Effects of short-term recombinant human growth hormone therapy on plasma leptin concentrations in dialysis patients

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Abstract

Background. Hyperleptinaemia is a well-known biochemical feature found in uraemic patients. However, little is known about the hormonal regulation of leptin in chronic renal disease. Recent studies have shown that circulating leptin levels are modified by treatment with recombinant human growth hormone (rhGH), by recombinant insulin-like growth factor I (rhIGF I), or by a combination of rhIGF I plus rhGH in patients with chronic renal failure. We performed a prospective study to assess plasma leptin concentrations in a group of dialysis patients both before and during short-term rhGH therapy.

Methods. We studied eight dialysis patients (four haemodialysis (HD) and four on continuous ambulatory peritoneal dialysis (CAPD); three female, five male; mean age 63.9 ± 3.1 years). All patients were instructed to maintain a stable diet (35 kcal/kg/day and 1 g protein/kg/day ideal body weight) and were treated with rhGH (0.2 IU/kg/day s.c.) for 4 weeks. Blood samples were taken at 0, 2, 4, and 8 weeks for determination of leptin, GH, and IGF I. Serum insulin concentrations were assessed at 0 and 4 weeks.

Results. Mean plasma leptin concentrations were elevated (36.2 ± 12.8 ng/ml) at study outset and increased progressively throughout the 4 weeks of rhGH therapy (43.7 ± 13.5 ng/ml (2 weeks, NS) and 70.6 ± 18.4 ng/ml (4 weeks, P < 0.0001)). These values returned to baseline levels (38.0 ± 12.0 ng/ml, NS) at 1 month after rhGH withdrawal. rhGH therapy was accompanied by the development of direct correlations between leptin and IGF I concentrations at 2 weeks (r = 0.86, P < 0.01), and with correlations between leptin and IGF I (r = 0.84, P < 0.01) and between leptin and insulin (r = 0.88, P < 0.01) after 4 weeks of rhGH administration.

Conclusion. These results confirm the presence of high circulating plasma leptin in dialysis patients and show that these levels are further increased by exogenous rhGH administration. The increase in plasma leptin after rhGH therapy may be related to the rhGH-induced changes in insulin in these patients.

Keywords: dialysis; growth hormone; insulin; insulin-like growth factor type I; leptin

Introduction

Leptin, the product of the mouse (ob) and human (OB) obese genes, is a 167-amino acid peptide secreted from fat cells [1]. Its main function is to act as a sensor of body fat stores. Serum leptin concentrations in humans are directly correlated with the amount of body fat and body mass index (BMI). Leptin acts by binding with specific brain receptors related to the regulation of feeding behaviour and energy balance. Besides its metabolic effects, leptin plays a role in the regulation of the growth hormone–insulin-like growth factor type I (GH–IGF I) axis [2]. Although there is a relationship between leptin and the GH–IGF I axis, it is not well-understood. By inducing lipolytic effects [3], GH plays an important role in the regulation of adipose tissue metabolism.

Recent studies have shown high plasma leptin concentrations in patients with chronic renal failure (CRF) undergoing conservative management, haemodialysis (HD), or continuous ambulatory peritoneal dialysis (CAPD) [4]. Several mechanisms have been proposed to explain this hyperleptinaemia, including increased production, secondary effects of uraemia on non-renal elimination of leptin, and decreased renal elimination [5]. Recently, it was reported that
recombinant human IGF I (rhIGF I) therapy or a combination of rhGH plus rhIGF I had effects on serum leptin levels in uraemic patients [5,6]. rhIGF I decreased serum leptin concentrations whereas the combination of rhIGF I plus rhGH caused an increase. More recently, the effect of rhGH therapy on leptin metabolism was evaluated in malnourished HD patients [7]. Although plasma leptin concentrations were unchanged following rhGH by itself, the combination of rhGH plus intra-dialytic parenteral nutrition (IDPN) produced increments in leptin concentrations. The purpose of this study was to evaluate the effect of short-term rhGH therapy on plasma leptin concentrations and to assess its relationship with the GH-induced modifications of other hormones, such as IGF I and insulin, in a group of dialysis (HD and CAPD) patients.

Subjects and methods

Subjects

Eight malnourished patients with CRF (three women, five men, mean age 63.9±3.1 years, range 52–78) undergoing dialysis (four on HD and four on CAPD) were studied. Patients were recruited to participate in a controlled randomized trial examining the effects of nutritional parameters in adult dialysis patients [see 8]. The study was approved by the Local Ethics Committee and informed written consent was obtained from all patients. The main clinical and analytical data of the patients are shown in Table 1. The aetiology of CRF included hypertensive nephroesclerosis (n = 3), chronic glomerulonephritis (n = 2), chronic pyelonephritis (n = 1), and unknown primary renal disease (n = 2). No patient had a history of diabetes mellitus. One patient was being treated with atenolol and another with clonidine to control hypertension. Seven patients were on regular erythropoietin therapy. During the study, there were no modifications in the dose of these drugs in any patient.

Study design

Details of the study design were reported in a previous prospective cross-over study with patients serving as their own controls [8]. All patients were instructed to prepare a stable diet (35 kcal/kg/day and 1 g of protein/kg/day ideal body weight) and were treated with s.c. injections of rhGH (Saizen, Serono, Spain), 0.2 IU/kg/day for 4 weeks. rhGH was administered every day at 08:30 in all patients, except on the day of dialysis in HD patients, where it was given immediately after the dialysis session. Blood samples were taken at 0, 2, 4, and 8 weeks for determination of leptin, GH, and IGF I. Serum insulin concentrations were assessed at 0 and 4 weeks.

Hormone assays

Blood samples were immediately centrifuged and the plasma or serum stored at −20°C until further assay. Plasma leptin concentrations were measured by using a polyclonal antibody radioimmunoassay (RIA) developed in rabbits against highly purified recombinant human leptin (Linco Research, St Louis, MO, USA). The intra- and inter-assay coefficients of variation were 4.8 and 3.5%, respectively. The sensitivity of the assay was 0.5 ng/ml. The normal range among 115 healthy subjects, aged 15–57 years, was 1–7.8 ng/ml. Human serum GH concentrations were determined using an automated immunoenzymatic assay (AIA 1200, Tosoh Corporation, Tokyo, Japan). Maximal intra- and inter-assay coefficients of variation were 5.4 and 3.3%, respectively. The sensitivity of GH assay was 0.1 ng/ml. Normal range was <5 ng/ml. Serum IGF I concentrations were measured by specific RIA after acid–ethanol extraction (Nichols Institute Diagnostics, San Juan Capistrano, CA, USA). The intra- and inter-assay coefficients of variation were 2.9 and 11.4%, respectively. The sensitivity of the assay was 3 μU/ml. The normal range in our laboratory was 5–25 μU/ml.

Statistical methods

Results are expressed as means±SEM. Analytical data at different time-points of the study were compared by using repeated measures analysis of variance. The Mann–Whitney test was used for comparisons between groups. Correlations between plasma leptin concentrations and the clinical and analytical parameters were evaluated using Pearson’s correlation test. Differences were considered significant when P<0.05.

Results

At the beginning of the study, mean plasma leptin concentrations were 36.2±12.8 ng/ml (range 6.9–95). These concentrations were slightly higher in CAPD than in HD patients (48.3±22.0 vs 24.0±13.5 ng/ml, NS), and slightly greater in women than in men (56.8±24.5 vs 23.8±13.3 ng/ml, NS). Mean baseline serum GH concentrations were normal or slightly increased, whereas mean serum IGF I and insulin concentrations were in the normal range (Table 2).

Plasma leptin concentrations progressively increased throughout the 4 weeks of rhGH therapy (43.7±13.5 (2 weeks, NS) and 70.6±18.4 ng/ml (4 weeks, P<0.0001)). These values returned to baseline levels

<table>
<thead>
<tr>
<th>Table 1. Clinical and analytical data</th>
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<tr>
<td>Number of patients</td>
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<tr>
<td>Age (year)</td>
</tr>
<tr>
<td>Sex (M/F)</td>
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<tr>
<td>Weight (kg)</td>
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<tr>
<td>BMI (kg/m²)</td>
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<tr>
<td>TSF (mm)</td>
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<tr>
<td>Type of dialysis (HD/CAPD)</td>
</tr>
<tr>
<td>Time on dialysis (months)</td>
</tr>
<tr>
<td>Glucose (mmol/l)</td>
</tr>
<tr>
<td>BUN (mmol/l)</td>
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<td>Creatinine (μmol/l)</td>
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Data are the number of patients or the mean±SEM.
Table 2. Hormone concentrations before and after rhGH therapy

<table>
<thead>
<tr>
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<th>Basal</th>
<th>4 Weeks</th>
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<tbody>
<tr>
<td>Leptin (ng/ml)</td>
<td>36.2±12.8</td>
<td>70.6±52.0***</td>
</tr>
<tr>
<td>Insulin (µU/ml)</td>
<td>16.9±3.0</td>
<td>36.6±9.4*</td>
</tr>
<tr>
<td>GH (ng/ml)</td>
<td>5.0±1.1</td>
<td>3.5±0.8</td>
</tr>
<tr>
<td>IGF I (ng/ml)</td>
<td>216.6±42.5</td>
<td>581.2±171.5*</td>
</tr>
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*p = 0.09; **p < 0.01; ***p < 0.0001.

Fig. 1. Leptin and IGF I responses to rhGH administration for 4 weeks in eight dialysis patients. Data are the mean±SEM. *p<0.05; **p<0.01; ***p<0.0001 vs baseline.

(38.0±12.0 ng/ml, NS) 1 month after finishing rhGH therapy (Figure 1). At 4 weeks, leptin increased from baseline by 37.7% in CAPD and by 21.4% in HD patients. At this point, plasma leptin concentrations were not different between HD and CAPD patients. GH levels remained constant during the study. Serum IGF I concentrations increased significantly from 216.6±42.5 ng/ml at baseline to 518.7±112.1 ng/ml (P<0.05) at 2 weeks, and to 581.2±171.5 ng/ml (P<0.01) after 4 weeks of rhGH administration. Serum insulin levels tended to increase during rhGH therapy; however, this increment was not statistically significant (16.9±3.0 to 36.6±9.4 µU/ml, P = 0.09).

Before rhGH therapy, plasma leptin concentrations were directly correlated with the BMI (r = 0.8, P<0.05) and triceps skinfold (TSF) (r = 0.86, P<0.01). These correlations were maintained at 2 weeks (BMI r = 0.8, P<0.05 and TSF r = 0.71, P<0.05), as well as at 4 weeks (BMI r = 0.84, P<0.01 and TSF r = 0.84, P<0.01) of rhGH therapy. This treatment was accompanied by significant increments in weight (65.6±4.9 kg, P<0.05) and BMI (26.9±2.3 kg/m², P<0.05), and by a non-significant decrease in TSF (to 15.3±4.0 mm).

At the study outset, there were no correlations between leptin and the other hormones (i.e. GH, IGF I, and insulin). However, rhGH treatment was accompanied by changes in the relationship between leptin and both IGF I and insulin. Further, there were additive effects on the relationship between IGF I and insulin via rhGH, being independent from GH serum levels. In this setting, rhGH treatment was associated with a direct correlation between leptin and IGF I concentrations at 2 weeks (r = 0.86, P<0.01), and with correlations between leptin and IGF I (r = 0.84, P<0.01) and between leptin and insulin (r = 0.88, P<0.01) at 4 weeks of rhGH administration. At this time, a strong positive correlation between IGF I and insulin (r = 0.98, P<0.0001) was also found (Table 3). On the contrary, plasma concentrations of leptin did not correlate with serum GH levels at any point of the study.

Table 3. Correlations between leptin, IGF I, and insulin at baseline and at 4 weeks of rhGH therapy in the eight dialysis patients

<table>
<thead>
<tr>
<th></th>
<th>At baseline</th>
<th>At 4 weeks</th>
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<tbody>
<tr>
<td>Leptin-IGF I</td>
<td>r = 0.41</td>
<td>r = 0.84*</td>
</tr>
<tr>
<td>Leptin-insulin</td>
<td>r = 0.11</td>
<td>r = 0.88*</td>
</tr>
<tr>
<td>IGF I-insulin</td>
<td>r = 0.48</td>
<td>r = 0.98**</td>
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*p<0.01; **p<0.0001.

Discussion

The present study documented elevated plasma leptin concentrations in dialysis patients. These values were closely correlated with adiposity parameters, such as BMI and TSF. Moreover, leptin levels increased significantly after a short-term course of rhGH therapy. This therapy was also associated with the development of direct relationships between plasma leptin concentrations and serum IGF I and insulin levels at the end of the 4 weeks of rhGH therapy.

GH has important effects on body composition, increasing lean body mass and decreasing fat mass. It would, therefore, be expected that rhGH therapy might affect leptin secretion. GH deficiency is associated with an increase in fat mass and elevated circulating leptin levels, and both alterations are diminished by rhGH [9,10]. Some authors have suggested that rhGH may indirectly influence serum leptin levels through its lipolytic action [10].

Leptin secretion is affected by hormones such as testosterone, glucocorticoids, and insulin [11–13]. A close positive correlation between circulating leptin and serum insulin concentrations has been documented in humans [11]. Moreover, GH affects carbohydrate metabolism by reducing glucose tolerance, impairing peripheral glucose utilization, increasing insulin secretion, and reducing insulin sensitivity. These effects would explain previous increases in leptin after short-term rhGH therapy in GH-deficient adults [14].

Leptin concentrations are markedly increased in patients with CRF, regardless of treatment modality [4]. Several factors have been evoked to explain hyperleptinaemia in CRF. Among them are an increased secretion rate, effects of uraemia, and a reduced capacity for renal elimination. However, as in healthy subjects, plasma leptin concentrations in uraemic patients are also influenced by other factors, including...
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total body fat and serum insulin concentrations. Positive correlations have been found between leptin levels and per cent body fat and BMI in uraemic patients [15–17]. On the other hand, the presence of a positive correlation between serum leptin and insulin levels in CRF patients has indicated that insulin resistance and hyperinsulinaemia might contribute to hyperleptinaemia in uraemic patients [16–18]. In this study, plasma leptin levels were high and were positively correlated with body fat mass estimated by BMI and TSF; however, there was no relationship between leptin plasma insulin levels before rhGH therapy. The absence of this correlation in our patients may be explained by the low degree of insulin resistance in our patients, as indicated by the BMI values as well as by the basal insulin concentrations.

There have been only a few clinical studies examining the effect of rhGH and/or rhIGF I therapy on leptin concentrations in CRF patients [5–7]. Dagogo-Jack et al. [5], evaluated the effects of rhIGF I (50 μg/kg/day, s.c. for 24 days) on plasma leptin in nine subjects with CRF. Before therapy, plasma leptin levels were correlated with BMI but not with plasma IGF I concentrations. Treatment with rhIGF I was accompanied by an increase in serum IGF I concentrations as well as by an early and sustained decrease in leptin levels. This finding suggested a possible inhibitory effect of IGF I on leptin secretion [5]. Fouque et al. [6], in a randomized cross-over trial, compared the effects of either rhIGF I alone (40 μg/kg/12 h s.c.) or a combination of rhIGF I (40 μg/kg/12 h) with rhGH (50 μg/kg/day s.c.) for 3 days on serum leptin in eight well-nourished chronic HD patients. Serum leptin was strongly correlated with body fat, and both treatments affected serum leptin in opposite manners. Whereas rhIGF I decreased serum leptin levels, the combination of rhGH plus rhIGF I increased them. In addition, there was a relationship between serum leptin and insulin variations after treatment. These findings demonstrated that both rhGH and rhIGF I acutely regulate serum leptin in dialysis patients [6]. More recently, Garibotto et al. [7] found in six malnourished HD patients that rhGH alone (5 mg s.c., at the end of each dialysis session for 6 weeks) caused no change in leptin levels, whereas rhGH plus IDPN was associated with ~50% increment in leptin levels. In this study combined therapy also caused a significant increase in insulin levels, and a positive correlation between leptin and insulin was found [7]. In our study, baseline values of leptin were increased and did not correlate with IGF I or with insulin. rhGH therapy was followed by increases in both leptin and IGF I levels with a strong positive correlation between these parameters after 2 and 4 weeks of rhGH therapy. Serum insulin increased beyond the normal range, but not significantly; however, after 4 weeks of therapy, there was a positive correlation between insulin leptin levels. Moreover, there was also a strong positive correlation between insulin and IGF I by the end of rhGH therapy. These findings indicate that rhGH therapy alone increases plasma leptin concentrations in dialysis patients, and that this effect is more potent than the described inhibitory effect of IGF I on leptin levels. In the same study by Garibotto et al. [7], short-term treatment with rhGH alone did not modify leptin. The difference between our study and Garibotto et al. [7] may be explained by the degree of patient malnutrition, type of dialysis, baseline leptin and insulin levels, and by the dose of rhGH. In accordance with previous studies, our results indicate that rhGH may increase through mechanisms involving concomitant insulin changes that reflect an increase in the insulin resistance state.

In summary, our data confirm the presence of high circulating plasma leptin in dialysis patients. These levels were further increased by exogenous rhGH administration. Hyperinsulinaemia and insulin resistance may contribute to the rise in leptin concentrations in dialysis patients treated with rhGH.

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References


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