Leptin in CAPD patients: serum concentrations and peritoneal loss

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Abstract

Background. To determine whether serum leptin concentrations in CAPD patients are influenced by peritoneal loss of leptin and to compare serum leptin levels of normal subjects with those of patients receiving renal replacement therapy such as haemodialysis (HD), CAPD, or kidney transplantation.

Results. Patients (men and women) on CAPD and after kidney transplantation exhibited significantly higher serum concentrations of leptin and leptin/BMI ratios than control subjects. These increased values did not reach statistical significance in HD patients. Serum leptin concentrations were correlated very significantly with BMI in all cases (r = 0.380, P < 0.001). Moreover, in CAPD patients (r = 0.630, P < 0.007) and in HD patients (r = 0.668, P < 0.005), but not in kidney transplant recipients or control subjects, significant correlations were observed between serum leptin and insulin concentrations. Residual renal function (RRF) in the range 0–12.8 ml/min and serum β2-microglobulin levels in the range 7.9–47.1 mg/l did not influence serum leptin levels in CAPD and HD patients. As expected, leptin was detected in the peritoneal fluid of CAPD patients. Twenty-four-hour peritoneal loss (30.95 ± 21.05 ng/min) and 24-h peritoneal clearance (0.01 ± 0.01 ml/kg/min) of leptin account for only 3.9% of estimated whole-body leptin production rate and 0.7% of leptin clearance from plasma respectively.

Conclusions. These findings suggest that serum leptin levels are not affected by continuous peritoneal loss of leptin during CAPD and that insulin resistance and hyperinsulinaemia contribute to elevated serum leptin concentrations in CAPD and HD patients. The aetiology of increased serum leptin levels in kidney transplant recipients is probably different from that in dialysis patients.

Key words: β2-microglobulin; body mass index; haemodialysis; insulin; kidney transplantation; leptin; peritoneal dialysis; peritoneal loss; residual renal function

Introduction

Leptin is a 16-kDa plasma protein that is encoded by the ob gene [1], synthesized in adipocytes and play an important role in regulation of food intake and energy expenditure in animal models [2,3]. The role of this hormone in humans is still unknown. However, in most of the clinical studies very significant positive correlations have been demonstrated between serum leptin concentrations and body mass index (BMI) or body fat mass [4–15]. Leptin in humans is partly cleared by the kidney [16,17]. Therefore, it is not surprising that increased plasma leptin concentrations in patients with chronic renal failure (CRF) pre-dialysis [4–6], during haemodialysis [4,5,7,8,16,18] or peritoneal dialysis [4,6,7] and after kidney transplantation [7] have been reported. However, there was no significant correlation between serum leptin levels and residual renal function (RRF) [4,6–8,18].

It has been suggested that elevated serum leptin concentrations in CRF are related to insulin resistance and hyperinsulinaemia [5] and that serum leptin concentrations could serve as a valuable clinical marker for the body fat content [4]. Continuous ambulatory peritoneal dialysis (CAPD) is accompanied by large losses of proteins of wide-ranging molecular mass [19], and thus peritoneal loss...
Serum levels and peritoneal loss of leptin during CAPD

of leptin could also influence the serum levels of this low molecular weight protein.

The aim of the present study was to evaluate the effect of peritoneal loss of leptin on serum levels of this protein in adult patients on CAPD and to compare the leptin profile of CAPD patients with those on haemodialysis, after kidney transplantation and normal subjects.

Subjects and methods

Eighty-four individuals were investigated: six female and 14 male undergoing standard CAPD receiving four exchanges of 2 litres of dialysis fluid (Dialine 1.5% dextrose; Travenol Labs., Ashdod, Israel) per day; 13 female and 13 male haemodialysis patients (HD) receiving three sessions of 4-h haemodialysis per week, using bicarbonate-buffered dialysate and cellulose acetate dialysers; 10 female and eight male kidney transplant recipients (Tr) receiving triple immunosuppressive therapy with varying daily doses of azathioprine (range 75–125 mg), cyclosporin (range 100–250 mg), and prednisone (range 7.5–10 mg); 10 female and 10 male normal subjects as controls. The CAPD and HD patients were receiving recombinant human erythropoietin in similar doses (females, 81.4 ± 17.8 and 172.9 ± 68.3; males, 76.0 ± 19.3 and 90.7 ± 23.3 respectively; mean ± SEM U/kg/week). The clinical characteristics of the studied individuals are shown in Table 1. The cause of chronic renal failure was diabetic nephropathy in seven CAPD and eight HD patients, chronic glomerulonephritis in four CAPD and seven HD patients, chronic interstitial nephritis in one CAPD, five HD and four Tr patients, nephrosclerosis in six CAPD, four HD and three Tr patients, polycystic kidney disease in two CAPD, one HD and three Tr patients and AA amyloidosis due to FMF in one HD and two Tr patients.

Venous blood was sampled at 0800 hours after 12 h of fasting; 24-h and 8-h (overnight) samples of peritoneal dialysate and 24-h urine samples were collected. Serum and dialysate concentrations of glucose, urea and creatinine were analysed by standard autoanalyser techniques. Albumin was determined in serum using the bromcresol green method and in dialysate and urine using an immunoturbidimetric method. Serum and dialysate concentrations of β₂-microglobulin (β₂MG) were measured in the IMX system (Microparticle Enzyme Immunoassay, Abbott Laboratories, Abbott Park, IL, USA). Serum insulin concentrations were measured by radioimmunoassay.

Residual renal function (RRF) was calculated as the sum of 24-h urinary creatinine and urea clearances divided by 2.

Concentrations of leptin in original serum and in dialysate and urine concentrated 6 to 20-fold by Centricon 3 (cutoff 3000 daltons) were measured with a commercial RIA (Linco Research Inc., St Charles, MO, USA).

Data were evaluated by Student’s t test, analysis of variance and linear regression and are expressed as mean ± SEM. The Mann–Whitney test was used for analysis of leptin values.

Results

Both CAPD and kidney transplant patients exhibited significantly higher serum concentrations of leptin and leptin/BMI ratios than control subjects (Tables 2 and 3). These increased values did not reach statistical significance in HD patients. As shown in Table 4, serum leptin concentrations correlated very significantly in HD patients. As shown in Table 4, serum leptin concentrations correlated very significantly in HD patients.

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Age (years)</th>
<th>BMI (kg/m²)</th>
<th>RRF (ml/min)</th>
<th>Time (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CL</td>
<td>Female 10</td>
<td>46.0 ± 4.5</td>
<td>25.7 ± 2.1</td>
<td>N</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>Male 10</td>
<td>48.0 ± 7.6</td>
<td>26.7 ± 1.0</td>
<td>N</td>
<td>–</td>
</tr>
<tr>
<td>HD</td>
<td>Female 13</td>
<td>61.1 ± 2.5*</td>
<td>24.1 ± 1.3</td>
<td>0</td>
<td>45.6 ± 10.8</td>
</tr>
<tr>
<td></td>
<td>Male 13</td>
<td>59.3 ± 3.4</td>
<td>25.1 ± 1.0</td>
<td>0</td>
<td>72.3 ± 15.0</td>
</tr>
<tr>
<td>Tr</td>
<td>Female 10</td>
<td>46.0 ± 3.8</td>
<td>26.7 ± 2.2</td>
<td>66.2 ± 10.2</td>
<td>84.2 ± 13.0</td>
</tr>
<tr>
<td></td>
<td>Male 8</td>
<td>44.8 ± 5.0</td>
<td>26.6 ± 1.6</td>
<td>64.5 ± 7.7</td>
<td>82.6 ± 10.6</td>
</tr>
<tr>
<td>PD</td>
<td>Female 6</td>
<td>60.8 ± 3.7*</td>
<td>23.7 ± 1.7</td>
<td>2.7 ± 1.1</td>
<td>14.0 ± 5.6***</td>
</tr>
<tr>
<td></td>
<td>Male 14</td>
<td>58.7 ± 3.2</td>
<td>24.1 ± 0.9</td>
<td>3.2 ± 1.2</td>
<td>13.5 ± 3.3**</td>
</tr>
</tbody>
</table>

CL, control; HD, haemodialysis; Tr, kidney transplant; PD, peritoneal dialysis; BMI, body mass index; RRF, residual renal function; Time, duration of renal replacement therapy (dialysis or transplantation); N: normal renal function (serum creatinine <115 μmol/l).

*P<0.05 vs CL and Tr; **P<0.001 vs HD and Tr; ***P<0.001 vs Tr.
Fig. 1. Relationship between serum insulin and leptin levels in CAPD and haemodialysis patients. The resulting regression equations were as follows: CAPD patients (open circles), $y = 2.37x + 17.70; r = 0.630; P < 0.007; n = 17$. Haemodialysis patients (closed circles), $y = 1.35x + 7.99; r = 0.668; P < 0.005; n = 16$. All patients (CAPD and haemodialysis; solid line), $y = 1.56x + 16.74; r = 0.572; P < 0.0001; n = 33$.

Table 2. Serum leptin concentrations in studied individuals (µg/l; mean ± SEM)

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Male</th>
<th>n</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>CL</td>
<td>10</td>
<td>4.8 ± 0.9</td>
<td>10</td>
<td>20.8 ± 3.8</td>
</tr>
<tr>
<td>HD</td>
<td>13</td>
<td>17.7 ± 6.0</td>
<td>13</td>
<td>35.6 ± 12.7</td>
</tr>
<tr>
<td>Tr</td>
<td>8</td>
<td>20.4 ± 7.9*</td>
<td>10</td>
<td>54.8 ± 9.2*</td>
</tr>
<tr>
<td>PD</td>
<td>14</td>
<td>26.1 ± 10.2*</td>
<td>6</td>
<td>89.4 ± 26.3*</td>
</tr>
</tbody>
</table>

CL, control; HD, haemodialysis; Tr, kidney transplant; PD, peritoneal dialysis. *P < 0.05 vs control group.

Table 3. Serum leptin to body mass index ratios in studied individuals (mean ± SEM)

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Male</th>
<th>n</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>CL</td>
<td>10</td>
<td>0.2 ± 0.03</td>
<td>10</td>
<td>0.8 ± 0.09</td>
</tr>
<tr>
<td>HD</td>
<td>13</td>
<td>0.6 ± 0.20</td>
<td>13</td>
<td>1.3 ± 0.43</td>
</tr>
<tr>
<td>Tr</td>
<td>8</td>
<td>0.7 ± 0.23*</td>
<td>10</td>
<td>2.0 ± 0.30*</td>
</tr>
<tr>
<td>PD</td>
<td>14</td>
<td>1.0 ± 0.32*</td>
<td>6</td>
<td>3.8 ± 1.15*</td>
</tr>
</tbody>
</table>

CL, control; HD, haemodialysis; Tr, kidney transplant; PD, peritoneal dialysis; *P < 0.05 vs control group.

Table 4. Relationship between serum leptin concentrations and body mass index

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>r</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>CL</td>
<td>20</td>
<td>0.542</td>
<td>0.014</td>
</tr>
<tr>
<td>HD</td>
<td>26</td>
<td>0.591</td>
<td>0.001</td>
</tr>
<tr>
<td>Tr</td>
<td>18</td>
<td>0.521</td>
<td>0.027</td>
</tr>
<tr>
<td>PD</td>
<td>20</td>
<td>0.545</td>
<td>0.013</td>
</tr>
<tr>
<td>Total*</td>
<td>84</td>
<td>0.380</td>
<td>0.000</td>
</tr>
</tbody>
</table>

*Female, $r = 0.408, P = 0.009$; male, $r = 0.530, P = 0.000$. CL, control; HD, haemodialysis; Tr, kidney transplant; PD, peritoneal dialysis.

Table 5. Eight-hour peritoneal clearances (ml/min) of solutes according to the molecular mass (daltons) in 17 CAPD patients (mean ± SEM)

<table>
<thead>
<tr>
<th>Solutes</th>
<th>Molecular mass</th>
<th>Peritoneal clearance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beta-2-microglobulin</td>
<td>11 800</td>
<td>1.12 ± 0.15</td>
</tr>
<tr>
<td>Leptin</td>
<td>16 000</td>
<td>0.77 ± 0.10</td>
</tr>
<tr>
<td>Albumin</td>
<td>69 000</td>
<td>0.12 ± 0.02</td>
</tr>
</tbody>
</table>

Discussion

The major new finding of this study was the detection of leptin, a protein of MW 16 kD, in urine and peritoneal fluid of CAPD patients. This has not been

albumin and $\beta_2$MG, consistent with its molecular mass (Table 5). These results fit the log-to-log function: $\ln$ (molecular mass) = 9.47 – 0.79 $\ln$ (peritoneal clearance) with correlation coefficient ($r$) = 0.999, and suggest that leptin is cleared peritoneally in the free form, and not in combination with its 90–100 or 280-kDa binding proteins [21]. The peritoneal clearance of leptin was correlated significantly with peritoneal clearances of albumin ($r = 0.541, P = 0.025$). The strong correlation was observed also between peritoneal clearances of $\beta_2$MG and leptin (Figure 3) suggesting passive diffusion of leptin rather than its local synthesis.

Twenty-four-hour urinary losses of leptin measured in 17 CAPD patients (11 males and 6 females) were $7.0 ± 3.6 \mu g/24\ h$, which accounts for $5.6 ± 1.8\%$ (range 0.3–15.2%) of total (peritoneal plus urinary) loss of this hormone. There was no significant correlation between 24-h urinary loss and serum levels of leptin ($r = 0.425, P = 0.089$).

Age, time on dialysis, serum glucose and albumin concentrations, as well as peritoneal glucose absorption and peritoneal albumin loss had no effect on serum leptin levels (results not shown).
previously reported. We found that peritoneal loss of leptin accounts for only 3.9% of mean estimated whole-body leptin production rate and 0.7% of mean leptin clearance from plasma when these results were compared with the results estimated in 14 male volunteers [22]. There are no previous reports about whole-body production rate and plasma clearance of leptin in uremic patients. A preliminary report showing down-regulation of the ob gene expression in CAPD patients with hyperleptinemia [23] suggests that these parameters in uremic patients may differ from those of healthy subjects. 24-h urinary losses of leptin were also very small, accounting for 5.6% of mean total (peritoneal plus urinary) loss of this hormone. These findings indicate that peritoneal and urinary losses of leptin have only a small or negligible effect on serum leptin concentrations in CAPD patients. The strong positive correlation between serum values and peritoneal loss of leptin also points to passive leakage of this low molecular weight protein from blood into peritoneal fluid. As expected [19], transperitoneal transport of leptin, together with albumin and β2-MG, was strongly correlated with molecular mass (r = 0.999). These kinetic parameters suggest that peritoneal transport of leptin is unlikely to occur in the form of a complex between leptin and its binding proteins [21] and that concentrations of leptin in peritoneal fluid are not due to its degradation or intraperitoneal production by adipocytes. Alternatively the expected decreased peritoneal clearance of leptin from plasma due to protein binding [21] may be balanced by the local intraperitoneal synthesis of leptin by omental adipocytes [24].

The absence of significant positive correlation between serum levels and urinary loss of leptin in CAPD patients support the conclusions of Meyer et al. [17] that renal leptin removal from plasma is due to renal tissue leptin uptake and degradation rather than glomerular filtration and urinary excretion.

The present results corroborate previous studies [4,6,7], which demonstrated that CAPD patients and kidney transplant recipients exhibited significantly higher serum leptin levels and leptin to BMI ratios than control subjects. In contrast to some studies published recently [5,7,8,16], HD patients showed serum leptin concentrations and leptin/BMI ratios in the high-normal range. One possible explanation for these discrepant results may be the fact that HD patients were older than control subjects. Two studies have shown that serum leptin concentrations are inversely correlated to age [14,15] showing a reduction in circulating leptin by 53% in subjects over age 60 years [14]. In addition, suppression of lepininaemia induced by long-term recombinant human erythropoietin therapy in haemodialysed uremic patients has not been excluded [25]. The HD patients in the present study were longer on dialysis than those on CAPD. In lean individuals with BMI less than 20 kg/m², Kennedy et al. [26] found that serum concentrations of leptin were similar in men and women. The percentage of these patients in our population was only 10.7% with similar distribution among the groups. Therefore, in accordance to the results reported by others [2,3], women in this study had values for leptin markedly higher than men, suggesting specific gender-related and/or hormonal effects [27,28].

BMI is a relative marker of obesity and percent of body fat mass [29]. In confirmation with previous observations [7–10,30], we found a strong positive correlation between serum leptin concentrations and BMI in individuals of all studied groups.

A long-term effect of insulin on leptin production has been demonstrated both in vitro and in vivo [31]. High leptin concentrations may in part be influenced by hyperinsulinaemia or impaired insulin sensitivity [30]. The significant association described between fasting plasma levels of leptin and insulin is independent of body adiposity [32]. On the other hand, leptin attenuates some insulin-induced signals in human hepatic cell lines raising the possibility that leptin modulates insulin activities in obese individuals [33]. Insulin resistance and hyperinsulinaemia are well-known features of chronic renal failure (CRF) [34,35]. Stenvinkel et al. [5] showed first that insulin resistance and hyperinsulinaemia contribute to elevated serum leptin concentrations in CRF patients. We extended this observation to patients undergoing HD and CAPD, in whom insulin levels correlate with serum leptin concentrations.

Our findings are in agreement with the results of Howard et al. showing markedly elevated serum concentrations of leptin in kidney transplant recipients [7]. Serum leptin concentrations in transplant subjects were affected by adiposity (BMI) but not by fasting insulin levels or kidney function. The role of steroid treatment in the increase of leptin levels in these patients has not been excluded. Indeed, steroids have been found to increase leptin mRNA expression and protein production by adipocytes in vitro [36] and in
References


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