Circulating resistin concentrations in children depend on renal function

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Abstract

Background. Resistin is a newly discovered peptide hormone that inhibits adipogenesis. Furthermore, it may be involved in regulative processes taking place in insulin resistance and inflammation. In human beings as well as in rodents, the exact physiological role of resistin is unknown. The objective of this study was to examine whether resistin serum concentrations in childhood are regulated by renal function.

Methods. Fifteen patients with end stage renal disease treated by haemodialysis (HD) (6m, 9f; age (median (25–75% percentile)) 16.1 (14.2–16.9) years; body mass index (BMI) 17.8 (17.6–20.6) kg/m²; glomerular filtration rate (GFR)<10 ml/min/1.73 m²), 11 patients with chronic renal failure (CRF) (4m, 7f; age 11.3 (9.2–16.9) years; BMI 17.9 (15.4–22.1) kg/m²; GFR 21.2 (19.8–39.4) ml/min/1.73 m²) and 23 healthy children (13m, 10f; age 7.8 (4.7–14.5) years; BMI 16.5 (14.9–18.1) kg/m²; GFR 168.5 (154.0–197.2) ml/min/1.73 m²) were included. Resistin concentration in serum was measured by ELISA, leptin by RIA.

Results. Resistin concentrations were significantly elevated in HD (6.6 (5.7–8.7) ng/ml) and CRF (4.8 (4.1–6.2) ng/ml) compared to healthy controls [2.7 (2.0–3.6) ng/ml; P < 0.0001 by multiple regression analysis]. Furthermore, resistin concentrations showed a tendency to be higher in children below the age of 5.5 years (youngest tertile) and above 12.5 years of age (oldest tertile), compared to children aged between 5.5 and 12.5 years (P = 0.053 and P = 0.043, respectively). Gender (P = 0.686) and BMI (P = 0.663) did not have a significant influence on resistin concentrations. Resistin and leptin serum concentrations correlated in HD patients only (r = 0.62, P = 0.013 by Spearman correlation). Haemodialysis did not eliminate resistin.

Conclusions. With decreasing renal function, resistin concentration in serum increases in our small paediatric cohort. Age possibly influences circulating resistin concentration. The hypothesis that elevated serum resistin in children with chronic renal failure or end stage renal disease may add to malnutrition and reduced BMI needs further investigation and is not supported by our data.

Keywords: children; chronic renal failure; end stage renal disease; haemodialysis; resistin

Introduction

Human resistin is a cysteine-rich, 108-amino-acid peptide hormone with a molecular weight of 12.5 kDa. In rodents, resistin is expressed and secreted almost exclusively by adipocytes [1]. It inhibits adipogenesis in cultivated 3T3-L1 adipocytes [2]. Serum concentrations are elevated in mouse models of genetic (ob/ob and db/db) and diet-induced obesity and diabetes [1,3]. mRNA expression in white adipose tissue is decreased in ob/ob-mice [4] and not altered in diet-induced obesity [4]. In wild-type mice, plasma resistin concentration decreases with age. Female mice show higher resistin levels than male [5]. In adult human beings, resistin expression is highest in bone marrow [6]. In adipocytes of lean subjects, resistin mRNA is almost undetectable [7]. In adiposity, plasma resistin concentrations are reported to be elevated [8] or unchanged [9]. Body mass index (BMI) is weakly positively correlated [10] or not correlated [9] with resistin concentrations in serum, and women may have higher resistin concentrations than men. Schäffler et al. [10] report that gender and age do not have any influence on circulating resistin concentrations in healthy or diabetic human beings aged 18–86 years. Elevated resistin mRNA expression in whole adipose
tissue in obese subjects is mainly due to preadipocytes [11] and mononuclear cells [7] and not due to adipocytes, which express resistin at very low levels [6]. Resistin mRNA expression in human peripheral blood mononuclear cells is increased by proinflammatory cytokines in vitro [12].

In human type 2 diabetes, circulating resistin concentrations are reported to be elevated [13], equal [14] or decreased [10] compared to healthy controls. Administration of resistin worsens, inactivation of resistin improves insulin sensitivity and glucose homeostasis in mice [1] and man [15]. In contrast to mice [4], Lee et al. [9] report that fasting and leptin administration did not influence resistin serum levels in humans. Resistin levels are not associated with circulating leptin concentrations or insulin resistance.

In children, it is reported that serum resistin concentrations of 46 girls aged 9.8–15.4 years do not correlate with serum leptin concentrations or skinfold thickness [16]. In obese, non-diabetic children (age 3.4–17.8 years) and healthy children (age 7.9–17.9 years), resistin concentrations in serum positively correlate with age, but not with BMI. Girls have higher concentrations than boys [17].

In summary, resistin may be involved in regulating processes occurring in adiposity, insulin resistance and inflammation. In humans, existing data is more controversial than in rodents. Relationships of murine resistin expression with obesity and insulin resistance cannot be translated to humans. The exact physiological role of resistin is unknown.

Data on the role of resistin in renal disease is scarce. In adults (age 43 ± 2 yr) with IgA-nephritis and a glomerular filtration rate (GFR) between 20 and 145 ml/min/1.73 m², resistin plasma concentrations are significantly negatively correlated with GFR and positively correlated with age, but not with BMI, fasting plasma insulin, glucose or leptin concentrations and insulin sensitivity [18]. Recently, Diez et al. reported significantly elevated resistin serum concentrations in adult haemodialysis (HD, age 60.8 ± 1.6 years) and peritoneal dialysis (PD, age 54.4 ± 1.8 years) patients, compared to ureaemic patients on conservative management [19].

The current study was performed to explore the role of renal function on serum resistin concentrations in children.

### Subjects and methods

#### Subjects

The study was approved by the ethics committee of the University of Erlangen-Nürnberg. Parents and all patients capable of understanding gave informed written consent to participate in the study.

Serum was obtained from three groups of individuals: 15 patients with end stage renal disease treated by haemodialysis (HD), 11 patients with chronic renal failure and conservative treatment (CRF) and 23 healthy children (C, Table 1).

End stage renal disease was caused by juvenile nephropathies (3 patients), renal dysplasia, obstructive nephropathy or focal segmental glomerulosclerosis (2 patients each) and cystinosis, familial hypomagnesaemia with hypercalciuria and nephrocalcinosis, reflux nephropathy, Senior–Loken syndrome, Alport syndrome or unknown origin (1 patient each). One HD patient had had renal transplantation and allograft failure before (second period of haemodialysis).

All patients were treated with antihypertensive drugs because of renal hypertension, 10 of them with one or two, five of them with three or more antihypertensives. Enalapril (8 patients), atenolol (8 patients), furosemide (9 patients), amloidine (3 patients), nifedipine (2 patients), prazosin (2 patients), captopril (1 patient) and/or catapressan (1 patient) were applied. Further medication was calcidiol (15 patients), calcitriol (14 patients), sodium bicarbonate (13 patients), phosphate binders (11 patients), intravenous iron(III)-sodium-gluconate (15 patients), growth hormone (4 patients), FK506, decorin, cysteamine or ethosuximide (1 patient each). Fourteen out of 15 patients received intravenous human recombinant erythropoetin.

### Table 1. Characteristics of patient groups and control group. Concentrations of resistin and leptin are shown

<table>
<thead>
<tr>
<th></th>
<th>End stage renal disease before haemodialysis (HD)</th>
<th>End stage renal disease after haemodialysis (HD)</th>
<th>Chronic renal failure (CRF)</th>
<th>Controls (C)</th>
<th>P-value (Kruskal–Wallis test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number (n)</td>
<td>15</td>
<td>11</td>
<td>23</td>
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<td></td>
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<tr>
<td>Gender (male, female)</td>
<td>6m, 9f</td>
<td>4m, 7f</td>
<td>13m, 10f</td>
<td>--</td>
<td></td>
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<tr>
<td>Age (years)</td>
<td>16.1 (14.2–16.9)</td>
<td>11.3 (9.2–16.9)</td>
<td>120.0 (105.0–174.5)</td>
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<tr>
<td>Height (m)</td>
<td>158.3 (147.3–168.5)</td>
<td>149.0 (143.5–163.8)</td>
<td>120.0 (105.0–174.5)</td>
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<tr>
<td>Height-SDS</td>
<td>−1.4 (−1.95–−0.25)</td>
<td>−0.9 (−2.0–0.55)</td>
<td>−0.3 (−2.45–−1.25)</td>
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<tr>
<td>BMI (kg/m²)</td>
<td>18.6 (17.7–20.5)</td>
<td>17.8 (17.6–20.6)</td>
<td>17.9 (15.4–22.1)</td>
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<tr>
<td>BMI-SDS</td>
<td>−0.2 (−1.1–0.0)</td>
<td>−0.3 (−1.0–0.1)</td>
<td>−0.3 (−1.2–0.3)</td>
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<tr>
<td>Weight for height (%)</td>
<td>105.0 (102.5–110.5)</td>
<td>105.0 (101.0–111.0)</td>
<td>105.0 (99.5–120.5)</td>
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<tr>
<td>GFR (ml/min/1.73 m²)</td>
<td>21.2 (19.8–39.4)</td>
<td>168.5 (154.0–197.2)</td>
<td>98.0 (90.0–108.0)</td>
<td>&lt;0.001</td>
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<tr>
<td>Resistin i.s. (ng/ml)</td>
<td>6.6 (5.7–8.7)</td>
<td>6.6 (5.4–8.8)</td>
<td>4.8 (4.1–6.2)</td>
<td>&lt;0.001</td>
<td></td>
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<tr>
<td>Leptin i.s. (ng/ml)</td>
<td>5.5 (1.7–15.4)</td>
<td>6.1 (4.0–13.1)</td>
<td>15.2 (2.7–56.6)</td>
<td>0.450</td>
<td></td>
</tr>
</tbody>
</table>

HD, haemodialysis; CRF, chronic renal failure; C, controls; BMI, body mass index; GFR, glomerular filtration rate; i.s., in serum; SDS, standard deviation score. Values expressed as median and (in brackets) 25–75% percentile.
Circulating resistin concentrations in children depend on renal function

Chronic renal failure was caused by renal dysplasia or focal segmental glomerulosclerosis (3 patients each) and juvenile nephronophthisis, obstructive nephropathy, familial hypomagnesemia with hypercalciuria and nephrocalcinosis, perinatal asphyxia or unknown origin (1 patient each). Nine out of 11 patients were treated with antihypertensive drugs because of renal hypertension, six of them with one or two, three of them with three or four antihypertensives. Enalapril (6 patients), atenolol (3 patients), furosemide (4 patients), captopril (2 patients), nifedipine (1 patient) and/or propranolol (1 patient) were administered. Further medication was calciold (9 patients), calcitriol (8 patients), sodium bicarbonate (7 patients), phosphate binders (2 patients), iron(II)-glycine-sulfate (6 patients), l-thyroxine (2 patients), pravastatin or trosplum chloride (1 patient each). Three out of 11 patients received subcutaneous human recombinant erythropoetin.

GFR was calculated by the Schwartz formula. In HD patients, GFR was <10.0 ml/min/1.73 m². CRF was defined as GFR between 15 and 70 ml/min/1.73 m². Controls were healthy, especially without any signs for renal disease. No subjects had clinical signs for inflammatory disease.

Haemodialysis regimen

Patients were dialyzed three times a week for 3 to 4.5 h. The ultrafiltration rate was between 0 and 300 ml per session in 12 patients and 2100 to 3500 ml in three anuric patients. Fresenius® Polysulfone High-Flux F60S Filters were used in two patients, Diacap® SMC 1.8 SD in six patients, Diacap® SMC 1.5 SD in one patient and Diacap® LO PS 18 in six patients.

Sample collection

Since fasting did not influence serum resistin levels in adolescents (age 17.7 ± 1.7 years) [9] and is not advised especially in young children, samples were taken without regard to previous meals. From haemodialysis patients, serum was obtained before HD (between 08:00 and 09:00 h or between 12:00 and 13:30 h) and after HD (immediately before the saline flush of the dialysis system) to evaluate the influence of HD (Table 2). In eight haemodialysis patients, samples were obtained during the first dialysis session of the week, in seven during the second or third dialysis sessions. From CRF patients, serum was obtained during routine outpatient clinic. In healthy children, serum was collected between 08:00 and 16:00 h to resemble the collection time of the patients. Samples were processed immediately and serum was stored at −20°C until measurement.

Resistin ELISA and leptin RIA

Human resistin levels were determined by a DuoSet ELISA Development Kit (R&D Systems, Wiesbaden, Germany). The analytical sensitivity was below 15 pg/ml. Samples were diluted 1:50 with reagent dilution. Intra- and inter-assay coefficients of variations were below 10.2% at 104 pg/ml (n = 7). There is no cross-reaction with recombinant human RELM-β or recombinant murine Resistin (no further cross-reactions tested).

Total leptin in serum was determined by specific RIA as described before [20].

Auxological data

BMI standard deviation score (BMI-SDS), height-SDS and weight for height were calculated using an auxology calculator (Pfizer, Karlsruhe, Germany).

Statistical analysis

Kruskal–Wallis test was used to compare age and BMI in the three groups (HD, CRF, C). Bivariate associations of resistin with age, BMI, BMI-SDS and leptin were analysed by Spearman correlation analysis, performed separately for each group. Since correlation of resistin with age seemed to be rather U-shaped than linear, with higher resistin values in young children and adolescents compared to middle-aged children, we sorted age according to age-tertiles (0–5.5 years, >5.5 to <12.5 years, 12.5–18 years). Because there were significant differences of age and BMI between the three groups (HD, CRF, C, by Kruskal–Wallis test) and both possibly influence resistin serum concentration, multiple regression analysis was carried out in all children to analyze resistin concentration and its dependence on the following variables: diagnosis (HD, CRF, C), gender, BMI and age terciles. By analysing age terciles, it was possible to answer the question whether there is a difference in resistin levels of individuals in the youngest tertile was compared with the oldest and middle terciles. Before multiple regression analysis, resistin was log-transformed to approximate normality. Resistin before and after haemodialysis was compared by Wilcoxon matched-pairs test.

In healthy children only, we performed a Kruskal–Wallis test to analyse the relationship of age terciles. The test was done for resistin concentrations, gender, BMI-SDS and GFR. BMI-SDS was chosen as the variable for the Kruskal–Wallis test instead of BMI because of age- and gender-dependency of BMI.

A P-value (2-tailed) of <0.05 was considered significant. All values are expressed as median and (in brackets) 25th percentile–75th percentile.

Results

Resistin serum concentrations in all individuals

Resistin was detected in all serum samples; concentrations ranged from 1.04 ng/ml to 12.30 ng/ml. Spearman correlation analyses of circulating resistin and BMI (HD: r = 0.01, P = 0.98; CRF: r = −0.04, P = 0.924; C: r = −0.06, P = 0.788) was compared with resistin and BMI-SDS (HD: r = −0.24, P = 0.394; CRF: r = 0.08, P = 0.820; C: r = −0.28, P = 0.203) showed no significant correlation.

Resistin concentrations were measured 6.6 (5.7–8.7) ng/ml in HD patients, 4.8 (4.1–6.2) ng/ml in CRF patients and 2.7 (2.0–3.6) ng/ml in healthy controls. Multiple regression analysis of all individuals showed that resistin concentration depended on renal function (Figure 1, Table 1) and was significantly
Leptin was detected in all serum samples; concentrations showed no correlation of resistin concentration with leptin in healthy controls ($r = -0.32$, $P = 0.131$) or chronic renal failure ($r = -0.35$, $P = 0.285$). In haemodialysis patients, there was a positive correlation between resistin and leptin before ($r = 0.62$, $P = 0.013$) and after HD ($r = 0.57$, $P = 0.025$).

**Discussion**

We show that resistin is significantly elevated in our paediatric patients with renal failure, with highest values in end stage renal disease. This confirms data recently shown in adults [18,19]. Haemodialysis does not eliminate resistin as expected for its molecular size of 12.5 kDa. The consequences of resistin elevation in renal failure need to be determined. So far, it has been demonstrated that there is neither an association with impaired insulin sensitivity [18] nor with atherosclerotic vascular disease [19] in adults with renal failure.

Resistin concentrations are elevated in mouse models of adiposity [1,3]. In human adiposity, there are reports that resistin concentrations are elevated [8] or not associated with BMI and fat mass [9]. Resistin is capable of inhibiting adipogenesis in cultivated 3T3-L1 adipocytes [2]. In childhood renal failure, it is of specific clinical interest to secure adequate physical development of the patients, including normal body weight after haemodialysis, when possible fluid excess has been eliminated. Therefore, we investigated the influence of BMI on resistin concentration. In our patients, who have had no apparent nutritional problems since mean BMI-SDS was $-0.37$ in HD patients, $-0.15$ in CRF and $-0.50$ in healthy children, BMI and BMI-SDS are not correlated with serum resistin concentrations, in healthy individuals or in renal failure. This is comparable to findings in adults with or without renal failure [9,18] as well as to data in large samples of healthy or obese children [16,17]. Therefore, resistin does not seem to be involved in the regulation of body weight in healthy children. The hypothesis that hyperresistinaemia may contribute to malnutrition and reduced body weight in uraemic paediatric patients is not supported by our data and needs further investigation.

Data in humans is controversial not only regarding the relationship between adiposity and BMI, but also type 2 diabetes and resistin serum concentrations. Elevated circulating resistin concentrations in human type 2 diabetes are reported by Fujinami et al. [13], who analysed patient and control groups matched by age, BMI and gender. Measurements were performed by self-developed resistin ELISAs. Equal resistin concentrations in patients and controls (ANOVA analysis), but higher resistin levels than measured by Fujinami et al. [13] are reported by Fehmann and Heyn [14], measured in subjects with higher BMI by an ELISA kit from Phoenix Pharmaceuticals. Decreased resistin serum concentrations compared to healthy controls [10] elevated in HD and CRF patients compared to healthy controls ($P < 0.0001$). Furthermore, resistin concentrations showed a tendency to be higher in children below the age of 5.5 years ($n = 9$) and above 12.5 years of age ($n = 26$), compared to children aged between 5.5 and 12.5 years ($n = 14$; $P = 0.053$ and $P = 0.043$, respectively). Gender ($P = 0.686$) and BMI ($P = 0.663$) did not have significant influence on resistin concentrations.

Wilcoxon matched pairs test revealed that haemodialysis did not eliminate resistin significantly [6.6 (5.7–8.7) mg/ml before HD versus 6.6 (5.4–8.8) ng/ml after HD, $P = 0.626$].

**Resistin serum concentrations and age in healthy children**

Additionally, we analysed the tertiles of our healthy control patients. In this small sample ($n = 23$), resistin and age showed a U-shaped relationship comparable to the adjusted analysis of all individuals. Kruskal–Wallis test showed that resistin in healthy controls had a tendency ($P = 0.066$) to be higher in young [3.3 (2.0–4.4) ng/ml; $n = 7$] and old [3.1 (2.3–4.8) ng/ml; $n = 8$] children compared to the middle-aged group [2.1 (1.7–2.6) ng/ml, $n = 8$]. There was no significant difference between BMI-SDS ($P = 0.724$), gender distribution ($P = 0.427$) or GFR ($P = 0.940$) between these age groups.

**Resistin and leptin**

Leptin was detected in all serum samples; concentrations ranged from 0.3 ng/ml to 132 ng/ml. Correlation analyses (Spearman) showed no correlation of resistin concentration with leptin in healthy controls ($r = -0.32$, $P = 0.131$) or chronic renal failure ($r = -0.35$, $P = 0.285$). In haemodialysis patients, there was a positive correlation between resistin and leptin before ($r = 0.62$, $P = 0.013$) and after HD ($r = 0.57$, $P = 0.025$).
have been found in a large number of diabetic patients, measured by the BioVendor human resistin ELISA kit. Total resistin levels determined in this study in patients with relatively high BMI are low compared to the other studies. The reason for this discrepancy may be that resistin measurements by different assay systems should not be compared because of the presence of different molecular isoforms of resistin in human blood [17]. This also may be one explanation for the discrepancy between the absolute resistin serum concentrations measured in healthy children by Gerber et al. [17] and our values, which are relatively low.

Another interesting observation of our study is the relationship between resistin concentrations and age in children. In adults, age does not influence circulating resistin concentrations, as demonstrated in a large number of healthy and diabetic subjects aged 18–86 years [10]. In contrast, our data recorded in a small sample of 23 healthy children (age 1.9–18.0 years) compared with all 49 children (age 1.7–18.0 years) suggests that their resistin concentrations may be influenced by age, with possibly higher values in early childhood and adolescents than in middle-aged children. Gerber et al. found a positive correlation between age and resistin serum concentrations in healthy (age 3.4–17.8 years) or obese children (age 7.9–17.9 years) [17]. We conclude that childhood may be a period of considerable resistin regulation with a unique, age-dependent concentration profile. Consequently, resistin data from adults cannot be transferred to paediatric patients. Data in children below the age of 3.4 years (four children in our study) is extremely rare and needs further evaluation.

There is no correlation of circulating resistin and leptin levels in healthy controls or chronic renal failure. This resembles data described by Kielstein et al. [18]. In end stage renal disease of paediatric patients treated by haemodialysis, we show a positive correlation between resistin and leptin concentrations before and after HD. We conclude that total loss of renal function can no longer be offset by regulative processes, so that loss of renal elimination becomes the main determinant of resistin and leptin concentrations in serum.

In summary, our findings are a first step to the understanding of resistin physiology in children with chronic renal failure or end stage renal disease. With decreasing renal function, resistin concentration in serum increases. Age possibly influences circulating resistin concentrations in children. There is no effect of gender and BMI in our small paediatric cohort. Regarding energy metabolism in renal failure, the hypothesis that elevated resistin levels in renal failure are associated to reduced BMI needs further investigation and is not supported by our data.

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