Renal loss of leptin in patients with nephrotic syndrome

Michael Schroth, Michael Gröschl, Helmut G Dörr, Werner F Blum1, Wolfgang Rascher and Jörg Dötsch

Klinik mit Poliklinik für Kinder und Jugendliche, Friedrich-Alexander-Universität Erlangen-Nürnberg, Loschgestraße 15, D-91054 Erlangen, Germany and 1Lilly Deutschland GmbH, Saalburgstraße 153, D-61350 Bad Homburg, Germany

(Correspondence should be addressed to Michael Schroth; Email: michael_schroth@yahoo.de)

Abstract

Objective: In humans, short term changes of serum leptin lead to alterations in food intake and energy expenditure. The objective of the present study was to relate urine leptin concentrations with the extent of proteinuria in patients with nephrotic syndrome (NS). A second goal was to investigate the impact of potential urinary leptin losses on serum leptin concentrations and body composition.

Design and Methods: Seventeen patients with proteinuria were compared with twenty patients with remission of NS and ten healthy children. Leptin was measured by radioimmunoassay.

Results: Urinary leptin excretion in proteinuric patients was significantly higher than in non-proteinuric patients with and without NS and in healthy controls (2.64±0.034 µg/g creatinine, and 0.026±0.05 µg/g creatinine, and 0.073±0.11 µg/g creatinine respectively; P < 0.001 and P < 0.01 respectively compared with controls). Urine leptin positively correlated with urine IgG concentration (r² = 0.36) in the proteinuric group. No difference in serum leptin values could be demonstrated between the three groups.

Conclusions: In summary, our data demonstrate a significant leptin excretion in children with severe proteinuria. Proteinuria, however, does not lead to changes in serum leptin, suggesting that the significant loss of leptin is compensated for by sustained up-regulation of leptin production.

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Introduction

Leptin, a 167 amino acid polypeptide with a molecular size of 16 kDa, is synthesized in adipose tissue (1, 2). Leptin, the product of the obese (ob) gene, plays an important role in the regulation of appetite and food intake in mice and humans (3–5). Mutations of the leptin gene or its receptor gene lead to obesity in mice and humans (1, 6). There is a positive correlation between serum leptin concentrations and body mass index (7–11). In adipose patients chronically raised levels of leptin do not lead to weight reduction possibly due to the set-point changes in the hypothalamic leptin sensor. Long-term leptin treatment, however, causes moderate weight reduction. Short-term changes in leptin serum concentrations may modulate appetite and energy expenditure in humans during fasting and after major surgery (12). Leptin is known to play an important role in the regulation of weight gain in early infancy and shows a relationship between its concentration and fetal growth (13–15).

The idiopathic nephrotic syndrome (INS) is an albumin-losing nephropathy in childhood often due to minimal change lesions of the kidneys. Severe proteinuria (urinary protein level exceeding 1 g/m² of body surface area per day) leads to hypoproteinemia (hypalbuminemia, albumin in serum <25 g/l) and chronically to a catabolic nutritional state (16). Investigations revealed that the defect in minimal change glomerulonephritis results mainly from a loss of charge selectivity, whereas the defect in membranous glomerulonephritis results from a loss of size selectivity (17, 18). As a consequence, the protein loss affects body composition. Children are often seen in an edemic state. Blood coagulation as well as the immunologic system can be affected due to the loss of components of the coagulation system or the loss of immunoglobulins.

Since proteinuria may be associated with urinary leptin wasting in renal protein-losing diseases, the present study was designed to examine the relationship of serum and urine leptin concentration and proteinuria in patients with nephrotic syndrome (NS). A second goal of the study was to examine whether or not an increased urinary leptin excretion may influence serum leptin concentrations and body composition.

Experimental subjects

Thirty-seven pediatric patients with NS were studied. Patients’ characteristics are shown in Table 1. In 17 patients (group I) proteinuria (urinary protein level exceeding 1 g/m² of body surface area per day) was present. Twenty patients were in remission (group II,
Table 1: Patient characteristics of 37 patients suffering from nephrotic syndrome and of 10 healthy children in the control group. Patient number, age, gender, BMI and pubertal stage (Tanner) as well as medication (prednison, cyclosporine, tacrolimus, β-blocking agents) and diagnoses of all patients are listed. Age and BMI are expressed as means ± standard deviation and median.

<table>
<thead>
<tr>
<th></th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number (n)</td>
<td>17</td>
<td>20</td>
<td>10</td>
</tr>
<tr>
<td>Age (years)</td>
<td>8.8 ± 3.8; 8</td>
<td>9.9 ± 3.6; 10</td>
<td>10.5 ± 4.2; 9.5</td>
</tr>
<tr>
<td>Gender (m/f)</td>
<td>8/9</td>
<td>17/3</td>
<td>5/5</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>18.1 ± 2.9; 18.1</td>
<td>18.3 ± 3.6; 17.45</td>
<td>17.2 ± 2.4; 17.15</td>
</tr>
<tr>
<td>Tanner 1/2</td>
<td>10/7</td>
<td>11/9</td>
<td>4/6</td>
</tr>
<tr>
<td>Patients receiving prednison</td>
<td>9</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Patients ever (actually) treated with cyclosporine A or tacrolimus</td>
<td>6 (2)</td>
<td>10 (7)</td>
<td>0</td>
</tr>
<tr>
<td>Patients ever treated with cyclophosphamid</td>
<td>10</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Patients receiving β-blocking agents</td>
<td>12</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Diagnosis</td>
<td>INS (8), FGGS* (1), FSGS* (3), MCGN (3), MPGN* (1), congenital nephrotic syndrome* (1)</td>
<td>INS (15), MCGN* (5), Healthy</td>
<td></td>
</tr>
</tbody>
</table>

*Diagnosis confirmed by histological examination.

FGGS, focal global glomerulosclerosis; FSGS, focal segmental glomerulosclerosis; MCGN, minimal change glomerulonephritis; MPGN, membrano proliferative glomerulonephritis.

no significant proteinuria). In this study group proteinuria did not exceed 100 mg/m² of body surface area per day. The period of remission was at least 6 months and was equal in all patients included. The patients’ groups were compared with 10 healthy children (group III).

Leptin concentrations in serum and urine were measured by radioimmunoassay (as shown in detail below). After centrifugation of blood samples, which were all drawn between 1000 and 1200 h, serum and urine samples were kept frozen for up to 8 weeks at −20 °C and were analyzed when all specimens had been obtained.

In all patients, body weight and body height were measured to obtain the body mass index (BMI). Subscapular and triceps skinfold thickness were determined with a caliper by the same trained investigator. No patient was fasting. All clinical and auxological data were obtained during routine visits and were recorded using standard data sheets. Details are shown in Table 1. The study was approved by the local ethics committee of the University of Erlangen. Informed, written consent was obtained from all parents and the older patients.

Materials and methods

Leptin was measured by a specific radioimmunoassay. Recombinant human leptin was used for raising a polyclonal antibody in rabbits, for the production of tracer by the Chloramine T method (Lilly, Bad Homburg, Germany), and for the production of antiserum. For the measurement of serum samples a standard curve from 18.75 to 0.049 ng/ml was prepared by geometrical dilution. The tracer activity was ~20 000 c.p.m./100 µl, the final dilution of the specific antibody was 1:50 000. In the assay, 100 µl of each of tracer, sample and antibody were added. Incubation lasted for 24 h at room temperature. Bound and non-bound fractions were separated by a goat-anti-rabbit IgG (DSL, Sinsheim, Germany) in 4% polyethylene glycol (PEG) at a final dilution of ~1:100. After 2-h incubation at room temperature the tubes were centrifuged (15 min, 3000 r.p.m., 4 °C). Activity of the pellet was counted for one min in a gamma counter. Fifty percent binding occurred at 0.9 ng/ml. The sensitivity of undiluted samples was 0.01 ng/ml. Inter- and intra-assay coefficients of variation (%) were 8.5 and 0.8 respectively.

Leptin in urine was measured by a highly sensitive variation of the assay. Tracer activity was 8000 c.p.m./25 µl. The antibody had a final dilution of 1:8000 in 25 µl. The standard curve expanded from 1250 to 9.8 pg/ml. The buffer and 2nd antibody dilutions were the same as described above. Urine leptin concentrations were related to the respective urine creatinine concentration (U leptin/U creatinine (µg/g creatinine)) and are also given as absolute values (µg/l). Leptin standard deviation scores (SDS) were calculated according to Blum et al. by the following equation: leptin SDS = [ln(leptin) − ln(a) − b*BMI]/day where a and b are constants and BMI, the body mass index (19).
with severe proteinuria, urinary protein level exceeding 1 g/m² of body surface area per day), group II (patients with NS without proteinuria) and group III (control group, healthy children). Data are shown as scattergrams and as boxes and whiskers. Significantly higher levels in group I (**P = 0.0005) were evaluated using one-way ANOVA, P values were corrected according to Bonferroni. There is no difference in urinary leptin between boys and girls and Tanner stages 1 and 2, as shown in Table 2.

To exclude the interference of massive proteinuria with leptin measurements we have demonstrated that in leptin-spiked urine samples even high IgG and albumin levels do not create non-specific effects leading to falsely high leptin levels in the urine. The amount of leptin spiked into the urine was 3 ng/ml. Albumin concentrations ranged from 3.13 to 100.0 mg/ml and the concentration of added leptin was 3 ng/ml. Albumin levels do not create non-specific effects leading in leptin-spiked urine samples even high IgG and albumin did not influence leptin concentrations; IgG was added concentration in the absence of albumin was 3.13 ng/ml, and in the absence of IgG it was 3.06 ng/ml. There was no statistical difference in measured leptin concentrations after adding albumin or IgG. In a further experiment, leptin at a starting concentration of 3.18 ng/ml was diluted 1:2, 1:4, 1:8, 1:16 up to 1:32 (0.53 ng/ml). Leptin dilution in urine showed linearity between calculated leptin and measured leptin concentrations (r² = 0.98, P < 0.001).

**Statistical analysis**

Data with Gaussian distribution were correlated by linear regression. Parametric data were compared by two-tailed t-test. In the case of multiple tests, data were compared using one-way ANOVA, and, in the case of significance, post-hoc t-test. P values were corrected according to Bonferroni. A P value <0.05 was considered statistically significant.

**Results**

Urinary excretion of leptin in proteinuric patients with nephrotic syndrome was significantly higher (2.64±0.34 µg/g creatinine, absolute value 100.3±21.23 µg/l) than in patients without proteinuria (0.026±0.047 µg/g creatinine, absolute value 2.37±1.15 µg/l, P < 0.001) or healthy controls (0.073±0.11 µg/g creatinine, absolute value 3.83±2.06 µg/l, P < 0.01) (P-values for all data related to creatinine as well as for absolute values) (Fig. 1). Urine leptin values were also related to the different pubertal stages and sex (data shown in correlation to individual creatinine values); there was no difference in urinary leptin excretion between boys and girls, nor between Tanner stages 1 and 2 (Table 2).

**Table 2** Urine leptin excretion in boys and girls, Tanner stages 1 and 2.

<table>
<thead>
<tr>
<th>Urine leptin concentrations</th>
<th>Urine leptin concentrations</th>
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<tbody>
<tr>
<td>in patients with nephrotic syndrome and with proteinuria (&gt;1 g/m²/day)</td>
<td>in patients with nephrotic syndrome without proteinuria</td>
</tr>
<tr>
<td>µg/g creatinine</td>
<td>µg/g creatinine</td>
</tr>
<tr>
<td><strong>Boys, Tanner 1</strong></td>
<td><strong>0.026±0.02</strong></td>
</tr>
<tr>
<td><strong>Boys, Tanner 2</strong></td>
<td><strong>0.023±0.03</strong></td>
</tr>
<tr>
<td><strong>Girls, Tanner 1</strong></td>
<td><strong>0.032±0.03</strong></td>
</tr>
<tr>
<td><strong>Girls, Tanner 2</strong></td>
<td><strong>0.026±0.02</strong></td>
</tr>
</tbody>
</table>

There is no difference between boys and girls, nor between Tanner stage 1 and 2.
A positive relationship was obtained between BMI and serum leptin in nephrotic patients with proteinuria (P = 0.032, r² = 0.27; P = 0.027, r² = 0.31), in patients with remission of nephrotic syndrome (P = 0.021, r² = 0.33; P < 0.0001, r² = 0.79) and in healthy controls (P < 0.0001, r² = 0.41; P = 0.028, r² = 0.29). No significant correlations could be demonstrated between the skinfold thicknesses and urine leptin concentrations in any group.

**Discussion**

Patients in groups I and II show similar auxological data including BMI (Table 1). The distribution of girls and boys and their respective pubertal stage (Tanner stage 1 or 2) in any of the groups nor between the three groups (Fig. 3) (19). The patients’ medication (cyclosporine, tacrolimus, cyclophosphamid, β-blocking agents, prednisone) did not significantly influence serum or urine leptin concentrations when groups receiving one of the medications were compared with non-treated patients.

A positive relationship was obtained between BMI and serum leptin in nephrotic patients with proteinuria (P = 0.016, r² = 0.36, y = 0.43x – 5.92), in patients with remission (P < 0.0001, r² = 0.82, y = 0.74x – 11.2) and in controls (P = 0.012, r² = 0.41). No significant correlations were found between BMI and urine leptin concentrations in any group. BMI correlated positively with triceps skinfold thickness in groups I and II (P = 0.006, r² = 0.52 and P = 0.0025, r² = 0.49 respectively) as well as with subscapular skinfold thickness in both groups (P < 0.0001, r² = 0.84 and P = 0.001, r² = 0.66 respectively). There was a positive correlation between triceps and subscapular skinfold thickness and serum leptin in nephrotic patients with proteinuria (P = 0.017, r² = 0.26), in patients with remission of nephrotic syndrome (P = 0.021, r² = 0.33; P < 0.0001, r² = 0.79) and in healthy controls (P < 0.0001, r² = 0.41; P = 0.028, r² = 0.29). No significant correlations could be demonstrated between the skinfold thicknesses and urine leptin concentrations in any group.

These data demonstrate a renal urinary leptin loss in prepubertal and early pubertal children suffering from active nephrotic syndrome with proteinuria >1 g/m². Urinary leptin loss disappeared after remission and was minimal in the control group. However, despite urinary leptin loss, serum leptin levels were similar in all three groups. Several studies have shown a relationship between body mass index, methods for body fat determination such as subscapular and triceps skinfold thickness measurements, and serum concentrations of leptin (8–11). These observations could be confirmed in the present study. However, our data did not reveal any relationship between urine leptin concentrations and BMI or triceps and subscapular skinfold thicknesses. Unexpectedly, serum leptin levels did not differ between groups despite a 100-fold increment in leptin excretion in group I. This finding strongly suggests that the renal loss of leptin is compensated for by a substantial increase in leptin production. The mechanisms that cause the up-regulation of leptin synthesis remain unclear (20–25).

To answer the question as to what degree of glomerular protein leakage allows for the loss of leptin, excretion of leptin was related to albumin and IgG in urine. Our data show that there is no significant difference in urinary leptin excretion in proteinuric patients with an IgG/albumin ratio lower or higher than 1. Consequently, glomerular albumin loss is associated with the loss of leptin. According to these data, we propose a glomerular loss of the 16 kDa peptide leptin in patients with proteinuria that is independent of the molecular size.
It would be of interest to establish data about leptin binding proteins, or data about free leptin concentrations in urine and serum in proteinuric patients in order to gain information about possible leptin binding protein excretion in urine. The clearance of leptin could help to estimate the extent of binding protein loss. However, determination of leptin clearance is limited by several factors: 24-h urine sampling is necessary, which requires a urinary catheter especially in very young children. Furthermore, from the literature we do not have any data about tubular reabsorption of leptin and its binding protein.

Recently published studies revealed that leptin is cleared by the kidney, and patients with end-stage renal disease show increased plasma leptin concentrations (26–28). Leptin promotes renal growth and fibrodisease show increased plasma leptin concentrations cleared by the kidney, and patients with end-stage renal its binding protein. Children. Furthermore, from the literature we do not have any data about tubular reabsorption of leptin and its binding protein.

Recently published studies revealed that leptin is cleared by the kidney, and patients with end-stage renal disease show increased plasma leptin concentrations (26–28). Leptin promotes renal growth and fibrosis in vivo and in vitro (29). Proliferation of endothelial cells stimulated by leptin may contribute to a progression of renal damage and, in consequence, may promote glomerulosclerosis. Leptin was shown to stimulate the expression of transforming growth factor-β1 (TGF-β1), a major modulator of renal fibrosis, in vitro and in vivo. This may activate the transcription of extracellular matrix proteins such as collagen type IV, which may eventually lead to damage of renal glomerular endothelial cells (29). It is well known that renal injury leads to an increased generation of angiotensin II, which induces an up-regulation of the TGF-β1 gene. As a consequence, tubular cells show hypertrophy which, together with increased synthesis of type IV collagen, leads ultimately to fibrogenesis and renal scarring (30).

Thus, grossly elevated glomerular and urinary leptin levels might accelerate the proliferation of endothelial and possibly tubular cells in renal glomerula, leading to an aggravation of (predominant) glomerulosclerosis and tubular damage. In consequence, continuous leptin excretion might be an additional mechanism in the progression of renal failure.

This hypothesis may be tested by long-term observation of children with severe proteinuria and leprotinuria in relation to the progression of renal failure. Furthermore, the effect of leptin on renal endothelial and tubular cell proliferation should be examined in vivo and in vitro.

In summary, our data demonstrate significant leptin excretion in children with severe proteinuria. Elevated urinary leptin concentrations may contribute to the proliferation of glomerular endothelial cells and glomerulosclerosis.

References
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