Inappropriate Elevation of Serum Leptin Levels in Children with Chronic Renal Failure

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Abstract. Decreased spontaneous nutrient intake is a frequent clinical problem in patients with chronic renal failure (CRF). Leptin, the recently characterized gene product of the obese gene, is produced by adipocytes and is thought to act as an afferent satiety signal on the appetite and satiety centers of the brain. Serum leptin levels were investigated in 134 pediatric patients in different stages of CRF to evaluate a possible relationship between leptin, GFR, and spontaneous energy intake. Serum leptin levels, measured by a specific RIA, were elevated above the 50th percentile of the normal range in 78% of CRF patients and above the 95th percentile in 45% of patients. Gel chromatography of CRF sera yielded only one single immunoreactive peak at 16 kD, indicating that the increase of immunoreactive leptin levels in CRF serum was not due to accumulation of leptin degradation products. Multiple stepwise regression analysis revealed the percentage of body fat as assessed from skinfold measurements ($r = 0.79$, $P < 0.0001$) and GFR ($r = -0.17$, $P < 0.005$) as independent predictors of serum leptin levels, accounting for 66% of total statistical variability. There was an inverse linear correlation between standardized leptin levels (leptin z-score) and the spontaneous energy intake quantified from written dietary diaries ($r = -0.36$, $P < 0.001$). These data suggest that the percentage of body fat remains the main determinant of serum leptin in CRF patients, but their levels increase with declining GFR, presumably by reduced renal clearance. Leptin levels in CRF serum that are inappropriately elevated in relation to the percentage of body fat might lead to a dysregulation of the normal peripheral–central leptin feedback loop, thereby contributing to decreased nutrient intake in uremia. (J Am Soc Nephrol 9: 1074–1079, 1998)

Chronic renal failure (CRF) is associated with a wasting syndrome characterized by inadequate nutrient intake and decreased tissue anabolism and/or increased catabolism (1). Despite improvements in the treatment of secondary complications of CRF such as anemia and metabolic acidosis, persistent anorexia remains a clinical problem, particularly in infants and children with end-stage renal disease (ESRD) (2). The pathophysiology of the reduced spontaneous nutrient intake of uremic patients has not yet been fully elucidated.

Leptin, the recently characterized gene product of the obese gene, plays an important role in the regulation of body weight by signaling the size of the adipose tissue mass to the hypothalamus (3). Leptin is released into the circulation by adipocytes dependent on their lipid content (4). It reaches the hypothalamus via the blood–brain barrier, where it binds to specific receptors, leading to satiety (5). Leptin-deficient mice exhibit an increased food intake resulting in gross obesity, whereas systemic application of leptin to these animals normalizes food consumption and body weight (6). A positive correlation between serum leptin levels and the percentage of body fat was found in humans (7), suggesting a role for leptin as an endogenous indicator of body fat mass and, possibly, as a mediator of the central nervous feedback inhibition of food intake.

The mechanism responsible for clearance of leptin from the circulation has not yet been determined. The molecular size of the hormone (16 kD) renders it likely that leptin is cleared from the circulation by glomerular filtration and tubular metabolism in the kidney. In CRF, retention of low molecular weight peptide hormones in the circulation as a result of reduced renal clearance is a well known feature (8). Therefore, we sought to determine whether serum leptin levels are elevated in CRF and whether this alteration plays a role in the pathogenesis of uremic anorexia.

Materials and Methods

Patients

Two cohorts of children with CRF were studied. The first group consisted of 108 children (75 boys, 33 girls) with preterminal CRF on
conservative treatment who were investigated prior to enrollment into a multicenter randomized study on the effect of a low-protein diet on the progression of CRF in childhood (9). These patients were selected to obtain a study population with a broad age range (10.6 ± 4.3 yr; range, 2.7 to 19.2 yr) and residual GFR (34.3 ± 18.5 ml/min per 1.73 m²; range, 6.5 to 77 ml/min per 1.73 m²). The second group consisted of 26 children (19 boys, seven girls) with ESRD treated either by hemodialysis (n = 8) or continuous cycling peritoneal dialysis (CCPD) (n = 18), aged 13.5 ± 5.6 yr (range, 1.4 to 23.3 yr). Hemodialysis was performed either as standard bicarbonate hemodialysis (n = 5) or hemodiafiltration (n = 3), with a weekly dialysis time of 12 h. CCPD was performed with 1.0 to 4.25% glucose solutions with four to 10 exchanges of 750 to 1000 ml/m² dwell volumes, resulting in a mean 24-h total dialysate volume of 6.4 ± 2.1 L/m² body surface area.

The patients had the following primary renal disease: obstructive or reflux uropathy (n = 41), renal hypoplasia/dysplasia (n = 36), chronic glomerulonephritis (n = 12), hemolytic-uremic syndrome (n = 10), polycystic kidney disease (n = 6), interstitial nephritis (n = 4), nephronophthisis (n = 4), infantile nephropathic cystinosis (n = 3), other hereditary nephropathies (n = 10), nephrocalcinosis (n = 2), renal artery stenosis (n = 1), and unknown (5). Not one of the patients had nephrotic syndrome. Excluded from the study were patients with additional thyroid, liver, or gastrointestinal disease; systemic diseases such as lupus erythematosus, amyloidosis, or oxalosis; or severe cardiac diseases. Also excluded were patients undergoing treatment with glucocorticoids or other immunosuppressive drugs during the previous 6 mo and patients with acute infections or severe anemia (hemoglobin, <8 g/dl). Hemodialysis patients were sampled immediately before a regular hemodialysis session in the morning. In CCPD an overnight fast. Hemodialysis patients were sampled immediately before a regular hemodialysis session in the morning. In CCPD an overnight fast. Hemodialysis patients were sampled immediately before a regular hemodialysis session in the morning. In CCPD an overnight fast. Hemodialysis patients were sampled immediately before a regular hemodialysis session in the morning. In CCPD an overnight fast. Hemodialysis patients were sampled immediately before a regular hemodialysis session in the morning. In CCPD an overnight fast. Hemodialysis patients were sampled immediately before a regular hemodialysis session in the morning. In CCPD an overnight fast.

GFR was estimated using the formula given by Schwartz et al.: $\text{GFR} = 0.55 \times \text{height (cm)}/\text{serum creatinine concentration (mg/dl)}$ (14). In patients with ESRD on dialysis treatment, no attempt was made to measure residual GFR, which is usually in the very low range between 0 and 5 ml/min per 1.73 m². Therefore, a value of 3 ml/min per 1.73 m² was arbitrarily entered.

**Assays**

Serum leptin concentrations were measured by a recently developed highly sensitive RIA (15). The sensitivity of the assay with undiluted samples was 0.03 µg/L corresponding to 0.003 ng per tube. The intra- and interassay coefficients of variation were 0.8 and 8.5%, respectively (15). The day-to-day coefficient of variance of serum leptin in healthy subjects was 12%. Sera of three hemodialysis patients were separated into molecular weight fractions by gel chromatography on a 1.5-x-80-cm column of Sephadex G-50 at 4°C. Leptin immunoreactivity was then determined in the individual fractions by RIA. Serum total protein, albumin, transferrin, creatinine, urea, and glucose were measured in one laboratory (Heidelberg) by automated procedures, using a Cobas autoanalyzer (Hoffmann-La Roche, Grenzach-Wyhlen, Germany).

**Statistical Analyses**

Reference ranges of plasma leptin levels referring to BMI in 713 healthy children, stratified according to gender and pubertal stage, have been published previously (15). The distribution of normal plasma leptin levels was log normal. Therefore, measured leptin values were transformed to their logarithms before calculating leptin z-scores. In a normal population, this score is normally distributed with a mean of zero and an SD of 1. The calculation of leptin z-scores was based on the means and associated SD from the control subject data grouped by gender and pubertal stage, using the equation: Leptin z-score = $\ln(\text{leptin}) - m_i/s_i$, where $m_i$ is the mean of the ln of the leptin levels in normal subjects of the same BMI, gender, and pubertal stage, and $s_i$ is the SD of the ln of the leptin levels in healthy subjects of the same BMI, gender, and pubertal stage.

Data are given as mean ± SD. The Shapiro–Wilk test was used to confirm normality of data (16). For comparison of more than two normally distributed groups, one-way ANOVA followed by pairwise multiple comparison (Scheffe test) was used. Correlations between variables were assessed using univariate linear regression analysis. Multiple stepwise linear regression analysis was used to identify independent predictors of serum leptin levels. Interaction between variables was assessed by Spearman correlation analysis. $P < 0.05$ was considered statistically significant.

**Results**

Individual serum leptin levels in children with CRF are depicted in Figure 1 in comparison to the respective normal range referring to BMI. For the group as a whole, 104 of 134 patients (78%) had leptin levels above the 50th percentile; 60 patients (45%) had clearly elevated levels above the 95th percentile. Serum leptin levels in CRF were not consistently elevated over the whole range of BMI examined; there was rather a tendency for increased leptin levels above a BMI of 18 to 20 kg/m², in particular in prepubertal boys (Figure 1A). The increase of immunoreactive leptin levels in CRF was not due to accumulation of leptin degradation products, because gel chromatography of sera from hemodialyzed patients (n = 3)
yielded only one single immunoreactive peak at 16 kD (Figure 2).

To investigate the influence of remnant renal function on serum leptin levels, values were standardized by calculation of a z-score. There was a stepwise increase of standardized leptin levels in the three stages of CRF (Figure 3). For the group as a whole, there was an inverse correlation between standardized leptin levels and GFR ($r = -0.20, P < 0.02$). This correlation remained significant ($r = -0.18, P < 0.05$) when patients with ESRD were excluded from the analysis. The relative increase of leptin was most pronounced in patients with ESRD ($2.37 \pm 1.80$ z-score) (Figure 3). The elevation of serum leptin was comparable in patients on hemodialysis and CCPD.

Next, we sought to determine which independent variables

\begin{figure*}[h]
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\includegraphics[width=\textwidth]{figure1.png}
\caption{Individual serum leptin levels in children with chronic renal failure (CRF) as a function of body mass index (BMI), stratified according to gender and pubertal stage, in comparison to the normal range given by the median and the 5th and 95th percentile. Closed symbols indicate patients with end-stage renal disease (ESRD); open symbols indicate patients with preterminal CRF. Note the logarithmic y-axis.}
\end{figure*}

\begin{figure*}[h]
\centering
\includegraphics[width=\textwidth]{figure2.png}
\caption{Representative elution profile of immunoreactive leptin in uremic serum that was separated into molecular weight fractions by gel chromatography on a Sephadex G-50 column. There was only one single immunoreactive peak at 16 kD. Immunoreactive serum leptin concentration in this patient with ESRD was 365 ng/ml.}
\end{figure*}

\begin{figure*}[h]
\centering
\includegraphics[width=\textwidth]{figure3.png}
\caption{Standardized serum leptin levels (z-score) in three different stages of CRF. Data are mean ± SD. The asterisk indicates a significant difference ($P < 0.01$) versus the normal range, defined as a $-2$ to $+2$ z-score.}
\end{figure*}
contribute statistically to the prediction of serum leptin levels in children with CRF. The percentage of body fat, BMI, GFR, gender, age, pubertal stage, fasting glucose concentration, serum albumin, protein, and transferrin were evaluated as possible predictors of serum leptin concentrations by multiple stepwise regression analysis. In this analysis, only the percentage of body fat ($r = 0.79, P < 0.0001$) and GFR ($r = -0.17, P < 0.005$) contributed significantly to the total variability of serum leptin levels. The following best-fitting prediction formula was calculated: Log leptin [ng/ml] = $-0.418 + 0.068 \times$ Percent fat mass [%] $- 0.0059 \times$ GFR [ml/min per 1.73 m$^2$] ($r = 0.81, P < 0.0001$) (Figure 4). The variables BMI, gender, age, pubertal stage, blood glucose, serum protein, transferrin, or albumin did not significantly add to the ability of the equation to predict plasma leptin and were not included in the final equation. However, when we used BMI as an estimate of body fat rather than the percentage of body fat calculated by skinfold measurements in this model, gender and pubertal stage emerged as significant independent predictors. The total explained variance with the BMI-based model ($r^2 = 0.54$) was lower than that achieved with the model based on the calculated percentage of body fat ($r^2 = 0.63$).

To test the hypothesis that elevated leptin levels in CRF contribute to the reduced nutrient intake, the relationship of standardized leptin levels with the spontaneous energy intake, expressed as % RDA, was examined. There was an inverse linear correlation between standardized leptin levels and % RDA ($r = -0.36, P < 0.001$) (Figure 5). There was also an albeit weaker correlation between the spontaneous protein intake, expressed as % RDA, and standardized leptin levels ($r = -0.26, P < 0.02$).

**Discussion**

The present study represents the first analysis of serum leptin levels in children with CRF. Using a recently established highly sensitive RIA, we describe a significant elevation of serum leptin levels in CRF that was clearly out of proportion with the patients’ respective percentage of body fat. These data are in line with recent reports of elevated serum leptin levels among adults with CRF (17-20).

Our observation of an inverse correlation of leptin levels with remnant GFR suggests that leptin accumulates in CRF serum as a consequence of reduced renal clearance. Although the precise mechanism of leptin clearance from the circulation remains to be determined, one can assume by comparison to other peptide hormones with similar molecular size (8) that leptin is substantially removed from the circulation by renal filtration and tubular metabolism. Indeed, it has recently been shown that plasma leptin is partly cleared by the kidney both in humans (19) and rats (21). Hence, the inverse relation of standardized leptin levels with remnant renal function in our study strongly indicates that elevated leptin levels in CRF are due to reduced renal filtration and metabolism by the diseased kidney. However, it remains to be determined whether an increased rate of leptin production also contributes to high serum leptin levels in CRF.

It was important to show that the increase of immunoreactive leptin levels in CRF was not due to accumulation of leptin degradation products, indicating that circulating leptin is likely

![Figure 4. Serum leptin levels (logarithmic scale) in children with CRF (n = 134) as a function of the percentage of body fat, as calculated from skinfold measurements and as a function of GFR. Log leptin could be predicted from a linear combination of the independent variables percentage of body fat and GFR: Log leptin [ng/ml] = $-0.418 + 0.068 \times$ Percent fat mass [%] $- 0.0059 \times$ GFR [ml/min per 1.73 m$^2$] ($r = 0.81, P < 0.0001$), adjusted $r^2 = 0.66$.](image1)

![Figure 5. Spontaneous energy intake (% RDA) in 134 children with CRF as a function of standardized serum leptin levels (z-score). There was an inverse linear correlation ($r = -0.36, P < 0.001$).](image2)
to be functional in CRF patients. The key question is whether the elevation of leptin levels in CRF has any functional consequences with respect to food intake. Leptin is thought to act as an afferent satiety signal in a feedback loop that affects the appetite and satiety centers of the brain, presumably by inhibition of hypothalamic neuropeptide Y release (5). When leptin is administered for long periods to leptin-deficient ob/ob mice, their food intake is reduced, as is their body weight (6). Administration of recombinant leptin to normal fasted mice led to a significant decrease in food intake (22). Also, changes of endogenous leptin levels discordant to changes in the adipose tissue mass appear to contribute to the regulation of feeding behavior in animals. The antidiabetic thiazolidinediones down-regulate the expression of the ob gene in rodents, associated with an increase of food intake and adipose tissue weight (23,24). Moreover, induction of ob gene expression by corticosteroids (25) or endotoxin and cytokines (26) is accompanied by body weight loss and reduced food intake. There is recent evidence that leptin contributes to the regulation of feeding behavior also in humans. In Pima Indians, a population prone to obesity, relatively low plasma leptin concentrations precede their weight gain, and may therefore play a role in the development of obesity (27). On the basis of these data in human and animal models, one could speculate that the inappropriately elevated leptin levels in CRF serum contribute to the decreased nutrient intake in these patients. This hypothesis is supported by our observation of an inverse relationship of BMI-adjusted leptin levels with the spontaneous energy intake in children with CRF. Our observational cross-sectional study does not, however, prove the pathogenic role of leptin for weight gain, and may therefore play a role in the development of obesity (27). On the basis of these data in human and animal models, one could speculate that the inappropriately elevated leptin levels in CRF serum contribute to the decreased nutrient intake in these patients. This hypothesis is supported by our observation of an inverse relationship of BMI-adjusted leptin levels with the spontaneous energy intake in children with CRF.

In summary, serum leptin levels are inappropriately elevated in children with CRF. The percentage of body fat remains the main determinant of serum leptin in CRF patients, but their levels increase with declining GFR. The inverse relationship between leptin levels and spontaneous energy intake suggests that accumulation of leptin in CRF might lead to a dysregulation of the normal adipocyte–hypothalamic leptin loop system, thereby contributing to decreased nutrient intake in uremia.

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Appendix


References

10. Committee on Dietary Allowances: Recommended dietary allowances, Washington DC, National Academy of Science, 1989

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