Serum leptin levels during recombinant human GH therapy in adults with GH deficiency

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Abstract

Objective: Recent studies suggest an involvement of the obese (ob) gene and its product leptin in the regulation of body fat. Since adults with growth hormone deficiency (GHD) have a high body fat mass which can be normalized with recombinant human (rh) GH therapy, we investigated whether GH influences serum leptin directly or indirectly via its lipolytic effect.

Design: Fourteen adults with GHD were treated with subcutaneous injections of rhGH given every evening for 52 weeks. Serum leptin, fat mass and body fat percentage were measured at baseline and after 4 and 52 weeks of treatment.

Methods: Serum leptin was measured with a commercially available RIA. Total body water was determined by dilution of deuterium. Fat free mass was estimated by assuming a hydration of 73%. Fat mass was estimated by subtracting fat free mass from weight.

Results: At baseline, serum leptin levels were exponentially related to body fat percentage (r=0.88; P<0.0005). rhGH treatment for 4 weeks did not significantly influence serum leptin levels, whereas treatment for 52 weeks significantly decreased serum leptin levels (15.6±2.9 to 10.8±2.1 μg/l; P = 0.020). Fat percentage was significantly decreased after 52 weeks of treatment (37.6±2.1 to 33.8±2.5%; P < 0.0005). The decrease in serum leptin could largely be explained by the decrease in body fat percentage, whereas the relation between leptin and body fat percentage did not change.

Conclusions: The influence of GH on serum leptin is indirect, via its effect on body fat percentage.

Introduction

The recently recognized obesity (ob) gene produces a 16 kDa protein called leptin. Leptin is secreted from adipose tissue and is presumed to function as part of a signalling pathway from adipose tissue to the brain and as a homoeostatic lipostat mechanism through modulation of satiety signals and energy expenditure (1–4). Mutations in the ob gene resulting in leptin deficiency are the cause of obesity in the ob/ob mouse. In contrast, the high leptin concentrations reported in obese subjects point to an insensitivity to endogenous leptin. Since adults with growth hormone (GH) deficiency (GHD) have an increased body fat mass and a decreased fat free mass (5, 6), one can expect high levels of serum leptin. GH therapy in adults with GHD decreases body fat mass (7–9) and thus might be expected to exert an effect on serum leptin levels. We therefore investigated whether GH influences serum leptin directly or indirectly via its lipolytic effect in adults with GHD at baseline and during recombinant human GH (rhGH) therapy for 4 and 52 weeks.

Subjects and methods

Subjects

Five males and nine females (mean age 48 years; range 33–67 years) with adult-onset GHD were studied. A peak serum GH response of less than 7 mU/l during insulin-induced hypoglycaemia confirmed GHD. None of the patients had a body mass index (BMI) larger than 32 kg/m². The aetiology of GHD was a pituitary adenoma in eight patients: non-functioning adenoma (n = 4), adrenocorticotropic adenoma (n = 3), prolactinoma (n = 1). In the other six patients the aetiology was trauma (n = 1), Sheehan’s syndrome (n = 1) or a craniopharyngeoma (n = 4). All patients had multiple pituitary deficiencies and received conventional replacement therapy as indicated. Replacement therapy was monitored during rhGH therapy and doses adjusted as required. Informed consent was obtained from all subjects, and the study was approved by the ethics committee of the Leiden University Hospital.
**Design of the study**

All GHD patients were treated with subcutaneous injections of rhGH (Genotropin, Pharmacia and Upjohn AB, Peptide Hormones, Uppsala, Sweden) given every evening for 52 weeks. All patients started with a dose of 0.6 IU per day (~0.2 mg/day) for the first 4 weeks. After 52 weeks the dose varied between 0.6 and 1.8 IU per day (~0.2–0.6 mg/day), mainly based on individual serum insulin-like growth factor-I (IGF-I) levels.

At the start of the study and after 4 and 52 weeks of rhGH therapy, body fat percentage and fat mass were estimated based on total body water as measured by dilution of deuterium.

**Body composition**

**Body weight and height** Body weight of subjects dressed in minimal clothing was measured to the nearest 0.1 kg. Height was measured barefoot to the nearest 0.001 m. BMI was calculated as weight/height$^2$ (kg/m$^2$).

**Dilution technique** Total body water was determined by the dilution of deuterium oxide, as described elsewhere (10). Fat free mass was estimated by assuming a hydration of 73% (11). Fat mass was estimated by subtracting fat free mass from weight.

**Assays**

Total serum IGF-I concentration was determined by RIA (Incstar, Stillwater, MN, USA) after extraction and purification on ODS-silica columns. Interassay coefficient of variation was less than 11%. The detection limit was 1.5 nmol/l. Age-related normal data were determined in the same laboratory. IGF-I was also expressed as a standard deviation score (S.D.-score) from age-related normal levels.

Serum leptin was measured by a highly specific RIA (Linco Research Inc, St Charles, MO, USA) as described by Ma et al. (12). In our hands, interassay coefficients of variation were 10.2, 5.3 and 5.9% at 4, 11 and 22 μg/l respectively, and the limit of detection was 0.5 μg/l.

**Statistical analysis**

Statistical analysis was performed using SPSS for Windows (version 7.0; SPSS, Chicago, IL, USA). Results are expressed as means ± S.E.M.

Paired t-tests were used to compare treatment data with baseline data. Unpaired t-tests were used to compare results for female patients with those for male patients. Pearson’s product–moment correlations were calculated. Analysis of covariance was used to investigate whether GH treatment and/or gender influences serum leptin levels independently of body fat percentage. The level of significance was set at 0.05.

**Results**

**Baseline**

In Table 1 the baseline characteristics of the GHD patients are shown. Serum leptin levels and body fat percentage were significantly higher in female than in male GHD adults ($P = 0.005$ and $P < 0.001$ respectively). Fat mass was not significantly different between female and male patients ($P = 0.235$).

The most significant relation with serum leptin was an exponential function with body fat percentage ($r = 0.88$; $P < 0.0005$; Fig. 1). Serum leptin was also correlated with fat mass ($r = 0.59$; $P = 0.025$; Fig. 1). The gender difference in serum leptin disappeared after correction for the exponential relation between body fat percentage and serum leptin ($P = 0.593$), but did not disappear after correction for the exponential relation between fat mass and serum leptin ($P = 0.015$).

Serum leptin levels correlated with neither BMI ($P = 0.492$) nor serum IGF-I levels ($P = 0.217$) and age of the patient ($P = 0.939$). Serum IGF-I and age also did not correlate with serum leptin when corrected for body fat percentage.

**Four weeks treatment**

Table 2 shows the data on serum IGF-I, serum leptin and parameters of body composition during rhGH therapy. Serum IGF-I significantly increased after 4 weeks of rhGH treatment ($P < 0.0005$). Weight and thus BMI of the patient significantly increased (both $P$ values 0.001).

Fat free mass significantly increased ($P = 0.002$) whereas fat mass did not change ($P = 0.359$). A small decrease in body fat percentage was therefore found ($P = 0.023$). Serum leptin levels did not change ($P = 0.733$).
Fifty-two weeks treatment

One female GHD patient was withdrawn from the study as she expressed the wish to become pregnant. After 52 weeks, the mean GH dose was 1.6 ± 0.8 IU/day, with a range of 0.6 to 1.8 IU/day. Serum IGF-I significantly increased to 22.6 ± 1.8 nmol/l (P < 0.0005), that is a mean S.D.-score of +0.9 ± 0.4. Fat free mass significantly increased and fat mass significantly decreased after 52 weeks of rhGH treatment (both P < 0.0005). Body fat percentage therefore significantly decreased (P < 0.0005). Serum leptin levels decreased significantly (P = 0.020) (Table 2).

GH therapy did not significantly influence the exponential relation between body fat percentage and serum leptin. For all measurements, 75.7% (r = 0.87; P < 0.0005) of the variance in serum leptin could be explained by body fat percentage.

The absolute change in the logarithmically transformed serum leptin concentration after 52 weeks of treatment significantly correlated with the absolute change in body fat percentage (r = 0.621; P = 0.024). No correlation was found between the absolute change in the logarithmically transformed serum leptin concentration and the absolute change in fat mass (r = 0.421; P = 0.152).

The percentage decrease in logarithmically transformed leptin levels significantly correlated with the percentage decrease in both body fat percentage (r = 0.762; P = 0.002) and body fat mass (r = 0.763; P = 0.002) (Fig. 2).

Table 2 Changes in IGF-I, leptin and body composition during rhGH treatment (mean ± S.E.M.).

<table>
<thead>
<tr>
<th></th>
<th>Baseline (n = 14)</th>
<th>4 weeks rhGH (n = 14)</th>
<th>52 weeks rhGH (n = 13)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GH dose (IU per day)</td>
<td>0</td>
<td>0.6 ± 0.9*</td>
<td>1.6 ± 0.8</td>
</tr>
<tr>
<td>IGF-I (nmol/l)</td>
<td>6.6 ± 1.1</td>
<td>12.6 ± 1.6*</td>
<td>22.6 ± 1.8*</td>
</tr>
<tr>
<td>Leptin (µg/l)</td>
<td>15.6 ± 2.9</td>
<td>15.2 ± 2.8</td>
<td>10.8 ± 2.1*</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>73.4 ± 3.6</td>
<td>74.6 ± 3.8*</td>
<td>75.3 ± 3.7*</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.7 ± 0.8</td>
<td>25.1 ± 0.9*</td>
<td>25.2 ± 0.8*</td>
</tr>
<tr>
<td>Total body water (l)</td>
<td>33.6 ± 2.2</td>
<td>34.7 ± 2.1*</td>
<td>36.5 ± 2.4*</td>
</tr>
<tr>
<td>Fat free mass (kg)</td>
<td>46.0 ± 3.0</td>
<td>47.5 ± 2.9*</td>
<td>50.0 ± 3.3*</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>27.4 ± 1.9</td>
<td>27.1 ± 2.0</td>
<td>25.3 ± 2.2*</td>
</tr>
<tr>
<td>Body fat percentage</td>
<td>37.6 ± 2.1</td>
<td>36.4 ± 2.0*</td>
<td>33.8 ± 2.5*</td>
</tr>
</tbody>
</table>

* The dose provided was not different between the subjects.
Data were analysed with two-tailed paired t-test: a, b, and c Significantly changed (P < 0.0005, P < 0.005 and P < 0.05 respectively) compared with baseline and 4 weeks treatment respectively.
Discussion

GH has both anabolic and lipolytic effects (13, 14). Adults with GHD therefore have a marked increase in total body fat, an abnormal fat distribution pattern, and a decreased muscle mass, which can be normalized with rhGH therapy (5–9). In agreement with these studies, we have shown a decrease in body fat percentage with rhGH therapy in patients with GHD, which is due to both an increase in fat free mass and a decrease in fat mass. Serum leptin levels did not change during short-term GH therapy (4 weeks) and decreased significantly during long-term therapy (52 weeks). The decrease in serum leptin, however, could be largely explained by the decrease in body fat percentage.

Circulating leptin levels have been shown to be strongly correlated with the degree of adiposity or fat mass. Several studies have shown a strong correlation between fat mass and serum leptin levels (15), whereas others (16–18) have shown the highest correlation between body fat percentage and serum leptin. These discrepancies are probably due to differences in the range of obesity and/or weight, the gender groups studied, and/or the method used to measure body composition (BMI or a more direct method).

With a relatively small number of patients, we found a strong exponential relation between body fat percentage and serum leptin concentration in adults with GHD. Body fat percentage was calculated from total body water, measured by deuterium dilution, assuming a hydration of 73% (11). Patients with GHD have low extracellular water compared with total body water which can be normalized by GH therapy (19). At baseline, fat free mass based on total body water could therefore be slightly underestimated and fat percentage thus overestimated. When the effect of GH on body weight is taken into account, the overestimation of body fat percentage at baseline is about 1–2%.

After 52 weeks of rhGH treatment, serum leptin levels decreased, which is largely explicable by the decrease in body fat percentage since the exponential function between body fat percentage and leptin did not change. We therefore consider the effect of GH on leptin to be indirect, via body fat percentage, which has also been suggested by Florkowski et al. (20). In another study, no change in serum leptin was found after 24 months of rhGH treatment (21). However, these authors did not publish any data on fat mass or body fat percentage for their patient group, and thus it is impossible to ascertain whether rhGH therapy had an impact on body fat percentage, or whether other mechanisms could have counteracted the decrease in serum leptin caused by a decrease in body fat percentage.

The decrease in body fat or body fat percentage with GH therapy correlates significantly with the decrease in logarithmically transformed serum leptin concentrations. The finding that changes in body fat and weight are accompanied by changes in leptin concentrations is mirrored by weight loss programmes of obese subjects and those of normal weight (16, 22–24).

Several investigators have studied the effect of insulin on serum leptin levels and found no (fat-independent) effect of insulin on circulating levels of leptin (15, 25–28). Saad et al. (29) calculated a 2% contribution of fasting insulin to serum leptin concentrations in addition to the 42% and 28% contribution of body fat and gender respectively. Moreover, an increased concentration of serum leptin was found during the final 24 h of a long-term (72 h) clamp study (30). The latter results and results of in vitro studies in both human and rodent fat cells (30) suggest that insulin may play some role in leptin production. Adults with GHD are resistant to insulin, but do not show a compensatory increase in fasting plasma insulin (31). An initial worsening in the insulin resistance with 6 weeks of rhGH therapy has been described (32). However, after 6 months of rhGH therapy, insulin levels were restored to baseline values (32), possibly as the result of the decrease in fat mass during rhGH therapy. Therefore we think that insulin per se does not explain the differences in leptin concentration, as found in this study during rhGH therapy for 52 weeks, although a minor role cannot be excluded at this time.

Our results indicate an exponential relation between body fat percentage and serum leptin which is independent of GH status.

Acknowledgements

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