Plasma Resistin Levels Associate with Risk For Hypertension among Nondiabetic Women

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ABSTRACT

Emerging evidence suggests a role for resistin in inflammation and vascular dysfunction, which may contribute to the pathogenesis of hypertension, but the association between resistin levels and incident hypertension is unknown. We examined the association between plasma resistin levels and the risk for incident hypertension among 872 women without a history of hypertension or diabetes from the Nurses’ Health Study. We identified 361 incident cases of hypertension during 14 years of follow-up. After adjustment for potential confounders, resistin levels in the highest tertile conferred a 75% higher risk for hypertension than the lowest tertile (relative risk [RR] 1.75; 95% confidence interval [CI] 1.19 to 2.56). Further adjustment for other adipokines did not change the RR substantially. In stratified analysis, resistin levels in the highest tertile significantly increased the risk for hypertension among women aged ≥55 years (adjusted RR 2.40; 95% CI 1.55 to 3.73) but not among women aged <55 years (adjusted RR 0.64; 95% CI 0.25 to 1.62). In a subset analysis of 362 women who also had measurements of inflammatory and endothelial biomarkers, plasma resistin levels significantly correlated with IL-6, soluble TNF receptor 2, intercellular adhesion molecule 1, vascular adhesion molecule 1, and E-selectin after controlling for age and body mass index. After further adjustment for these biomarkers and C-reactive protein, resistin levels remained significantly associated with incident hypertension. In conclusion, higher plasma resistin levels independently associate with an increased risk for incident hypertension among women without diabetes.
dicator of both systolic (SBP) and diastolic BP (DBP) in patients with diabetes, even after adjustment for body mass index (BMI) and fasting glucose.\textsuperscript{14} A cross-sectional study from Greece demonstrated that “healthy” individuals with prehypertension had significantly higher plasma resistin levels compared with healthy individuals with normotension.\textsuperscript{15} To the best of our knowledge, however, no study has prospectively investigated whether plasma resistin levels influence the risk for developing hypertension; therefore, we prospectively investigated the association between plasma resistin levels and the risk for incident hypertension among 872 women without diabetes or hypertension from the Nurses’ Health Study (NHS).

RESULTS

During the 14 years (9398 person-years) of follow-up of 872 women without hypertension, 361 incident cases of physician-diagnosed hypertension were reported. Participant characteristics by tertiles of plasma resistin level are presented in Table 1. Women with higher plasma resistin were younger; had higher BMI; were less physically active; and had lower intakes of alcohol, magnesium, and calcium. Current smoking was more frequent within higher tertiles of resistin. Although the high molecular weight (HMW)—total adiponectin ratio and leptin were associated with resistin at baseline in crude analysis (Table 1), these adipokines were no longer associated with resistin levels after adjustment for age and BMI (data not shown).

Plasma resistin levels were positively associated with the risk for incident hypertension in age-adjusted and multivariable-adjusted analyses (Table 2). Compared with those in the bottom tertile of resistin, the multivariable relative risk (RR) for incident hypertension for those in the top tertile of resistin was 1.75 (95% confidence interval [CI] 1.19 to 2.56; \( P < 0.001 \)) for trend). Further adjustment for other adipokines did not change the RRs substantially (1.78 [95% CI 1.20 to 2.65] for the top tertile). To avoid misclassification by including women with undiagnosed hypertension at baseline, we performed two additional analyses. First, we excluded women who never had a physical examination for screening purposes during the follow-up period (\( n = 70 \)). The results were not markedly changed, and the multivariable RR for the top tertile of resistin was 1.79 (95% CI 1.19 to 2.70). Second, we limited our primary analysis to participants who reported hypertension 1 year after 1990 (\( n = 36 \)). The multivariable RR for the top tertile of resistin attenuated slightly, which was 1.73 (95% CI 1.16 to 2.60).

After adjustment for age and BMI, total adiponectin (RR for bottom compared with top tertile 0.95 [95% CI 0.65 to 1.38]), the HMW–total adiponectin ratio (RR for bottom compared with top tertile 0.83 [95% CI 0.57 to 1.21]), and leptin (RR for top compared with bottom tertile 1.22 [95% CI 0.72 to 2.06]) were not associated with risk for incident hypertension. The results remained NS after additional adjustment for other covariates.

The association between plasma resistin level and risk for incident hypertension was greater among women who were aged \( \geq 55 \) years (\( P = 0.05 \) value for interaction; Figure 1). The multivariable RR for the top tertile of resistin was 2.40 (95% CI 1.55 to 3.73) among older women and was 0.64 (95% CI 0.25 to 1.62) among women who were younger than 55 years (Table 2). We did not observe effect modification by BMI (\( P = 0.35 \) value for interaction).

Altogether, 362 women were included in the secondary analysis, with 145 incident hypertension cases through 14 years of follow-up. At baseline, plasma resistin levels were correlated with levels of IL-6 (correlation coefficient 0.24; \( P < 0.001 \)) and TNF receptor 2 (TNF R2; \( r = 0.22, P < 0.001 \)) after controlling for age and BMI but not with C-reactive protein (CRP; \( r = 0.07, P = 0.18 \); Table 3). Plasma

| Table 1. Baseline characteristics by tertiles of resistin |
|-------------|---------------|---------------|----------------|
| Variable    | Tertile 1     | Tertile 2     | Tertile 3     | \( P \)       |
| No. of participants | 310           | 289           | 273           |             |
| Age (years; median [IQR]) | 57 (49 to 61) | 55 (49 to 62) | 53 (48 to 59) | 0.008       |
| BMI (kg/m\(^2\); median [IQR]) | 23.6 (21.6 to 25.8) | 24.8 (22.5 to 28.3) | 25.7 (22.8 to 30.9) | <0.001 |
| Alcohol consumption (g/d; median [IQR]) | 1.8 (0.0 to 7.0) | 1.1 (0.0 to 5.8) | 1.1 (0.0 to 4.7) | 0.04        |
| Physical activity (METs/wk; median [IQR]) | 11.5 (5.1 to 25.7) | 9.2 (3.4 to 21.5) | 7.9 (3.1 to 17.3) | <0.001 |
| Family history of hypertension (%) | 43.2          | 47.4          | 43.2          | 0.97        |
| Current smoker (%) | 8.7           | 10.4          | 15.8          | 0.009       |
| Postmenopausal (%) | 78.1          | 72.0          | 70.7          | 0.04        |
| Dietary factors (median [IQR]) |
| Folate (\( \mu \)g/d) | 372 (276 to 590) | 366 (279 to 573) | 344 (251 to 562) | 0.17        |
| Sodium (mg/d) | 1856 (1645 to 2045) | 1815 (1623 to 2027) | 1797 (1601 to 2044) | 0.37        |
| Potassium (mg/d) | 2897 (2522 to 3261) | 2824 (2544 to 3138) | 2809 (2450 to 3114) | 0.09        |
| Magnesium (mg/d) | 303 (264 to 344) | 300 (255 to 347) | 287 (246 to 330) | 0.009       |
| Calcium (mg/d) | 713 (563 to 900) | 691 (550 to 885) | 656 (523 to 866) | 0.08        |
| Total adiponectin (\( \mu \)g/ml; median [IQR]) | 19.1 (13.7 to 22.9) | 18.4 (13.2 to 22.6) | 17.1 (12.9 to 22.8) | 0.33        |
| HMW–total adiponectin ratio (median [IQR]) | 0.41 (0.33 to 0.48) | 0.39 (0.32 to 0.47) | 0.37 (0.30 to 0.47) | 0.04        |
| Leptin (\( \mu \)g/ml; median [IQR]) | 14.2 (8.1 to 24.9) | 17.0 (10.4 to 27.0) | 20.5 (10.3 to 32.9) | <0.001       |

IQR, interquartile range; MET, metabolic equivalent.
resistin levels were significantly correlated with biomarkers of endothelial dysfunction including intercellular adhesion molecule 1 (ICAM-1; $r = 0.15, P = 0.005$), vascular adhesion molecule 1 (VCAM-1; $r = 0.10, P = 0.05$), and E-selectin ($r = 0.12, P = 0.02$; Table 3).

In this subset of 362 women, the highest compared with the lowest tertile of plasma resistin was associated with an increased risk for incident hypertension after multivariable adjustment (RR 2.78 [95% CI 1.07 to 7.23]; Table 4). Further adjustment for either CRP or all inflammatory biomarkers (CRP, IL-6, and TNF R2) simultaneously did not materially alter the RR (Table 4). Similarly, adjustment for endothelial markers including ICAM-1, VCAM-1, and E-selectin did not change RR markedly (RR 2.99 [95% CI 1.09 to 8.16] for the top tertile). Adding all inflammatory and endothelial biomarkers simultaneously into the multivariable model resulted in an RR for the top tertile of 3.01 (95% CI 1.06 to 8.83; Table 4). Because we observed effect modification by age in the primary analysis, we also performed a stratified analysis in this subset of women despite that the $P$ value for interaction was not statistically significant ($P = 0.67$). After adjustment for all inflammatory and endothelial biomarkers, the RR for the top tertile of resistin was 4.03 (95% CI 1.03 to 18.84) among women aged $\geq 55$ years.

**DISCUSSION**

We present the first prospective study to examine the relation between plasma resistin levels and the risk for incident hypertension. We found that higher plasma resistin levels were independently associated with the risk for incident hypertension. The association was statistically significant only among women aged $\geq 55$ years in stratified analysis. Finally, the association between resistin and hypertension was robust even after controlling for inflammatory and endothelial biomarkers.

Experimental studies suggest a possible role for resistin in the pathogenesis of hypertension. Resistin is primarily involved in the inflammatory process by strongly up-regulating IL-6 and TNF-α, probably via the NF-κB pathway. Resistin exerts direct effects to promote endothelial cell activation by inducing endothelin 1 release and also the expression of adhesion molecules such as ICAM-1 and VCAM-1. Furthermore, resistin leads to proliferation of human vascular smooth muscle cells. All of these processes can induce inflammation within the vascular wall and lead to vascular remodel-
ing and injury. At the functional level, resistin has been shown to reduce endothelium-dependent and -independent vasorelaxation, possibly by increasing superoxide radical production and by decreasing endothelial nitric oxide synthase expression in endothelial cells.\(^\text{16}\)

In this study, plasma resistin levels were strongly and independently correlated with IL-6 and TNF R2, as well as biomarkers of endothelial dysfunction, which corresponds with results of \textit{in vitro} studies. In contrast, plasma CRP was not correlated with resistin after adjustment for age and BMI in our study, which is consistent with another report of participants without diabetes,\(^\text{17}\) yet adding these biomarkers to our multivariable models did not eliminate the association between plasma resistin and risk for hypertension, suggesting that other mechanisms explaining the resistin–hypertension link might exist.

Human studies of the association between resistin and hypertension are limited. A cross-sectional study of patients with diabetes from Japan\(^\text{14}\) revealed that serum resistin levels were higher in patients with hypertension compared with those without hypertension. Moreover, resistin was independently associated with both SBP and DBP. In another small cross-sectional study from Japan, 33 patients with essential hypertension were classified into as having insulin resistance or not.\(^\text{18}\) Their plasma resistin levels were compared with 18 volunteers with normotension.\(^\text{18}\) Plasma resistin levels were nonsignificantly slightly higher in the groups that had essential hypertension without insulin resistance (21.9 \pm 8.9 \mu g/L) and with insulin resistance (20.2 \pm 8.2 \mu g/L) than in the volunteer group (19.0 \pm 9.6 \mu g/L). A report from Greece\(^\text{15}\) indicated that individuals without diabetes and with prehypertension had significantly higher plasma resistin levels compared with healthy individuals with normotension (10.62 \pm 3.17 \textit{versus} 6.72 \pm 3.15 \mu g/L). Furthermore, plasma resistin levels were found to be higher in healthy offspring of patients with essential hypertension compared with those without a family history of hypertension. These findings suggest that resistin might be associated with hypertension in the population without diabetes and that increased resistin levels may exist before the occurrence of clinical hypertension. Results from this study provide the first prospective evidence of the association between plasma resistin and risk for incident hypertension during 14 years of follow-up.

Although classified as an adipokine, resistin in humans is mainly produced by inflammatory cells, both within and outside the adipose tissue,\(^\text{1,5}\) distinguishing resistin biologically from adiponectin and leptin. After controlling for total adiponectin, the HMW–total adiponectin ratio (reflecting the proportion of biologically most active form of adiponectin) and leptin, the association between plasma resistin and hypertension did not change markedly, suggesting the resistin–hypertension link is independent of other adipokines.

The association was statistically significant only among women aged \(\geq 55\) years in stratified analysis. Because the prevalence of hypertension in women who are older than 55 to 60 years is markedly increased and exceeds the prevalence of hypertension in men,\(^\text{19}\) the strong association among older women may have significant public health implications. The mechanisms that may underlie this finding are unclear, although sex hormones might be one explanation. For example,

### Table 3. Correlation between plasma resistin levels and other biomarkers

<table>
<thead>
<tr>
<th>Parameter</th>
<th>(r^a)</th>
<th>(P)</th>
<th>(r^b)</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRP (mg/L)</td>
<td>0.18</td>
<td>&lt;0.001</td>
<td>0.07</td>
<td>0.18</td>
</tr>
<tr>
<td>IL-6 (pg/ml)</td>
<td>0.30</td>
<td>&lt;0.001</td>
<td>0.24</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>TNF R2 (pg/ml)</td>
<td>0.26</td>
<td>&lt;0.001</td>
<td>0.22</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ICAM-1 (ng/ml)</td>
<td>0.16</td>
<td>0.003</td>
<td>0.15</td>
<td>0.005</td>
</tr>
<tr>
<td>VCAM-1 (ng/ml)</td>
<td>0.10</td>
<td>0.07</td>
<td>0.10</td>
<td>0.05</td>
</tr>
<tr>
<td>E-selectin (ng/ml)</td>
<td>0.18</td>
<td>&lt;0.001</td>
<td>0.12</td>
<td>0.02</td>
</tr>
</tbody>
</table>

\(^a\)Controlled for age.  
\(^b\)Controlled for age and BMI.

### Table 4. Plasma resistin levels and risk for incident hypertension adjusting for other inflammatory biomarkers

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Plasma Resistin Levels</th>
<th>Tertile 1</th>
<th>Tertile 2</th>
<th>Tertile 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Person-years</td>
<td>1431</td>
<td>1372</td>
<td>1053</td>
<td></td>
</tr>
<tr>
<td>No. of cases</td>
<td>49</td>
<td>43</td>
<td>53</td>
<td></td>
</tr>
<tr>
<td>Model A (RR [95% CI])(^a)</td>
<td>1.00 (reference)</td>
<td>1.34 (0.56 to 3.19)</td>
<td>2.78 (1.07 to 7.23)</td>
<td></td>
</tr>
<tr>
<td>Model A + CRP (RR [95% CI])</td>
<td>1.00 (reference)</td>
<td>1.36 (0.57 to 3.27)</td>
<td>2.98 (1.12 to 7.94)</td>
<td></td>
</tr>
<tr>
<td>Model A + IL-6 + TNF R2 (RR [95% CI])</td>
<td>1.00 (reference)</td>
<td>1.34 (0.55 to 3.25)</td>
<td>2.74 (0.99 to 7.56)</td>
<td></td>
</tr>
<tr>
<td>Model A + endothelial biomarkers (RR [95% CI])(^b)</td>
<td>1.00 (reference)</td>
<td>1.71 (0.65 to 4.47)</td>
<td>2.99 (1.09 to 8.16)</td>
<td></td>
</tr>
<tr>
<td>Model A + other biomarkers (RR [95% CI])(^c)</td>
<td>1.00 (reference)</td>
<td>1.71 (0.65 to 4.53)</td>
<td>3.01 (1.06 to 8.83)</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\)Model A was adjusted for age; BMI; physical activity; family history of hypertension; current smoking; menopause status; fasting status; and intakes of alcohol, sodium, potassium, magnesium, calcium, and folate.  
\(^b\)Endothelial biomarkers include ICAM-1, VCAM-1, and E-selectin.  
\(^c\)Biomarkers include CRP, IL-6, TNF R2, ICAM-1, VCAM-1, and E-selectin.
studies have documented higher resistin levels in premenopausal women compared with men and with postmenopausal women. Although speculative, the possibility that sex hormones also influence the function of resistin could not be excluded. Unfortunately, there were too few premenopausal women in our study to be able to analyze effect modification by menopausal status.

In our secondary analysis (n = 362), the RRs were larger and the CIs were wider compared with the RRs and CIs in the primary analysis (n = 872). This seeming discrepancy may simply be due to the smaller sample size and smaller number of cases, increasing the instability of the model. Indeed, the RR estimates from the secondary analysis were within the CIs from the primary analysis, so we believe the results were consistent.

Our study has limitations. First, our study population was derived from a preexisting case-control study of diabetes. Although we only included control subjects to avoid bias, the generalizability of results from this study might be limited. Second, BP was not directly measured and hypertension was self-reported; however, all participants are registered nurses, and we demonstrated that hypertension reporting by participants of this cohort is highly sensitive. Another concern might be ascertainment bias. For example, overweight and obese individuals, who are susceptible to developing health problems, would have been more likely than normal-weight participants to be screened for and receive a diagnosis of clinical hypertension; however, excluding participants who never had a screening physical examination did not change the results in our analysis. Third, we lacked information on renal function. It has been reported that higher plasma resistin levels were associated with lower estimated GFR, yet, because all women in our study were free from diabetes and hypertension at baseline, it is unlikely that many women had impaired renal function. Indeed, other studies have indicated a very low prevalence of renal dysfunction in the NHS. Fourth, we had a single measurement of plasma resistin. Nonetheless, a pilot study indicated that individual plasma resistin levels do not significantly change during at least a period of 1 year. Although during 14 years the changes in resistin levels may be more substantial, this type of misclassification would tend to bias our study toward not finding an association; therefore, it is possible that we underestimated the true association. Fifth, because our study was observational, the possibility of residual confounding by some unmeasured covariate exists; however, the association between resistin and hypertension was robust after adjustment for numerous lifestyle and dietary covariates, as well as inflammatory and endothelial biomarkers. Finally, our study was entirely female and mostly white; therefore, the results may not be generalizable to nonwhite individuals or to men.

In conclusion, our prospective analysis suggests that higher plasma resistin levels are independently associated with an increased risk for incident hypertension among women without diabetes. We further confirmed the association noted in experimental studies between resistin and inflammation and endothelial dysfunction; however, these pathways do not fully explain the association between resistin and hypertension. Our findings may increase the understanding of the pathophysiology of hypertension in older individuals and should be tested in other cohorts.

**CONCISE METHODS**

**Source Population**

The NHS cohort was assembled in 1976, when 121,700 female nurses aged 30 to 55 years returned a mailed questionnaire. Participants are followed via biennial questionnaires that gather updated information on health-related behaviors and medical events. From 1989 to 1990, 32,826 consenting women provided blood samples returned with a cold pack by overnight mail; 97% of samples were received within 24 hours of collection. All blood samples were stored in liquid nitrogen (≤ −130°C) until laboratory analysis. This study was approved by the institutional review board at Brigham and Women’s Hospital. Receipt of each questionnaire implied participant’s consent.

**Study Population**

In a nested case-control study in the NHS designed to examine incident diabetes, participants had resistin measured and were therefore included in this study (Figure 2). The exclusion criteria were (1) women who developed diabetes between 1990 and June 2004 (the
Assessment of Plasma Resistin Levels
Resistin concentration was measured by using an ELISA (Linco Research, St. Charles, MO) with a minimum detectable limit of 0.16 ng/ml. On the basis of blinded quality-control samples, the coefficient of variation (CV) for resistin was 2.5%. One study indicated that the overall intraclass correlation between resistin measured on blood samples frozen at −70°C for 4 years, 2 years, and 1 year was 0.95, suggesting that long-term storage might not have a major effect on resistin levels.

Assessment of Other Covariates
BMI (calculated as weight in kilograms divided by height in meters squared), physical activity (metabolic equivalent tasks), smoking status, and menopause status were ascertained by questionnaire at baseline. Intakes of alcohol, sodium, potassium, calcium, magnesium, and folate were ascertained from a food frequency questionnaire in 1990. Except for intake of alcohol, nutrient values were adjusted for total energy intake by the residual method. The reproducibility and validity of the questionnaire have been documented. Other plasma adipokines, including total adiponectin, HMW adiponectin, and leptin, were also measured. The methods for measuring these adipokines are described elsewhere. In blinded quality-control samples, the CVs for total adiponectin, HMW adiponectin, and leptin were 8.9, 9.9, and 8.3%, respectively.

For the secondary analysis, other measured biomarkers included CRP, IL-6, soluble TNF R2, ICAM-1, VCAM-1, and E-selectin. Assays used for measuring these biomarkers are described elsewhere, and CVs ranged from 2.1% for CRP to 9.8% for VCAM-1.

Assessment of Hypertension
The baseline and biennial follow-up questionnaires inquired about physician-diagnosed hypertension and the year of diagnosis. Self-reported hypertension was found to be highly reliable in the NHS. In a subset of women (n = 51) who reported hypertension, medical record review confirmed a documented SBP ≥ 140 mmHg or DBP ≥ 90 mmHg in 100% of participants. Among another subset of women without previous self-reported hypertension (n = 161), only 6.8% of them had recorded BP ≥ 140/90 mmHg, and none of them had BP ≥ 160/95 mmHg. A participant was considered to have prevalent hypertension when she reported this diagnosis on any questionnaire up to and including the 1990 questionnaire and therefore was excluded from this study. Incident cases included individuals who first reported hypertension on subsequent questionnaires and whose year of diagnosis was after the return of the 1990 questionnaire.

Statistical Analysis
Person-time was truncated at the date of hypertension diagnosis, at the date of death, or at the date of cancer diagnosis (except for nonmelanoma skin cancer), whichever came first. Plasma resistin levels were analyzed in tertiles, using the lowest tertile as the reference group. The relationship between resistin and other covariates at baseline (in 1990) were analyzed using the Kruskall-Wallis test for continuous variables and the Mantel-Hanzel χ² test of trend for categorical variables. The association between resistin and hypertension through 14 years of follow-up was analyzed using Cox proportional hazards regression models to estimate RRs and 95% CIs. Multivariable models were constructed to adjust for potential confounding variables that have been previously associated with incident hypertension: Age (continuous); BMI (six categories); current smoking (cigarettes per day); family history of hypertension (yes/no); menopause status (yes/no); physical activity (quintiles); fasting status (<8 versus ≥8 hours since last meal); and intakes of alcohol (continuous), sodium, potassium, calcium, magnesium, and folate (all continuous). Total adiponectin, the HMW–total adiponectin ratio, and leptin (all in tertiles) were further added to the multivariable model. We determined P values for trend for each of the exposures of interest by using the median for each category.

We also investigated whether the association between plasma resistin and hypertension varied according to age (<55 or ≥55 years) and BMI (<25 or ≥25 kg/m²). Stratified multivariable analyses were performed, and appropriate interaction terms were generated to test whether interactions were statistically significant.

In the secondary analyses limited to the 362 women who also had inflammatory and endothelial biomarkers measured, we used age- and BMI-controlled Spearman correlation coefficients to evaluate associations between resistin and other biomarkers. We then added CRP, IL-6, and TNF R2, as well as ICAM-1, VCAM-1, and E-selectin individually and together (all as continuous variables) in the multivariate model of resistin to observe whether the association between plasma resistin levels and incident hypertension changed and became insignificant after controlling for these biomarkers.

All P values are two-tailed. Statistical tests were performed using SAS 9.1 for UNIX statistical software package (SAS Institute, Cary, NC).

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DISCLOSURES
None.
REFERENCES


See related editorial, “Hypertension: Shall We Focus on Adipose Tissue?” on pages 1067–1068.