Peritoneal clearance of leptin in CAPD patients: impact of local insulin administration

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Abstract

Introduction. The ob gene product leptin is secreted by fat cells and the serum leptin levels reflects the body fat content. Markedly elevated serum leptin levels have been reported in patients with chronic renal failure. The aim of the present study was to assess if the dialysate leptin levels in peritoneal dialysis are similar to what can be expected from passive diffusion or if intraperitoneal synthesis of leptin may occur.

Methods. We studied 39 patients (20 males), mean age 54 ± 12 years, who had been treated with peritoneal dialysis for 17 ± 12 months. Ten of the patients were diabetics of which seven used intraperitoneal insulin. A 24-h collection of dialysate was performed and dialysate and fasting blood samples were analysed for leptin, albumin and β2-microglobulin, and the peritoneal clearances (PCl) were calculated for these solutes.

Results. Serum leptin (mean 47 ± 76, range 3–350 ng/ml) was related to body mass index (r = 0.35, P < 0.05). In multiple regression analysis, serum leptin also correlated to serum TNF-α. Although dialysate leptin levels correlated to serum leptin, they were higher than expected from the molecular weight of 16 kD. PCl of leptin was 1.3 ml/min (range 0.2–5.9 ml/min), which was 1.6 times higher than expected from the molecular weight of leptin and PCl for albumin and β2-microglobulin, not taking the protein binding of leptin into account. A strong correlation was found between PCl for albumin and β2-microglobulin (r = 0.68, P < 0.0001) but neither PCl albumin, nor PCl β2-microglobulin correlated to PCl leptin. The PCl of leptin was markedly higher in diabetics using intraperitoneal insulin (n = 7) compared to the other 32 patients (2.6 ± 2.0 vs 1.1 ± 0.7 ml/min, P < 0.05).

Conclusion. Serum leptin is locally produced in the peritoneal cavity, and intraperitoneal insulin enhances local production of leptin.

Key words: leptin; peritoneal dialysis; insulin; TNF-α

Introduction

The obesity (ob) gene protein, known as leptin is secreted mainly by white fat adipocytes [1], and regulates food intake and energy expenditure in animal models. Leptin reaches the brain by a saturable transport mechanism via the blood-brain-barrier. Via direct effects on the hypothalamus leptin decreases appetite and increases metabolism [2]. In subjects without renal failure the circulating leptin levels are closely related to the body fat content and markedly elevated leptin levels have been reported in patients with obesity [3,4].

It has recently been demonstrated that the leptin levels are closely related to the body fat mass also in patients with chronic renal failure (CRF) [5,6]. However, in CRF patients the leptin levels are usually markedly elevated compared to patients with similar body fat content but without CRF [5–9], although elevated serum leptin levels are not a universal finding in advanced CRF [10]. The cause(s) of elevated serum leptin levels in uremia are probably multifactorial and there are data suggesting that decreased glomerular filtration rate [8,9,11–13], inflammation [5,11] and hyperinsulinaemia [10,13] all may increase serum leptin levels in CRF. In contrast to obesity, where the ob-gene expression is increased, we have recently demonstrated that the ob-gene expression is decreased in patients with CRF and hyperleptinemia, suggesting that decreased catabolism/elimination of leptin inhibits ob gene expression by a feed-back mechanism [11]. Since leptin is thought to be an inhibitor of appetite, it has been speculated that elevated serum leptin levels could contribute to anorexia and poor nutrition in patients with renal failure [6].

We have previously reported that 12 months of CAPD treatment is associated with a marked increase in both serum leptin levels and body fat content, in contrast to unchanged serum leptin levels observed after 12 months of hemodialysis treatment [5]. These findings may suggest that the continuous carbohydrate
load in CAPD increases the body fat mass and consequently also the serum leptin levels [5]. The molecular weight of leptin (16 kD) suggests that it will not be cleared by ordinary synthetic hemodialysis membranes and only to a minor degree by peritoneal dialysis. Accordingly, several studies have demonstrated that leptin levels are not reduced by hemodialysis with low permeable membranes [7–9], whereas a decrease in plasma leptin is seen after hemodialysis with high flux membranes [14]. In contrast, little is known about the elimination of leptin by peritoneal dialysis. However, as the omental adipocytes synthesize leptin (although to a lesser degree compared to subcutaneous adipocytes) [15], it is likely that local intraperitoneal synthesis of leptin will occur in peritoneal dialysis patients. Furthermore, long-term (72 h) insulin infusion [16] stimulates leptin secretion in humans without renal failure, and serum leptin levels in patients with CRF are related to fasting insulin levels [10,13,16]. Therefore, one may also speculate that intraperitoneal insulin therapy may stimulate local intraperitoneal leptin synthesis.

The aim of the present study was at first to evaluate if the peritoneal clearance of leptin in CRF patients is similar to what can be expected from the molecular weight of leptin (16 kD) or, alternatively, if higher dialysate leptin levels (suggesting intraperitoneal synthesis of leptin) are observed. Secondly, we wanted to investigate if the peritoneal clearance of leptin was higher in patients using intraperitoneal insulin.

Patients and methods

A cross-sectional study was performed in 39 clinically stable peritoneal dialysis patients (20 males), with mean age 54 ± 12 years, who had been treated with peritoneal dialysis for at least 3 months (on average 17 ± 12 months; range 3–49 months). Thirty patients were treated with continuous ambulatory peritoneal dialysis (CAPD) whereas nine patients were treated with continuous cyclic peritoneal dialysis (CCPD). The causes of renal failure among these patients varied between chronic glomerulonephritis (n = 18), diabetic nephropathy (n = 9), chronic interstitial nephritis (n = 5) and other or unknown causes (n = 7). One patient with interstitial nephritis also had diabetes. All the diabetics (n = 10) were treated with insulin, three used subcutaneous insulin only, four used intraperitoneal insulin only and three used a combination of intraperitoneal and subcutaneous insulin.

A 24-h collection of dialysate and urine was performed and blood samples were drawn in the morning before the first dialysis exchange after an overnight oral fast. The patients height and weight were recorded. The study was approved by the local ethics committee of the Karolinska Institute at Huddinge Hospital.

Dialysate and serum samples were stored in −70°C until analysis. Serum and dialysate leptin were analysed using a commercially available radioimmunoassay kit (Linco Research Inc., St Charles, MO, USA). This leptin assay is completely homologous, since the antibody used was raised against highly purified human leptin and both standard and tracer were prepared with human leptin. The coefficient of variation within in the sample is 6%. Determination of creatinine and urea in serum, dialysate and urine were performed in the Department of Clinical Chemistry, Huddinge Hospital, using routine methods. Albumin was determined in serum with the bromcresol purple method and in dialysate with an immunoturbidometric method. β₂-microglobulin was determined in serum and dialysate using a RIA method. Serum levels of IL-1ß, TNF-α, hyaluronic and CRP were evaluated as markers of inflammation. IL-1ß and TNF-α were measured by a photometric ELISA method (Boehringer Mannheim, Mannheim, Germany) and dialysate and serum levels of hyaluronic were measured by a RIA method (Pharmacia Diagnostics AB, Uppsala, Sweden). Serum CRP was measured using an immunonephelometric method (Tina-quant®, Boehringer Mannheim, Mannheim, Germany). CRP-values below the detection limit of 10 mg/l were in the statistical analysis treated as 9 mg/l. Values below the detection limit of 5 ng/l for IL-1ß and TNF-α (one case each), were excluded.

Body Mass Index was calculated as weight (kg)/height (m)². Peritoneal clearances (PCI) for leptin, β₂-microglobulin and albumin were calculated from the respective serum concentrations (C₀), dialysate concentrations (Cₐ), and the drained 24-h dialysate volume (Vₑ) using the equation:

\[ \text{PCI} = \frac{\text{C₀} \times \text{Vₑ}}{\text{Cₐ}} \]

The expected peritoneal leptin clearance (ExpPCI leptin) was calculated in each patient from the peritoneal clearances of albumin (PCI alb) and β₂-microglobulin (PCI β₂m) and the molecular weights of leptin (16 kD), albumin (69 kD), and β₂-microglobulin (12 kD) using the equation:

\[ \text{ExpPCI leptin} = \frac{\text{PCI β₂m} - [(69–12)](\text{PCI β₂m} - \text{PCI alb})}{(16–12)} \]

Values are expressed as mean ± SD or as medians (with range). Linear regression was used to evaluate the correlation coefficients between different parameters and differences between patient groups were evaluated using the Mann-Whitney U-test.

Results

The mean serum leptin level was 47 ± 76 ng/ml (range 3–350 ng/ml). Serum leptin was significantly related to BMI (r = 0.35, P < 0.05) and did also tend to correlate to serum TNF-α levels (r = 0.29, P = 0.08). On the other hand, no correlations were found between serum leptin vs CRP (r = 0.083, NS), IL-1ß (r = 0.054, NS), or hyaluronic (r = 0.046, NS). The possible relationships between serum leptin and BMI and between leptin and serum TNF-α were further analysed with multiple linear regression demonstrating that in this statistical model both these parameters correlated significantly to serum leptin (both P < 0.05).

Although the dialysate leptin levels among the CAPD patients correlated to the serum leptin levels (r = 0.87, P < 0.001), they were much higher than was expected from the peritoneal albumin and β₂-microglobulin clearances (Table 1). Peritoneal clearance of leptin was on average 1.3 ml/min (range 0.2–5.9 ml/min), which is 1.6 times higher than the expected peritoneal leptin clearance. Peritoneal leptin clearance did not correlate to peritoneal albumin clearance nor to peritoneal β₂-microglobulin clearances (r = 0.25; NS and r = 0.08, NS, respectively), whereas a significant correlation was found between peritoneal
Table 1. Serum levels of leptin and inflammatory markers and peritoneal clearances (PCl) of albumin, \( \beta_2 \)-microglobulin and leptin

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean ± SD (range)</th>
</tr>
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<tbody>
<tr>
<td>Body mass index (kg/m(^2))</td>
<td>24.4 ± 4.4 (16.6–35.6)</td>
</tr>
<tr>
<td>Serum leptin (ng/ml)</td>
<td>19* (3–350)</td>
</tr>
<tr>
<td>Serum CRP (mg/l)</td>
<td>&lt;10* (&lt;10–76)</td>
</tr>
<tr>
<td>Serum IL-1( \beta ) (ng/l)</td>
<td>11.2* (&lt;5–65.9)</td>
</tr>
<tr>
<td>Serum TNF-( \alpha ) (ng/l)</td>
<td>12* (&lt;5–85)</td>
</tr>
<tr>
<td>Serum hyaluronan (μg/l)</td>
<td>219 ± 170 (55–955)</td>
</tr>
<tr>
<td>Dialysate leptin (ng/ml)</td>
<td>5.7 ± 8.2 (0.3–41.0)</td>
</tr>
<tr>
<td>PCl albumin (ml/min)</td>
<td>0.094 ± 0.033 (0.040–0.180)</td>
</tr>
<tr>
<td>PCl ( \beta_2 )-microglobulin (ml/min)</td>
<td>0.83 ± 0.35 (0.20–1.76)</td>
</tr>
<tr>
<td>PCl leptin (ml/min)</td>
<td>1.3 ± 1.2 (0.2–5.9)</td>
</tr>
<tr>
<td>Expected PCl leptin (ml/min)</td>
<td>0.8 ± 0.3 (0.2–1.7)</td>
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*Median values.

Discussion

In general, the transport of solutes over the peritoneal barrier can be described as a process of passive diffusion (and to some extent convection) through pores of three different sizes [17]. There is a close relationship between the peritoneal transport characteristics for solutes with different molecular weight up to the size of albumin [17]. However, there is a large variation between different patients in transport rates for solutes of this wide range of sizes [18]. A markedly higher dialysate appearance rate than predicted from the molecular weight of the solute is a strong indicator of local intraperitoneal production [19]. Furthermore, the protein binding of leptin in serum [8,20] was not taken into account in the calculation of peritoneal clearance for leptin. As only the free fraction of leptin can pass the peritoneal barrier, the peritoneal leptin clearance (calculated from the measured dialysate and total serum concentration of leptin) should in fact be lower than predicted from the molecular weight and the total serum concentration. The chief finding in the present study is that a markedly higher intraperitoneal leptin clearance than expected was found, without relation to the clearances of albumin and \( \beta_2 \)-microglobulin. This suggests intraperitoneal synthesis of leptin from the intraperitoneal adipocytes. It has recently been described that omental adipocytes synthesize leptin, although the synthesis rate is slightly lower compared to subcutaneous adipocytes [15]. Although the dialysate leptin levels are markedly higher than expected they still correlate to the serum leptin, This finding is most likely due to a possible correlation between the intraperitoneal fat volume and the total fat mass.

The second main finding in this study is that local administration of insulin enhances the intraperitoneal

![Fig. 1. Peritoneal clearance of leptin (ml/min) in CAPD patients without and with intraperitoneal insulin therapy (P<0.05).](image-url)
clearance of leptin in patients treated with peritoneal dialysis suggesting that insulin stimulates local leptin production in the intra-abdominal fat tissue. This finding cannot be explained by altered peritoneal permeability due to diabetic vasculopathy as this would also result in increased peritoneal clearances of albumin and β2-microglobulin, which were used to calculate the expected peritoneal leptin clearance. Thus, a potentially altered peritoneal permeability is already taken into account in the calculations of the expected peritoneal leptin clearance. Furthermore, the patients using intraperitoneal insulin did not have increased peritoneal clearances of albumin or β2-microglobulin compared to the other patients. However, due to the small number of patients using intraperitoneal insulin in the present study, our findings must still be interpreted with caution. Prospective studies of patients starting treatment with intraperitoneal insulin are needed to clarify the relation between intraperitoneal insulin and peritoneal leptin clearance.

Several recent studies have addressed the possible impact of insulin on serum leptin. We have previously demonstrated that serum leptin concentrations correlate to plasma insulin concentrations independently of body fat content in chronic renal failure [10]. Moreover, whereas short-term insulin administration does not increase leptin secretion in humans [16,21,22], long-term insulin infusion (72 h) does [16]. It has been suggested that insulin stimulates leptin secretion by a direct throphic effect on the adipocytes. One could therefore speculate that intraperitoneally administrated insulin stimulates the increase of intra-abdominal fat content in CAPD-patients. It has been suggested that the use of intraperitoneal insulin could be involved in the development of subcapsular steatosis of the liver in peritoneal dialysis patients [23,24]. Furthermore, the increase in intra-abdominal fat content could perhaps increase the risk of an adverse clinical outcome in this patient population [25], like in patients without renal failure [26,27].

Finally, the present study have demonstrated that serum leptin, in the multiple regression analysis, correlates significantly to TNF-α which suggests that this cytokine may contribute to hyperleptinaemia in peritoneal dialyse patients. However, in the present study we could not demonstrate any correlation between serum leptin levels and CRP, another indicator of inflammation, like we previously have done in predialysis patients [5]. It has previously been demonstrated that cytokines, such as TNF-α and interleukin-1, induce both increased leptin mRNA levels and anorexia in animal models [28,29] and it has been suggested that leptin may be a mediator of anorexia in inflammatory disease. Moreover, Mantzoros et al. [30] have recently demonstrated a significant relation between serum leptin and the levels of soluble TNF-receptor (TNF-α-R55) in humans and Zumbach et al. [31] have reported that infusion of TNF-α increases serum leptin levels in humans. Furthermore, Kirchgesner et al. [32] have provided data suggesting that TNF-α can act directly on adipocytes to regulate the release of leptin. As elevated levels of cytokines is a common phenomenon in chronic renal failure [33,34] one could speculate that a continuous low-grade inflammation could contribute to elevated serum leptin levels in patients with chronic renal failure.

In summary, the peritoneal leptin clearance is higher than expected from the peritoneal albumin and β2-microglobulin clearances, which suggests a local intraperitoneal leptin production in CAPD patients. Moreover, the markedly enhanced peritoneal leptin clearance in diabetic patients treated with intraperitoneal insulin suggests that insulin stimulates the intraperitoneal leptin production.

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