puberty was assessed according to Tanner and Whitehouse (13) for breast and pubic hair development, and according to Zachmann *et al.* (14) for testicular volume, measured using an orchidometer. When adrenarche and gonadarche differed, the pubertal stage was rated according to the development of gonadarche: breast development in girls and testicular volume in boys. The mean (s.d.) chronological age at the start of GH treatment was 11.5 (1.6) years (range, 7.8 to 14.9 years), and the mean (s.d.) height was -2.33 (0.38) s.d. scores (SDS; range, -3.05 to -1.70 SDS). Height and weight at the time of the study were expressed as SDS compared with Swedish reference values (15). The index weight for height SDS was expressed in SDS ($WH_{SDS}SDS$) (16, 17). All children were healthy and well nourished but differed in their GH secretory capacities. Thyroid, kidney and liver functions were normal, and none of the children had coeliac disease.

Study protocols

Pretreatment investigation of GH secretion A standard arginine-insulin tolerance test (AITT) was

performed during the pretreatment year in 16 children according to a protocol described previously (18). Eleven of these children had a maximal GH response (GH_{max}) below 32 mU/l ($10 \mu\text{g/l}$), corresponding to standard 80/505, which is equal to 20 mU/l with the old standard, 66/217. (The WHO International Reference Preparation (IRP) for hGH 80/505 was used as the standard). Spontaneous 24-h GH secretion was estimated in 32 children in terms of both the secretory rate and pulsatile pattern, as previously reported (17). Briefly, plasma GH concentrations from 24-h profiles were transformed to GH secretion rates (GH_t) by means of a deconvolution technique. For these children, the mean GH_t was 0.63 (0.34) U/24 h and the mean area under the curve above the baseline (AUC_b) was 114.0 (68.9) mU/l/24 h. Seven out of eleven children who had a GH_{max} below 32 mU/l ($10 \mu\text{g/l}$) in response to an AITT also had a maximal peak GH level below 32 mU/l ($10 \mu\text{g/l}$) during 24-h GH profiles.

Treatment follow-up Ten children were treated for GH deficiency (GHD) within the Swedish National Registry and twenty-three children were treated within a randomized trial of GH treatment for ISS. Recombinant human GH (Pharmacia & Upjohn) was administered by daily subcutaneous injections. Twenty-six children (4 girls and 22 boys) were treated with GH at a dose of 0.1 IU/kg ($33 \mu\text{g/kg}$) body weight per day and seven children (1 girl and 6 boys) were treated with GH at a dose of 0.2 IU/kg ($66 \mu\text{g/kg}$) body weight per day. Blood samples for measurement of leptin were collected between 1000 and 1400 h at the start of treatment and after 10 and 30 days, and 3, 6 and 12 months of treatment. Bone mineral density (BMD) and body composition measurements were taken at baseline and 12 months after the onset of GH treatment. Characteristics of the children in this study are given in Table 1.

The study was approved by the ethical committee of the

Table 1 Clinical and biochemical characteristics at the start of the investigation in short children with various GH secretory capacities. GH was administered at a daily dose of 0.1 ($n=26$ (4 girls, 22 boys)) or 0.2 ($n=7$ (1 girl, 6 boys)) IU/kg body weight.

	All ($n=33$)			Girls ($n=5$)			Boys ($n=28$)		
	<i>n</i>	Mean	S.D.	<i>n</i>	Mean	S.D.	<i>n</i>	Mean	S.D.
Age (years)	33	11.5	1.6	5	10.8	2.7	28	11.6	1.4
Height SDS	33	-2.3	0.4	5	-2.4	0.4	28	-2.3	0.4
Weight SDS	33	-1.5	0.8	5	-1.8	0.5	28	-1.5	0.9
$WH_{SDS}SDS$	33	0.5	1.2	5	-0.2	0.8	28	0.6	1.2
BMI (kg/m^2)	33	16.7	1.9	5	16.1	0.5	28	16.8	2.0
AITT, GH_{max} (mU/l)	16	27.4	14.3	2	26.1	7.7	14	27.6	15.2
24-h GH profile									
GH_{max} (mU/l)	32	39.2	25.2	4	28.9	10.9	28	40.6	26.4
Baseline (mU/l)	32	0.7	0.5	4	1.3	1.0	28	0.7	0.4
AUC_b (mU/l/24 h)	32	114.0	68.9	4	110.0	48.6	28	114.6	72.0
AUC_t (mU/l/24 h)	32	131.7	76.4	4	140.3	65.7	28	130.5	78.8
GH_b (U/24 h)	32	0.55	0.32	4	0.48	0.25	28	0.55	0.33
GH_t (U/24 h)	32	0.63	0.34	4	0.62	0.36	28	0.63	0.34

AUC_t , area under the curve above the zero line; GH_b , excreted amount of GH above the calculated baseline.

Medical Faculty, University of Göteborg. Informed consent was obtained from the children and their parents.

Hormone analysis

GH concentrations were measured using a polyclonal immunoradiometric assay (hGH-kit, Pharmacia & Upjohn, Uppsala, Sweden) with WHO International Reference Preparation 80/505 as the standard.

Serum leptin concentrations were determined in duplicate by radioimmunoassay (human leptin RIA kit, Linco Research, Inc., St Charles, MO, USA). The assay had a detection range of 0.2–100 µg/l, with an intra-assay coefficient of variation of 7.0% at 2.4 µg/l, and 4.9% at 14.0 µg/l. The corresponding values for the interassay coefficients of variation were 9.0% and 5.0% (19).

Measurements of BMD and body composition

BMD and body composition were measured by dual-energy X-ray absorptiometry (DXA; Lunar, DPX-L, Scan Export Medical, Helsingborg, Sweden) in the whole body, trunk and upper and lower limbs. This system uses a constant potential X-ray source and a K-edge filter to achieve a congruent beam of stable dual-energy radiation. Total body scans were performed and body fat, lean mass, BMD and bone mineral content (BMC) were analysed using the extended research mode of software version 1.31. The coefficients of variation for the scanner used, as determined from duplicate measurements in ten healthy volunteers, were 1.7% for fat mass, 0.7% for lean tissue mass, 1.5% for BMD and 1.9% for BMC. Total body BMD results were compared with the age- and sex-matched Dutch reference values ($n = 500$, 4–20 years of age) of Boot *et al.* (20, 21) and expressed as SDS. As BMD measurements are influenced by bone size, we also calculated bone mineral apparent density (BMAD), using the DXA-derived bone area and other skeletal measurements to estimate bone volume (22, 23). The following formula was used to derive BMAD of the whole body: $\text{BMAD} = \text{BMC}/(\text{projected area}^2/\text{height})$. Body composition was measured as lean tissue mass (g), fat mass (g) and BMC (g). Lean body mass was taken as the sum of lean tissue mass and BMC, and total tissue mass was the sum of the three variables. Body fat is given as a percentage of the total tissue mass.

Statistical methods

Results are expressed as means, with s.d. values in parentheses unless otherwise stated. The Wilcoxon signed rank test was used to compare paired samples, and the Wilcoxon rank sum test was used to compare non-paired samples. The Spearman rank correlation coefficient was used to determine correlations. To assess

the independent effects of variables on changes in fat and leptin, a multiple stepwise linear regression analysis was performed. In this analysis, leptin concentrations and GH secretion variables were log-transformed to normalize the distribution. Differences were considered significant at a P value of 0.05 or less.

Results

Growth response to GH therapy

The change in height SDS per year (Δ height SDS) was used to describe the growth response. The mean Δ height SDS for the year before the start of GH treatment was -0.04 (0.22). This increased to 0.60 (0.30) during the year of GH therapy ($P < 0.001$). Consequently, the mean height attained increased from -2.33 (0.38) SDS at the start of treatment to -1.73 (0.55) SDS at the end of 1 year of GH treatment.

The absolute values for BMD of the total body, trunk, arms and legs increased significantly ($P < 0.001$) during the year of GH treatment. However, there was no significant difference in total body BMD SDS (-0.70 (1.24) to -0.91 (1.40)), whereas total body BMAD decreased significantly ($P < 0.001$) from 0.096 (0.005) to 0.090 (0.005).

During the year of GH treatment, body fat decreased significantly ($P < 0.001$), in the total body by 3.0 (3.3)%, in the trunk by 1.7 (3.4)%, in the arms by 3.5 (4.9)% and in the legs by 4.6 (3.4)% (Fig. 1). Lean tissue mass increased significantly ($P < 0.001$), in the total body by 2.9 (3.3)%, in the trunk by 1.6 (3.4)%, in the arms by 3.3 (4.7)% and in the legs by 4.3 (3.3)%.

Relationship between serum leptin levels and body composition

Serum leptin levels decreased to 78.9%, 73.6%, 73.9%, 74.3% and 87.4% of baseline values after 10 and 30 days and 3, 6 and 12 months respectively (Fig. 2a,b).

Serum leptin levels at the start and after 1 year of GH treatment correlated with the percentage body fat ($r = 0.55$, $P < 0.01$, and $r = 0.71$, $P < 0.001$ respectively) (Fig. 3a,b). A significant correlation was observed between the 1-year change in absolute leptin concentration and the 1-year change in absolute total body fat ($r = 0.40$, $P < 0.05$) (Fig. 4). This correlation was lost when the leptin concentration was expressed per unit fat mass (Fig. 5). The 1-year Δ height SDS correlated with the 10-day change in leptin concentration ($r = -0.54$, $P < 0.01$).

Relationship between body composition, serum leptin and GH secretion

To explain both the variabilities in change in fat mass (%) and change in serum concentration of leptin during 1 year of GH therapy, multiple stepwise linear regression

Figure 1 Effect of GH therapy for 1 year on total body and regional fat distribution. Box and whisker plots indicate median, lower, and upper quartiles, and the whiskers show the 5th and 95th percentiles. The mean values are depicted as solid squares in each box plot. ** $P < 0.001$ compared with baseline levels (Wilcoxon signed rank test).

analyses were applied to fat (%), 1 year change in fat (%), leptin, changes in leptin (after 10 and 30 days and 3, 6 and 12 months) and auxological variables available at the start of GH therapy: age, sex, height SDS, weight SDS, WH_{SDS} and body mass index (BMI), as well as variables from measurements of spontaneous 24-h GH secretion, i.e. GH_t , GH_{max} (Table 2).

In the first model to explain the 1-year change in fat (%), only the variables available at the start of GH therapy and the baseline leptin level were analysed. Leptin and age were the only two significant parameters, and these accounted for 40.4% of the variance in the 1-year change in fat (%) (analysis 1). When changes in leptin (at 10 and 30 days and 3, 6 and 12 months) were available, they were not selected.

In order to explain the 1-year change in leptin, the variables available at the start of GH therapy and the baseline fat (%) were used as explanatory variables (analysis 2a). GH_t was the only significant parameter, accounting for 22.9% of the variance in the 1-year change in leptin. With the addition of 1-year change in fat (%) to those parameters as explanatory variables, 1-year change in fat (%), baseline fat (%) and GH_t were

Figure 2. Changes in serum leptin levels at various times after the start of GH treatment in short children with various GH secretory capacities. (a) Individual serum leptin levels. Thick line shows mean values. (b) Percentage decreases (means $\pm$ S.D.). * $P < 0.05$, ** $P < 0.01$ compared with baseline (Wilcoxon rank sum test).

selected and accounted for 24.0%, 11.5% and 12.2% of the variability respectively (total 47.7%) (analysis 2b).

Discussion

In this study we have demonstrated that the change in serum leptin levels during 1 year of GH treatment

Figure 3 Relationship between serum leptin concentrations and total body fat mass (%) in short children with various GH secretory capacities (a) at the start of GH therapy ($r = 0.54$, $P < 0.01$), and (b) after 1 year of GH therapy ($r = 0.73$, $P < 0.001$; Spearman rank correlation coefficient).

correlates with the change in fat mass in short children with various GH secretory capacities. This is in agreement with previous findings in adults with GHD (24). Furthermore, we have shown that the magnitude of changes in both fat mass and leptin correlates with the endogenous GH secretion rate.

Figure 4 Relationship between changes in serum leptin concentrations and changes in total body fat mass (kg) in short children with various GH secretory capacities during 1 year of GH therapy ($r = 0.40$, $P < 0.05$; Spearman rank correlation coefficient).

DXA is a relatively new method of assessing body composition. It provides measurements of body fat mass as well as lean tissue and BMC. The main advantage of this technique is its precision compared with other clinically available methods. Although the accuracy of DXA is still not fully validated, it has been shown to provide accurate measurements of body composition in adults with GHD (25, 26). The possibility of obtaining regional as well as total body measurements is another advantage of DXA. As the procedure is associated with a very low radiation dose, it can be used safely for repeated measurements. However, DXA cannot provide information on the composition of lean tissue, for example changes in extracellular volume. Taking these points together, we regard this technique as preferable for monitoring changes in body fat, lean tissue and bone mass.

GH administration for 1 year resulted in no change in the total body BMD SDS after the onset of GH treatment in this group of short children with a broad range of GH secretory levels. This absence of change in total body BMD SDS and the decrease in total body BMAD after 1 year of GH treatment may reflect a faster rate of bone expansion than of mineral acquisition. Similar changes in BMD during GH treatment have been reported in previous studies in children with GHD (21) and short stature children without GHD (27).

Figure 5 Comparison of individual serum leptin levels, expressed as leptin concentration per unit fat mass (kg). Horizontal bars represent the mean and s.d. for leptin secretion per unit fat mass. There was no significant difference between mean values at the start and after 1 year of GH therapy (Wilcoxon rank sum test).

Although there was a significant reduction in the percentage body fat at all sites studied, regional differences in the effect of GH on body composition were demonstrated. These regional differences may suggest that GH has site-selective lipolytic actions, and the redistribution of body fat may have been due to the net effect of interactions with other hormones, the activity of which may be different at different sites (24, 26). Because all the children remained prepubertal during the period of GH treatment, we could exclude changes due to sex-steroid hormones, such as testosterone, which have been shown to have a direct inhibitory action on leptin production by adipose tissue (28, 29).

GH treatment decreased serum leptin levels signifi-

cantly. At both baseline and 1 year after the onset of GH treatment, serum leptin levels were highly correlated with the percentage total body fat. Circulating leptin levels are positively correlated with measures of obesity including BMI and percentage body fat, and are elevated in obesity (11). Ferron *et al.* reported that reduced leptin levels in women with eating disorders, such as anorexia nervosa and bulimia nervosa, are reduced, and that leptin levels are unrelated to the specific pathology but correlated with the individual BMI (30). Progressive weight loss during a hypocaloric diet is accompanied by a decline in circulating leptin levels (9, 31). In adults with GHD, the percentage body fat is significantly decreased after 1 year of GH treatment, whereas the

Table 2 Stepwise multiple linear regression analyses for 1-year change in fat and leptin.

	Dependent variables	n	Variables in equation	Coefficient	Standard error	P value	Model R ²
Analysis 1	1 year change in fat (%)	33	(1) Leptin	-8.33	1.95	0.00	(0.34)
			(2) Age	-0.65	0.32	0.05	0.40
Analysis 2a	1 year change in leptin	33	GH _t	6.89	2.19	0.00	0.23
Analysis 2b	1 year change in leptin	33	(1) Change in fat (%)	0.59	0.16	0.00	(0.24)
			(2) Fat (%)	0.22	0.07	0.00	(0.35)
			(3) GH _t	5.61	2.04	0.01	0.48

Leptin, GH_t, GH_{max} data were log₁₀ transformed.

Values for the model R² in brackets represent the values on completion of each step of the regression analysis and those in bold are the final R² when all variables in the equation had been entered.

relationship between leptin and the percentage body fat does not change (32). In the present study, there was a significant correlation between the change in total body fat and the change in leptin during 1 year of GH treatment; however, the leptin concentration per unit fat mass did not change, suggesting that GH does not have an independent effect on leptin, other than via a reduction in fat mass. The mean WH_{SDS} was 0.5 (1.2) in the children studied, and only 3 of the 33 children had a WH_{SDS} above 2.0. Despite the limited range of body composition in our population, the correlation between the degree of fat mass and leptin was highly significant, emphasizing that subtle changes in fat mass influence leptin levels in short children. In summary, GH has an effect on fat mass, and leptin concentrations change in proportion to the change in absolute body fat mass. It therefore seems likely that leptin is a fat messenger rather than a controller of fat mass.

In obese adults without GHD, the abnormal GH response to GH-releasing stimuli is normalized after weight reduction (33). In normally growing children, increased adiposity is associated with reduced GH secretion (4), implying a reciprocal relationship between the rate of GH secretion and adiposity. However, it is not clear how body composition alters GH secretion. Females have a greater degree of adiposity for the same level of GH secretion (4), suggesting the presence of regulatory mechanisms that account for gender differences in body composition. This is consistent with previous reports (34, 35) that there are gender differences in leptin concentrations, with higher levels in women than men. Leptin concentrations are higher in hypopituitary patients than in weight-matched healthy controls (36, 37). These increases in leptin concentrations are greater than would be expected from the degree of obesity. In experimental animal models, Carro *et al.* (38) have suggested that physiological levels of leptin are needed to ensure normal spontaneous GH secretion. Furthermore, leptin levels are closely correlated with those of GH-binding protein in prepubertal children, suggesting that leptin may mediate the effects of body fat mass on GH sensitivity (39, 40). In the present study, when the 1-year change in leptin was used as the dependent variable in stepwise regression analyses, the estimated GH secretion rate was selected and accounted for some portion of the variability in both analyses 2a and 2b. A similar finding has been reported previously in normal elderly subjects by Gill *et al.* (37), who found a significant inverse correlation between the integrated GH concentration over 24 h (IGHC) and both leptin and percentage body fat, and that 28% of the variability in IGHC could be explained by leptin levels. These observations may indicate that leptin is a metabolic signal involved in the regulation of GH secretion in humans.

In conclusion, our data show that there is a significant correlation between the change in total

body fat and the change in leptin during 1 year of GH treatment; however, the leptin concentration per unit fat mass does not change. There are also significant correlations between changes in leptin and fat and endogenous GH secretion in short children with various GH secretory capacities. Leptin may be the messenger by which adipose tissue affects hypothalamic regulation of GH secretion.

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