Association of Plasma Resistin With Glomerular Filtration Rate and Albuminuria in Hypertensive Adults

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Abstract—Resistin, a recently discovered proinflammatory cytokine, has been variably associated with insulin resistance, inflammation, and renal dysfunction. We investigated the association of plasma resistin with estimated glomerular filtration rate and albuminuria in 1575 hypertensive adults without known coronary heart disease or stroke (857 blacks and 718 non-Hispanic whites). Resistin was measured by a solid phase sandwich immunoassay, estimated glomerular filtration rate was estimated from serum creatinine, and albuminuria was expressed as urine albumin:creatinine ratio. After adjustment for coronary heart disease risk factors (age, sex, body mass index, smoking history, systolic blood pressure, diabetes, and total and high-density lipoprotein cholesterol) and use of renin-angiotensin blockers and statins, higher plasma resistin levels were associated with lower estimated glomerular filtration rate in both ethnic groups (each \( P<0.0001 \)); the association remained significant after further adjustment for a marker of insulin resistance (homeostasis model assessment for insulin resistance) and a marker of inflammation (plasma C-reactive protein) and was seen in subjects with and without diabetes (each \( P<0.0001 \)) in both ethnic groups. Higher plasma resistin levels were associated with a higher urine albumin:creatinine ratio in black subjects with diabetes (\( P<0.0001 \)) and non-Hispanic white subjects with diabetes (\( P=0.032 \)), independent of coronary heart disease risk factors, hypertension medication use, and statin use; the association remained significant after additional adjustment for homeostasis model assessment for insulin resistance and C-reactive protein. In adults with hypertension, higher circulating resistin levels were associated with a lower estimated glomerular filtration rate and with increased urine albumin:creatinine ratio in the presence of concomitant diabetes. This association was independent of coronary heart disease risk factors and markers of insulin resistance and inflammation. (Hypertension. 2007;50:708-714.)

Key Words: glomerular filtration rate ■ albuminuria ■ resistin ■ insulin resistance ■ inflammation

Resistin is a recently discovered cysteine-rich plasma protein that belongs to a family of polypeptides called resistin-like molecules.\(^1\) Although classified as an adipokine, resistin in humans is mainly produced by blood-derived leukocytes and mononuclear cells, both within and outside the adipose tissue.\(^2,3\) The physiological role and pathophysiological importance of resistin in humans are unclear. Resistin antagonizes the effects of insulin on glucose metabolism in liver and skeletal muscle, interacts with and reinforces inflammatory pathways, and may promote endothelial cell activation.\(^1,2,4,5\) Increased resistin levels have been associated with obesity,\(^6\) insulin resistance,\(^7\) metabolic syndrome,\(^8\) type 2 diabetes,\(^9\) and increased cardiovascular risk,\(^8,10\) although the evidence is not consistent.\(^11-13\) Recently, plasma resistin levels have been associated with markers of chronic kidney disease,\(^14-16\) and it is speculated that inflammatory, metabolic, and vascular abnormalities associated with increased circulating resistin levels may have a pathogenic role in chronic kidney disease.

Hypertension is a major risk factor for coronary heart disease (CHD), stroke, and chronic kidney disease. Reduced glomerular filtration rate (GFR)\(^17\) and increased urinary albumin excretion\(^18\) are manifestations of target organ damage in hypertension. Whether resistin is associated with GFR and albuminuria in the setting of essential hypertension is not known. The present study was undertaken to investigate the association of plasma resistin levels with estimated GFR (eGFR) and albuminuria in a biethnic cohort of adults with hypertension without known CHD or stroke. We sought to answer whether increased circulating resistin levels are associated with reduced eGFR and increased urinary albumin excretion and whether any such association is confounded by insulin resistance or inflammation.
Methods

Study Population
Subjects included participants in the Genetic Epidemiology Network of Arteriopathy Study, a multicenter, community-based study that aims to identify genes influencing blood pressure. In Phase 1 of the study, sibships with ≥2 individuals diagnosed with essential hypertension before the age of 60 years were enrolled in Jackson (black subjects, n=1854) and in Rochester (non-Hispanic white subjects, n=1577). During the second phase, participants were invited to return for another clinic visit with participation by 1482 black subjects and 1239 non-Hispanic white subjects. The present study was limited to 1575 of the Phase 2 subjects (857 blacks and 718 non-Hispanic whites) who had hypertension but no history of myocardial infarction, coronary revascularization, or stroke. The study was approved by the institutional review board of the Mayo Clinic and the University of Mississippi Medical Center, and the subjects gave informed consent.

General Examination
Standardized protocols were used by trained observers in all of the examinations. Height measured by stadiometer and weight measured by electronic balance were used to calculate body mass index (BMI; kilograms per meter squared). Resting systolic and diastolic blood pressure (BP) levels were measured in the right arm with a random 0 sphygmomanometer. The diagnosis of hypertension was based on either BP measurements (systolic BP ≥140 mm Hg or diastolic BP ≥90 mm Hg) or previous diagnosis of hypertension and current treatment with antihypertensive medications. Diabetes was considered present if a subject was receiving treatment with insulin or an oral antidiabetic agent or had fasting serum glucose levels ≥126 mg/dL. Information about the use of renin-angiotensin blockers and statins was obtained from questionnaires completed by the subjects.

Laboratory Analysis
Blood was collected by venipuncture after an overnight fast, and the plasma/serum samples were stored at −80°C until analyzed. Serum total cholesterol, high-density lipoprotein (HDL) cholesterol, glucose, and creatinine were measured by standard enzymatic methods. Plasma C-reactive protein (CRP) was quantified by immunoturbidimetry on a Hitachi 911 Chemistry Analyzer (Roche Diagnostics) with interassay coefficient of variation of 1.0% to 8.0%. Plasma insulin was measured by an enzymatic immunoassay (Access System, Beckman Coulter). The sensitivity of the assay was 0.03 μU/mL with an interassay coefficient of variation of <10%. Homeostasis model assessment for insulin resistance (HOMA-IR) was calculated as fasting plasma insulin (micromunits per milliliter) × fasting plasma glucose (millimoles per liter)/22.5. Resistin was measured by solid-phase sandwich ELISA (BioVendor). Intra-assay coefficients of variation were 10.3%, 8.9%, and 6.7% at 3.01, 4.97, and 15.38 ng/mL, respectively. Interassay coefficients of variation were 12.9% and 11.8% at 6.6 and 17.48 ng/mL, respectively.

Assessment of GFR and Albuminuria
GFR was estimated using the abbreviated equation from the Modification of Diet in Renal Disease Study using standardized serum creatinine values. Albuminuria was assessed by urinary albumin/creatinine ratio (UACR). The first voided urine was collected on the morning of the study visit and stored at −80°C until analyzed. Urine albumin, urine creatinine, and serum creatinine concentrations were measured by standard methods on a Hitachi 911 Clinical Chemistry Analyzer (Roche Diagnostics), and UACR was expressed as milligrams of albumin per gram of creatinine.

Statistical Analysis
All of the statistical analyses were performed with SAS version 9.1 (SAS Institute). Descriptive statistics are expressed as mean±SD (and Q1, Q3) for continuous variables or as number (percentage) for categorical variables. Values for HDL cholesterol, resistin, CRP, HOMA-IR, eGFR, and UACR were log transformed (after adding 1 to urine albumin in the case of UACR) to minimize skewness. Because of sibships in the sample, we used population-based generalized estimating equations to account for intrafamilial correlations. Separate linear generalized estimating equation models were constructed within each ethnic group to assess whether circulating resistin was associated with eGFR and UACR. We assessed the association of resistin with eGFR and UACR, first in univariable models and then in models that adjusted for age, sex, and BMI. Next, multivariable regression models were fitted to assess whether circulating resistin was associated with eGFR and UACR, independent of conventional CHD risk factors (age, sex, BMI, smoking history, systolic BP, diabetes, total cholesterol, and HDL cholesterol) and the use of statins and renin-angiotensin blockers.

Because resistin has been associated with insulin resistance and inflammation, we investigated whether HOMA-IR (a marker of insulin resistance) and plasma CRP level (a marker of inflammation) confounded the association of resistin with eGFR and UACR. For this purpose, HOMA-IR and CRP were added to the multivariable models for eGFR and UACR, and stepwise backward elimination models were fitted for eGFR and UACR. Finally, additional adjustment for eGFR was also done to investigate whether any association of resistin with UACR was influenced by eGFR.

Subgroup analyses were performed to determine whether the association between resistin and renal function was present in strata defined by the presence or absence of diabetes. Statistical significance was determined at a 2-sided P value of <0.05.

Results
The characteristics of the study participants are summarized in Table 1. Black participants were older than their non-Hispanic white counterparts and had significantly higher mean systolic BP and a higher prevalence of diabetes. On the other hand, non-Hispanic white subjects had a greater prevalence of past history of smoking and had lower HDL cholesterol but a greater frequency of statin use. Mean eGFR (74.6±20.1 versus 63.5±13.8 mL/min) and UACR (51.8±206.0 versus 10.5±58.2 mg/g) were higher in blacks (each P<0.0001). Plasma resistin levels did not differ significantly between the ethnic groups (P=0.36).

In blacks, after adjustment for age, plasma resistin was correlated positively with BMI (r=0.08; P=0.019), HOMA-IR (r=0.11; P=0.003), CRP (r=0.19; P<0.001), and UACR (r=0.19; P<0.001) and inversely with HDL cholesterol (r=−0.15; P<0.001) and eGFR (r=−0.32; P<0.001). In non-Hispanic whites, after adjustment for age, plasma resistin was correlated positively with BMI (r=0.12; P=0.002), HOMA-IR (r=0.09; P=0.015), and CRP (r=0.19; P<0.001) and inversely with eGFR (r=−0.23; P<0.001).

Plasma resistin levels were inversely correlated with eGFR in both ethnic groups (each P<0.0001; Figure 1). The association of resistin with eGFR remained significant after the following: (1) adjustment for age, sex, and BMI (each P<0.0001; model 2; Table 2); (2) further adjustment for CHD risk factors and use of renin-angiotensin blockers and statins (each P<0.0001; model 3; Table 2); and (3) additional adjustment for HOMA-IR and plasma CRP (each P<0.0001; model 4; Table 2). In both ethnic groups, plasma resistin was significantly associated with lower eGFR in subjects with diabetes (n=243 for blacks, P<0.0001; and n=119 for non-Hispanic whites, P=0.001) and subjects without diabetes (n=499 for blacks and n=579 for non-Hispanic whites; each P<0.0001), independent of other determinants of eGFR and
independent of HOMA-IR and plasma CRP (analyses not shown).

In blacks, resistin levels were positively correlated with UACR \( P<0.0001 \); Figure 2). The association remained significant after the following: (1) adjustment for age, sex, and BMI \( P<0.0001 \); model 2; Table 3); (2) further adjustment for CHD risk factors and use of renin-angiotensin blockers and statins \( P<0.0001 \); model 3; Table 3); and (3) additional adjustment for HOMA-IR and CRP \( P=0.001 \); model 4; Table 3). Higher resistin levels were associated with higher UACR even after adding eGFR to model 4 (\( \beta\pm SE=0.28\pm 0.12; \ P=0.017 \)). In non-Hispanic whites, plasma resistin was positively associated with UACR in univariable analyses \( P=0.025 \); Figure 2) but not after further adjustment for CHD risk factors and medication use \( P=0.12 \); Table 3).

In subgroup analyses, higher plasma resistin was independently associated with higher UACR in subjects with diabetes within each ethnic group \( P=0.0002 \) and \( P=0.048 \) for blacks \( n=243 \) and non-Hispanic whites \( n=113 \), respectively); in subjects without diabetes, resistin was not associated with UACR in either ethnic group \( n=499, \ P=0.60 \) and \( n=544, \ P=0.44 \), respectively; analyses not shown).

Discussion
To the best of our knowledge, the present study is the first to report an association of plasma resistin levels with eGFR and albuminuria in adults with hypertension without known CHD or stroke. Higher plasma resistin was associated with lower eGFR in blacks and non-Hispanic whites with hypertension and with higher urinary albumin excretion in subjects with
hypertension and diabetes in both ethnic groups. These associations were independent of other known determinants of GFR and albumin excretion. These findings indicate that elevated levels of resistin may be a risk factor for kidney disease or may even represent overt kidney damage in asymptomatic adults with essential hypertension.

In previous smaller studies, resistin levels have been shown to be elevated in chronic kidney disease, with levels correlating inversely with GFR. In 238 Japanese adults with type 2 diabetes, higher resistin levels were associated with presence and severity of nephropathy. In a posthoc analysis of 239 patients with chronic kidney disease, Axelsson et al found significantly elevated serum resistin levels compared with 24 control subjects, with a significant inverse association between eGFR and serum resistin, independent of age, sex, lean body mass, HDL cholesterol, blood leukocyte count, and CRP. Association of resistin levels with GFR has also been reported in children with end-stage kidney disease, adults with immune-mediated kidney disease, renal allograft recipients, and patients undergoing evaluation for CHD. The results of our study confirm an independent association between resistin and eGFR and extend this to community-based subjects with hypertension without known CHD or stroke.

Several mechanisms can be postulated to explain the association of plasma resistin with eGFR. Resistin is a low molecular weight plasma protein (12.5 kDa), and polypeptides of comparable molecular weights are thought to be freely filtered at the glomerulus. In addition, the kidneys play an important role in the catabolism of small plasma polypeptides. Thus, a decrease in functional renal parenchyma could potentially lead to elevation in plasma resistin levels through several mechanisms.

We cannot exclude a potential role of resistin as a contributory factor for chronic kidney disease, as has been suggested for leptin, another adipocytokine. It is possible that the roles of resistin in mediating insulin resistance and inflammation or some other biological effects of this cytokine underlie the association between resistin and GFR. Chronic kidney disease occurs in insulin-resistant states like obesity, metabolic syndrome, and diabetes, and insulin resistance in the setting of high resistin levels could have potential detrimental

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<th>Table 2. Association of Resistin With Log eGFR in Linear Regression Models</th>
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RAS indicates renin-angiotensin system. Model 1 is unadjusted; model 2 is adjusted for age, sex, and BMI; and model 3 is adjusted for age, sex, BMI, diabetes, smoking history, systolic BP, total cholesterol, HDL cholesterol, RAS blocker use, and statin use. Model 4 was built by adding log HOMA-IR and log CRP to model 3 and performing stepwise elimination of variables until only variables significant at P<0.1 remained in the model, with age, sex, log resistin, log HOMA-IR, and log CRP forced into the model. For model 4, n=742 for blacks and n=698 for non-Hispanic whites.

Figure 2. Scatter plots showing the correlation of plasma resistin levels with albumin excretion in blacks (A) and non-Hispanic whites (B). The P values are derived from unadjusted Pearson product-moment correlation coefficients.
effects on kidney function. In the present study, a significant (albeit modest) positive correlation of plasma resistin with BMI and HOMA-IR was noted. However, even after adjustment for these variables, resistin remained significantly associated with eGFR in both blacks and non-Hispanic whites. Furthermore, higher resistin levels were independently associated with lower eGFR in both subjects with and without diabetes of either ethnic group. Recently, in a preliminary report of 630 postmenopausal women, plasma resistin was associated with serum creatinine, independent of CRP and white blood cell count. Taken together, these findings suggest that insulin resistance may not explain all of its association with urinary albumin excretion.

Thus, our findings support the notion that the association of resistin with eGFR is independent of metabolic effects of this cytokine on glucose metabolism.

Resistin is known to stimulate the expression of other proinflammatory cytokines and adhesion molecules, and several studies have found circulating resistin levels to correlate with markers of inflammation. Low-grade inflammation has been proposed to play a role in the progression of chronic kidney disease. In the present study, as in some previous studies, plasma levels of resistin and CRP were correlated. However, the association between resistin and eGFR remained significant when CRP was included in the regression models. In a previous smaller study, Fagerberg et al. found plasma resistin to be associated with serum creatinine, independent of CRP and white blood cell count. Taken together, these findings suggest that the association between resistin and eGFR is unlikely to be mediated entirely through modulation of inflammatory pathways.

Increased urinary albumin excretion in the setting of systemic hypertension may occur with or without a decline in GFR, is a marker of target organ damage, and is associated with increased CHD risk. Both increased glomerular filtration of albumin because of hypertension-related intrarenal hemodynamic abnormalities and defective proximal tubular reclamation, possibly mediated by effects of cytokines, have been proposed to increase urinary albumin excretion in essential hypertension. In the present study, higher plasma resistin was associated with higher UACR in subjects with diabetes but not in subjects without diabetes, in both blacks and non-Hispanic whites, independent of CHD risk factors, medication use, and eGFR. The mechanism underlying the association of plasma resistin with albuminuria in subjects with hypertension and diabetes is not clear. Insulin resistance is known to be associated with increased albumin excretion and could potentially explain the association between resistin and albuminuria. However, after adjustment for HOMA-IR, the association of plasma resistin with UACR was not altered, suggesting that insulin resistance may not be a major mediator of the association between resistin and albuminuria.

A role for inflammation has also been proposed in urinary albumin excretion. The humoral factors induced by resistin and their downstream effectors could potentiate mesangial proliferation and interstitial fibrosis, thereby affecting both glomerular and tubular processes. In the present study, plasma resistin remained associated with UACR even after inclusion of plasma CRP in the multivariable regression model, suggesting that any proinflammatory effects of resistin may not explain all of its association with urinary albumin excretion. However, CRP is an acute phase reactant, and a possible involvement of local or low-grade systemic inflammatory pathways in the association of resistin with albuminuria (as well as that with eGFR) cannot be excluded.
Finally, reduced GFR and increased urine albumin in essential hypertension are believed to be manifestations of preglomerular microvasculopathy. Resistin is known to increase the expression of endothelin, adhesion molecules, matrix metalloproteinases, and other mediators of endothelial activation and inflammation. Such humoral mediators induced by resistin may promote systemic vascular dysfunction, which, in turn, could affect GFR and albumin excretion. Resistin has been associated with microvascular complications in patients with diabetes. Whether circulating resistin levels mediate target organ damage in essential hypertension needs to be confirmed.

A limitation of the present study is its cross-sectional design, precluding interpretations about the sequence of changes in resistin levels and markers of kidney disease. Prospective studies will be needed to confirm whether increased resistin levels lead to impairment in GFR or albuminuria. We measured plasma resistin but not different isoforms, which could potentially differ in their biological activity. It is also possible that differences in the molecular masses of the isoforms of the circulating resistin may influence the elimination mechanisms for resistin and, therefore, its association with markers of kidney disease. Furthermore, GFR was not directly measured but was instead estimated on the basis of plasma creatinine levels using the Modification of Diet in Renal Disease formula, which may weaken estimated associations with predictors.

Perspectives

Essential hypertension is a major risk factor for chronic kidney disease. We demonstrate that in community-based subjects with hypertension without known myocardial infarction or stroke, higher circulating resistin levels are associated with a lower eGFR and with increased UACR in the presence of concomitant diabetes, independent of CHD risk factors and medication use. The association of resistin with eGFR and UACR was independent of inflammatory markers and insulin resistance, suggesting that the association is mediated, at least in part, through processes other than the metabolic or proinflammatory effects of resistin. Future studies should aim at understanding any direct or indirect effects of resistin on cellular and humoral pathways relevant to glomerular or tubular function. Such knowledge could be potentially useful in identifying novel targets for intervention to reduce target organ damage in individuals with hypertension.

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Disclosures

None.

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