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# 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

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**Resistin is a novel adipocyte-secreted hormone proposed to link obesity with diabetes. Studies in mice have revealed conflicting data however, and the physiological role of circulating resistin in humans remains unknown. We conducted cross-sectional studies in 123 middle-aged women and 120 healthy young subjects and found that serum resistin levels did not correlate with markers of adiposity, including body mass index, waist-to-hip ratio, or fat mass, or insulin resistance assessed by homeostasis model, lipid profile, or serum leptin levels; but females had higher resistin levels than males ($P < 0.02$). We also found no difference in serum resistin levels between lean healthy and obese insulin-resistant nondiabetic and type 2 diabetic adolescents. Finally, to evaluate the effect of food deprivation and/or leptin administration on resistin levels, we performed interventional studies that revealed no significant difference in resistin levels after 48 h of fasting and/or leptin administration at either physiological or pharmacological doses. We conclude that circulating resistin is unlikely to play a major role in insulin resistance or energy homeostasis in humans. (*J Clin Endocrinol Metab* 88: 4848–4856, 2003)**

RESISTIN, ALSO CALLED adipocyte-secreted factor (ADSF) or found in inflammatory zone 3 (F1ZZ3), is a novel hormone secreted by adipocytes that has been proposed to link obesity with insulin resistance and diabetes (1–3). Resistin belongs to a family of cysteine-rich secreted proteins along with resistin-like molecule $\alpha$(RELM-$\alpha$), expressed in adipose tissue, heart, lung, and tongue, and RELM$\beta$, which is expressed in the intestine (3, 4). Initial studies in mice suggested that resistin mediates insulin resistance by antagonizing insulin action and modulating one or more steps in the insulin-signaling pathway (1, 5). However, conflicting animal data have since been presented regarding whether resistin production is increased (1, 5) or decreased (6–10) in obesity and decreased (1, 5, 11–13) or increased (6, 14) by thiazolidinediones (TZDs), drugs known to reduce insulin resistance.

Importantly, the relevance and physiological role of resistin in humans remain unknown. Given the incomplete homology (59%) between human and mouse resistin (4) and the absence in humans of one of the three murine resistin isoforms, resistin in humans may have a different physiological role than that in mice. Studies of genetic variations in the resistin gene, including single-nucleotide polymorphisms, are controversial regarding the role of resistin in obesity and insulin sensitivity (15–20). Moreover, resistin mRNA expression is very low in isolated human adipocytes and does not correlate consistently with insulin resistance or obesity (21–23), making the role of human resistin in insulin resistance unclear.

To gain insight into the predictors and physiological role of circulating resistin in humans, we conducted a series of cross-sectional and interventional studies. To evaluate whether resistin correlates with obesity, insulin resistance, fasting lipid profile, and/or leptin (another adipocyte-secreted hormone that has important associations with obesity and insulin resistance in rodents and leptin-deficient humans), we studied cross-sectionally anthropometric, metabolic, and hormonal predictors of serum resistin levels in 123 middle-aged women and 120 healthy young subjects. To assess whether resistin levels are higher in insulin-resistant states, we compared serum resistin levels in a separate study of 19 normal-weight healthy adolescents and 19 adolescents with insulin resistance, *i.e.* obese nondiabetic subjects and obese type 2 diabetic (T2DM) subjects. Finally, to determine whether, similar to studies in mice (1, 2), resistin levels are

Abbreviations: BIA, Bioelectrical impedance analysis; BMI, body mass index; CV, coefficient of variation; DEXA, dual-energy x-ray absorptiometry; FM, fat mass; %FM, percent fat mass; HOMA, homeostasis model assessment; IR, insulin resistance; RELM, resistin-like molecule; r-metHuLeptin, recombinant methionyl human leptin; T2DM, type 2 diabetes; TZD, thiazolidinedione; WC, waist circumference; WHR, waist-hip ratio.

regulated by fasting (a low-insulin and low-leptin state) and/or leptin administration, we measured resistin levels in healthy subjects in response to fasting alone, fasting with physiologic-dose leptin administration, and pharmacological doses of leptin in the fed state.

## Subjects and Methods

### *Cross-sectional studies*

One hundred thirty Greek middle-aged women [age 49.4 ± 9.2 yr, body mass index (BMI) 30.9 ± 5.5 kg/m$^2$], without known history of diabetes, and 120 Greek students (age 17.7 ± 1.7 yr, BMI 22.3 ± 3.6 kg/m$^2$) were consecutively enrolled in two separate studies approved by the Ethics Committee at Harokopio University in Athens, Greece, and the Institutional Review Board of Beth Israel Deaconess Medical Center. Use of two studies (relatively older and overweight *vs.* younger and lean subjects) allowed us to determine whether there is a delayed effect of resistin on insulin resistance, *i.e.* a low-penetration phenomenon, and/or whether there is a role of resistin in the absence of obesity. Subjects (and parents of the students) gave written informed consent to participate in the studies. Subjects completed a self-administered questionnaire on demographic characteristics, general health status, smoking history, and current medications, including hormone replacement therapy and oral contraceptive treatment for the middle-aged women. The women were classified as premenopausal if they had regular monthly periods, perimenopausal if they had irregular periods or elevated FSH levels, and postmenopausal if they reported no periods for 6 or more months. Blood samples were collected from all subjects after an overnight fast (≥10 h) between 0830 and 1030 h, and the sera were stored at −70 C. Analysis was restricted to 123 subjects for whom resistin data were available.

*Anthropometry and body composition.* Anthropometric and body composition measurements were performed in all study participants before breakfast, with the subject wearing light clothing without shoes. For all subjects, weight and height were measured to the nearest 0.5 kg and 0.5 cm, respectively, and the BMI was calculated as weight (kilograms) divided by height squared (square meters). Waist circumference (WC) and hip circumference were also measured by the same observer to a precision of 0.1 cm, and the waist-to-hip ratio (WHR) was calculated. Triceps, biceps, subscapular, and suprailiac skinfolds were measured twice by one observer on the right side of the body to a precision of 0.2 mm using a Lange skinfold caliper (Cambridge Scientific Instruments, Cambridge, MA) with the average of the two measurements reported. Body composition was assessed for all subjects by bioelectrical impedance analysis (BIA), using a single frequency bioimpedance analyzer (model 101, RJL Systems, Mt. Clemens, MI). Fat-free mass and percent fat mass (%FM) were calculated by anthropometric and BIA variables using the fatness-specific regression equations developed by Segal *et al.* (24) for the group of middle-aged women, and using the age- and gender-specific equations developed by Deurenberg *et al.* (25) for the students.

To validate the BIA and anthropometry measurements, 60 of the middle-aged women also underwent a dual-energy x-ray absorptiometry (DEXA) scan using a Lunar DPX densitometer (Lunar Corp., Madison, WI; software version 4.7e) for measurement of total body and trunk fat mass (expressed in kilograms and percent body weight). Estimates of fat mass (FM) and %FM obtained from the two methods correlated strongly with each other (FM by BIA and DEXA: Pearson's coefficient, $r = 0.91$, $P < 0.001$; %FM by BIA and DEXA: $r = 0.88$, $P < 0.001$). Moreover, FM and %FM by BIA and DEXA correlated with BMI (both FM and %FM by BIA and BMI: $r = 0.94$, $P < 0.001$; both FM and %FM by DEXA and BMI: $r = 0.84$, $P < 0.001$). In addition, trunk fat, a measurement of central adiposity, obtained by DEXA correlated highly with WC ($r = 0.90$, $P < 0.001$) and WHR ($r = 0.54$, $P < 0.001$). Thus, in our analysis, we used BMI and FM calculated by BIA as markers of overall adiposity, and WC and WHR as markers of central obesity. Secondary analysis was also performed using DEXA-derived measurements of FM and central obesity.

*Study of lean subjects vs. obese, insulin-resistant, nondiabetic and diabetic subjects.* In a separate cross-sectional study, 19 normal-weight healthy adolescents and 19 obese, insulin-resistant adolescents (10 nondiabetic subjects and nine T2DM subjects) were enrolled from the endocrinology clinic at Connecticut Children's Medical Center (Hartford, CT), in a study approved by the Connecticut Children's Medical Center Institutional Review Board. Subjects and their parents gave written informed consent to participate in the study. Subjects were classified as obese if their BMI was over the 95th percentile for age and gender (26). All of the diabetic adolescents were classified as having T2DM by criteria established by the National Diabetes Data Group (27). The mean BMI was 22.2 ± 2.1 kg/m$^2$ for the normal-weight subjects; 40.3 ± 7.3 kg/m$^2$ for the obese, nondiabetic subjects; and 43.5 ± 10.5 kg/m$^2$ for the obese T2DM subjects. There was no significant difference in age among the three groups: 14.5 ± 1.6 yr, 12.9 ± 1.1 yr, and 14.8 ± 1.2 yr, respectively. The male:female ratios were similar, *i.e.* 10:9, 4:6, and 5:4, respectively. Fasting blood samples were obtained between 0800 and 1000 h, and the plasma was stored at −70C until analyses of resistin and insulin concentrations were performed. Of the subjects with T2DM, three were on insulin, four were on metformin, and the other two were not on pharmacotherapy. For the seven subjects on pharmacotherapy, insulin administration the evening before blood sampling was decreased to one third the normal dose and metformin was held the morning of blood sampling.

### *Interventional studies*

Two interventional studies were performed to investigate the effect of fasting and/or leptin administration on resistin levels. The Beth Israel Deaconess Medical Center Institutional Review Board approved the studies, and all subjects gave written informed consent to participate in their respective study. Clinical-quality recombinant methionyl human leptin (r-metHuLeptin) was supplied by Amgen, Inc. (Thousand Oaks, CA) and was administered under an investigator-initiated investigational new drug application.

*Administration of physiological doses of r-metHuLeptin in the fasted state.* Six normal-weight men (age 22.0 ± 2.1 yr, BMI 24.2 ± 1.2 kg/m$^2$) were screened for any medical problems and admitted to the General Clinical Research Center under three different conditions: baseline fed state, 48-h fasting with placebo administration, and 48-h fasting with physiologic-dose r-metHuLeptin administration. The three admissions were scheduled at least 7 wk apart from each other to allow for return of body weight to baseline, avoidance of residual effects of administered r-metHuLeptin, and recovery of hematocrit. Subjects were admitted to the General Clinical Research Center the evening before d 1 for each admission. Fasting blood samples for resistin and leptin measurements were obtained at 0800 h on d 1 and 3 of each admission.

During the baseline fed state, subjects were placed on an isocaloric diet to maintain their admission body weight, with four standardized meals per day: 20% of calories from breakfast (0800 h), 35% from lunch (1300 h), 35% from dinner (1800 h), and 10% from a snack (2200 h). During both fasting studies, subjects received only caffeine-free and calorie-free liquids, NaCl (500 mg/d), KCl (40 meq/d), and a standard multivitamin with minerals daily. R-metHuLeptin was administered sc at a dose of 0.04 mg/kg·d on the first day and 0.1 mg/kg·d on the second day, which were designed to achieve physiological serum leptin levels similar to the fed state. The total daily dose of r-metHuLeptin was divided into four equal doses given every 6 h, starting at 0800 h on the first day after baseline blood samples were obtained. During the fasting with placebo administration admission, buffer solution was administered sc according to the same schedule as r-metHuLeptin.

*Administration of pharmacological doses of r-metHuLeptin in the fed state.* Five normal-weight healthy men (BMI < 25 kg/m$^2$), five obese healthy men (BMI > 29 kg/m$^2$), and five normal-weight healthy women (BMI < 25 kg/m$^2$) were admitted to the General Clinical Research Center during the evening and received one pharmacological dose of r-metHuLeptin (0.3 mg/kg) the following morning at 0800 h. Fasting blood samples were collected in the morning before r-metHuLeptin administration (time 0) and again 5 h after the dose. During the study, subjects received an isocaloric diet, as described above.

*Hormone measurements.* Resistin levels were measured using an ELISA (Biovendor Laboratory Medicine Inc., Brno, Czech Republic; supplied by Alpco Diagnostics, Windham, NH) as previously described (28, 29). The limit of detection is 0.2 ng/ml, intraassay coefficient of variation (CV)

**TABLE 1.** Cross-sectional study of 130 middle-aged women: demographic, anthropometric, metabolic, and hormonal parameters (mean ± SD)

| | | | |
|---|---|---|---|
| Age (y) | 49.4 ± 9.2 | Estradiol (pg/ml) | 188.1 ± 114.4 |
| Menopausal status (n = 121) | | (pmol/liter) | 690.5 ± 420.0 |
| Premenopausal | 44 (36.4%) | Free testosterone (pg/ml) | 1.4 ± 0.9 |
| Perimenopausal | 12 (9.9%) | (pmol/liter) | 4.85 ± 3.12 |
| Postmenopausal | 65 (53.7%) | Fasting glucose (mg/dl) | 99.8 ± 16.3 |
| Smoking status (n = 121) | | (mmol/liter) | 5.54 ± 0.90 |
| Nonsmoker | 86 (71.1%) | Fasting insulin (μIU/ml) | 7.9 ± 4.1 |
| Present smoker | 31 (25.6%) | (pmol/liter) | 47.4 ± 24.6 |
| Past smoker | 4 (3.3%) | HOMA-IR | 2.0 ± 1.3 |
| Weight (kg) | 78.4 ± 15.6 | Total cholesterol (mg/dl) | 216.1 ± 42.5 |
| BMI (kg/m$^2$) | 30.9 ± 5.5 | (mmol/liter) | 5.59 ± 1.10 |
| WC (cm) | 88.6 ± 12.9 | LDL cholesterol (mg/dl) | 141.0 ± 37.1 |
| WHR | 0.80 ± 0.06 | (mmol/liter) | 3.65 ± 0.96 |
| FM (kg) | 32.9 ± 9.8 | HDL cholesterol (mg/dl) | 54.7 ± 12.2 |
| FM (%) | 41.3 ± 4.2 | (mmol/liter) | 1.41 ± 0.32 |
| Resistin (ng/ml) | 9.3 ± 7.7 | ApoA1 lipoprotein (mg/dl) | 114.7 ± 12.0 |
| Leptin (ng/ml) | 23.4 ± 13.7 | (mmol/liter) | 2.97 ± 0.31 |
| Cortisol (μg/dl) | 9.5 ± 4.1 | ApoB lipoprotein (mg/dl) | 87.3 ± 21.6 |
| (nmol/liter) | 262.1 ± 113.1 | (mmol/liter) | 2.26 ± 0.56 |
| | | Fasting triglyceride (mg/dl) | 102.1 ± 56.1 |
| | | (mmol/liter) | 2.64 ± 1.45 |

5.2% for low levels and 3.4% for high levels, and inter-assay CV 7.2% and 6.3%, respectively. The antibodies in the ELISA have no detectable cross-reactivity to mouse resistin or other cytokines in human serum. To validate the reliability of the resistin ELISA, we assayed serial dilutions of recombinant human resistin (PeproTech, Inc., Rocky Hill, NJ) between 3.13 ng/ml and 50.0 ng/ml using the Biovendor ELISA on two separate occasions. We found an excellent correlation between calculated and observed resistin concentrations each time ($r^2$ = 0.99 and 0.98). To analyze the spiking recovery of the assay, serum from two subjects with baseline resistin levels of 5.52 ng/ml and 8.59 ng/ml were spiked with increasing amounts of recombinant human resistin (between 3.13 ng/ml and 50.0 ng/ml) and assayed. Similar to the spiking recovery data presented by the manufacturer, our analysis revealed high correlations ($r^2$ = 0.9996 and 0.999). Moreover, the linearity data presented by the manufacturer show that the assay is highly linear ($r^2$ = 0.999). Lastly, we studied the effect of repeated freezing and thawing of samples on resistin levels. Serum from four subjects, with resistin levels ranging from 6.77 ng/ml to 19.98 ng/ml, were each divided into two aliquots, one of which was frozen/thawed five times and the other that was thawed once and stored at 4 C for the 2 d before being assayed. No significant difference in resistin levels between the two groups was detected (12.2 ± 8.1 *vs.* 11.0 ± 6.1 ng/ml, $P$ = 0.31, by paired $t$ test).

Additional hormone concentrations were measured using commercially available RIAs as follows: leptin (Linco Research, St. Charles, MO; sensitivity 0.5 ng/ml; intraassay CV 8.3%); cortisol [Diagnostics Systems Laboratories (DSL), Webster, TX; sensitivity 0.5 μg/dl (13.8 nmol/liter); intraassay CV 5.3–8.4%]; insulin [DSL; sensitivity 1.3 μIU/ml (7.8 pmol/liter); intraassay CV 8.3%]; estradiol [Diagnostic Products Corp. (DPC), Los Angeles, CA; sensitivity 8.0 pg/ml (29.4 pmol/liter); intraassay CV 4.3–7.0%]; and free testosterone [DPC; sensitivity 0.15 pg/ml (0.52 pmol/liter); intraassay CV 8.0%]. For the Connecticut Children's Medical Center adolescents, insulin was measured via ELISA (DSL; sensitivity 0.26 μIU/ml (1.56 pmol/liter); intraassay CV 2.6% for low levels and 1.3% for high levels; interassay CV 5.2% for low levels and 6.2% for high levels). The ELISA has no cross-reactivity with proinsulin or C peptide. Cross-validation with the DSL insulin RIA yielded a high correlation (r = 0.96). To minimize variability, hormone levels were measured in one assay for all subjects participating in each cross-sectional study and in one assay for each subject in the interventional studies. Glucose and lipid profiles were measured using a photometric method with liquid reactants (Hitachi 917, Indianapolis, IN). Insulin resistance (IR) was estimated using the homeostasis model assessment (HOMA) using the formula: IR = fasting insulin × fasting glucose/22.5 (30).

### *Statistical analysis*

SPSS 8.0 software (SPSS Inc., Chicago, IL) was used for statistical analysis, and $P$ less than 0.05 (two-tailed) was considered statistically significant for all analyses. In the cross-sectional studies of middle-aged women and young subjects, we calculated Pearson's correlation coefficients and then performed bivariate and multivariate regression analyses. We evaluated for associations between serum resistin levels and anthropometric (BMI, FM, WC, WHR), metabolic (fasting glucose, insulin, HOMA-IR, lipid profile), and hormonal (leptin, cortisol, estradiol, free testosterone) parameters, all expressed as continuous variables. Analyses were controlled for potential confounders, including age, menopausal status, FM, and estrogen levels for the middle-aged women, and gender, age, and FM for the young population. Several variables were logarithmically transformed to obtain a normal distribution. Subjects taking hormone replacement (n = 5) or oral contraceptive (n = 2) treatment at the time of the evaluation were included. In addition to adjustment for circulating estrogen levels, multivariate regression analysis models were also run to adjust for estrogen replacement using a dummy variable (yes/no) and after stratification for hormone replacement (*i.e.* by excluding women on hormone replacement). We used $t$ tests to analyze for differences in body composition, Tanner stage, and hormonal parameters between the normal-weight and obese adolescents from the Connecticut Children's Medical Center. We used one-way ANOVA with *post hoc* (least significant difference) analysis to assess for differences in resistin and insulin levels among the normal-weight, obese nondiabetic, and obese T2DM adolescents. For the interventional studies, because the data were not normally distributed, we used Wilcoxon signed ranks tests to evaluate for changes in resistin levels between d 1 and 3 of each fed or fasting admission and between time 0 and 5 h after administration of a pharmacological dose of leptin.

The size of each cross-sectional study of 123 middle-aged women and 120 young subjects provided more than 80% power, and the combination of both studies provided more than 96% power, to detect clinically-significant associations ($r \geq 0.22$) at the conventional $\alpha = 0.05$ level. Comparing two groups of 19 subjects each (Connecticut Children's Medical Center subjects) provided more than 80% power at the conventional $\alpha = 0.05$ level to detect a difference in mean resistin levels 80% or more of the corresponding SD.

## Results

Table 1 summarizes the demographic, anthropometric, metabolic, and hormonal parameters of the 130 middle-aged women enrolled in the first cross-sectional study. The mean WC was 88.6 ± 12.9 cm, FM 32.9 ± 9.8 kg, and %FM 41.3 ± 4.2%. Subjects had a mean resistin level of 9.3 ± 7.7 ng/ml, mean leptin level of 23.4 ± 13.7 ng/ml, and mean fasting insulin level of 7.9 ± 4.1 μIU/ml (47.4 ± 24.6 pmol/liter).

Figure 1 illustrates the positive association between serum

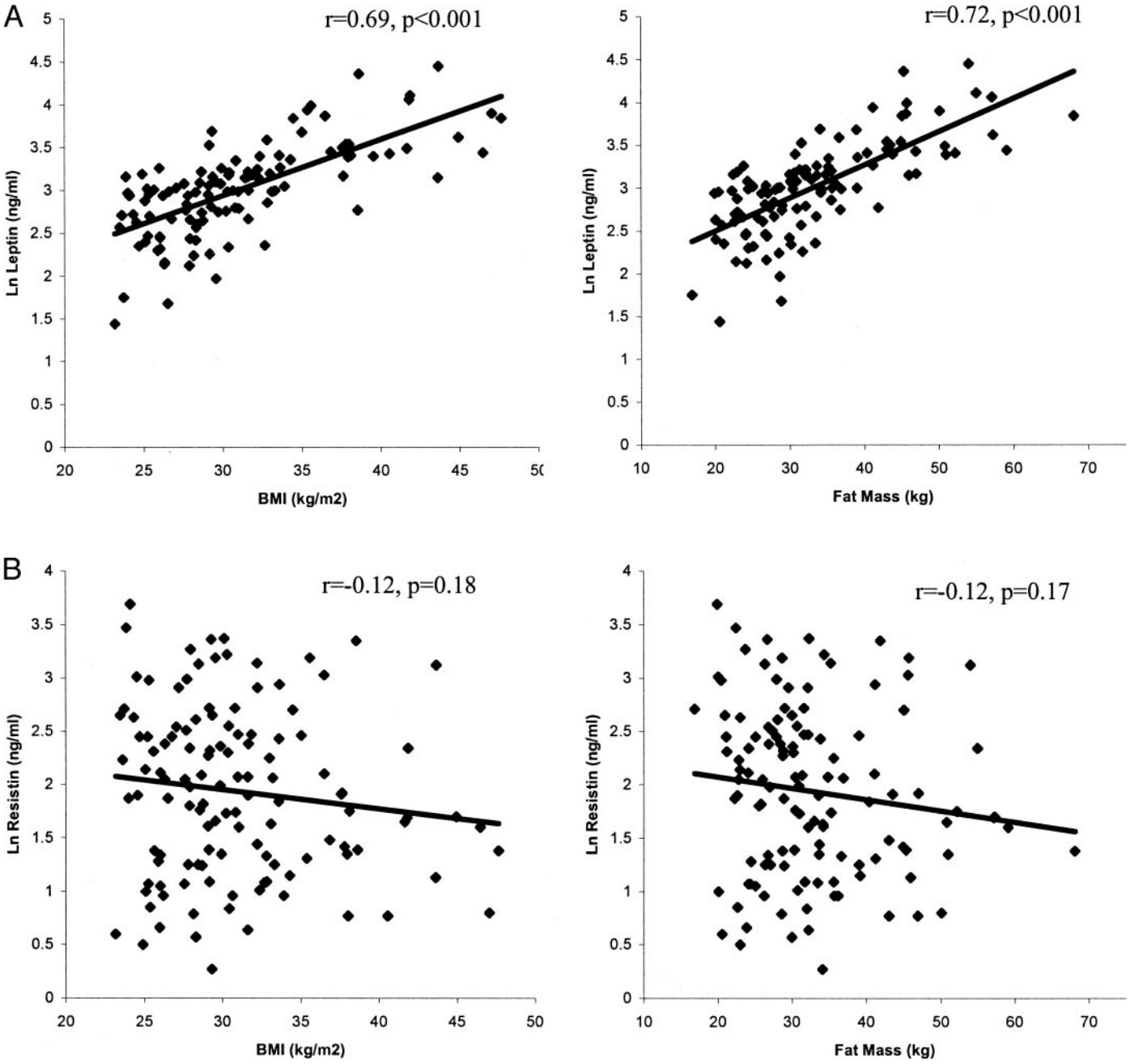


FIG. 1. Association of BMI (kg/m$^2$) and fat mass (kg) with logarithmically transformed serum leptin (A) and resistin (B) in 123 middle-aged women.

leptin levels and BMI (Pearson's correlation coefficient r = 0.69, $P < 0.001$) and FM (r = 0.72, $P < 0.001$), and the lack of association between resistin levels and BMI (r = −0.12, $P$ = 0.18) or FM (r = −0.12, $P$ = 0.17). To evaluate potential predictors of resistin, we performed bivariate regression analyses of resistin and several anthropometric, metabolic, and hormonal factors. We found no correlation of resistin with any markers of adiposity or insulin resistance, including BMI, WC, WHR, FM, insulin and HOMA-IR (Table 2). In addition, there was no association between resistin levels and lipid (total cholesterol, high-density lipoprotein, low-density lipoprotein, triglyceride) or hormone levels (leptin, cortisol, estradiol, free testosterone) (Table 2). To adjust for potential confounders, we then performed multivariate regression analyses, adjusting for successively introduced covariates, *i.e.* age, menopausal status, estradiol level (or use of estrogen replacement therapy), and FM. With these adjustments, we again found no association between resistin and markers of adiposity or insulin resistance, lipid levels, or hormone levels (Table 2). Similar multivariate models adjusting for age and menopausal status but excluding perimenopausal women or women on hormone replacement therapy yielded similar results (data not shown). Secondary analysis using bivariate and multivariate models assessing DEXA-derived measure-

**TABLE 2.** Cross-sectional study of 123 middle-aged women: bivariate and multivariate regression analysis of anthropometric, metabolic, and hormonal factors as predictors of serum resistin levels

| | $\beta$1 | $\beta$2 | $\beta$3 | $\beta$4 | $\beta$5 |
|---|---|---|---|---|---|
| Age | 0.02 | N/A | 0.04 | 0.03 | 0.04 |
| BMI (kg/m$^2$) | 0.10 | 0.10 | 0.10 | 0.11 | 0.04 |
| WC (cm) | 0.09 | 0.10 | 0.10 | 0.12 | −0.05 |
| WHR | 0.01 | 0.01 | 0.01 | 0.01 | −0.04 |
| FM (kg)[a] | 0.10 | 0.10 | 0.11 | 0.11 | N/A |
| FM (%) | 0.09 | 0.09 | 0.09 | 0.10 | N/A |
| Total cholesterol (mg/dl) | −0.17 | −0.18 | −0.17 | −0.17 | −0.18 |
| LDL cholesterol (mg/dl) | −0.13 | −0.14 | −0.13 | −0.13 | −0.15 |
| HDL cholesterol (mg/dl) | −0.18 | −0.18 | −0.19 | −0.19 | −0.19 |
| Triglyceride (mg/dl)[a] | −0.14 | −0.15 | −0.13 | −0.13 | −0.21 |
| Fasting glucose (mg/dl) | 0.11 | 0.11 | 0.12 | 0.13 | 0.02 |
| Fasting insulin ($\mu$IU/ml)[a] | 0.18 | 0.18 | 0.18 | 0.19 | 0.05 |
| HOMA-IR[a] | 0.20 | 0.20 | 0.20 | 0.21 | 0.05 |
| Leptin (ng/ml)[a] | 0.07 | 0.07 | 0.07 | 0.08 | 0.01 |
| Cortisol ($\mu$g/dl)[a] | 0.00 | 0.01 | 0.01 | 0.00 | −0.00 |
| Estradiol (pg/ml)[a] | −0.01 | −0.01 | −0.01 | N/A | −0.04 |
| Free testosterone (pg/ml)[a] | 0.05 | 0.05 | 0.05 | 0.05 | 0.06 |

$\beta$1, Bivariate standardized linear regression coefficient; $\beta$2, multivariate standardized regression coefficient adjusted for age; $\beta$3, multivariate standardized regression coefficient adjusted for age and menopausal status; $\beta$4, multivariate standardized regression coefficient adjusted for age, menopausal status, and estradiol level; $\beta$5, multivariate standardized regression coefficient adjusted for age, menopausal status, estradiol level, and FM ($P > 0.05$ for all values).

[a] Natural logarithmic transformation was performed before analysis.

ments of FM and central obesity also revealed similar results (data not shown). Because of the impact of tobacco use on cardiovascular risk, we also performed multivariate analyses adjusting for age and smoking and did not find a correlation between resistin and any of the variables (data not shown). Furthermore, we explored nonlinear correlations between resistin and both FM and HOMA-IR and did not find significant associations (data not shown). Finally, we compared resistin levels between obese (BMI $>$ 30 kg/m$^2$, n = 59) and nonobese women (BMI $<$ 30 kg/m$^2$, n = 64) and found no significant difference between the two groups (8.7 ± 7.3 ng/ml *vs.* 9.9 ± 8.1 ng/ml, $P = 0.41$ by *t* test).

Table 3 summarizes the demographic, anthropometric, metabolic, and hormonal parameters of the 120 young subjects enrolled in the second cross-sectional study. The male: female ratio was 56:62. The population was lean (BMI 22.3 ± 3.6 kg/m$^2$, WC 74.3 ± 10.5 cm, %FM 22.7 ± 7.9%), and none of the subjects had diabetes mellitus. Subjects had a mean resistin level of 4.5 ± 1.9 ng/ml, mean leptin level of 6.8 ± 5.9 ng/ml, and mean fasting insulin level of 5.6 ± 3.2 $\mu$IU/ml (33.6 ± 19.2 pmol/liter).

Figure 2 depicts the positive association between leptin and BMI (r = 0.30, $P = 0.001$) and FM (r = 0.65, $P < 0.001$), and lack of association between resistin and BMI (r = −0.02, $P = 0.83$) or FM (r = 0.15, $P = 0.11$). By bivariate and multivariate regression analysis, females had higher resistin levels, compared with males ($\beta = -0.29$, $P = 0.002$), even after adjustment for age and FM (Table 4). Females had a mean resistin level of 4.9 ± 1.6 ng/ml, compared with 4.1 ± 2.1 ng/ml for males ($P = 0.02$ by *t* test). Although there were weak associations between resistin and sum of skinfolds ($\beta = 0.20$, $P = 0.03$), %FM ($\beta = 0.25$, $P < 0.01$), and free testosterone ($\beta = -0.28$, $P = 0.002$) by bivariate analysis, these correlations decreased and lost significance after adjustment for gender, indicating that these associations are due to confounding by gender. Similarly, there was a significant positive association between resistin and leptin levels ($\beta = 0.32$, $P = 0.001$) that achieved only borderline statistical significance after adjustment for gender (Table 4). There were also no significant associations between resistin levels and WC, WHR, FM, or insulin by bivariate and multivariate regression analysis (Table 4). Lastly, there was no association between resistin levels and either smoking or exercise status (data not shown).

To further investigate whether resistin correlates with insulin resistance and/or obesity, we compared resistin levels in 19 normal-weight and 19 obese insulin-resistant adolescents. There was no significant difference in Tanner stage between the two groups (data not shown), but the two groups had significant differences in weight (61.0 ± 7.5 kg *vs.* 111.4 ± 27.0 kg, $P < 0.001$); BMI (22.2 ± 2.1 kg/m$^2$ *vs.* 41.8 ± 8.8 kg/m$^2$, $P < 0.001$); insulin levels [9.0 ± 6.7 $\mu$IU/ml *vs.* 45.4 ± 32.7 $\mu$IU/ml (54.0 ± 40.2 pmol/liter *vs.* 272.4 ± 196.2 pmol/liter), $P < 0.001$]; and HOMA-IR (2.2 ± 0.9 *vs.* 6.3 ± 4.6, $P = 0.001$). Resistin levels did not differ significantly between the two groups (5.6 ± 1.9 ng/ml *vs.* 6.7 ± 3.3 ng/ml, $P = 0.21$).

To evaluate whether diabetes mellitus *per se* is associated with resistin levels, we compared the obese insulin resistant nondiabetic subjects with the T2DM subjects. Fasting insulin levels were 52.5 ± 40.5 $\mu$IU/ml (315.0 ± 243.0 pmol/liter) for the nondiabetic subjects and 37.6 ± 20.9 $\mu$IU/ml (225.6 ± 125.4 pmol/liter) for the T2DM subjects, compared with 9.0 ± 6.7 $\mu$IU/ml (54.0 ± 40.2 pmol/liter) for the normal-weight subjects ($P < 0.001$ by one-way ANOVA). By *post hoc* (least significant difference) analysis, there was a significant difference in insulin levels between the normal-weight subjects and both obese nondiabetic subjects ($P < 0.001$) and obese T2DM subjects ($P = 0.005$). Insulin levels and HOMA-IR were not different among patients on insulin, metformin, or no pharmacotherapy. There was no significant difference in resistin levels between the obese nondiabetic subjects (5.8 ± 1.6 ng/ml), obese T2DM subjects (7.8 ± 4.6 ng/ml), and normal-weight subjects (5.6 ± 1.9 ng/ml) ($P = 0.13$ by one-way ANOVA).

We then evaluated whether fasting and/or leptin administration regulates resistin levels in healthy subjects. In the fed state, leptin and resistin levels remained stable between d 1 and d 3 (leptin 1.6 ± 1.6 ng/ml *vs.* 1.7 ± 0.8 ng/ml, $P = 0.31$; resistin 9.5 ± 2.4 ng/ml *vs.* 10.4 ± 4.3 ng/ml, $P = 0.53$). After 48 h of fasting plus placebo administration, weight decreased from 75.7 ± 7.1 kg on d 1 to 73.4 ± 7.2 kg on d 3 ($P = 0.03$), and leptin levels declined by 67% (2.1 ± 1.8 ng/ml *vs.* 0.7 ± 0.9 ng/ml, $P = 0.01$). Leptin replacement during fasting restored the decrease in leptin levels to levels higher than baseline but within the normal physiological range for lean men (2.3 ± 1.9 ng/ml *vs.* 4.8 ± 2.1 ng/ml, $P = 0.06$). However, resistin levels remained unchanged after 48 h of fasting with placebo administration (11.2 ± 3.2 ng/ml *vs.* 10.6 ± 2.1 ng/ml, $P = 0.34$), and after 48 h of fasting plus physiologic-dose r-metHuLeptin administration (8.9 ± 2.4 ng/ml *vs.* 9.9 ± 2.3 ng/ml, $P = 0.50$).

**TABLE 3.** Cross-sectional study of 120 young subjects: demographic, anthropometric, metabolic, and hormonal parameters (mean ± SD)

| | | | |
|---|---|---|---|
| Age (yr) | 17.7 ± 1.7 | WC (cm) | 74.3 ± 10.5 |
| Gender | | | |
| Males | n = 56 (47.5%) | WHR | 0.76 ± 0.1 |
| Females | n = 62 (52.5%) | FM (kg) | 14.4 ± 6.2 |
| Smoking status | | % FM | 22.7 ± 7.9 |
| Nonsmoker | n = 67 (57.3%) | Resistin (ng/ml) | 4.5 ± 1.9 |
| Light smoker | n = 9 (7.7%) | Leptin (ng/ml) | 6.8 ± 5.9 |
| Moderate smoker | n = 31 (26.5%) | Cortisol ($\mu$g/dl) | 11.1 ± 4.4 |
| Heavy smoker | n = 10 (8.5%) | (nmol/liter) | 306.2 ± 121.4 |
| Exercise status | | Estradiol (pg/ml) | 60.3 ± 52.6 |
| Sedentary | n = 7 (6.0%) | (pmol/liter) | 221.4 ± 193.1 |
| Light | n = 31 (26.5%) | Free testosterone (pg/ml) | 17.9 ± 17.4 |
| Moderate | n = 50 (42.7%) | (pmol/liter) | 62.1 ± 60.3 |
| Vigorous | n = 29 (24.8%) | Fasting insulin ($\mu$IU/ml) | 5.6 ± 3.2 |
| Weight (kg) | 63.4 ± 13.7 | (pmol/liter) | 33.6 ± 19.2 |
| BMI (kg/m$^2$) | 22.3 ± 3.6 | | |

Because there was no effect of physiological levels of leptin on resistin levels, we studied whether resistin levels are affected by pharmacological leptin levels by measuring leptin and resistin levels immediately before and 5 h after a pharmacological dose of r-metHuLeptin was administered to 15 healthy subjects, *i.e.* five normal-weight men (age 22.2 ± 2.0 yr, BMI 22.0 ± 1.0 kg/m$^2$), five obese men (age 23.4 ± 3.4 yr, BMI 32.0 ± 2.3 kg/m$^2$), and five normal-weight women (age 20.4 ± 1.5 yr, BMI 21.9 ± 1.5 kg/m$^2$). Leptin levels increased more than 20-fold (10.6 ± 7.6 ng/ml *vs.* 243.7 ± 114.2 ng/ml, $P = 0.001$), but resistin levels did not change significantly (6.2 ± 2.4 ng/ml *vs.* 5.8 ± 1.8 ng/ml, $P = 0.21$) over this period of elevated leptin levels. Separate analyses within each subgroup (lean men, obese men, and lean women) also showed no significant difference in resistin levels at baseline and 5 h after r-metHuLeptin administration (data not shown).

## Discussion

We present one of the first human studies on circulating resistin levels and, using a highly specific ELISA, find no evidence supporting a role for serum resistin in mediating insulin resistance or reflecting obesity in humans. Specifically, we found no independent associations between resistin and markers of obesity, central adiposity, insulin resistance, hyperlipidemia, leptin, or sex hormones in healthy overweight middle-aged women and healthy lean young men and women. Additionally, we found no difference in resistin levels between normal-weight adolescents and obese insulin resistant nondiabetic and T2DM adolescents, despite a distinct elevation in insulin levels in the insulin resistant nondiabetic and diabetic groups, compared with the control group. Similar to leptin, resistin levels exhibit a sexual dimorphism, with women having higher levels than men (~20% higher), but unlike leptin, circulating resistin levels are not affected by acute fasting and/or by r-metHuLeptin administration at either physiological or pharmacological doses.

Prior studies in mice have shown conflicting results regarding the link between resistin and insulin resistance and/or obesity. Initial studies supported a role for resistin in mediating insulin resistance. Circulating resistin levels were found to be elevated in genetic (*ob/ob* and *db/db*) and diet-induced models of obesity (1). Exogenous resistin administration to healthy mice worsened glucose tolerance and induced insulin resistance, but neutralizing antiresistin antibodies in obese, insulin-resistant mice improved insulin sensitivity and glucose tolerance (1, 5). Interestingly, infusion of either resistin or RELM$\beta$ induced severe hepatic but not peripheral insulin resistance, including inhibition of glucose metabolism and increased glucose production (31). TZDs, which lower insulin resistance by binding to peroxisome proliferator activated receptor-$\gamma$ (PPAR$\gamma$) receptors, decreased resistin levels in *ob/ob* mice and diabetic fatty rats (1, 5, 11, 12). Furthermore, tissue-cultured murine adipocytes treated with TZDs decreased resistin mRNA expression (1, 11, 13, 32). In contrast, resistin expression in 3T3-L1 adipocytes and adipose tissue was increased by high glucose concentrations and dexamethasone (13, 33). Finally, serum resistin levels in mice as measured by immunoblot decreased after a 48-h fast, with similar changes in resistin mRNA and protein expression in adipocytes (1, 2, 33).

Other studies, however, have not supported a role for resistin in mediating insulin resistance in rodents. TZD treatment increased resistin expression in both lean and obese rodents, including *ob/ob* mice and Zucker diabetic fatty rats (6, 14), and resistin protein expression was increased by 66% in *db/db* mice treated with metformin (34). Others have shown that adipose tissue of obese mice has decreased expression of resistin mRNA (6–8, 10, 33) and that resistin gene expression in the adipose tissue of insulin-resistant Fischer 344 rats is similar to that of age-matched, insulin-sensitive Sprague Dawley rats (35). Additionally, resistin gene expression in 3T3-L1 adipocytes was inhibited by insulin and TNF$\alpha$, which are increased in obesity (13, 32, 33, 36). The inconsistencies between animal studies may be related to methodological limitations, including different methods used to measure resistin, and/or variation among serum concentrations, mRNA, and protein levels, or may indicate that resistin does not play a significant role in the pathophysiology of insulin resistance (37).

Studies of resistin mRNA and protein expression in adipose tissue of humans have provided additional support that resistin may not play a role in insulin resistance or obesity in humans. Resistin mRNA and protein expression are increased in abdominal fat depots (23, 38) and whole adipose

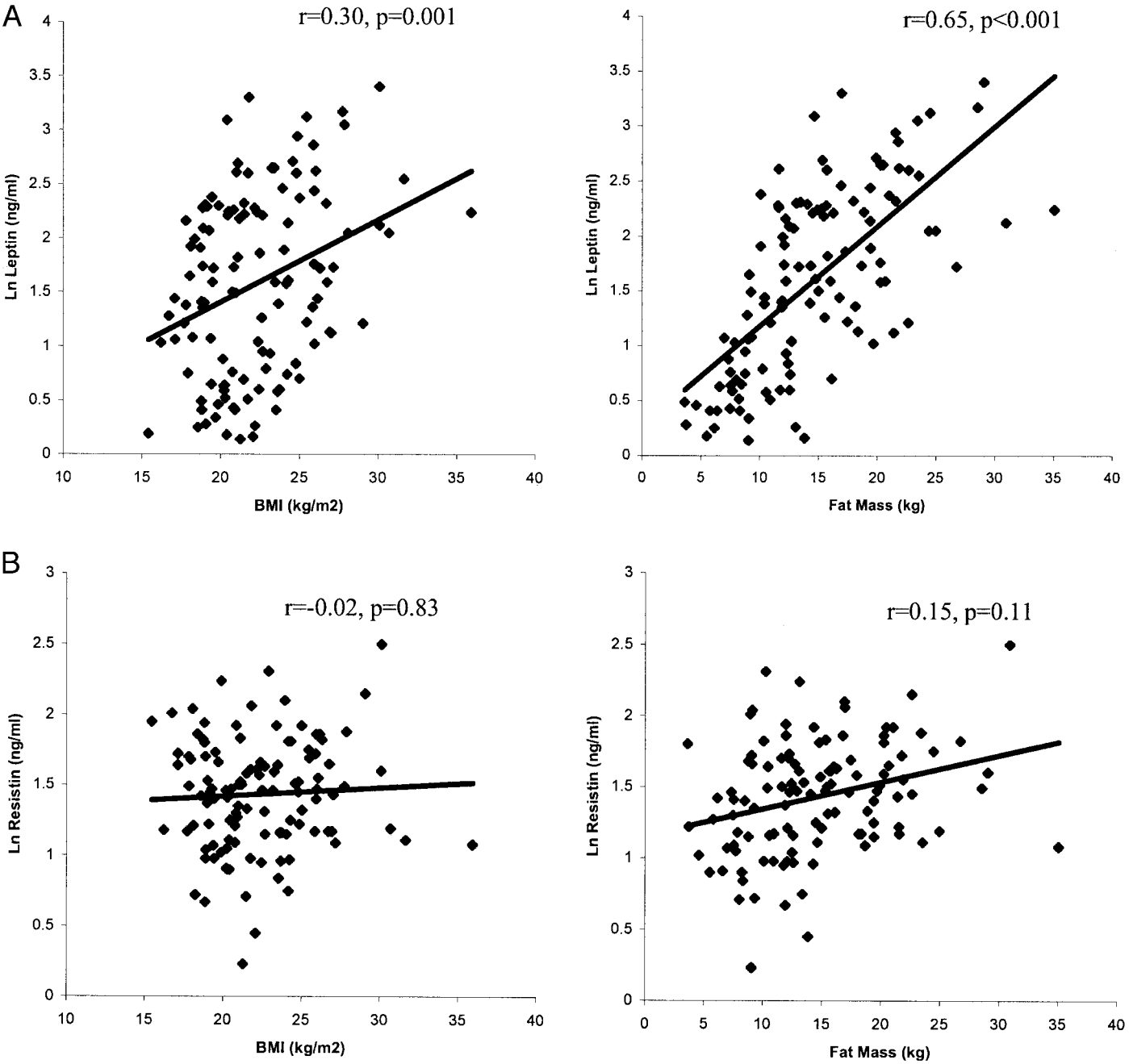


FIG. 2. Association of BMI (kg/m$^2$) and fat mass (kg) with logarithmically transformed serum leptin (A) and resistin (B) in 120 young subjects.

tissue of obese individuals, but isolated human adipocytes have very low resistin mRNA levels (21). Furthermore, previous studies have reported no differences in resistin expression in human fat and muscle cells when healthy, insulin-resistant, and T2DM subjects were compared (22), no correlation between resistin gene expression in human adipocytes and insulin resistance (39) or BMI (21), and, in contrast to mice studies (1, 5, 6, 11–14, 32), no change in resistin expression in human mononuclear cells in response to peroxisome proliferator activated receptor-$\gamma$ agonists (21). Finally, studies on the potential association between genetic variations of the resistin gene and obesity or insulin resistance have revealed inconsistent results (15–20). Thus, human studies have not provided consistent evidence that tissue resistin expression plays a key role in the development of insulin resistance (37).

The cross-sectional and interventional studies we report herein do not support a major role for circulating resistin in mediating obesity or insulin resistance and/or linking obesity to diabetes in humans. Given the incomplete homology between human and mouse resistin (4) and the differences in energy metabolism between mice and humans, the physiology of resistin in mice may differ from that in humans (37, 40). Furthermore, in humans, resistin lies on chromosome 19p13.3, a region that has not been linked with susceptibility to obesity or insulin resistance (40). Our findings are con-

**TABLE 4.** Cross-sectional study of 120 young subjects: bivariate and multivariate regression analysis of anthropometric, metabolic, and hormonal factors as predictors of serum resistin levels

| | $\beta 1$ | $\beta 2$ | $\beta 3$ | $\beta 4$ |
|---|---|---|---|---|
| Gender | $-0.29^a$ | N/A | $-0.30^b$ | $-0.28^a$ |
| Age | 0.07 | −0.10 | N/A | 0.09 |
| BMI (kg/m$^2$) | −0.02 | 0.08 | 0.07 | N/A |
| WC (cm) | −0.05 | 0.19 | 0.17 | 0.48 |
| WHR | −0.11 | 0.18 | 0.16 | 0.13 |
| Sum of skinfolds (mm) | $0.20^c$ | 0.13 | 0.13 | N/A |
| FM (kg) | 0.15 | 0.11 | 0.09 | N/A |
| FM (%) | $0.25^a$ | 0.10 | 0.09 | N/A |
| Fasting insulin ($\mu$IU/ml) | −0.02 | −0.01 | −0.02 | −0.07 |
| Leptin (ng/ml)$^e$ | $0.32^b$ | $0.23^d$ | $0.23^d$ | $0.30^d$ |
| Cortisol ($\mu$g/dl) | −0.05 | 0.05 | 0.04 | 0.05 |
| Estradiol (pg/ml) | 0.18 | 0.04 | 0.05 | 0.06 |
| Free testosterone (pg/ml) | $-0.28^a$ | −0.12 | −0.14 | −0.15 |

$\beta 1$, Bivariate standardized linear regression coefficient; $\beta 2$, multivariate standardized regression coefficient adjusted for gender; $\beta 3$, multivariate standardized regression coefficient adjusted for gender and age; $\beta 4$, multivariate standardized regression coefficient adjusted for gender, age, and FM.

[a] $0.001 < P < 0.01$.

[b] $P = 0.001$.

[c] $0.01 < P < 0.05$.

[d] $0.05 < P < 0.10$.

[e] Natural logarithmic transformation was performed before analysis.

sistent with prior studies that demonstrated no association between human resistin mRNA and protein expression and insulin resistance or obesity (21, 22, 39). Additionally, we previously found no association between serum resistin levels and either total energy or macronutrient intake (28), and a recent study, using the same resistin ELISA as in our studies, found no difference in serum resistin levels between insulin-resistant nondiabetic partial lipodystrophy patients and healthy controls (29).

Although there is evidence for higher resistin mRNA in visceral, compared with peripheral, fat (23, 38, 41), our data do not reveal a significant association between circulating resistin and central obesity in humans and thus do not support a role for resistin in mediating the effect of central obesity on insulin resistance or diabetes. Similar to leptin, resistin levels in mice decrease in response to fasting and increase with feeding or insulin administration (1, 2). In contrast to mice, however, we detected no changes in circulating resistin levels after 2 d of fasting in healthy humans. Furthermore, we found no significant correlation between resistin and leptin after adjustment for gender. Lastly, neither restoring the fasting-induced decrease in leptin levels to physiological levels nor achieving pharmacological leptin levels with r-metHuLeptin administration altered resistin levels.

This study was adequately powered to demonstrate clinically significant associations and differences between groups and used state-of-the-art methodology. We evaluated not only linear correlations of resistin with the outcomes of interest but also the possibility that nonlinear associations may exist between resistin and its putative effects, as previously suggested (42). We have studied different groups with varying degrees of insulin resistance and obesity and included a nonobese group of young subjects to evaluate the hypothesis that the impact of resistin may be more important in the absence of severe obesity (42) and/or that the effect of resistin on insulin resistance may be delayed, reflecting a low-penetration phenomenon.

Although our data do not support the notion that circulating resistin plays a critical role in insulin resistance and/or energy homeostasis in humans, it remains possible that resistin acting in a paracrine or autocrine manner may play a relatively more important role. Future research on the biology of circulating resistin in humans and the role of adipose tissue expression of resistin and RELMs in adipogenesis, obesity, and insulin resistance would shed more light on resistin physiology in humans. Interventional studies including recombinant human resistin administration in the context of well-designed clinical trials or the identification and detailed study of humans with congenital resistin deficiency would be invaluable. Finally, identification of the receptor system for resistin, relevant signaling pathway(s), and their sensitivity state are needed for a complete evaluation of the role of the resistin system in humans (42).

In summary, we present cross-sectional and interventional studies on the physiology of circulating resistin in humans and demonstrate that resistin levels are not associated with markers of insulin resistance and/or obesity and are not regulated by fasting or leptin administration. We conclude that circulating resistin is unlikely to play a major role in obesity, insulin resistance, or energy homeostasis in humans. Further detailed studies are needed to conclusively address these topics.

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## References

1. **Steppan 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
2. **Kim KH, Lee K, Moon YS, Sul HS** 2001 A cysteine-rich adipose tissue-specific secretory factor inhibits adipocyte differentiation. J Biol Chem 276:11252–11256
3. **Holcomb IN, Kabakoff RC, Chan B, Baker TW, Gurney A, Henzel W, Nelson C, Lowman HB, Wright BD, Skelton NJ, Frantz GD, Tumas DB, Peale Jr FV, Shelton DL, Hebert CC** 2000 FIZZ1, a novel cysteine-rich secreted protein associated with pulmonary inflammation, defines a new gene family. EMBO J 19:4046–4055
4. **Steppan 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
5. **Steppan CM, Lazar MA** 2002 Resistin and obesity-associated insulin resistance. Trends Endocrinol Metab 13:18–23
6. **Way JM, Gorgun CZ, Tong Q, Uysal KT, Brown KK, Harrington WW, Oliver WR, Willson TM, Kliewer SA, Hotamisligil GS** 2001 Adipose tissue resistin expression is severely suppressed in obesity and stimulated by peroxisome proliferator-activated receptor gamma agonists. J Biol Chem 276:25651–25653

7. **Le Lay S, Boucher J, Rey A, Castan-Laurell I, Krief S, Ferre P, Valet P, Dugail I** 2001 Decreased resistin expression in mice with different sensitivities to a high-fat diet. Biochem Biophys Res Commun 289:564–567
8. **Juan CC, Au LC, Fang VS, Kang SF, Ko YH, Kuo SF, Hsu YP, Kwok CF, Ho LT** 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. **Masuzaki H, Paterson J, Shinyama H, Morton N, Mullins J, Seckl J, Flier J** 2001 A transgenic model of visceral obesity and the metabolic syndrome. Science 294:2166–2170
10. **Milan G, Granzotto M, Scarda A, Calcagno A, Pagano C, Federspil G, Vettor R** 2002 Resistin and adiponectin expression in visceral fat of obese rats: effect of weight loss. Obes Res 10:1095–1103
11. **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
12. **Hartman HB, Hu X, Tyler KX, Dalal CK, Lazar MA** 2002 Mechanisms regulating adipocyte expression of resistin. J Biol Chem 277:19754–19761
13. **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
14. **Fukui Y, Motojima K** 2002 Expression of resistin in the adipose tissue is modulated by various factors including peroxisome proliferator-activated receptor alpha. Diabetes Obes Metab 4:342–345
15. **Wang H, Chu WS, Hemphill C, Elbein SC** 2002 Human resistin gene: molecular scanning and evaluation of association with insulin sensitivity and type 2 diabetes in Caucasians. J Clin Endocrinol Metab 87:2520–2524
16. **Sentinelli F, Romeo S, Arca M, Filippi E, Leonetti F, Banchieri M, Di Mario U, Baroni MG** 2002 Human resistin gene, obesity and type 2 diabetes: mutation analysis and population study. Diabetes 51:860–862
17. **Engert JC, Vohl MC, Williams SM, Lepage P, Loredo-Osti JC, Faith J, Dore C, Renaud Y, Burtt NP, Villeneuve A, Hirschhorn JN, Altshuler D, Groop LC, Despres JP, Gaudet D, Hudson TJ** 2002 5′ flanking variants of resistin are associated with obesity. Diabetes 51:1629–1634
18. **Osawa H, Onuma H, Murakami A, Ochi M, Nishimiya T, Kato K, Shimizu I, Fujii Y, Ohashi J, Makino H** 2002 Systematic search for single nucleotide polymorphisms in the resistin gene: the absence of evidence for the association of three identified single nucleotide polymorphisms with Japanese type 2 diabetes. Diabetes 51:863–866
19. **Ma X, Warram J, Trischitta V, Doria A** 2002 Genetic variants at the resistin locus and risk of type 2 diabetes in Caucasians. J Clin Endocrinol Metab 87:4407–4410
20. **Pizzuti A, Argiolas A, Di Paola R, Baratta R, Rauseo A, Bozzali M, Vigneri R, Dallapiccola B, Trischitta V, Frittitta L** 2002 An ATG repeat in the 3′-untranslated region of the human resistin gene is associated with a decreased risk of insulin resistance. J Clin Endocrinol Metab 87:4403–4406
21. **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-gamma action in humans. Diabetes 50:2199–2202
22. **Nagaev 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
23. **McTernan CL, McTernan PG, Harte AL, Levick PL, Barnett AH, Kumar S** 2002 Resistin, central obesity, and type 2 diabetes. Lancet 359:46–47
24. **Segal KR, Van Loan M, Fitzgerald PI, Hodgdon JA, Van Itallie TB** 1998 Lean body mass estimation by bioelectrical impedance analysis: a four-site cross-validation study. Am J Clin Nutr 47:7–14
25. **Deurenberg P, van der Kooy K, Leenen R, Weststrate JA, Seidell JC** 1991 Sex and age specific prediction formulas for estimating body composition from bioelectrical impedance: a cross-validation study. Int J Obes Relat Metab Disord 15:17–25
26. **Kuczmarski RJ, Ogden CL, Grummer-Strawn LM, Flegal KM, Guo SS, Wei R, Mei Z, Curtin LR, Roche AF, Johnson CL** 2000 CDC growth charts: United States. Adv Data 314:1–27
27. **American Diabetes Association** 1998 Committee report: report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Diabetes Care 21(S1):S5–S19
28. **Yiannakoulia M, Yiannakouris N, Bluher S, Matalas A, Klimis-Zakas D, Mantzoros C** 2003 Body fat mass and macronutrient intake in relation to circulating soluble leptin receptor, free leptin index, adiponectin, and resistin concentrations in healthy humans. J Clin Endocrinol Metab 88:1730–1736
29. **Hegele RA, Kraw ME, Ban MR, Miskie BA, Huff MW, Cao H** 2003 Elevated serum C-reactive protein and free fatty acids among nondiabetic carriers of missense mutations in the gene encoding lamin A/C (*LMNA*) with partial lipodystrophy. Arterioscler Thromb Vasc Biol 23:111–116
30. **Howard G, Bergman R, Wagenknecht LE, Haffner SM, Savage PJ, Saad MF, Laws A, D'Agostino Jr RB** 1998 Ability of alternative indices of insulin sensitivity to predict cardiovascular risk: comparison with the "minimal model." Ann Epidemiol 8:358:369
31. **Rajala M, Obici S, Scherer P, Rossetti L** 2003 Adipose-derived resistin and gut-derived resistin-like molecule-$\beta$ selectively impair insulin action on glucose production. J Clin Invest 111:225–230
32. **Haugen F, Jorgensen A, Drevon CA, Trayhurn P** 2001 Inhibition by insulin of resistin gene expression in 3T3-L1 adipocytes. FEBS Lett 507:105–108
33. **Rajala M, Lin Y, Ranalletta M, Yang X, Qian H, Gingerich R, Barzilai N, Scherer P** 2002 Cell type-specific expression and coregulation of murine resistin and resistin-like molecule-$\alpha$ in adipose tissue. Mol Endocrinol 16:1920–1930
34. **Fujita H, Fujishima H, Morii T, Koshimura J, Narita T, Kakei M, Ito S** 2002 Effect of metformin on adipose tissue resistin expression in *db/db* mice. Biochem Biophys Res Commun 298:345–349
35. **Levy JR, Davenport B, Clore JN, Stevens W** 2002 Lipid metabolism and resistin gene expression in insulin-resistant Fischer 344 rats. Am J Physiol Endocrinol Metab 282:E626–E633
36. **Fasshauer M, Klein J, Neumann S, Eszlinger M, Paschke R** 2001 Tumor necrosis factor $\alpha$ is a negative regulator of resistin gene expression and secretion in 3T3-L1 adipocytes. Biochem Biophys Res Commun 288:1027–1031
37. **Ukkola O** 2002 Resistin—a mediator of obesity-associated insulin resistance or an innocent bystander? Eur J Endocrinol 147:571–574
38. **McTernan PG, McTernan CL, Chetty R, Jenner K, Fisher FM, Lauer MN, Crocker J, Barnett AH, Kumar S** 2002 Increased resistin gene and protein expression in human abdominal adipose tissue. J Clin Endocrinol Metab 87:2407
39. **Janke J, Engeli S, Gorzelniak K, Luft FC, Sharma AM** 2002 Resistin gene expression in human adipocytes is not related to insulin resistance. Obes Res 10:1–5
40. **Shuldiner A, Yang R, Gong D** 2001 Resistin, obesity, and insulin resistance—the emerging role of the adipocyte as an endocrine organ. N Engl J Med 345:1345–1346
41. **Gabriely I, Ma X, Yang X, Atzmon G, Rajala M, Berg A, Scherer P, Rossetti L, Barzilai N** 2002 Removal of visceral fat presents insulin resistance and glucose intolerance of aging. Diabetes 51:2951–2958
42. **Hotamisligil GS** 2003 The irresistible biology of resistin. J Clin Invest 111:173–174