# Plasma Resistin Levels and Risk of Myocardial Infarction and Ischemic Stroke

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**Context:** Resistin is a hormone that has been linked to insulin resistance, inflammatory processes, and coronary heart disease in case-control studies; however, prospective data on the association between plasma resistin levels and future risk of cardiovascular disease are lacking.

**Objective:** The objective of the study was to investigate the association between plasma resistin levels and risk of future myocardial infarction (MI) and ischemic stroke (IS) in a large prospective cohort.

**Methods:** We investigated the association between plasma resistin levels and risk of MI and IS in a case-cohort design among 26,490 middle-aged subjects from the European Investigation into Cancer and Nutrition-Potsdam Study without history of MI or stroke at time of blood draw. Plasma resistin levels were measured in baseline blood samples of 139 individuals who developed MI, 97 who developed IS, and 817 individuals who remained free of cardiovascular events during a mean follow-up of 6 yr.

**Results:** After multivariable adjustment for established cardiovascular risk factors including C-reactive protein, individuals in the highest compared with the lowest quartile of plasma resistin levels had a significantly increased risk of MI (relative risk 2.09; 95% confidence interval 1.01–4.31; *P* for trend = 0.01). In contrast, plasma resistin levels were not significantly associated with risk of IS (relative risk 0.94; 95% confidence interval 0.51–1.73; *P* for trend = 0.88).

**Conclusion:** Our data suggest that high plasma resistin levels are associated with an increased risk of MI but not with risk of IS. Further studies are needed to evaluate the predictive value of plasma resistin levels for cardiovascular disease. **(*J Clin Endocrinol Metab* 93: 2647–2653, 2008)**

Resistin is a 114-amino acid polypeptide (12.5 kDa) hormone that belongs to a family of resistin-like molecules (1). Resistin was reported to be secreted by adipocytes and to cause insulin resistance in animal models (2), which fueled the hypothesis that this hormone may play a role in the pathogenesis of obesity-mediated insulin resistance and diabetes. However, structure and biology of resistin differ substantially between species, and many aspects, specifically its association with obesity and its effects on insulin sensitivity in humans, remain controversial (1). In contrast to mice, human resistin is expressed at lower levels in adipocytes but at higher levels in circulating blood monocytes (3–5). *In vitro*, resistin activated human endothelial cells, leading to increased expression of adhesion molecules, and induced human aortic muscle cell proliferation (6, 7). Further-

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Abbreviations: BMI, Body mass index; CHD, coronary heart disease; CI, confidence interval; CRP, C-reactive protein; CV, coefficient of variation; CVD, cardiovascular disease; EPIC, European Prospective Investigation into Cancer and Nutrition; HDL-C, high-density lipoprotein-cholesterol; ICD-10, *International Classification of Diseases*, 10th revision; IS, ischemic stroke; MI, myocardial infarction; RR, relative risk; TC, total cholesterol.

more, some studies described positive associations between plasma levels of resistin and C-reactive protein (CRP) (8–10). These findings suggest that resistin may contribute to the development of atherosclerosis and may thereby be linked to cardiovascular disease (CVD) in humans (11). Recent cross-sectional and case-control studies found higher plasma resistin levels in subjects with coronary heart disease (CHD) when compared with controls (12, 13). However, data from prospective studies are lacking. Therefore, we conducted a case-cohort study nested in the European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam Study to assess the association between plasma resistin levels and risk of incident myocardial infarction (MI) and ischemic stroke (IS) over a mean follow-up period of 6 yr.

## Subjects and Methods

### Study population

The EPIC-Potsdam Study is part of a large-scale European-wide prospective cohort study and includes 27,548 individuals (16,644 women and 10,904 men). Participants were recruited between 1994 and 1998 from the general population with the preferred ages 35–65 yr in women and 40–65 yr in men (14). The baseline examination included standardized blood pressure measurements, anthropometric measurements, self-administered questionnaires on diet and lifestyle, personal computer-guided interviews, and blood sampling. Blood was collected from 95% of participants at the Potsdam center. All participants gave written informed consent, and the Ethics Committee of the Federal State Brandenburg approved all study procedures. Information about incident diseases and changes in lifestyle is biennially assessed by self-administered questionnaires (15).

After exclusion of subjects with a history of a MI or stroke, we identified 156 individuals with incident MI and 132 individuals with incident stroke (103 IS, 25 hemorrhagic strokes, and four strokes with undefined etiology) among 26,490 participants during a mean follow-up of 6.0 ± 1.5 yr. We restricted the analysis for stroke to individuals with IS because ischemic and nonischemic stroke may differ in etiology and because of the low number of nonischemic strokes observed in this cohort. The association of resistin levels with risk of MI or IS was analyzed using a case-cohort design (16, 17). With this established type of study design (18), the results are expected to be generalizable to the entire cohort without the need to measure biomarker levels in the entire cohort (16). A random sample (subcohort) of about 3% of the EPIC-Potsdam cohort was selected. The selection of the subcohort size was based on both cost and power calculations. Using this cohort size, a relative risk of 2 or above can be detected with sufficient power (80%) based on a number of 150 cases (type 1 error of 0.05). For these purposes a random sample (subcohort) comprising 851 individuals was selected among those participants in the EPIC-Potsdam Study without prevalent stroke or MI who had provided sufficient blood samples for measurement of a predefined set of biomarkers including total cholesterol, high-density lipoprotein-cholesterol, CRP, and IL-6. In agreement with the case-cohort design, five of the 156 MI cases and five of the 103 cases of IS were part of the subcohort. For the present analyses, we excluded eight cases of MI and six cases of IS because blood specimens were not available. Furthermore, eight MI cases and 24 participants of the subcohort had to be excluded due to insufficient blood volume for resistin measurements. Thus, the final case-cohort sample comprised a total of 1053 participants including 139 cases of MI and 97 cases of IS.

### Ascertainment of MI and stroke

Potential cases were identified based on self-reports on one of the four follow-up questionnaires of MI or stroke or based on death certificates. To increase sensitivity, the questionnaire included additional questions about cerebral ischemia and stroke symptoms (19). All potential incident cases were verified by contacting the patients' attending physician or by review of death certificates according to World Health Organization Monitoning Trends and Determinants in Cardiovascular Disease (MONICA) criteria, and only confirmed cases were considered for analysis. According to *International Classification of Diseases*, 10th revision (ICD-10), cases were classified as incident MI (ICD-10, I21), IS (ICD-10, I63.0-I63.9), intracerebral (ICD-10, I61.0-I61.9), or subarachnoidal hemorrhage (ICD-10, I60.0-I60.9) or undetermined stroke (ICD-10, I64.0-I64.9) by two physicians in the study center (20).

### Assessment of risk factors and covariates

Lifestyle characteristics, including regular physical exercise and smoking history, were documented at baseline by trained interviewers during a personal computer-guided interview. Physical exercise was defined as the mean time spent on leisure time physical activities during the summer and winter seasons (hours per week). Anthropometric data and blood pressure were measured by trained and quality-monitored personnel (21). The body mass index (BMI) was calculated as body weight divided by height squared (kilograms per square meter). Hypertension was defined as systolic blood pressure 140 mm Hg or greater or diastolic blood pressure 90 mm Hg or greater (based on the mean of the second and third measurement) or self-reporting of a diagnosis or use of antihypertensive medication. History of diabetes was evaluated by a physician using information on self-reported medical diagnosis, medication records, and dieting behavior. Uncertainties regarding the diagnosis were clarified by contacting the participant or treating physician. Dietary habits including alcohol consumption during the preceding year were assessed by a validated self-administered food frequency questionnaire (22).

### Blood collection and laboratory analysis

A total of 30 ml of venous blood was collected at baseline from each study participant at the Potsdam center; fractioned into serum, plasma, buffy coat, and erythrocytes; and was aliquoted into straws and stored in liquid nitrogen at −196 C for conservation. Total cholesterol (TC), high-density lipoprotein-cholesterol (HDL-C), and CRP were measured using standard methods with reagents from Horiba ABX (Shefford, UK), with intraassay coefficients of variation (CVs) of 0.9, 1.2, and 2.6, respectively, and interassay CVs of 4.7, 5.2, and 7.9%, respectively. IL-6 was measured by an ELISA (R&D Systems, Minneapolis, MN). The intraassay CV ranged between 3.8 and 11.1%, and the interassay CV was 9.9%. Creatinine was measured enzymatically using an automated assay (Roche Molecular Biochemicals, Mannheim, Germany) with an intraassay CV of 0.7% and an interassay CV of 1.1%. Resistin levels were measured in citrate plasma by ELISA using a commercial test assay (BioVendor Laboratory Medicine Inc., Modrice, Czech Republic) and intra- and interassay CVs of 3.2 and 5.1%, respectively.

### Statistical analysis

Statistical analysis was performed using SAS software package, release 9.1 (SAS Institute, Cary, NC). All tests were performed two sided with $P < 0.05$ considered as statistically significant. Plasma levels of resistin, high-sensitivity CRP, creatinine, and IL-6 were log transformed to normalize their distributions. Age- and sex-adjusted baseline characteristics and geometric mean resistin levels were compared between cases and noncases using analysis of covariance. Correlations between resistin levels and potential cardiovascular risk factors were assessed using Pearson's age- and sex-adjusted partial correlation coefficient.

We examined the association of plasma resistin levels with risk of MI and IS by calculating relative risks (RRs) using Cox proportional-hazards regression, modified according to the method of Prentice (16) to account for the case-cohort design. Using this approach, participants within the subcohort are given a weight of 1 at all times, whereas cases outside the subcohort are assigned a weight of 1 at time of event and have weight 0 at all other times. Age was used as the underlying time variable in the

counting process with entry and exit time defined as the subject's age at recruitment and age at MI or IS diagnosis or censoring, respectively. We considered only the first event because of concern that this event would lead to changes in the subjects' risk factors. The risk of MI and IS was analyzed in separate regression models for quartiles of resistin levels based on the subcohort.

We present four regression models: The first, crude model includes age, sex, and resistin levels. The second model further includes smoking status (never smoker, past smoker > 5 yr, past smoker ≤ 5 yr, current smoker less than 20 cigarettes per day, current smoker ≥ 20 cigarettes/d); sports (less than 2 h/d *vs.* ≥ 2 h/d); education (vocational school or no vocational training, technical school, university); BMI (continuously); waist circumference (continuously); and alcohol consumption (men: < 2 g/d, 2–15 g/d, > 15 g/d; women: < 1 g/d, 1–7.5 g/d, > 7.5 g/d); history of diabetes; history of hypertension; and HDL-C and TC. The third model additionally includes CRP, and the fourth model additionally includes creatinine. To test whether the associations of resistin levels with cardiovascular events differ between MI and IS, we also evaluated these relationships for log-transformed resistin levels on a continuous scale in a common regression model using the data augmentation method described previously (23, 24). In this analysis, each subject has a separate observation for each outcome. We assumed different associations of covariates with the two outcomes by including interaction terms between each covariate with type of outcome in each model. To be less sensitive against violations of the proportional hazards assumption, we stratified the analysis by age at recruitment and type of event. Interactions between resistin levels and subgroups were tested with a cross-product term (subgroup × log transformed resistin levels) in the fully adjusted model.

## Results

Subjects with incident MI or IS were older and were more likely to be male, to have a history of diabetes and hypertension, and to have ever smoked (Table 1). In analyses adjusted for age and sex, individuals with subsequent MI or stroke had significantly higher levels of CRP and IL-6. HDL-C levels were lower among subjects with MI but not among those with stroke when compared with individuals without events. Compared with subjects without cardiovascular events, resistin levels were significantly higher among individuals with incident MI, whereas no such difference was observed for IS.

Table 2 shows age- and sex-adjusted Pearson correlation coefficients between plasma resistin levels and selected cardiovascular risk factors for the subcohort. Resistin was weakly correlated with creatinine ($r = 0.19$, $P < 0.0001$), CRP ($r = 0.08$, $P = 0.02$), and IL-6 levels ($r = 0.08$, $P = 0.02$), respectively. A weak inverse correlation between resistin levels and TC ($r = -0.13$, $P < 0.001$) and HDL-C ($r = -0.09$, $P < 0.01$) was observed.

Table 3 shows the estimated RRs of MI and IS during follow-up across quartiles of resistin levels at baseline. After adjustment for age and sex, individuals in the highest quartile of resistin levels had significantly increased risk of MI [RR 2.03;

**TABLE 1.** Characteristics of subjects who developed MI or IS and subjects who remained free of those cardiovascular events during follow-up in EPIC-Potsdam[a]

| Characteristics | Subjects without incident cardiovascular events | Subjects with incident cardiovascular events | | | |
|---|---|---|---|---|---|
| | | MI | $P^b$ | IS | $P^b$ |
| n | 817 | 139 | | 97 | |
| Men, % | 39.2 | 76.3 | <0.0001 | 56.7 | 0.0007 |
| Age, yr, mean ± SE | 49.9 ± 0.28 | 55.4 ± 0.69 | <0.0001 | 56.3 ± 0.82 | <0.0001 |
| Education, % | | | 0.37 | | 0.33 |
| Vocational school or no vocational training | 36.2 | 40.6 | | 40.3 | |
| Technical school | 24.6 | 26.6 | | 28.7 | |
| University | 39.2 | 32.7 | | 31.0 | |
| Smoking status, % | | | <0.0001 | | 0.048 |
| Never smokers | 45.5 | 26.8 | | 36.9 | |
| Past smokers, >5 yr | 27.0 | 15.5 | | 35.9 | |
| Past smokers, ≤5 yr | 7.9 | 6.7 | | 1.5 | |
| Current smokers, <20 cigarettes/d | 14.6 | 25.5 | | 18.1 | |
| Current smokers, ≥20 cigarettes/d | 5.0 | 25.6 | | 7.7 | |
| Diabetes, % | 1.0 | 14.2 | <0.0001 | 15.4 | <0.0001 |
| Hypertension, % | 46.5 | 67.4 | <0.0001 | 67.6 | <0.0001 |
| BMI, kg/m$^2$, mean ± SE | 25.9 ± 0.1 | 26.7 ± 0.3 | <0.02 | 26.7 ± 0.4 | 0.05 |
| Waist circumference, men, cm, mean ± SE | 95.0 ± 0.5 | 97.0 ± 0.9 | 0.06 | 96.5 ± 1.3 | 0.27 |
| Waist circumference, women, cm, mean ± SE | 79.2 ± 0.4 | 83.9 ± 1.7 | 0.0061 | 81.5 ± 1.5 | 0.13 |
| Sports ≥ 2 h, % | 21.4 | 14.6 | 0.08 | 19.4 | 0.65 |
| Alcohol, g/d, geometric mean (CI) | 7.5 (6.8–8.2) | 4.6 (3.7–5.8) | 0.0001 | 7.2 (5.5–9.4) | 0.78 |
| TC, mmol/liter, mean ± SE | 4.62 ± 0.03 | 5.02 ± 0.07 | <0.0001 | 4.66 ± 0.08 | 0.59 |
| HDL-C, mmol/liter, mean ± SE | 1.21 ± 0.01 | 1.11 ± 0.02 | <0.0001 | 1.20 ± 0.03 | 0.72 |
| CRP, mg/liter, geometric mean (CI) | 0.62 (0.56–0.68) | 1.31 (1.05–1.63) | <0.0001 | 1.17 (0.90–1.52) | <0.0001 |
| IL-6, pg/ml, geometric mean (CI) | 1.21 (1.15–1.26) | 1.68 (1.50–1.88) | <0.0001 | 1.59 (1.39–1.83) | 0.0002 |
| Creatinine, μmol/liter, geometric mean (CI)[c] | 65.2 (64.5–66.0) | 65.8 (64.0–67.6) | <0.56 | 63.5 (61.4–65.6) | 0.13 |
| Resistin, ng/ml, geometric mean (CI)[c] | 2.42 (2.36–2.47) | 2.66 (2.52–2.81) | 0.0016 | 2.34 (2.19–2.49) | 0.36 |

[a] All variables other than number of individuals, gender, and age were adjusted for gender and age. Number of individuals and gender were unadjusted. Age was adjusted for gender only. The results were obtained using analysis of covariance.

[b] *P* value for the difference between subjects with incident events and subjects without incident events.

[c] Gemoetric means and CIs are based on reduced numbers of cases or noncases. IL-6 values are based on 95 cases of IS and 138 cases of MI. Creatinine values are based on 788 noncases and 96 stroke cases.

**TABLE 2.** Age- and sex-adjusted Pearson correlation coefficients between log-transformed plasma resistin levels and selected cardiovascular risk factors in the subcohort

| | Subcohort | | |
|---|---|---|---|
| | n | R | *P* |
| Age[a] | 827 | 0.07 | 0.05 |
| BMI | 827 | 0.02 | 0.58 |
| Waist | 827 | 0.01 | 0.85 |
| TC | 827 | −0.13 | <0.001 |
| HDL-C | 827 | −0.09 | <0.01 |
| Log-CRP | 827 | 0.08 | 0.02 |
| Log-IL-6 | 827 | 0.08 | 0.02 |
| Log-creatinine | 798 | 0.19 | <0.0001 |
| Systolic blood pressure | 802 | −0.08 | 0.02 |
| Diastolic blood pressure | 802 | −0.04 | 0.27 |

[a] Only sex adjusted.

95% confidence interval (CI) 1.16–3.56; *P* trend across quartiles = 0.022], compared with participants in the lowest quartile of resistin levels. Further adjustment for smoking status, BMI, waist circumference, alcohol consumption, education, leisure time physical activity, hypertension, diabetes, and plasma levels of TC, HDL-C, CRP, and creatinine did not substantially change these results (RR: 2.05; 95% CI 0.97–4.30; *P* trend = 0.021). In contrast, plasma resistin levels were not related to risk of IS (RR in the fully adjusted model, 0.99; 95% CI 0.53–1.84; *P* trend = 0.96, Table 3). We further evaluated these associations in competing risk analyses using log-transformed resistin on a continuous scale. Similar to the quartile analysis, resistin was positively related to risk of MI (multivariable adjusted RR = 1.32; 95% CI 1.07–1.63 per 1 SD increase) but not to risk of IS (RR 0.89; 95% CI 0.70–1.12, *P* difference between end points, *P* = 0.006). Additional adjustment for IL-6 also did not change the results (data not shown). The associations of resistin levels with risk of cardiovascular events was not significantly different between men and women (*P* for interaction of log-transformed resistin levels with sex, 0.49 for MI, and 0.73 for IS).

Table 4 shows the multivariable-adjusted RR of MI per increase of log-transformed resistin levels by 1 SD in subgroups of the study population defined by the presence or absence of selected cardiovascular risk factors. The relative risk estimates were generally similar across most strata but lacked statistical significance in many subgroups, likely due to small sample size. In participants without hypertension we observed a null association between resistin levels and risk of MI (RR = 1.09; 95% CI 0.70–1.72; *P* = 0.70), whereas the association between resistin and MI was pronounced in participants with a history of hypertension (RR = 1.46; 95% CI 1.12–1.91; *P* = 0.005). However, the interaction term for resistin and hypertension was only borderline significant (*P* = 0.06). With respect to this result, we also investigated the association between plasma resistin levels and risk of IS among participants with prevalent hypertension in a subgroup analysis. However, we did not observe an association between resistin levels and risk of IS (RR = 0.95; 95% CI 0.70–1.27; *P* = 0.72) among hypertensive subjects in the multivariable adjusted model.

Because our subset included only a limited number of participants with diabetes at baseline, we were not able to calculate effect estimates within this subgroup; however, exclusion of these participants did not substantially alter the results. Thus, when participants with diabetes were excluded, the RR for MI in the fully adjusted model across quartiles of resistin levels were 1 (reference); 1.19 (95% CI 0.54–2.26); 1.86 (0.88–3.95); and 2.06 (0.98–4.33); *P* for trend = 0.018. Multivariable adjusted RR per 1 SD increase of log-transformed resistin was 1.34 (95% CI 1.07–1.66, *P* = 0.01).

**TABLE 3.** Relative risks of MI and IS according to quartiles of plasma resistin levels[a]

| | Quartiles of resistin levels | | | | |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | *P* for trend |
| Subcohort participants (n) | 206 | 207 | 207 | 207 | |
| Resistin ng/ml | <2.00 | 2.00–2.42 | 2.43–2.95 | >2.95 | |
| MI | | | | | |
| Cases (n) | 24 | 38 | 34 | 43 | |
| Sex adjusted | 1 (Reference) | 1.54 (0.87–2.72) | 1.48 (0.83–2.73) | 2.03 (1.16–3.56) | 0.022 |
| Model 2[b] | 1 (Reference) | 1.43 (0.69–2.96) | 2.08 (1.03–4.22) | 2.37 (1.18–4.73) | 0.0047 |
| Model 3[c] | 1 (Reference) | 1.29 (0.61–2.73) | 2.06 (1.01–4.31) | 2.09 (1.01–4.31) | 0.013 |
| Model 4[d] | 1 (Reference) | 1.42 (0.66–3.04) | 2.19 (1.07–4.51) | 2.05 (0.97–4.30) | 0.021 |
| IS | | | | | |
| Cases, n | 24 | 26 | 21 | 26 | |
| Sex adjusted | 1 (Reference) | 0.87 (0.48–1.58) | 0.83 (0.45–1.55) | 1.07 (0.60–1.92) | 0.85 |
| Model 2[b] | 1 (Reference) | 1.08 (0.58–2.01) | 1.12 (0.59–2.11) | 1.08 (0.59–1.99) | 0.80 |
| Model 3[c] | 1 (Reference) | 0.99 (0.53–1.84) | 1.05 (0.55–1.99) | 0.94 (0.51–1.73) | 0.88 |
| Model 4[d] | 1 (Reference) | 1.06 (0.56–2.06) | 1.07 (0.55–2.05) | 0.99 (0.53–1.84) | 0.96 |

[a] All models were derived from Cox proportional hazard regression with age as underlying time variable. Quartiles are based on the distribution of plasma resistin levels in the subcohort.

[b] Model 2 adjusted for sex, smoking status, BMI, waist circumference, alcohol intake, education, sports, TC, HDL-C, prevalent hypertension, and prevalent diabetes.

[c] Model 3 adjusted as model 2 plus CRP.

[d] Model 4 adjusted as model 3 plus creatinine (based on 788 noncases, all MI cases, or 96 IS cases).

**TABLE 4.** Multivariable adjusted RRs of MI per increase of log-transformed resistin levels by 1 SD for subgroups[a]

| Group | Cases | Controls | RR (95% CI) | *P* value | *P* value for interaction[b] |
|---|---|---|---|---|---|
| All | 139 | 793 | 1.32 (1.07–1.63) | 0.009 | |
| Age, yr | | | | | |
| < 60 | 93 | 310 | 1.24 (0.96–1.61) | 0.011 | 0.96 |
| ≥ 60 | 46 | 483 | 1.81(1.20–3.31) | 0.05 | |
| Sex | | | | | |
| Men | 106 | 310 | 1.29 (1.00–1.68) | 0.05 | 0.49 |
| Women | 33 | 483 | 1.26 (0.82–1.94) | 0.29 | |
| Hypertension | | | | | |
| No | 35 | 440 | 1.09 (0.70–1.72) | 0.70 | 0.06 |
| Yes | 104 | 353 | 1.46 (1.12–1.91) | 0.005 | |
| Overweight | | | | | |
| No (BMI < 25 kg/m$^2$) | 32 | 353 | 1.32 (0.77–2.27) | 0.32 | 0.86 |
| Yes (BMI ≥ 25 kg/m$^2$) | 107 | 440 | 1.34 (1.03–1.73) | 0.03 | |
| Current smoking | | | | | |
| No | 70 | 633 | 1.42 (1.11–1.81) | 0.005 | 0.97 |
| Yes | 69 | 160 | 1.28 (0.87–1.88) | 0.21 | |
| TC, mmol/liter | | | | | |
| < 5.2 | 82 | 639 | 1.45 (1.10–1.91) | 0.008 | 0.67 |
| ≥ 5.2 | 57 | 154 | 1.38 (1.03–1.83) | 0.03 | |
| HDL, mmol/liter | | | | | |
| < 1.0 | 76 | 631 | 1.20 (0.92–1.56) | 0.19 | 0.94 |
| ≥ 1.0 | 63 | 162 | 1.11 (0.72–1.72) | 0.64 | |
| CRP, mg/liter | | | | | |
| <1.0 | 53 | 498 | 1.41 (0.97–2.04) | 0.07 | 0.76 |
| ≥1.0 | 86 | 295 | 1.15 (0.81–1.63) | 0.43 | |
| Alcohol intake | | | | | |
| < 7.5 g/d in women, < 15 g/d in men | 84 | 432 | 1.23 (0.96–1.57) | 0.11 | 0.40 |
| ≥ 7.5 g/d in women, ≥ 15 g/d in men | 55 | 361 | 1.40 (1.00–1.95) | 0.05 | |

[a] All models were derived from Cox proportional hazard regression with age as underlying time variable. All models are adjusted for sex, smoking status, BMI, waist circumference, alcohol intake, education, sports, TC, HDL-C, CRP, prevalent hypertension, prevalent diabetes, CRP, and creatinine.

[b] *P* values for interaction were calculated using dichotomous variables (which were used for stratification of risk factors) and log-transformed resistin levels.

## Discussion

The results of this prospective case-cohort study suggest that high plasma resistin levels are related to an increased risk of MI, whereas no such relationship was observed between resistin and the risk of IS. This association was independent of other established cardiovascular risk factors, including age, sex, smoking status, hypertension, diabetes, BMI, waist circumference, education, leisure time physical activity, alcohol, TC, HDL-C, CRP, and creatinine.

To our knowledge, this is the first prospective study reporting on an association between resistin levels and future risk of MI in a general population without history of major CVD. Our findings are in line with results from previous case-control and cross-sectional studies (11–13, 25, 26). These studies either observed higher resistin levels in CHD cases, compared with noncases (11, 12, 27), or reported on correlations between resistin levels and coronary artery calcification (26). Thus, a case-control study in women found that resistin levels were significantly higher in subjects with CHD, compared with controls, independent of traditional cardiovascular risk factors, such as hypertension, diabetes, smoking, and BMI (13). However, in that case-control study, the relationship was substantially attenuated and no longer significant after adjustment for CRP levels (13). In contrast, although in our study the association between resistin levels and risk of MI was attenuated after adjustment for CRP, it remained statistically significant, suggesting that the association between resistin levels and risk of MI can only partly be explained by elevated CRP. Our findings are supported by data from Reilly *et al.* (26), who reported significant associations between resistin levels and coronary artery calcification independent of CRP levels.

Resistin was suggested to affect endothelial dysfunction and the migration of vascular smooth muscle cells, (28), which are key pathophysiological mechanisms of atherosclerosis (29). Resistin may thus be involved in pathways that lead to CVD. In humans, resistin was found to be expressed mainly in inflammatory cells, such as macrophages (5), and activation of the inflammatory cascade has been shown to induce the expression of resistin (30). Some studies found positive relationships of resistin levels with plasma inflammatory markers, such as IL-6, soluble TNF-$\alpha$ receptor 2, or adhesion molecules; however, complex interactions exist between resistin and inflammatory cytokines (6, 28, 30), and the precise role of resistin in the context of inflammation and cardiovascular disease remains to be fully clarified (9, 26, 31).

Recent data from an investigation in American Indians suggest that the relationship of resistin with cardiovascular disease observed in case-control studies may be explained by its association with diabetic nephropathy (25). However, although in our study resistin levels were correlated with plasma creatinine lev-

els, the association between resistin and risk of MI was not substantially affected by adjustment for creatinine levels.

We found a stronger association between plasma resistin levels and risk of MI among hypertensive subjects. It is unclear whether this reflects a true relationship or the play of chance in the light of multiple comparisons. Therefore, this findings need to be confirmed in future studies.

Because atherosclerosis is a major precursor of both MI and IS, it was surprising that we found a strong association between resistin levels and risk of MI but no significant association for risk of IS. However, other established risk factors of atherosclerosis differ considerably in their importance for the development of MI or IS, including hypertension, smoking, and cholesterol (19, 27, 32, 33). Thus, it is conceivable that specific processes linked to resistin may be more important for coronary artery disease but less relevant for the development of cerebrovascular events. This notion is underlined by a number of previous studies, which found no association of resistin levels with intima media thickness of carotid arteries (9, 34, 35). These putative pathogenic differences between cerebrovascular and coronary events are supported by the fact that coronary atherosclerosis manifests earlier in life than cerebral atherosclerosis (36). Moreover, ischemic stroke comprises different subtypes, *i.e.* large-artery sclerosis, cardioembolic infarction, and small vessel-disease, which may considerably differ in their risk factor profile. Our study lacks information on subtypes of IS, and we were not able to investigate associations between resistin and risk of particular subtypes.

Among the strengths of this study are the prospective study design; the measurements of biomarkers, which was blinded with respect to the case-control status; and the comprehensive data on study participants allowing for adjustment for other risk factors. All cases of MI and stroke were validated by medical records, treating physicians, or death certificates, thus providing a high specificity in identifying incident cases. Nevertheless, some limitations of our study should be discussed. Our results are based on single measurements of resistin levels. However, data from a recent reproducibility study suggest that a single resistin measurement may be sufficient for risk assessment (37). Resistin levels were measured in fasting and nonfasting individuals. Although a recent study reported that resistin levels may increase after a standardized liquid meal challenge (38), other studies found no substantial effects of fasting status on resistin levels (37, 39). Furthermore, recent studies suggest that resistin may exist in different isoforms, which were not considered in our investigation (40). We can therefore not exclude the possibility that the association of resistin with cardiovascular events vary for these different isoforms. Although randomly selected from the source population, our subcohort included only a small number of subjects with prevalent diabetes and may therefore not be entirely representative for the source population. However, results were similar when participants with prevalent diabetes were excluded from the analyses. Furthermore, the biological relationship between resistin and incidence of cardiovascular disease should be similar to men and women in the general population.

In conclusion, our data suggest that plasma resistin levels may be associated with the risk of MI but not IS. According to our data, this association is largely independent of CRP and other established risk factors of cardiovascular diseases. However, the pathogenic mechanisms underlying the observed association remain to be elucidated. Moreover, it is unclear why resistin plasma levels may relate to the risk of myocardial infarction but being less relevant for ischemic stroke.

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