

Serum Resistin Levels of Obese and Lean Children and Adolescents: Biochemical Analysis and Clinical Relevance

M. Gerber, A. Boettner, B. Seidel, A. Lammert, J. Bär, E. Schuster, J. Thiery, W. Kiess, and J. Kratzsch

Institute of Laboratory Medicine (M.G., A.L., J.T., J.K.), Clinical Chemistry and Molecular Diagnostics, and Institute of Biochemistry (J.B.), Faculty of Medicine, University of Leipzig, D-04103 Leipzig, Germany; Hospital for Children and Adolescents (A.B., B.S., W.K.), Faculty of Medicine, University of Leipzig, D-04317 Leipzig, Germany; and Institute for Medical Informatics (E.S.), Statistics and Epidemiology, University of Leipzig, Leipzig, D-04107 Leipzig, Germany

Objective: It was hypothesized that resistin links obesity with diabetes, but this has not been studied in children and adolescents to date.

Patients: We determined serum resistin levels of 135 obese (body mass index, 32.0 ± 6.2 kg/m²; age, 12.6 ± 3.4 yr) and 201 lean children (body mass index, 18.7 ± 2.4 kg/m²; age, 12.5 ± 2.5 yr) by a newly developed and extensively evaluated in-house immunoassay. These results were controlled for their association with markers of puberty, obesity, and insulin sensitivity.

Results: The analytical evaluation of our assay revealed different resistin isoforms with major peaks of higher than 660 and 55 kDa in the size exclusion chromatography. Using this assay system we found no difference in the resistin levels of obese compared with lean subjects ($P = 0.48$). However, resistin was significantly higher in girls

than in boys (6.74 ± 2.42 vs. 5.79 ± 2.45 ; $P < 0.001$). Interestingly, in both obese and lean children, resistin correlated with age ($P < 0.01$), Tanner stage, and testosterone and estradiol levels ($P < 0.05$). In contrast, no significant correlation was found with parameters of insulin resistance such as homeostasis model assessment, insulin sensitivity index, or insulin, proinsulin, and glucose concentrations in obese subjects.

Conclusions: Resistin appears to be not the main link between obesity and insulin resistance in children and adolescents but because of its association with Tanner stage, it may be related to the maturation of children during pubertal development. Additionally, we have demonstrated the presence of different molecular isoforms of resistin in human blood, and this may raise problems in comparing data from diverse assay systems. (*J Clin Endocrinol Metab* 90: 4503–4509, 2005)

RESISTIN IS A novel adipocytokine and belongs to the family of cysteine-rich resistin-like molecules (RELM) (1), together with RELM- α and RELM- β . Because resistin serum levels were found to be elevated in mouse models of obesity where they could antagonize insulin action, resistin was firstly described as a potential link between obesity and insulin resistance (2). Furthermore, the fact that thiazolidinediones (TZD), a group of pharmaceuticals that improve insulin sensitivity, down-regulated resistin expression in rodents (2–4) underlines its potential role in the pathomechanism of insulin resistance. However, some studies investigating resistin mRNA and protein levels in different rodent models of obesity and diabetes could not clearly confirm resistin as a mediator of insulin resistance. Findings from the literature show increased resistin expression related to insulin resistance (5, 6) or impaired glucose homeostasis as a result of increased hepatic glucose production after resistin administration in rats (7). But down-regulation of resistin in

the insulin resistance state (8) and up-regulation of resistin expression after administration of TZD in mice (9, 10) was also reported.

The situation in humans is even more controversial. In contrast to mice, human resistin is barely detectable in adipose tissue (11, 12), and no correlation was found between resistin expression of isolated adipocytes and obesity or type 2 diabetes (11–13). Resistin serum levels were found to be related to body mass index (BMI) in human subjects (14–16), but other studies did not reveal a correlation between body mass and resistin levels in blood (17–19).

A considerable number of studies failed to detect an association between resistin concentration and markers for insulin sensitivity (14, 17–21). In contrast, Silha *et al.* (22) described an association between resistin in serum and homeostasis model assessment for insulin resistance (HOMA-R) in a small group of subjects. Additionally, the treatment of 13 patients with type 2 diabetes mellitus (T2DM) by TZD for 16 wk in another study led to decreased resistin serum levels (23). Both findings support a possible impact of resistin on insulin sensitivity.

The prevalence of childhood and adolescent obesity has increased over the past three decades such that obesity is now a worldwide pediatric health risk factor (24). In parallel, the incidence of obesity-associated diseases such as T2DM and dyslipidemia is rising dramatically. Because children are relatively free from comorbidity compared with adults, we

First Published Online May 31, 2005

Abbreviations: BMI, Body mass index; DHEA-S, dehydroepiandrosterone sulfate; HOMA-R, homeostasis model assessment for insulin resistance; ISI, insulin sensitivity index; oGTT, oral glucose tolerance test; RELM, resistin-like molecules; rh, recombinant human; SDS, sd score(s); SEC, size exclusion chromatography; T2DM, type 2 diabetes mellitus; TZD, thiazolidinediones.

JCEM is published monthly by The Endocrine Society (<http://www.endo-society.org>), the foremost professional society serving the endocrine community.

examined the role of serum resistin levels as a marker of juvenile obesity and insulin resistance. For this use, we extensively evaluated a new in-house immunoassay for its determination of resistin serum levels in children.

Subjects and Methods

Antiresistin antibodies

Anti-human-resistin antibodies were raised in rabbits against an N-terminal peptide (amino acids 1–20) and the recombinant human (rh) protein (Pepro-Tech, Rocky Hill, NC). The IgG fraction was purified from both antisera using an in-house protein A-Sepharose column (Amersham Biosciences, Freiburg, Germany). Anti-rh-resistin IgG was biotinylated with D-biotinyl- ϵ -aminocaproic acid *N*-hydroxysuccinimide ester (Roche, Mannheim, Germany) according to the product instruction sheet.

Partial purification of resistin from human serum by affinity chromatography with anti-rh-resistin antibodies

An affinity chromatography column was prepared by coupling 5 mg anti-rh-resistin IgG to 1 ml of CNBr-activated Sepharose (Amersham) according to the manufacturer's instructions. A pool of 40 ml of human serum obtained from healthy blood donors in a dilution of 1:2 in PBS was subjected to this column. The resistin level of this pool was 10 ng/ml and was reduced to undetectable levels by passage through the column. After extensive washing with PBS, bound resistin was eluted from the column with 0.1 M glycine buffer at pH 2.7. The eluate was immediately neutralized and stored at -20°C .

Size exclusion chromatography (SEC)

SEC experiments were performed using a Superdex 200 (16/60) (Amersham). The Superdex 200 column was equilibrated with PBS at 4 C with a flow rate of 1 ml/min. Loaded sample volume was 1 ml, and fractions of 1 ml were collected. The SEC system was adjusted by measuring the absorbance of commercially available calibration proteins (Amersham). Before assaying resistin levels, individual SEC fractions were concentrated to 4-fold of the original volume.

Western blotting

Samples of partially purified resistin or controls were mixed with Laemmli buffer either with or without mercaptoethanol and subjected to 15% SDS-PAGE. Thereafter, proteins were transferred to a nitrocellulose membrane (Bio-Rad, Munich, Germany). This membrane was blocked with 2% nonfat dry milk (Bio-Rad) in Tris-buffered saline. We then incubated the membrane with the antipeptide antiserum or with the purified antibody against rh-resistin followed by incubation with horseradish peroxidase-conjugated goat antirabbit IgG (Pierce, Rockford, IL). Signal development was performed by ECL kit (Pierce). For the negative control, we incubated the nitrocellulose membrane with rabbit normal serum instead of the first antibody.

In-house assay for the measurement of human resistin

Wells of a microtiter plate (Maxisorb; Nunc, Wiesbaden, Germany) were coated with 100 μl of the IgG fraction of the anti-rh-resistin antiserum in carbonate buffer overnight at 4C. Nonspecific binding sites were blocked with 1% BSA (Sigma Chemical Co., St. Louis, MO) in PBS for 1 h at room temperature. In the next step, 25 μl of resistin standards ranging from 0.6–20 ng/ml and 1:4 prediluted samples were pipetted in duplicate to the wells. Thereafter, 75 μl assay buffer was added to the wells followed by an overnight incubation at 4 C. For detection, wells were incubated with 100 μl biotinylated anti-rh-resistin IgG in assay buffer containing 1% rabbit γ -globulin (Calbiochem, Darmstadt, Germany) for 2 h at room temperature. After adding Europium-labeled streptavidin (Wallac, Turku, Finland), fluorescence signals were measured (Victor 1420 multilabel counter; Wallac). Between coating, blocking, incubation, and detection, plates were washed four times with PBS, 0.05% Tween 80.

Commercially available resistin assay for comparison

The resistin ELISA from Biovendor (Brno, Czech Republic) was performed according to the manufacturer's instructions. In our hands, the limit for detection of this assay was 0.2 ng/ml; intra- and interassay coefficients of variation were 4.0 and 7.2%. Biovendor found a recovery of dilution and spiking experiments of $99.8 \pm 6.8\%$ and $104.8 \pm 3.4\%$, respectively.

Subjects

Fasting serum samples from obese, nondiabetic children and adolescents ($n = 135$; age range, 3.4–17.8 yr; BMI range, 21.5–56.3 kg/m²) were obtained from patients of the obesity outpatient clinic at the University Hospital for children and adolescents; University of Leipzig (Table 1). Six patients in this cohort were found to have impaired glucose tolerance by an oral glucose tolerance test (oGTT) and received, therefore, metformin treatment. Five patients were treated with L-T₄ and were euthyroid. Twelve girls took oral contraceptives. Polycystic ovary syndrome was diagnosed in 22 female subjects based on clinical criteria of hirsutism, oligo-/amenorrhea, and hyperandrogenemia.

As control group, we used serum samples of healthy children and adolescents ($n = 201$; age range, 7.9–17.9 yr; BMI range, 14.5–25.5 kg/m²) of the Leipziger Schoolchildren Project (25) (Table 1).

Written informed consent for measurements and blood analyses was obtained from all guardians of the children. The study was approved by the ethical committee of the University of Leipzig.

Biochemical measurements and tests

Adiponectin was measured with a RIA (Linco Research, St. Charles, MO) according to the manufacturer's instructions. The intra- and interassay coefficients of variation were less than 14% in our hands, and sensitivity was calculated to be 1 ng/ml. Leptin was measured by an in-house RIA (26). For the leptin RIA, sensitivity was 0.2 ng/ml, and intra- and interassay coefficients of variation were lower than 12.5%.

The Elecsys immunoassay system (Roche) was used to measure testosterone and estradiol. Sensitivity for both assays was 0.42 nmol/liter and 55 pmol/liter, and the coefficient of variation was less than 5% for each parameter. Dehydroepiandrosterone sulfate (DHEA-S) was determined by Advantage immunoassay system (Nichols Institute, Bad Vilbel, Germany). For DHEA-S, a sensitivity of 30 nmol/liter was calculated, and intra- and interassay coefficients of variation were less than 7%. Measurements of insulin were performed on the Auto DELFIA system (Perkin-Elmer Wallac, Freiburg, Germany) with a sensitivity of 3 pmol/liter and intra- and interassay coefficients of variation less than 5%. For determination of proinsulin, a commercially available ELISA (IBL, Hamburg, Germany) was used. We found intra- and interassay coefficients of variation less than 12% and a sensitivity of 1 pmol/liter. Glucose was measured by the Modular system (Roche).

In obese children, an oGTT was performed according to the guidelines of the American Diabetes Association (27). The HOMA-R was calculated as previously described (28). Calculation of the insulin sensitivity index (ISI) was performed as indicated by Matsuda *et al.* (29).

TABLE 1. Anthropometric data of the obese and lean children and adolescents

	Obese group	Lean group
No. (male/female)	135 (65/70)	201 (99/102)
Age (yr)	12.6 \pm 3.4	12.5 \pm 2.5
Weight (kg)	84.4 \pm 28.8	46.6 \pm 12.6 ^a
BMI (kg/m ²)	32.0 \pm 6.2	18.7 \pm 2.4 ^a
BMI (SDS)	2.75 \pm 0.62	−0.04 \pm 0.82 ^a
Waist circumference (cm)	94.7 \pm 14.0	66.2 \pm 7.5 ^a
Hip circumference (cm)	107.1 \pm 15.7	83.5 \pm 9.5 ^a
WHR	0.88 \pm 0.06	0.79 \pm 0.05 ^a

Both subgroups are significantly different in all parameters except age. WHR, Waist to hip ratio.

^a $P < 0.001$.

Anthropometrical measurements

Height and weight were determined to the nearest of 0.1 cm and 0.1 kg using precision stadiometers and scales. National reference data (30) were used to standardize BMI as SD score (SDS). Pubertal stage was documented during physical examination according to the classification of Tanner by three experienced pediatric endocrinologists (31).

Statistical analysis

Because some of the investigated parameters, such as leptin, insulin, HOMA, estradiol, and testosterone, show a nonnormal distribution, nonparametric statistical methods (descriptive statistics by medians and quartiles, correlations according to Spearman ρ test, and comparison of variables by Mann-Whitney U test) were applied for all analyses. All results, including stepwise forward multiple regression analysis, were calculated using Statistica 6.0 (Statsoft, Tulsa, OK). Power analysis was performed by the SamplePower 1.0 software (SPSS, Inc., Chicago, IL) to calculate the probability of significance for parameters, which appear to be not related to resistin.

Results

Validation of the resistin in-house assay

Quality control data. The lowest detectable concentration, defined as 2 SD above the blank value, was calculated to be 0.4 ng/ml. Intra- and interassay coefficients of variation after 12 measurements for each of three control samples (23.6, 6.7, and 1.9 ng/ml) were on average 6.9 and 8.1%, respectively. In dilution experiments, we found a recovery of $100.5 \pm 11.0\%$ (mean \pm SD) for the respective resistin levels. Measuring of serum samples spiked with the resistin standard 5 ng/ml revealed a recovery of $91.1 \pm 7.6\%$. Neither human RELM- β (Pepro-Tech) nor mouse resistin (Biocat, Heidelberg, Germany) showed any signal in our in-house assay. No changes were found for resistin serum levels ($n = 6$) after repeated freezing and thawing ($n = 8$) compared with the basal levels ($P = 0.39$).

Characterization of the antiresistin antibody. To control the specificity of the anti-rh-resistin antibody used in the assay we enriched and partially purified resistin from a 40-ml serum pool by affinity chromatography with the same antibody. The eluted material was subjected to Western blot and detected with the in-house antiresistin antibody directed against the N terminus of the protein (Fig. 1). With different antibodies against resistin, specific immunoreactivity was

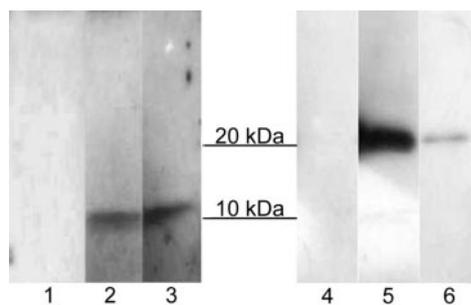


FIG. 1. Resistin purified from human serum in Western blot under reducing conditions detected with an antiresistin peptide (amino acids 1–20) antibody (lanes 1–3) and under nonreducing conditions detected with an antibody against dimeric resistin (lanes 4–6). Lanes 3 and 6, resistin from human blood; lanes 2 and 5, positive control with recombinant resistin; lanes 1 and 4, negative control with nonspecific rabbit IgG as detection antibody.

found at a molecular weight of 10 and 20 kDa under reducing and nonreducing conditions, respectively.

Characterization of serum isoforms by SEC. A buffered solution of rh-resistin was administered to a Superdex 200 column. Individual SEC fractions were measured for their resistin level by the in-house assay. The elution profile of these results revealed a major peak in the void volume corresponding to a molecular mass of more than 660 kDa. Moreover, a discrete maximum was observed at around 55 kDa (Fig. 2A). Human serum pools fractionated by the same system revealed comparable peaks (Fig. 2B). Additionally, the 55-kDa peak appeared to have a shoulder at approximately 45 kDa. Some resistin immunoreactivity was also found in fractions of a molecular mass between 10 and 20 kDa. Spiking of human serum with rh-resistin led to a significant increase only in the peak of the void volume (Fig. 2B). These results indicate that resistin occurs in high molecular mass isoforms in human blood.

Comparison with an commercially available assay. We compared serum levels measured by our in-house assay with data obtained by a commercially available ELISA from Biovendor.

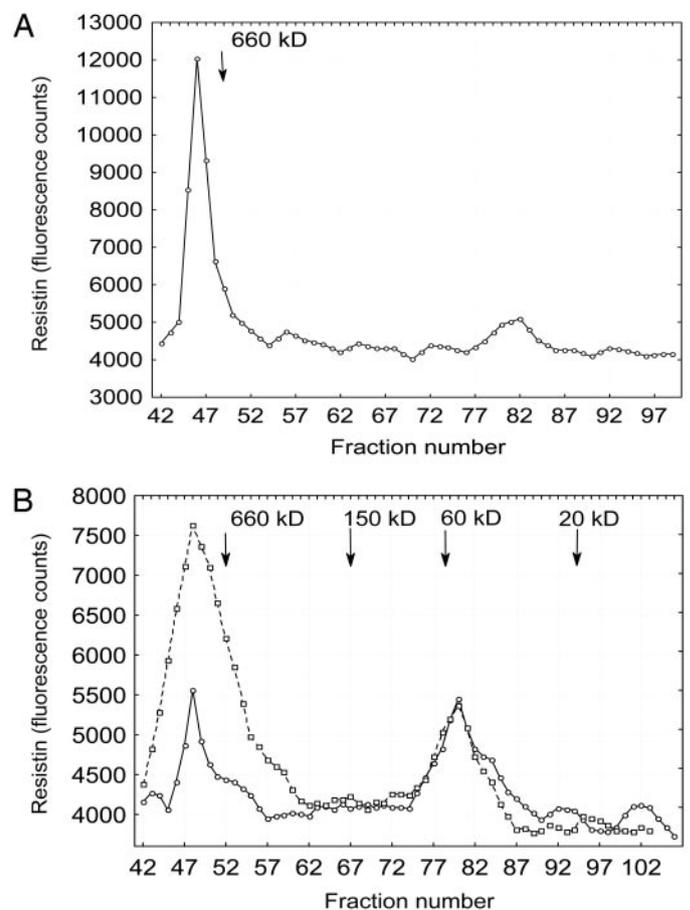


FIG. 2. Recombinant resistin (A) and pools of human serum (B) were fractionated by SEC (Superdex 200; 16/60 column). Thereafter, resistin levels were measured in the individual fractions by the in-house assay. A, rh-resistin in a protein-rich buffer with 4% human serum albumin and 1% human γ -globulin (\circ); B, resistin levels of a serum pool (\circ) spiked with and without rh-resistin (\square). The spiking experiment led to an increased peak of the void volume.

The Biovendor assay measured significantly lower resistin serum levels than the in-house assay (in-house result = $0.61 \times \text{Biovendor result} + 4.29 \text{ ng/ml}$). The coefficient of correlation between the two assays was 0.57 ($P < 0.001$) for the measurement of our 135 sera of obese children and adolescents.

Clinical results

Anthropometric and biochemical data of 135 obese and 201 lean children and adolescents are shown in Tables 1 and 2. All parameters except age are significantly different between lean and obese individuals.

Obese children

Resistin levels were significantly higher in girls ($6.73 \pm 2.70 \text{ ng/ml}$) compared with boys ($5.74 \pm 2.62 \text{ ng/ml}$) ($P = 0.016$) as measured by our in-house assay, although age and BMI did not demonstrate any dependence on gender.

Correlation analysis revealed a significant age dependence of resistin serum levels in both girls and boys (Table 3). Additionally, we observed a close correlation between resistin levels and pubertal stage as well as with testosterone of boys and estradiol and DHEA-S of girls. The association of serum resistin with markers of obesity such as weight, BMI, waist and hip measurements was weakly significant and lost significance in the male subgroups. No significant correlation ($P > 0.05$) and, consequently, a relatively low power for an association was found between serum resistin levels and parameters of insulin resistance and glucose homeostasis such as insulin (15%), glucose (6%), proinsulin (9%), HOMA (15%), and ISI (24%).

However, a potential association between resistin and indicators for insulin resistance cannot be completely excluded as seen by our results from the oGTT; serum maximal insulin levels ($r = 0.18$; $P < 0.05$) and the insulin levels at time point 60 min after start ($r = 0.19$; $P < 0.05$) were correlated weakly with resistin.

Control group of lean children

Resistin levels of the lean group were significantly higher in girls ($6.75 \pm 2.22 \text{ ng/ml}$) than in boys ($5.83 \pm 2.35 \text{ ng/ml}$) ($P < 0.01$). This result agreed with our finding from obese

TABLE 3. Correlation coefficients for anthropometrical and biochemical parameters

Parameter	Coefficient of correlation r	
	Whole group	Male/female group
Age	0.29 ^c	0.28 ^b /0.29 ^b
Weight	0.20 ^b	0.22/0.21
BMI	0.21 ^b	0.23/0.18
BMI (SDS)	0.09	0.12/0.06
Waist circumference	0.21 ^b	0.19/0.38 ^c
Hip circumference	0.21 ^b	0.17/0.32 ^b
WHR	-0.07	-0.02/-0.02
Tanner stage	0.34 ^d	0.29 ^b /0.29 ^b
Testosterone	0.27 ^b	0.27 ^b /-
Testicular volume	0.22 ^a	0.22 ^a /-
Estradiol	0.31 ^b	-/0.30 ^b
DHEA-S	0.22 ^b	0.14/0.26 ^b
Leptin	0.05	0.07/0.02
Adiponectin	-0.22 ^b	-0.22/-0.20

Resistin serum levels of obese children and adolescents were correlated with anthropometrical and biochemical parameters. No significant correlation was found between resistin serum levels and parameters of diabetes and metabolism such as insulin ($r = 0.08$), glucose ($r = 0.02$), proinsulin ($r = -0.05$), HOMA ($r = 0.08$), and ISI ($r = -0.11$). WHR, Waist to hip ratio.

^a $P < 0.10$.

^b $P < 0.05$.

^c $P < 0.01$.

^d $P < 0.001$.

children. There were no differences between obese and lean children matched for gender and age ($P = 0.48$) (Table 2).

Resistin levels of the whole group correlated with Tanner puberty stage ($r = 0.18$; $P < 0.05$) and age ($r = 0.17$; $P < 0.02$). Additionally, testosterone ($r = 0.21$; $P < 0.05$), testicular volume ($r = 0.21$; $P < 0.05$), and DHEA-S ($r = 0.22$; $P < 0.05$) were associated with resistin serum levels in males. In the female subgroup, no correlation was seen with any of those parameters except age.

Discussion

This is, to the best of our knowledge, the first study investigating the role of resistin serum levels in children and adolescents. For this study, we established an in-house assay and evaluated its analytical quality to measure resistin. Quality control measurements showed that this system is reliable, and coefficients of variation as well as experiments of dilu-

TABLE 2. Biochemical data of 135 obese and 201 lean children and adolescents

	Obese children		Lean children	
	Male	Female	Male	Female
Glucose (mmol/liter)	4.66 ± 0.42	4.65 ± 0.44		
Insulin (pmol/liter)	76.9 (45.7/113.6)	78.8 (57.2/113.5)		
HOMA	2.65 (1.56/3.93)	2.82 (1.91/3.95)		
Leptin (ng/ml)	34.3 (24.2/44.4)	36.7 (28.2/46.2)	3.02 ^b (1.88/4.37)	6.51 ^b (4.02/8.93)
Adiponectin (μg/ml)	5.53 ± 2.04	5.58 ± 2.38	6.72 ± 2.21 ^b	7.30 ± 2.07 ^b
Resistin (ng/ml)	5.74 ± 2.62	6.79 ± 2.67	5.83 ± 2.35	6.75 ± 2.22
Resistin, Biovendor (ng/ml)	4.11 ± 3.37	4.10 ± 2.52		
DHEA-S (nmol/liter)	4.72 ± 2.93	5.39 ± 3.77	4.94 ± 3.63	3.16 ± 2.66 ^b
Testosterone (nmol/liter)	2.40 (0.47/6.87)		9.64 ^a (1.16/16.94)	
Estradiol (pmol/liter)		103.2 (49.4/154.7)		102.2 (60.7/169.0)

Data were expressed as mean ± SD or median (quartiles) for nonnormal distributed parameters (insulin, HOMA, leptin, testosterone, and estradiol). Significant differences between obese and lean group are indicated.

^a $P < 0.05$.

^b $P < 0.001$.

tion and spiking were in an expected range. We excluded cross-reactivity with other members of the human RELM family and mouse resistin, because no signal was seen when samples with RELM- β or mouse resistin were measured with our assay (data not shown). By Western blotting experiments we clearly demonstrated that the antibody of our assay system detects specifically the resistin molecule. This finding is supported by the fact that both antibodies, the anti-N-terminus peptide antibody and the antibody raised against the whole molecule, marked comparable bands in serum resistin and in the positive control. The peptide antibody proved a molecular mass at 10 kDa for the monomer; the rh-resistin antibody demonstrated 20 kDa for the dimer. This is in agreement with expected molecular mass deduced from the amino acid sequence. Accordingly, resistin expressed in *Escherichia coli* migrated at 20 kDa in nonreduced Western blot experiments (32, 33).

In SEC experiments, we found increased immunoreactivity of rh-resistin in fractions corresponding to a high molecular mass of more than 660 kDa. Spiking of serum with rh-resistin led to an increase of this peak (Fig. 2B) and underlined its specificity. The unexpected high molecular mass may be explained by oligomerization of resistin, which is consistent with findings of Aruna *et al.* (34). In their experiments, the resistin protein was also found in the void volume but additionally in fractions corresponding to molecular masses of 50 and 25 kDa. The SEC profile of our human serum pool revealed comparable peaks at 55 kDa. This molecular mass is also in accordance with data of the Patel group, which showed that resistin from mouse serum forms hexamers with a molecular mass of 55 kDa (32). Furthermore, we observed a small shoulder of this major peak at 45 kDa. This shoulder may correspond to the trimeric form as described for the mouse protein (32). Immunoreactivity found in fractions of lower molecular mass consists probably of monomeric and dimeric fragments of the resistin molecule.

We hypothesize therefore, that resistin may circulate in different molecular isoforms in human peripheral blood. It is probable that these isoforms consist of oligomerized resistin molecules as seen in experiments with recombinant human resistin (33) or an aggregation with members of the RELM family as proposed by Chen *et al.* (35). This phenomenon has also been shown for the structurally related cytokines, such as erythropoietin (36). However, we cannot fully exclude complexes between resistin and other potential binding proteins. The function of oligomerization and/or a potential binding protein remains to be determined. Isoforms with higher molecular mass may prolong the half-life of circulating proteins and deliver more biologically active forms after dissociation. This fact is supported by the finding that murine resistin isoforms with lower molecular masses (around 46 kDa or trimers) revealed a significantly higher bioactivity than high molecular mass forms (32). The presence of different molecular isoforms may raise problems for the determination of resistin levels in serum samples and, especially, for the comparison of levels measured with different assay systems. Therefore, assays for the determination of resistin describe considerable discrepancies in the measuring range for resistin serum levels. Mean concentrations less than 1 ng/ml, measured by an ELISA system using monoclonal

antibodies (17), are in conflict with levels of about 15 ng/ml (ELISA; Phoenix Pharmaceuticals, San Francisco, CA) (19). The comparison of the data of our in-house assay with results of the commercially available Biovendor method revealed similar differences, reflected by a coefficient of correlation r of 0.57. Because the standard levels of both assays found the expected concentrations in a crossover determination, calibration problems can be ruled out as a cause for these differences. Our results coincide well with a recently published study reporting a coefficient of correlation r of 0.66 for the comparison between the Biovendor method and data of the Linco method (37). Therefore, we suggest that resistin assays should characterize the isoforms recognized by the individual antibodies. Moreover, data arrived at using resistin assays should be interpreted very carefully.

Resistin and gender

We found higher resistin levels in girls compared with boys of corresponding age and pubertal stage in a large cohort of children and adolescents. This is in accordance with some studies carried out in adults (21, 22). Other investigators did not detect any differences between genders using various assays (17–19). Because resistin levels of male subjects are in absolute values only marginally lower than those of females, these differences may be related to the relatively low number of subjects in the latter mentioned studies. Besides, the gender dependency may be more pronounced at a younger age. Lee *et al.* (21) confirmed this difference in subjects with a mean age of 17.7 yr; in contrast, this association was absent in adults (age > 40 yr) (17–19).

Resistin and Tanner stage

In our recent study, resistin levels of children and adolescents demonstrated a close correlation with pubertal stage and age, in analogy with other adipocytokines such as adiponectin and leptin (38, 39). Lean boys showed an increase of resistin levels according to pubertal stage, but resistin remained unchanged in girls when different Tanner stages were compared. However, when both obese and lean girls were included into the statistical analysis, Tanner stage ($r = 0.22$; $P < 0.01$) and age ($r = 0.25$; $P < 0.01$) correlated with resistin because of higher variation in the whole data set. These results are confirmed by the correlation of resistin with testosterone in lean and obese boys and with estradiol in obese girls. A similar effect was reported for adiponectin, where an association with markers of pubertal development was stronger in boys than in girls (38). An increase of resistin levels during pubertal maturation is also supported by a stepwise forward multiple regression model including age, Tanner stage, estradiol, testosterone, waist and hip circumference, BMI, weight, and height. Tanner stage was the only significant independent predictor for resistin, explaining 11% ($P < 0.001$) of its variance.

Resistin and markers of obesity and insulin resistance

Correlations between resistin and BMI were of weak significance for the obese group of our study. This finding is consistent with data obtained in adults (14–16). The statis-

tical significance was absent for the lean group. We suggest that parameters of pubertal maturation are stronger predictors of resistin variations, and therefore, only states of morbid obesity are associated with elevated resistin levels (11). Alternatively, the distribution of body fat could play a role in determination of resistin plasma levels as proposed by McTernan *et al.* (40), who found higher resistin mRNA expression in abdominal fat than in thigh. Therefore, the weak association that we found between resistin and waist or hip circumference in obese children may reflect different amounts of abdominal fat in morbidly obese individuals.

Our study did not, however, show any difference in resistin levels between obese and lean subjects as demonstrated in a few studies with adult subjects (14–16). Unfortunately, we were not able to ensure that all blood withdrawals from lean children were done in the fasting state. Therefore, the intake of nutrition may influence the levels of the lean group in a subtle way. However, resistin levels measured during the course of an oGTT of our obese patients ($n = 10$) did not reveal significant changes as a result of the glucose intake (data not shown). This finding is supported by two studies that did not find changes in resistin serum levels through modification of caloric intake (21, 41).

Finally, we could not find any significant correlation of resistin levels in obese children with markers of insulin resistance and glucose homeostasis such as HOMA, ISI, insulin, glucose, and proinsulin. This result is in accordance with findings in most adult studies (14, 17–21). However, our data of 5–24% probability for the significance of these correlations as determined by the Power analysis demonstrate that we cannot generally rule out positive associations between resistin and insulin sensitivity in children. This argument is supported by our results from the oGTT. Respective data for the presence of such a relationship were published also for adults by Silha *et al.* (22) or Bajaj *et al.* (23).

In summary, we hypothesize that resistin is not the main link between obesity and insulin resistance in children and adolescents, but because of its association with Tanner stage, it may be related to the maturation of children during pubertal development. Additionally, we have shown that different molecular isoforms of resistin in human blood may raise problems in comparing data from diverse immunoassay systems.

Acknowledgments

Received February 28, 2005. Accepted May 24, 2005.

Address all correspondence and requests for reprints to: J. Kratzsch, Institute of Laboratory Medicine, Clinical Chemistry and Molecular Diagnostics, University of Leipzig, Paul-List-Strasse 13–15, D-04103 Leipzig, Germany. E-mail: kraj@medizin.uni-leipzig.de.

This work was supported by grants of the Interdisciplinary Center for Clinical Research at the University of Leipzig to J.K. (B15) and A.B. (B21).

References

1. Stepan CM, Brown EJ, Wright CM, Bhat S, Banerjee RR, Dai CY, Enders GH, Silberg DG, Wen X, Wu GD, Lazar MA 2001 A family of tissue-specific resistin-like molecules. *Proc Natl Acad Sci USA* 98:502–506
2. Stepan CM, Bailey ST, Bhat S, Brown EJ, Banerjee RR, Wright CM, Patel HR, Ahima RS, Lazar MA 2001 The hormone resistin links obesity to diabetes. *Nature* 409:307–312
3. Shojima N, Sakoda H, Ogihara T, Fujishiro M, Katagiri H, Anai M, Onishi Y, Ono H, Inukai K, Abe M, Fukushima Y, Kikuchi M, Oka Y, Asano T 2002

Humoral regulation of resistin expression in 3T3 L1 and mouse adipose cells. *Diabetes* 51:1737–1744

4. Moore GB, Chapman H, Holder JC, Lister CA, Piercy V, Smith SA, Clapham JC 2001 Differential regulation of adipocytokine mRNAs by rosiglitazone in db/db mice. *Biochem Biophys Res Commun* 286:735–741
5. Chen L, Nyomba BL 2003 Glucose intolerance and resistin expression in rat offspring exposed to ethanol *in utero*: modulation by postnatal high-fat diet. *Endocrinology* 144:500–508
6. Hirosumi J, Tuncman G, Chang L, Gorgun CZ, Uysal KT, Maeda K, Karin M, Hotamisligil GS 2002 A central role for JNK in obesity and insulin resistance. *Nature* 420:333–336
7. Rajala MW, Obici S, Scherer PE, Luciano R 2003 Adipose-derived resistin and gut-derived resistin-like molecule- β impair insulin action on glucose production. *J Clin Invest* 111:225–230
8. Juan CC, Au LC, Fang VS 2001 Suppressed gene expression of adipocyte resistin in an insulin-resistant rat model probably by elevated free fatty acids. *Biochem Biophys Res Commun* 289:1328–1333
9. Way JM, Gorgun CZ, Tong Q, Uysal KT, Brown KK, Harrington WW, Oliver Jr WR, Willson TM, Kliewer SA, Hotamisligil GS 2001 Adipose tissue resistin expression is severely suppressed in obesity and stimulated by peroxisome proliferator-activated receptor γ agonists. *J Biol Chem* 276:25651–25653
10. Fukui Y, Motojima K 2002 Expression of resistin in the adipose tissues modulated by various factors including peroxisome proliferator-activated receptor γ agonists. *Diabetes Obes Metab* 4:342–345
11. Savage DB, Sewter CP, Klenk ES, Segal DG, Vidal-Puig A, Considine RV, O'Rahilly S 2001 Resistin/FIZZ3 expression in relation to obesity and peroxisome proliferator-activated receptor γ actions in humans. *Diabetes* 50:2199–2201
12. Nagae I, Smith U 2001 Insulin resistance and type 2 diabetes are not related to resistin expression in human fat cells or skeletal muscle. *Biochem Biophys Res Commun* 285:561–564
13. Janke J, Engeli S, Gorzelnik K, Luft FC, Sharma AM 2002 Resistin gene expression in human adipocytes is not related to insulin resistance. *Obes Res* 10:1–5
14. Azuma K, Katsukawa F, Oguchi S, Murata M, Yamazaki H, Shimada A, Saruta T 2003 Correlation between serum resistin level and adiposity in obese individuals. *Obes Res* 11:997–1001
15. Degawa-Yamauchi M, Bovenkerk JE, Juliar BE, Watson W, Kerr K, Jones R, Zhu Q, Considine RV 2003 Serum resistin (FIZZ3) protein is increased in obese humans. *J Clin Endocrinol Metab* 88:5452–5455
16. Fujinami A, Obayashi H, Ohata K, Ichimura T, Nishimura M, Matsui H, Kawahara Y, Yamazaki M, Ogata M, Hasegawa G, Nakamura N, Yoshikawa T, Nakano K, Ohta M 2004 Enzyme-linked immunosorbent assay for circulating human resistin: resistin concentrations in normal subjects and patients with type 2 diabetes. *Clin Chim Acta* 339:57–63
17. Youn BS, Yu KY, Park HJ, Lee NS, Min SS, Youn MY, Cho YM, Park YJ, Kim SY, Lee HK, Park KS 2004 Plasma resistin concentrations measured by enzyme-linked immunosorbent assay using a newly developed monoclonal antibody are elevated in individuals with type 2 diabetes mellitus. *J Clin Endocrinol Metab* 89:150–156
18. Heilbronn LK, Rood J, Janderova L, Albu JB, Kelley DE, Ravussin E, Smith SR 2004 Relationship between serum resistin concentrations and insulin resistance in nonobese, obese, and obese diabetic subjects. *J Clin Endocrinol Metab* 89:1844–1848
19. McTernan PG, Fisher FM, Valsamakis G, Chetty R, Harte A, McTernan CL, Clark PMS, Smith SA, Barnett AH, Sudhesh K 2003 Resistin and type 2 diabetes: regulation of resistin expression by insulin and rosiglitazone and the effects of recombinant resistin on lipid and glucose metabolism in human differentiated adipocytes. *J Clin Endocrinol Metab* 88:6098–6106
20. Volarova de Courten VB, Degawa-Yamauchi M, Considine MV, Tataranni PA 2004 High serum resistin is associated with an increase in adiposity but not a worsening of insulin resistance in Pima Indians. *Diabetes* 53:1279–1284
21. Lee JH, Chan JL, Yiannakouris N, Kontogianni M, Estrada E, Seip R, Orlova C, Mantzoros CS 2003 Circulating resistin levels are not associated with obesity or insulin resistance in humans and are not regulated by fasting or leptin administration: cross-sectional and interventional studies in normal, insulin-resistant, and diabetic subjects. *J Clin Endocrinol Metab* 88:4848–4856
22. Silha JV, Kresk M, Skrha JV, Sucharada P, Nyomba BLG, Murphy LJ 2003 Plasma resistin, adiponectin and leptin levels in lean and obese subjects: correlations with insulin resistance. *Eur J Endocrinol* 149:331–335
23. Bajaj M, Suraamornkul S, Hardies LJ, Pratipanawat T, DeFronzo RA 2004 Plasma resistin concentration, hepatic fat content and hepatic and peripheral insulin resistance in pioglitazone-treated type II diabetic patients. *Int J Obes Relat Metab Disord* 28:783–789
24. Freedman MR, Stern JS 2004 The role of optimal healing environments in the management of childhood obesity. *J Altern Complement Med* 10(Suppl 1): S231–S244
25. Reich A, Mueller G, Gelbrich G, Deutscher K, Goedicke R, Kiess W 2003 Obesity and blood pressure: results from the examination of 2365 schoolchildren in Germany. *Int J Obes Relat Metab Disord* 27:1459–1464
26. Kratzsch J, Berthold A, Lammert A, Reuter W, Keller E, Kiess W 2002 A rapid

- quantitative immunofunctional assay for measuring human leptin. *Horm Res* 57:127–132
27. **American Diabetes Association** 2000 Type 2 diabetes in children and adolescents. American Diabetes Association. *Diabetes Care* 23:381–389
 28. **Mathews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC** 1985 Homeostasis model assessment: insulin resistance and β -cell function from plasma glucose and insulin concentration in man. *Diabetologia* 28:412–419
 29. **Matsuda M, DeFronzo RA** 1999 Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp. *Diabetes Care* 22:1462–1470
 30. **Kromeyer-Hauschild K, Wabitsch M, Geller F, Ziegler A, Geiss HC, Hesse V, v. Hippel, Jäger U, Johnsen D, Korte W, Kunze D, Menner K, Müller M, Niemann-Pilatus A, Remer T, Schäfer F, Wittchen HU, Zabransky S, Zellner K, Hebebrand J** 2001 Perzentile für den Body Mass Index für das Kindes- und Jugendalter unter Heranziehung verschiedener deutscher Stichproben. *Monatsschr Kinderheilkd* 149:807–818
 31. **Tanner JM** 1962 *Growth at adolescence*, 2nd ed. Oxford, UK: Blackwell Scientific
 32. **Patel SD, Rajala MW, Rossetti L, Scherer PE, Shapiro L** 2004 Disulfide-dependent multimeric assembly of resistin family hormones. *Science* 304:1154–1158
 33. **Raguh P, Ghosh S, Soundarya K, Haseeb A, Aruna B, Ehtesham NZ** 2003 Dimerization of human recombinant resistin involves covalent and noncovalent interactions. *Biochem Biophys Res Commun* 313:642–646
 34. **Aruna B, Ghosh S, Singh AK, Mande SC, Srinivas V, Chauhan R, Ehtesham NZ** 2003 Human recombinant resistin protein displays a tendency to aggregate by forming intermolecular disulfide linkages. *Biochem* 42:10554–10559
 35. **Chen J, Wang L, Boeg YS, Xia B, Wang J** 2002 Differential dimerization and association among resistin family with implications for functional specificity. *J Endocrinol* 175:499–504
 36. **Cotes PM, Canning CE, Gaines Das RE** 1983 Modification of a radioimmunoassay for human serum erythropoietin to provide increased sensitivity and investigate non-specific serum responses. In: Hunter WM, Corrie JET, eds. *Immunoassays for clinical chemistry*. Edinburgh: Churchill Livingstone; 106–112
 37. **Lehrke M, Reilly MP, Millington SC, Iqbal N, Rader DJ, Lazar MA** 2004 An inflammatory cascade leading to hyperresistinemia in humans. *PLoS Med* 1:e45
 38. **Böttner A, Kratzsch J, Müller G, Kapellen TM, Blüher S, Keller E, Blüher M, Kiess W** 2004 Gender differences of adiponectin levels develop during the progression of puberty and are related to serum androgen levels. *J Clin Endocrinol Metab* 89:4053–4061
 39. **Blum WF, Engaloro P, Hanitsch S, Juul A, Hertel NT, Mueller J, Skakkebaek NE, Heiman ML, Birkett M, Attanasio AM, Kiess W, Rascher W** 1997 Plasma leptin levels in healthy children and adolescents: dependence on body mass index, body fat mass, gender, pubertal stage, and testosterone. *J Clin Endocrinol Metab* 82:2904–2910
 40. **McTernan PG, McTernan CL, Chetty R, Jenner K, Fisher FM, Lauer MN, Crocker C, Barnett AH, Kumar S** 2002 Increased resistin gene and protein expression in human abdominal adipose tissue. *J Clin Endocrinol Metab* 87:2407–2410
 41. **Silha J, Murphy L** 2004 Resistin is not responsive to short-term changes in caloric intake in mice. 13th European Congress on Obesity, Prague, Czech Republic. *Int J Obes Relat Metab Disord* 28(Suppl 1):S97

JCEM is published monthly by The Endocrine Society (<http://www.endo-society.org>), the foremost professional society serving the endocrine community.