Leptin elimination in hyperleptinaemic peritoneal dialysis patients

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Abstract

Background. Elevated plasma concentrations of leptin, a hormone thought to regulate body composition by influencing food intake/metabolic rate, are prevalent in renal failure patients. The mechanism for these increases is not known, but evidence suggests that simple accumulation due to decreased elimination is insufficient explanation.

Methods. We studied the incidence of hyperleptinaemia in 28 end-stage renal disease patients treated with continuous ambulatory peritoneal dialysis (CAPD), compared with body-mass-index-and sex-matched controls. Results were separated by gender because women have higher leptin concentrations than men. Excretion of leptin and other substances in dialysis fluid was also studied.

Results. Hyperleptinaemia was prevalent in women CAPD subjects, but not in men. Plasma leptin concentrations correlated strongly with the daily excretion of leptin in dialysis fluid. Clearance of leptin in dialysis fluid was greater in men than women CAPD subjects. Single regression analysis found that fasting insulin, glucose content of dialysis fluid, plasma albumin, C-reactive protein, erythropoietin dose, urinary creatinine clearance and plasma β₂-microglobulin were not determinants of plasma leptin concentrations. Stepwise forward multiple regression, examining the dependence of plasma leptin on body mass index, renal creatinine clearance, plasma albumin, daily dialysis fluid glucose load, daily leptin in dialysis fluid, erythropoietin dose and plasma C-reactive protein found only erythropoietin dose as a consistent negative predictor of plasma leptin concentrations.

Conclusions. The results suggest that hyperleptinaemia of CAPD was due to predisposing loss of renal elimination capacity combined with increased production due to obesity (more prevalent in women subjects of this study) and potentially female gender.

Introduction

Leptin, the hormone produced by adipose tissue and thought to be involved in regulating body composition by influencing food intake/metabolic rate [1–4], has been shown in several studies to be elevated in the plasma of renal failure patients [5–10], particularly in end-stage renal disease (ESRD) treated with haemodialysis [5–9] and peritoneal dialysis [7–10]. Although plasma leptin concentrations vary directly with percent body fat in normal lean and obese subjects, such that obese subjects have very high leptin concentrations [11,12], the elevations seen in many renal failure patients are much greater than predicted by their adiposity. The kidney appears to be the principal site of elimination of circulating leptin in healthy subjects and in rats, perhaps by a mechanism involving cellular uptake [6,13–16].

Elevated leptin concentrations in renal failure patients cannot be fully explained by simple accumulation due to decreased elimination; the degree of elevation does not generally correlate with indices of renal impairment and many patients with severe renal disease (i.e. most hemodialysis patients) have plasma leptin concentrations that are appropriate for their adiposity [5,6]. An alternative explanation is that leptin production is increased, perhaps as a secondary effect of underlying disease or in response to uraemia. Recent studies have reported a significant correlation of plasma leptin with fasting insulin concentrations [17] and with C-reactive protein [8] in renal failure patients. Chronic elevated insulin concentrations have been shown to increase plasma leptin concentrations in normal subjects [18–20]. Administration of endotoxin or tumour necrosis factor (TNF)α to rodents produces a strong elevation of plasma leptin, along with decreased food intake [21,22]. Elevated plasma leptin concentrations seen in many renal failure patients could result from increased production in response to accumulated insulin or to increased levels of cytokines, which might be reflected as elevated C-reactive protein [8,17].

In an effort to explain the hyperleptinaemia of renal failure, we have studied potential correlates of leptin metabolism and particularly leptin elimination by dialysis in peritoneal dialysis patients. These patients have little residual renal function and many have grossly elevated plasma leptin levels.
Materials and methods

Subjects

Twenty-eight adult patients (14 men; age 54.5 ± 17.7 years, range 21.5–81 years) receiving continuous ambulatory peritoneal dialysis (CAPD) treatment for ESRD gave written informed consent to a protocol approved by the Human Studies Committee of Washington University. Renal failure was due to diabetes mellitus in 12 (10 were receiving insulin), glomerulonephritis in seven, hypertension in five and HIV infection, lupus, reflux nephropathy and polycystic kidney disease in one subject each. Medications received by the subjects included erythropoietin by 23, antihypertensives by 19, warfarin by three, thyrroxine by four and anti-depressants by four; one patient was receiving prednisone and two were receiving replacement estrogens. Insulin therapy for the diabetics was administered by standard sub-cutaneous regimens (mean dosage 55 ± 31 U/day, range 8–95 U/day). All patients were performing four exchanges daily, except for two who were performing five exchanges; exchange volumes were 2.0 or 2.5 l per exchange. Patients had been treated with peritoneal dialysis for 18 ± 20 months (range 0.5–94 months). Dialysis adequacy was assessed by measuring Kt/V of urea, which was determined by collecting 24-h drained dialysate and urine specimens followed by blood samples. Kt/V urea, calculated by standard methods [23], averaged 2.09 ± 0.53 (range 1.31–3.99) in these patients. The same specimens were used for determination of leptin clearance. Normal adults (n = 28; age 36.8 ± 8.0 years, range 24–52 years) paired to match the CAPD subjects for sex and body mass index (BMI) served as controls.

Specimens for leptin analysis were drawn during daylight hours when subjects appeared for routine office assessment of dialysis therapy; the subjects were receiving their normal diet. Specimens for combined leptin and insulin determination were drawn after the subjects drained their dialysis fluid without overnight replacement and an overnight fast. None of the subjects were studied during intercurrent illness (except for the one HIV-infected subject), including peritonitis, and none were enrolled in active weight-management programs.

Analytical methods

Plasma leptin concentrations were measured with a commercial radioimmunoassay (Linco Research, St. Charles, MO, USA), which was based on polyclonal antibody to recombinant human leptin, recombinant human leptin for standards and tracer, and a second antibody method for separation of bound and free leptin. This method, which will detect 0.5 μg/l leptin in plasma, has been characterized extensively [24]. Long-term interassay coefficients of variation are 11.0% at 2.6 μg/l and 10.9% at 13.5 μg/l. Urea, creatinine, albumin and total protein analyses were performed by Spectra Laboratories (Freemont, CA, USA). C-Reactive protein analyses were performed on a Beckman Array 360 (Brea, CA, USA) using reagents provided by the manufacturer.

Statistical methods

Results

Plasma leptin concentrations ranged from 1.3 to 505 μg/l in the 28 study subjects. Because of the well-known sexual dimorphism in leptin levels [25–28], all of the results concerning leptin concentrations are separated by sex for presentation. Because leptin levels increase in normals and renal failure patients with increasing adiposity [11,12], results from CAPD subjects were compared with sex- and BMI-matched controls (Table 1). Plasma leptin concentrations in male and female CAPD subjects were three-fold higher but not statistically different from those of BMI-matched controls (P = 0.078). In females, leptin concentrations were seven-fold higher than in BMI-matched controls (P < 0.001), and 12 of the 14 women had concentrations exceeding 130 μg/l. The highest leptin concentration in a control was 82.2 μg/l (BMI 42.5 kg/m²). Thus frank hyperleptinaemia of renal failure was more pronounced in women. Repeated measurements of leptin over months showed that concentrations in CAPD subjects were stable, with modest variation consistent with previously [24]. The relationship of plasma leptin with BMI was disrupted in female and decreased in male CAPD subjects; whereas the control groups had strong correlation of daily excretion with plasma leptin concentration for both women (r = 0.774, P = 0.001 for men; P = 0.055 and r = 0.155, P = 0.596, respectively).

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Plasma leptin concentrations in CAPD subjects and BMI-matched control subjects

<table>
<thead>
<tr>
<th>BMI (kg/m²)</th>
<th>Leptin (μg/L)</th>
<th>Correlation of BMI with leptin</th>
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<tr>
<td></td>
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<tr>
<td>Control men</td>
<td>25.7 ± 4.5</td>
<td>6.1 ± 3.4 (2.4–13.4)</td>
</tr>
<tr>
<td>CAPD men</td>
<td>25.7 ± 4.4</td>
<td>18.2 ± 23.6** (1.3–89.7)</td>
</tr>
<tr>
<td>Control women</td>
<td>30.1 ± 6.5</td>
<td>30.5 ± 2.5 (8.5–82.2)</td>
</tr>
<tr>
<td>CAPD women</td>
<td>30.1 ± 6.4**</td>
<td>215.5 ± 120.1*** (27.1–505)</td>
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</tbody>
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Versus paired controls: *P = 0.993, **P = 0.998, ***P = 0.078, ****P = 0.001.

Table 2. Clearance of leptin and other solutes by peritoneal dialysis

<table>
<thead>
<tr>
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<th>Men (ml/min)</th>
<th>Women (ml/min)</th>
<th>P</th>
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</thead>
<tbody>
<tr>
<td>Creatinine</td>
<td>5.4 ± 0.8</td>
<td>5.5 ± 1.0</td>
<td>0.71</td>
</tr>
<tr>
<td>β2-Microglobulin</td>
<td>0.63 ± 0.31</td>
<td>0.57 ± 0.14</td>
<td>0.59</td>
</tr>
<tr>
<td>Leptin</td>
<td>1.7 ± 0.9</td>
<td>0.7 ± 0.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total protein</td>
<td>0.09 ± 0.04</td>
<td>0.07 ± 0.02</td>
<td>0.065</td>
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Substances was similar in women (P = 0.161) but the much higher clearance of leptin resulted in a highly significant difference in men (P = 0.001). Also, clearances of leptin and β2-microglobulin were highly correlated in women (r = 0.764, P = 0.004), but unrelated in men (r = 0.024, P = 0.940). Several parameters related to dialysis efficiency/renal function were compared between men and women CAPD subjects. Kt/V, daily glucose load in dialysate, creatinine clearance, age and length on dialysis were similar between the two groups (all P > 0.17). Dialysate Kt/V was significantly higher (P = 0.01) in women (1.88 ± 0.22) than men (1.60 ± 0.24); however, this difference was opposite to the difference in leptin clearance.

Fasting insulin and leptin concentrations were measured in 12 nondiabetic CAPD subjects; results were analyzed after segregation by sex. Insulin levels did not correlate with leptin in either men (n = 5, r = 0.312, P = 0.609) or women (n = 7, r = 0.253, P = 0.584). A related concern is the potential of the glucose content of the dialysis fluid to chronically increase insulin, and thereby increase circulating leptin concentrations. To test this hypothesis, we sought to correlate the total glucose content of dialysis fluid administered daily (range 125–425 g/day) with plasma leptin concentrations; in both men and women CAPD subjects there was no significant correlation (r = 0.168, P = 0.566 and r = 0.404, P = 0.151, respectively). Plasma albumin concentrations are frequently low in CAPD subjects, and are thought to reflect nutritional state. Since nutritional state can strongly influence leptin concentrations [29–31], we correlated plasma leptin with albumin concentrations in the same specimens; there was no significant correlation in either men (r = 0.298, P = 0.301) or women (r = 0.116, P = 0.692). Because inflammation has been shown to increase leptin concentrations [21,22], we measured C-reactive protein levels in the CAPD cohort; there was no significant correlation of C-reactive protein with plasma leptin in either men (r = 0.124, P = 0.685) or women (r = 0.393, P = 0.184). Because long-term erythropoietin (Epo) therapy has been reported to be a negative modifier of plasma leptin concentrations [32] we correlated plasma leptin with Epo weekly dose. The correlation for women (r = 0.275) was not statistically significant (P = 0.34), but the correlation for men (r = 0.520) approached significance (P = 0.057). Residual renal function, measured as urinary creatinine clearance, was examined in relation to plasma leptin concentrations; there was no significant correlation in male CAPD subjects (r = 0.134, P = 0.648) but there was a positive correlation in women (r = 0.634, P = 0.015). This positive correlation is unlikely to explain hyperleptinaemia since decreasing renal function (not the increasing relationship suggested by the correlation) is associated with elevated leptin concentrations. Also, plasma leptin was not significantly related to plasma β2-microglobulin concentrations in either men (r = 0.347, P = 0.27) or women (r = 0.398, P = 0.20). Repeating these cor-
relations, using ln leptin in place of raw plasma leptin concentrations, yielded very similar results (data not shown).

As a final examination of the data for determinants of hyperleptinaemia in CAPD subjects, stepwise forward multiple regression was performed with plasma leptin as the dependent variable, and the following independent variables: BMI, urinary creatinine clearance, plasma albumin concentration, daily dialysis fluid glucose load, daily leptin in dialysis fluid, plasma C-reactive protein and Epo dose per week. In the model derived for male CAPD subjects, Epo dose explained 25% and BMI 22% of the variation in leptin between subjects, the other variables were not significantly contributory. For the female subjects, the model found that urinary creatinine clearance (39% of variation), C-reactive protein concentration (23%) and Epo dose (7%) were significant determinants of leptin concentration. The effect of Epo in both of the models was negative, in keeping with an earlier report [32]. As in single regression analysis (see above), increasing creatinine clearance was associated with increasing plasma leptin concentrations and is not likely to be meaningful. The association of C-reactive protein in the model derived for women PD subjects was negative, and therefore the opposite of the relationship predicted by preceding work. Thus, Epo dose was the only consistent determinate of leptin concentrations among these variables. Repeating the multiple regression analyses, replacing plasma leptin with ln leptin as the dependent variable, generated very similar findings except that epo dose was no longer a significant determinant of leptin concentration for either men or women (data not shown).

Discussion

The hyperleptinaemia of ESRD has resisted convincing analysis of causation; the idea that leptin simply accumulates as renal elimination decreases is untenable since many individuals without renal function (including surgically anephric subjects) have leptin concentrations appropriate for their degree of adiposity, and leptin concentrations do not correlate strongly with measures of residual renal function such as urinary creatinine clearance [5–10]. Leptin production may decrease in ESRD to compensate for reduced elimination capacity, but there is no evidence currently available to suggest reduced production in hyperleptinaemic states, and the high leptin levels observed in obesity argue against such negative regulation of production [11,12]. Two recent studies of nephrectomized rats demonstrated rapid adaptation to the loss of leptin elimination, which restored prenephrectomy leptin concentrations within 24 h [33,34]. The studies suggested that loss of renal function in ESRD subjects may result in compensating leptin elimination by other tissues.

The results presented here advance the hypothesis that the hyperleptinaemia observed in many CAPD subjects results from a predisposing loss of renal elimination combined with increased production, the increased production likely overwhelms the adaptive elimination capacity and results in the hyperleptinemic state. Hyperinsulinemia or inflammation are potential important causes of increased production in ESRD, based on correlations of leptin concentrations with plasma insulin or C-reactive protein [8,17], but no evidence for these causes was found in the CAPD cohort studied here. Previous studies have noted that hyperleptinemia is much more prevalent in obese ESRD subjects (defined as BMI >28 kg/m²) after taking into account the increased levels seen in obesity in normals [5,6]. The women in the present study had an average BMI of 30.1 kg/m², which was significantly greater (P = 0.042) than that of male subjects (25.7 kg/m²). Also, BMI was a significant determinant of plasma leptin concentrations in forward stepwise multiple regression for men CAPD subjects. The higher plasma concentrations of leptin seen in obese subjects are due to increased production of leptin [35], so the obese state is a high-production state. Several studies have documented that women have higher leptin concentrations than men, even after adjusting for the differences in adiposity between men and women [25–28]; this sexual dimorphism likely results from effects of sex hormones on leptin production, since testosterone and estrogen have been shown to regulate leptin production [35–38]. Also, women CAPD subjects have higher percent body fat than men at the same BMI. Although use of direct measures of body composition such as dual excitation X-ray absorptiometry would likely reduce the sexual dimorphism found in the BMI-based data presented here [8,39], women CAPD subjects still produce more leptin as a consequence of higher percent body fat. These data and recent studies suggest that obesity, inflammation, hyperinsulinism and potentially female gender are causes of higher production of leptin in the hyperleptinaemia of ESRD.

The clearance of leptin in dialysate of CAPD subjects is readily measurable but accounts for only a tiny fraction of the leptin produced daily, based on measurements of leptin production in normals. If the half-life of leptin in plasma in CAPD subjects is similar to that of normals (about 25 min) [35], and the volume of distribution is the intravascular plasma volume (about 3 l), then a patient with a plasma leptin concentration of 140 µg/l would produce about 10 mg/day of leptin [40]. Postulating a larger volume of distribution requires even higher daily leptin production. Daily leptin cleared in dialysis fluid in our CAPD subjects never exceeded 350 µg per day, even in subjects with very high (>500 µg/l) plasma leptin concentrations. In male subjects plasma leptin concentrations were much lower (18.2 ± 23.6 µg/l) but so was average daily leptin in dialysate (34.1 ± 34.4 µg/l/day). Thus leptin elimination in dialysate is not the major elimination route in CAPD subjects, and CAPD subjects likely possess alternative means of eliminating leptin from circulation.

The dialytic clearance rates for leptin between men...
and women CAPD subjects were very different. Also, the dialysis clearance rates of $\beta_2$-microglobulin and leptin, which differ in molecular weight by a factor of only 1.4, were similar in CAPD women, but unrelated in CAPD men. Men have a higher fraction of their body fat in the omental depot, which is in direct contact with the dialysis fluid. The higher leptin clearance rates for men may reflect greater efficiency of dialytic transfer of local (omentum) leptin production: the correlation of $\beta_2$-microglobulin dialytic clearance with leptin dialytic clearance would be expected to be disrupted by such a mechanism, which was the case in this study. Leptin in circulation is known to be present in bound and free forms [41], and ESRD increases the fraction of leptin in free form [6]. Variation in the free-leptin fraction in ESRD could result in differences in the clearance of leptin by peritoneal dialysis because bound and free leptin likely differ greatly in molecular size and therefore permeability. Higher free-leptin concentrations should result in more efficient clearance of leptin to peritoneal dialysis and should make the clearance of the women subjects in our study, who had much higher total leptin concentrations, larger than that for men. The opposite was true, suggesting that the observed differences in clearance is not the result of variation in bound/free forms of leptin.

Several characteristics previously reported to correlate with leptin concentrations in normals or ESRD subjects were not correlated in this study. Indices of adiposity such as BMI usually correlate strongly with plasma leptin concentrations, which was true with the control subjects in this study. But this relationship was weaker or absent in the CAPD subjects, presumably because other factors obscured the relationship. The possible roles of inflammation (measured as C-reactive protein) and hyperinsulinemia were also difficult or impossible to detect in this study, even using stepwise regression, but the small numbers of subjects studied limited the statistical power of the correlations. While one might anticipate that hyperleptinaemia would correlate with residual renal function, we observed weak correlations of creatinine clearance with plasma leptin and these were positive in direction, suggesting statistical anomalies. Nevertheless, it is clear that reduced renal elimination capacity has a predisposing effect for hyperleptinaemia; renal transplant rapidly corrects the hyperleptinaemic state, demonstrating that it is not a secondary effect of underlying disease(s) that caused ESRD [42].

Acknowledgements. The authors wish to thank Barbara Hartman for her assistance in preparing this manuscript. This research was aided by support from Diabetes Research and Training Center grant P60 DK20579.

References
25. Havel PJ, Kasim-Karakas S, Dubuc GR, Mueller W, Phinney
Leptin elimination by peritoneal dialysis


Received for publication: 27.6.98
Accepted in revised form: 10.11.98