Circulating leptin level and growth hormone response to stimulation tests in obese and normal children

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Abstract
Objective: Growth hormone secretion is decreased in obese subjects, and their GH response to stimulation tests is blunted. The mechanisms relating excess adipose mass and GH secretion are unknown. We hypothesized that leptin might be a signal linking adipose mass to GH secretion.

Design: We measured serum leptin levels and the GH response to stimulation tests in 42 obese and 40 lean short normal prepubertal children.

Results: The mean serum leptin concentrations were 23.8 ± 1.7 ng/ml and 3.6 ± 0.4 ng/ml in obese and lean children respectively, and were found to be inversely related to GH peak in both groups. After adjusting for body fat data, leptin was still an independent predictor of GH peak. Multiple stepwise regression analysis identified both leptin (regression coefficient = -0.78, P = 0.001), and insulin (regression coefficient = -0.03, P = 0.009) as negative determinants of GH response to the GHRH test in obese children (multiple R = 0.64), and only leptin in lean children (r = -0.51, P = 0.001). No correlation was observed between leptin and IGF-I or IGBP binding protein-3.

Conclusion: These results are consistent with the hypothesis that leptin could contribute to the regulation of GH secretion.

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Introduction
Growth hormone (GH) secretion is influenced by many neuroendocrine, hormonal and metabolic factors (1). In humans, amino acids (2), sex steroids (3, 4), and thyroid hormones (5) elicit GH secretion whereas glucose (6), free fatty acids (7), glucocorticoids (8), insulin (9), insulin-like growth factor (IGF-I) (10), and GH itself suppress GH release. Most of these agents do not act directly on the pituitary to release GH, but promote or inhibit the secretion of hypothalamic growth hormone-releasing hormone (GHRH) and/or somatotrophin release-inhibiting factor.

A negative relationship between GH secretion and adiposity has long been recognized. Obese children and adults have a decreased spontaneous GH secretion as well as a blunted response of GH to stimulation tests (11–15). Body mass index (BMI), even within the normal range, was found to influence GH secretion in children (16) and adults (17). The defect in GH secretion is partially reversed after weight reduction (11) or short term caloric diet (18), suggesting that it is a secondary response to metabolic signals (11) rather than a primary defect associated with obesity. Low spontaneous GHRH secretion, high somatostatin secretion (19, 20), and increased negative feedback of IGF-I (10) were previously considered as possible causes of the blunted GH secretion in obese subjects. However, the molecular mechanisms which link adipose mass to the inhibition of the hypothalamic-pituitary somatotrophic axis are currently unknown. We hypothesized that leptin might be a signal linking adipose mass to GH secretion.

Leptin, the 16 kDa product of the ob gene (21), is secreted by adipocytes and the circulating leptin is closely related to BMI and absolute fat mass (22). Leptin signals the amount of adipose tissue stored in the body to the central nervous system (23). Leptin is important to several neuroendocrine pathways in rodents (24). It has a major role on female reproductive function (25). Leptin administration modifies spontaneous GH secretion in fasted rats (26), and increases IGF-I gene expression by liver independently of GH (27). In humans, plasma leptin levels are pulsatile (28), with higher levels during the night (29), inversely related to pituitary-adrenal function (28). A rise in plasma levels may trigger the onset of puberty in boys (30), and a critical blood leptin level may trigger reproductive ability in women (31).

To study the relationship between leptin and the somatotrophic axis in growing children, we determined GH response to stimulation tests in prepubertal obese
Subjects and methods

Subjects

We recruited 82 prepubertal children aged 5–13 years: 33 girls (16 non-obese and 17 obese) and 49 boys (24 non-obese and 25 obese). Obesity was defined as a body weight greater than 120% of the ideal value for age and height (32). Children were all at Tanner stage I, with serum testosterone <0.23 nmol/l in boys and oestradiol <18 pmol/l in girls. The main characteristics of the subjects are presented in Table 1. Body mass index (BMI) was defined as the weight in kilograms divided by the square of the height in metres. None of the obese children had any metabolic or endocrine disease other than obesity. Lean children were normal short children (-1 to -2 S.D.) who were primarily referred for assessment of growth hormone secretion using a conventional stimulation test. They were all in good health. None of them had growth hormone deficiency (33), chromosomal abnormalities, dysmorphic syndromes, skeletal dysplasia, chronic illness, or hypothyroidism. None was taking medication. Protocols were approved by our institutional review board. All subjects and families gave their informed consent.

Study design

A standardized GH stimulation test was performed in the obese children after a physiological overnight fast. GHRH was chosen because it stimulates GH secretion more efficiently than conventional GH stimulation tests in obese children (34). After placement of a catheter in a peripheral vein, the children rested in bed for 1 h. GHRH (1–44) NH2 (Sanofi Recherche, Toulouse, France) was injected at a dose of 60 μg/m² intravenously between 0800 and 0830 h. Ornithine hydrochloride i.v. (15 g/m²) was used in lean children, according to a similar protocol (35). Blood samples were collected at 0, 30, 45, and 60 min for GH measurements. Blood was also collected for basal measurements of leptin, IGF-I, IGF binding protein-3 (IGFBP3), insulin, thyroxine, cortisol, glucose and free fatty acids concentrations.

Assays

Serum GH concentration was measured by RIA using kits supplied by BioMerieux (Marcy-l’Etoile, France), calibrated against the first International Reference Preparation (IRP 66/217). The intra- and interassay coefficients of variation were 2.1% and 3.9% respectively. The plasma GH results are expressed in IRP 66/217 units, for which 2 mU = 1 ng. GH was expressed in ng/ml. Total IGF-I measurements were performed by IRMA after acid-ethanol extraction (Diagnostic System Laboratories, Webster, TX, USA), and IGFBP3 measurements by IRMA (Diagnostic System Laboratories). The intra- and interassay coefficients of variation were respectively <4% and 15% for IGF-I, and 1.9% and 3.9% for IGFBP3. Leptin was measured in plasma or serum samples by radioimmunoassay using reagents supplied by Linco Research Inc. (Saint Louis, Missouri, USA) (22). The intra-assay coefficients of variation at 1.4 and 14.2 ng/ml were 3.6 and 3.3% respectively. The interassay coefficients of variation at 1.4 and 14.2 ng/ml were 4.6 and 3.7%.

Table 1 Clinical and biological characteristics of the lean and obese prepubertal children. Values are means ± S.E.M.

<table>
<thead>
<tr>
<th></th>
<th>Obese</th>
<th>Lean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boys/girls</td>
<td>25/17</td>
<td>24.16</td>
</tr>
<tr>
<td>Age (years)</td>
<td>9.6 ± 0.3</td>
<td>9.1 ± 0.3</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>141.1 ± 1.8</td>
<td>123 ± 1.6</td>
</tr>
<tr>
<td>(SDS)</td>
<td>1.6 ± 0.2</td>
<td>-1.5 ± 0.05</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>51.9 ± 2</td>
<td>24.0 ± 0.9</td>
</tr>
<tr>
<td>% IBW</td>
<td>151.6 ± 2</td>
<td>100.1 ± 1.3</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.6 ± 0.4</td>
<td>16.1 ± 0.3</td>
</tr>
<tr>
<td>Body fat (kg)</td>
<td>22.8 ± 1.4</td>
<td>ND</td>
</tr>
<tr>
<td>Growth velocity (cm/year)</td>
<td>5.8 ± 0.2</td>
<td>4.6 ± 0.1</td>
</tr>
<tr>
<td>Serum leptin (ng/ml)</td>
<td>23.8 ± 1.7</td>
<td>3.8 ± 0.4</td>
</tr>
<tr>
<td>Male/female</td>
<td>23.5 ± 2.3/24.1 ± 2.3</td>
<td>3.4 ± 0.4/4.5 ± 0.6</td>
</tr>
<tr>
<td>Plasma GH (ng/ml)</td>
<td>11.6 ± 1.5</td>
<td>14.9 ± 1.2</td>
</tr>
<tr>
<td>Serum IGF-I (ng/ml)</td>
<td>256 ± 18</td>
<td>139 ± 10</td>
</tr>
<tr>
<td>Serum IGFBP3 (ng/ml)</td>
<td>3534 ± 83</td>
<td>2780 ± 106</td>
</tr>
<tr>
<td>Plasma insulin (mU/ml)</td>
<td>10.5 ± 0.6</td>
<td>5.7 ± 0.4</td>
</tr>
<tr>
<td>Plasma free thyroxine (pmol/l)</td>
<td>14 ± 0.6</td>
<td>13 ± 0.5</td>
</tr>
<tr>
<td>Plasma glucose (mmol/l)</td>
<td>3.96 ± 0.04</td>
<td>4.33 ± 0.06</td>
</tr>
<tr>
<td>Plasma FFA (mmol/l)</td>
<td>0.65 ± 0.03</td>
<td>ND</td>
</tr>
<tr>
<td>Cortisol (μg/dl)</td>
<td>16.4 ± 0.8</td>
<td>12.6 ± 1</td>
</tr>
</tbody>
</table>

IBW, ideal body weight; SDS,; ND, not determined.
respectively. Insulin was measured by radioimmunoassay using reagents provided by CIS Bionternational (Gif sur Yvette, France). Cortisol was measured using an enzyme-linked immunoassay (Abbott Laboratories, North Chicago, CA, USA). Testosterone and oestradiol measurements were performed by RIA (Coat-a-count, Diagnostic Products Corp., Los Angeles, CA, USA, detection limit 0.23 nmol/l and ImmunoDagnostic Systems, Boldon, Tyne and Wear, UK, detection limit 18 pmol/l respectively). Free thyroxine was measured by RIA (Clinical Assays, Baxter, Cambridge). Plasma glucose was measured with a glucose analyser (YSI, Yellow Springs, OH, USA). Plasma free fatty acids (FFA) were measured using a colorimetric method (22).

Other analyses
The percentage of fat-free mass and body fat was determined by bioelectric impedance analysis, a method previously validated in children (36). Tetrapolar whole-body bioelectric impedance was measured with a body composition analyser (Eugedia, France) using an alternating electrical current of 0.8 mA and 50 kHz. The measurements were standardized according to the procedural guidelines of Lukaski et al. (37). The electrodes were placed at defined positions on the right wrist and ankle. The mean of three sequential readings was used as the measurement value.

Statistical analysis
Values are expressed as means ± S.E.M. The effects of BMI and gender on leptin concentration were examined by simple as well as multiple regression analysis.

A model for predicting growth hormone response to stimulation test (GH peak) was constructed. First, simple linear regression analyses were performed, using auxological and biological variables including leptin concentration. GH peak is defined as the maximal GH value in response to the stimulation test. Given the extreme colinearity of leptin and BMI (or fat mass), we then performed a standard multiple regression analysis with leptin and BMI (or fat mass) as independent variables. To evaluate the determinants of GH peak, we finally examined the significant independent variables of the simple regression analyses by forward stepwise multiple regression analysis, in the obese and lean subjects respectively. Due to the skewness of serum leptin, body fat data, and GH peak, we used the log₁₀ transformed variables to increase the normality of their distribution.

Simple linear regression analyses were also performed using IGF-I and IGFBP3 concentrations as the dependent variables. Significance was defined as P < 0.05. Computations were made with Statview-4 (Abacus Concept, Berkeley, CA, USA) Statistical Software.

Results
Clinical and biological characteristics of the lean and obese prepubertal children are presented in Table 1. Mean serum leptin concentration was 3.8 ± 0.4 ng/ml in the 40 lean children and 23.8 ± 1.7 ng/ml in the 42 obese children. Serum leptin correlated with BMI in obese (r = 0.64, P < 0.0001) as well as in lean (r = 0.47, P = 0.002) children.

After adjustment for age and BMI, leptin was found to be increased independently by female sex (P = 0.002).

Serum leptin, GH secretion and IGF-I in obese children
Table 2 shows the results of univariate analyses of regression between GH peak value in response to

| Table 2 | Simple regression analysis of select variables with GH peak (log₁₀) in response to stimulation tests in obese (n = 42) and lean (n = 40) children. |
|---------|-------------------------------------------------|-------------------------------------------------|
|          | Obese Data                                     | Lean Data                                      |
|          | r      | P value | r      | P value |
| Age (years) | −0.43  | 0.004   | −0.25  | 0.12   |
| BMI (kg/m²)* | −0.54  | 0.0002  | −0.25  | 0.13   |
| Body fat (kg)* | −0.55  | 0.0002  |        |        |
| Height (cm)  | −0.47  | 0.002   | −0.20  | 0.22   |
| Serum IGF-I (ng/ml) | −0.27  | 0.08    | −0.22  | 0.20   |
| Serum IGFBP3 (ng/ml) | −0.18  | 0.26    | −0.25  | 0.13   |
| Serum leptin (ng/ml)* | −0.55  | 0.0001  | −0.51  | 0.001  |
| Plasma insulin (mU/l) | −0.46  | 0.002   |        | 0.31   |
| Glucose (mmol/l) | −0.27  | 0.08    |        | 0.97   |

Sex, growth velocity, cortisol, plasma thyroxine, and plasma FFA were not significantly correlated with GH peak.

* Data for these variables were transformed (log₁₀) before statistical analysis.
log10 leptin; r

coefficient

GH peak as the dependent variable. Leptin (regression

insulin) in a stepwise multiple regression analysis with

independent variables (age, height, fat mass, leptin,

variables (Table 3). We finally entered the significant

regression analysis with BMI (or fat mass) as independent

predictor of GH peak when included in a multiple

analysis with GH peak.

Relation between serum leptin (log 10 ) and GH peak (log 10 )

Figure 1

Serum leptin, GH secretion and IGF-I in lean

short normal children. This observation supports the

hypothesis that serum leptin could contribute as a

negative feedback regulator to the control of GH

secretion.

Discussion

The results of our study confirm that prepubertal obese

children have a blunted response of GH to stimulation

tests. Their GH response to GHRH correlated with

their BMI, fat mass, age, height, plasma insulin, and

serum leptin concentrations. The negative effect of

serum leptin concentrations on GH response was still

significant when the influence of adiposity was

controlled statistically. Stepwise multiple regression

analysis identified two independent factors, leptin

and insulin, as potent negative determinants of GH

response. A negative correlation between serum leptin

and GH response to ornithine was also found in lean

short normal children. This observation supports the

hypothesis that serum leptin could contribute as a

negative feedback regulator to the control of GH

secretion.

This study was addressed to prepubertal children

in order to avoid the confounding role of sex steroids

on serum leptin as well as on the GH response to

stimulation tests (3, 4, 19, 38). Whether the nega-
tive effect of serum leptin on GH secretion is also

shown during or after puberty remains to be deter-

mined. However, we observed a sexual dimorphism of

leptin concentrations in both groups of prepubertal

children.

In the obese subjects, the negative association

between fat mass and GH secretion has long been

recognized (11–17). The negative effect of insulin on

GH secretion has also been described previously (9). In

our study, serum leptin was more strongly related to GH

secretion than insulin and fat mass, suggesting a truly

negative effect of this hormone on the somatotrophic

axis. Moreover, this negative effect was also observed in

our lean children, despite a narrow range of leptin

concentrations.

Serum leptin, GH secretion and IGF-I in lean

short normal children

Univariate as well as multivariate analyses (including

leptin and BMI) showed that serum leptin was the only

variable correlated to GH response to the stimulation

test (Fig. 1, Tables 2 and 3). Therefore, no stepwise

regression analysis was performed. No correlation was

found between serum leptin and serum IGF-I or IGFBP3

in the lean children.

<table>
<thead>
<tr>
<th>Group</th>
<th>GH peak</th>
<th>Variables</th>
<th>β</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Obese children (model 1)</td>
<td>Multiple R = 0.60</td>
<td>Leptin*</td>
<td>-0.51</td>
<td>-0.84 to -0.17</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td></td>
<td>BMI*</td>
<td>-2.43</td>
<td>-5.02 to 0.16</td>
<td>0.06</td>
</tr>
<tr>
<td>Obese children (model 2)</td>
<td>Multiple R = 0.62</td>
<td>Leptin*</td>
<td>-0.62</td>
<td>-1.17 to -0.08</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fat mass*</td>
<td>-0.73</td>
<td>-1.41 to -0.06</td>
<td>0.03</td>
</tr>
<tr>
<td>Lean children</td>
<td>Multiple R = 0.51</td>
<td>Leptin*</td>
<td>-0.51</td>
<td>-0.84 to -0.17</td>
<td>0.004</td>
</tr>
<tr>
<td></td>
<td></td>
<td>BMI*</td>
<td>-0.01</td>
<td>-48.07 to 47.52</td>
<td>0.98</td>
</tr>
</tbody>
</table>

* Data were log transformed (log_{10}) before analysis.
children. However, amino acids stimulate GH release at least in part through an acute release of hypothalamic GHRH (39), and the response observed in lean children is consistent with the hypothesis that serum leptin could diminish the physiological response of pituitary somatotrophs to GHRH.

Since the GH response to stimulation tests may not be a reliable index of endogenous GH secretion (40, 41), the physiological relevance of our findings is not established. In humans, growth hormone is secreted episodically in intermittent short bursts, mainly during sleep, with the higher burst after the onset of deep sleep (42). It is interesting in this respect that leptin levels also are pulsatile, with high nocturnal values (28, 29).

In humans, a negative relationship between circulating leptin and growth hormone secretion has also been found in elderly subjects (43), as well as in post menopausal women (44), although the mechanism by which leptin and GH are inversely related has not been determined. Leptin may inhibit GH secretion, GH may inhibit leptin secretion, or they may be independently regulated covariables.

Animal data suggest that somatotrophs may be important target cells of leptin to regulate GH secretion. In mice, leptin receptor is present in the anterior pituitary, and its expression has been increased by constitutive high expression of GHRH/GH (45). However, in rats leptin administration reversed the fasting-induced decrease in GH secretion. Conversely, leptin administration to normally fed rats did not modify GH secretion whereas administration of leptin antiserum decreased spontaneous GH secretion (26). The effect of leptin administration was dependent on the fasting or fed state in rodents, suggesting that the leptin signalling pathway interacts with other metabolic/hormonal signalling pathways in order to control GH secretion. This observation is consistent with the previous observation that leptin administration corrects many of the neuroendocrine changes that occur as a result of food deprivation in rodents (24). In humans, the factors controlling GH secretion are clearly different, since fasting is associated with stimulation of GH secretion (46). Thus, a reversal of the fasting-induced neuroendocrine changes by leptin would lead to a decrease in GH secretion. Leptin might interact with different metabolic/hormonal pathways and have opposite effects on human and murine somatotrophs. Alternatively, leptin might stimulate GH secretion in humans as well as in rodents, whereas its effect would be overcome by metabolic/hormonal inhibiting factors (including insulin). Finally, the apparent negative relationship between circulating leptin and GH secretion might only reflect the stimulating effect of insulin or other factors on leptin (47).

Administration of recombinant leptin to juvenile rats stimulates activation of nuclear stat-3 and stat-1, and IGF-I gene expression in hepatic extracts, even in hypophysectomized rats (27). In our obese and lean short normal children, we found no correlation between circulating leptin and circulating IGF-I or IGFBP3 concentrations.

The influence of nutrition on growth and the GH–IGF-I axis has long been recognized (46). Chronic overnutrition and obesity result in low GH secretion, presumably to limit the metabolic consequences of obesity, while stature is generally increased in prepubertal children (48). During chronic undernutrition, GH secretion is high and IGF-I low, likely to favour lipolysis and make fatty acids available to peripheral tissues (46). Our observations, together with the animal studies, are consistent with the hypothesis that leptin is part of the mechanisms relating storage of energy and growth hormone secretion.

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