Resistin, exercise capacity, and inducible ischemia in patients with stable coronary heart disease: Data from the Heart and Soul study

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\textbf{A R T I C L E   I N F O}

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\textbf{A B S T R A C T}

\textbf{Objective:} Resistin is an adipocytokine involved in insulin resistance, inflammation, and atherosclerosis. Its role in the development and progression of coronary heart disease (CHD) is not yet well-characterized. We performed a cross-sectional study to evaluate the association between serum resistin levels, exercise capacity, and exercise-induced cardiac ischemia among patients with stable CHD.

\textbf{Methods and results:} We measured serum resistin concentrations and determined treadmill exercise capacity and inducible ischemia by stress echocardiography in 899 outpatients with documented CHD. Of these, 215 (24%) had poor exercise capacity (<5 metabolic equivalent tasks), and 217 (24%) had inducible ischemia. As compared with participants who had resistin levels in the lowest quartile, those with resistin levels in the highest quartile were more likely to have poor exercise capacity (33% versus 16%, odds ratio [OR] 2.68, \(P = 0.0001\)) and inducible ischemia (30% versus 17%, OR 2.08, \(P = 0.001\)). Both associations remained robust after adjusting for numerous clinical risk factors, metabolic variables, and markers of insulin resistance (poor exercise capacity adjusted OR 1.73, \(P = 0.04\); inducible ischemia adjusted OR 1.82, \(P = 0.01\)). However, further adjustments for C-reactive protein, interleukin-6, and tumor necrosis factor-\(\alpha\) eliminated the association with poor exercise capacity (adjusted OR 1.50, \(P = 0.14\)) and substantially weakened the association with inducible ischemia (adjusted OR 1.72, \(P = 0.03\)).

\textbf{Conclusions:} Elevated serum resistin is associated with poor exercise capacity and exercise-induced cardiac ischemia in patients with stable coronary disease. Adjustment for inflammatory markers attenuated these associations, suggesting a possible role for resistin in inflammation and CHD pathophysiology.

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1. Introduction

Resistin is a signaling protein that has generated much interest as a potential mediator of insulin resistance and atherosclerosis. Initially characterized in mice, resistin is secreted almost exclusively by adipocytes and causes obesity-related diabetes [1,2]. However, studies in humans have revealed that resistin is not well-conserved structurally or functionally between mice and humans [3,4]. In humans, resistin is expressed primarily in monocytes and was found to be associated with inflammatory markers, but not with measures of insulin resistance [5–8]. Proinflammatory cytokines such as interleukin-1, interleukin-6, C-reactive protein, and tumor necrosis factor-\(\alpha\) appear to be associated with increased resistin expression, but resistin itself stimulates the production of tumor necrosis factor-\(\alpha\), monocyte chemotactic protein-1, brain-type natriuretic peptide, and endothelial adhesion molecules [9–12]. Resulting endothelial dysfunction and ischemia–reperfusion injury have been implicated as possible mechanisms by which resistin mediates atherosclerotic change and contributes to the development of coronary heart disease (CHD) [10–12].

Clinical studies have produced inconsistent results regarding the links between resistin, obesity, insulin resistance, and CHD. Some have shown elevated resistin levels to be associated with obesity and insulin resistance [13–15] in both normal and diabetic subjects while others have not [6,7]. Recent studies of resistin and coronary disease are also equivocal. One early study found elevated resistin levels to be associated with the presence and severity of coronary disease as determined by angiography [16], but other studies failed to find an association in patients with known CHD [6,17]. To date, no study has directly evaluated the relationship between resistin and cardiac functional status. To determine whether resistin level is predictive of poor exercise capacity and
exercise-induced ischemia, we measured serum resistin levels and performed treadmill stress echocardiography in a cross-sectional study of 899 outpatients with stable CHD.

2. Methods

2.1. Study participants

The Heart and Soul study is a prospective cohort study investigating the effects of psychosocial factors on health outcomes in patients with stable CHD. Methods have been previously described [18,19]. We recruited outpatients identified through administrative databases from 2 Veterans Affairs hospitals, 1 university medical center, and 9 community clinics in the San Francisco Bay Area. All patients had CHD documented by a history of myocardial infarction, coronary revascularization, angiographic evidence of at least 50% stenosis in one or more coronary vessels, or exercise-induced ischemia on treadmill electrocardiogram (ECG) or nuclear perfusion imaging. They had stable disease as defined by the absence of acute coronary syndromes within the previous six months. Patients were excluded if they were unable to walk one block or were likely to move out of the area within three years. Institutional review boards at each site approved the study protocol, and all participants provided written informed consent.

Between September 2000 and December 2002, 1024 participants enrolled. All participants completed a full-day baseline study that included a comprehensive medical history and physical examination, health status questionnaires, and an exercise treadmill test that included a comprehensive medical history and physical examination results.

2.2. Serum resistin

We used the Linco Adipokine Panel A multiplex immunoassay to determine the resistin level from thawed fasting serum samples (Millipore, St. Charles, MO). The lowest detectable concentration for resistin was 6.7 pg/mL. The inter-assay coefficient of variation for this multiplexed immunoassay was 19.7–20.4%, and the intra-assay coefficient of variation was 24.48%. We assayed each sample in duplicate and reported the average of the two measurements as the result. No significant antibody cross-reactivity was observed within the panel. The laboratory technicians who performed the assays were blinded to patient characteristics and the cardiac evaluation results.

2.3. Cardiac evaluation and stress echocardiography

Participants underwent a symptom-limited, graded exercise treadmill test based on a standard Bruce protocol with continuous 12-lead ECG monitoring until the subject experienced dyspnea, symptom-limited fatigue, chest discomfort, or ECG changes suggestive of ischemia [20]. To achieve maximal heart rate, participants who were unable to continue the standard Bruce protocol for orthopedic or other reasons were switched to lower treadmill settings and encouraged to exercise for as long as possible. Exercise capacity was calculated as the total number of metabolic equivalents (1 MET = 3.5 mL of oxygen uptake/kg/min) achieved at peak exercise. Using previously published criteria, we defined those achieving <5 METs as having poor exercise capacity for our analysis [21].

Complete resting two-dimensional echocardiograms with all standard views and subcostal imaging of the inferior vena cava were performed immediately before and after exercise treadmill testing using an Acuson Sequoia ultrasound system (Siemens Medical Solutions, Mountain View, CA) with a 3.5-MHz transducer and Doppler ultrasound examination. Standard resting two-dimensional parasternal short-axis and apical two- and four-chamber views were obtained during held inspiration. We used a computerized digitization system (Tom Tec Corporation, Boulder, CO) that performed the binocular method of disks to planimeterize these views and measure end-diastolic and end-systolic left ventricular volume [22]. We calculated left ventricular ejection fraction as: (end-diastolic volume – end-systolic volume)/end-diastolic volume. Left ventricular mass was calculated using the truncated ellipsoid method and indexed to body surface area. At peak exercise, precordial long- and short-axis and apical two- and four-chamber views were obtained to assess for wall motion abnormalities. Based on prior work, we defined exercise-induced cardiac ischemia as the presence of one or more new wall motion abnormality at peak exercise that was not present at rest [18,19]. This assessment of inducible ischemia has been associated with other cardiac biomarkers and validated as a predictor of cardiovascular events. A single experienced cardiologist (NBS), who was blinded to the results of the resistin assays and clinical histories, interpreted all echocardiograms. His intra-observer consistency was 85% using blinded duplicated studies.

2.4. Other patient characteristics

Age, sex, ethnicity, medical history, and current smoking status were determined by patient questionnaires. We measured weight and height and calculated the body mass index (BMI) (kg/m^2). Systolic and diastolic blood pressures were measured with a standard sphygmomanometer. Participants were instructed to bring their medication bottles to the study appointment, and study personnel recorded all current medications. Medications were categorized using Epocrates Rx (San Mateo, CA).

Total cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides, glucose, glycylated hemoglobin, insulin, and serum creatinine were measured from 12-h fasting serum samples. C-reactive protein (CRP), interleukin-6 (IL-6), and tumor necrosis factor-α (TNFα) were measured by immunoassay of the serum samples. Because the levels were not normally distributed, these variables were log-transformed for our analysis.

2.5. Statistical analysis

Because normal ranges for serum resistin levels are not yet established, we divided participants into quartiles on the basis of their plasma resistin level for our analyses. We compared baseline participant characteristics between the quartiles using analysis of variance (ANOVA) for continuous variables and χ² test for dichotomous variables. We performed logistic regression analysis with serum resistin level as the predictor variable and poor exercise capacity and inducible ischemia as categorical outcomes. We compared the rates of poor exercise capacity and inducible ischemia across quartiles of resistin levels. We further determined the odds associated with the continuous value of resistin, which we log-transformed to normalize the skewed distribution and expressed as odds per standard deviation (SD) increase in log resistin level. To evaluate the independent association of resistin with poor exercise capacity and inducible ischemia, we adjusted for known clinical risk factors, metabolic variables, and markers of insulin resistance and inflammation. We further tested for statistical interactions of resistin with age, sex, race, BMI, serum creatinine, history of diabetes, and use of diabetes medications to explore potential modifying effects of these factors. Analyses were performed using Statistical Analysis Software (version 9; SAS Institute Inc, Cary, NC).
Abbreviations

3. Results

The 899 study participants had a median resistin level of 8.48 ng/mL and an interquartile range of 5.84–11.88 ng/mL. Compared with those in the lowest quartile of resistin (<5.84 ng/mL), participants in the highest quartile (>11.88 ng/mL) were older and more likely to be white (Table 1). Although those with higher levels of resistin did not differ significantly in their medical histories, medication use, or metabolic characteristics, they had higher levels of serum creatinine, CRP, IL-6, and systolic blood pressure.

Of the 899 participants, 215 (24%) had poor exercise capacity (<5 METs), and 217 (24%) had inducible ischemia. The proportion of participants with both poor exercise capacity and inducible ischemia ranged from 17% in the lowest quartile to 30% in the highest quartile of resistin (P for trend = 0.0001). The proportion of participants with ischemia ranged from 17% in the lowest quartile to 33% in the highest quartile of resistin (P = 0.0002). Participants with both poor exercise capacity and inducible ischemia had the highest levels of serum resistin (Table 2).

3.1. Exercise capacity

When analyzed as a continuous variable, each SD (0.58 ng/mL) increase in log resistin was associated with a 51% greater odds of having poor exercise capacity (odds ratio [OR], 1.51; 95% confidence interval [CI], 1.28–1.78; P = 0.0001) (Table 3). This association remained robust after adjustment for age, sex, race, systolic blood pressure, serum creatinine, BMI, total cholesterol, HDL cholesterol, non-HDL cholesterol, and triglycerides (OR, 1.27; 95% CI, 1.06–1.53; P = 0.01). Further adjustment for measures of insulin resistance (serum insulin, fasting blood glucose, glycosylated hemoglobin, history of diabetes, and 3.2. Cardiac function

LV ejection fraction (%)
62 ± 9
63 ± 10
61 ± 10
62 ± 9
0.42
LV mass index
95 ± 25
97 ± 24
98 ± 23
98 ± 27
0.57
Heart rate at rest (beats/min)
68 ± 12
67 ± 12
68 ± 12
69 ± 12
0.63
Systolic blood pressure (mmHg)
129 ± 19
130 ± 18
133 ± 18
134 ± 18
0.004
Diastolic blood pressure (mmHg)
75 ± 11
74 ± 10
73 ± 10
74 ± 10
0.24

Serum resistin

Fig. 1. Proportion of poor exercise capacity and inducible ischemia by quartile of resistin in 899 participants with coronary heart disease.
Inducible ischemia (OR, 1.26; 95% CI, 1.08–1.48; *P* = 0.02) was associated with 26% greater odds of poor exercise capacity in the 664 participants under age 75 (OR, 1.94; 95% CI, 1.04–3.61; *P* = 0.07). As compared with participants who had resistin levels in the lowest quartile, those with resistin levels in the highest quartile had a 77% greater odds of poor exercise capacity (OR, 1.77; 95% CI, 1.30–2.36; *P* = 0.001) (Table 3). This association remained significant after sequential adjustment for clinical risk factors, metabolic variables, insulin resistance, and markers of inflammation (OR, 1.72; 95% CI, 1.05–2.81; *P* = 0.03). We observed no evidence of interaction between resistin and age, sex, race, BMI, serum creatinine, or use of diabetes medications (all *P* values for interaction >0.10). However, the association between resistin and poor exercise capacity appeared to differ by age (Table 4). This association was mildly attenuated after each step of adjustment for clinical risk factors, metabolic variables, markers of insulin resistance, and markers of inflammation, with comparable contribution by each step. The final adjustment model demonstrated a non-significant association between resistin and inducible ischemia (OR, 1.18; 95% CI, 0.98–1.41; *P* = 0.08).

We observed no evidence of interaction between resistin and age, sex, race, BMI, serum creatinine, diabetes, or use of diabetes medications (all *P* values for interaction >0.10). However, the association of resistin with poor exercise capacity appeared to differ by age (Table 4). Elevated resistin levels were more strongly associated with poor exercise capacity in the 664 participants under age 75 (OR, 1.94; 95% CI, 1.04–3.61; *P* = 0.04) than in the 235 participants age 75 or older (OR, 1.46; 95% CI, 0.59–3.04; *P* = 0.41) (Table 4).

### 3.2. Inducible ischemia

When analyzed as a continuous variable, each SD (0.58 ng/mL) increase in log resistin was associated with a 26% greater odds of inducible ischemia (OR, 1.26; 95% CI, 1.08–1.48; *P* = 0.004) (Table 3). This association was mildly attenuated after each step of adjustment for clinical risk factors, metabolic variables, markers of insulin resistance, and markers of inflammation, with comparable contribution by each step. The final adjustment model demonstrated a non-significant association between resistin and inducible ischemia (OR, 1.18; 95% CI, 0.98–1.41; *P* = 0.08).

### 4. Discussion

In this cross-sectional study of 899 ambulatory outpatients with stable CHD, we found that elevated serum resistin levels were associated with poor exercise capacity and inducible cardiac ischemia. Participants with resistin levels in the highest quartile had almost
double the odds of poor exercise capacity and inducible ischemia as compared with those who had resistin levels in the lowest quartile. These associations were modestly weakened by adjustment for clinical risk factors and insulin resistance but were eliminated after adjustment for greater inflammation in those with high resistin levels. Although our cross-sectional study cannot determine the causal pathways, our findings suggest that resistin is associated with worse cardiac function, and that inflammation may contribute to this association.

Resistin was initially isolated from mice adipocytes but has since been found to be secreted predominantly by monocytes in humans [3,4]. Therefore, the degree to which resistin interacts with metabolic versus inflammatory mechanisms remains an active area of research. Our findings suggest that while both may be involved, resistin likely exerts its predominant effects on coronary disease via inflammatory pathways. Consistent with laboratory and animal studies on the feedback interactions between resistin and other inflammatory cytokines [9], we found serum resistin cannot be strongly associated with inflammatory biomarkers CRP and IL-6. By contrast, participants with elevated serum resistin did not have significantly different lipid profiles or markers of impaired glucose metabolism. Adjustment for insulin resistance moderately attenuated the association of high serum resistin levels with poor exercise capacity and inducible ischemia to comparable degrees. However, adjustment for CRP, IL-6, and TNFα substantially attenuated these associations, suggesting that inflammatory pathways may contribute more significantly to the link between resistin and cardiovascular disease.

Clinical studies on the relationship between resistin, diabetes, and CHD have produced ambiguous results. Resistin has been shown to be associated with obesity and insulin resistance in some studies [13–15] but not in others [6,7]. Elevated resistin levels have been associated with CHD in patients with premature coronary disease, in angiography patients, in patients with end-stage renal disease, and in women [11,16,23,24], yet some patients with established CHD did not have high levels of serum resistin [6,17]. Consistent with prior studies [6,24,25], we found that our participants in the highest quartile of serum resistin also had the worst renal function as measured by serum creatinine. Therefore, one possible explanation for elevated serum resistin is the reduced renal clearance of this molecule. However, the association between serum resistin and poor cardiac function remained robust after adjustment for serum creatinine and other basic clinical risk factors, suggesting that renal dysfunction does not adequately explain our findings.

Our study is the first to directly evaluate the association between resistin and cardiac function in patients with stable CHD. Treadmill exercise capacity is a well-validated measure of cardiac functional status that is commonly used in clinical practice [21,26]. Inducible ischemia by stress echocardiography has also been validated as a predictor of poor cardiovascular outcome and mortality [19,27]. Evaluating for ventricular dysfunction on echocardiogram after treadmill testing with both normal and reduced exercise capacity improves prognostication and risk stratification for future cardiovascular events [28,29]. Because serum resistin was found to be associated with both poor exercise capacity and inducible ischemia, resistin may be a mediator of adverse cardiovascular outcomes and/or a predictor of CHD severity and prognosis. However, given our still limited understanding of this novel molecule, it would be premature for serum resistin to replace more established biomarkers, such as CRP, or noninvasive cardiac imaging for risk stratification and disease monitoring in CHD [30,31]. Further research is needed to characterize the clinical significance of the serum resistin level and its role in diagnostic testing.

We found that the association between resistin and exercise capacity was modified by age. Elevated resistin was significantly associated with poor exercise capacity in participants younger than 75 years of age, but not in participants 75 years of age or older. This is likely because exercise capacity declines with age [32,33], and poor exercise capacity was extremely common among our older patients with diabetes. Of note, there were only 227 participants with age ≥75 (N = 235) and only 235 of our 899 participants were 75 years of age or older. This is likely because exercise capacity declines with age [32,33], and poor exercise capacity was extremely common among our older patients with diabetes.

Table 4

<table>
<thead>
<tr>
<th>Resistin category</th>
<th>Proportion with poor exercise capacity</th>
<th>Odds ratio (95% CI) quartile IV versus I of resistin†</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Participants with age ≥75 (N = 235)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quartiles I (N = 38)</td>
<td>29% (11/38)</td>
<td>1.46 (0.59–3.64)</td>
<td>0.41</td>
</tr>
<tr>
<td>Quartile IV (N = 73)</td>
<td>42% (31/73)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Participants with age &lt;75 (N = 664)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quartiles I (N = 187)</td>
<td>13% (24/187)</td>
<td>1.94 (1.04–3.61)</td>
<td>0.04</td>
</tr>
<tr>
<td>Quartile IV (N = 151)</td>
<td>28% (43/151)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

† Adjusted for age, sex, race, systolic blood pressure, serum creatinine, BMI, total cholesterol, HDL-cholesterol, non-HDL cholesterol, triglycerides, serum insulin, fasting blood glucose, glycosylated hemoglobin, history of diabetes, and use of diabetes medications.

Table 5

<table>
<thead>
<tr>
<th>Resistin category</th>
<th>Proportion with inducible ischemia</th>
<th>Odds ratio (95% CI) quartile IV versus I of resistin†</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Participants with a history of diabetes mellitus (N = 227)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quartiles I (N = 58)</td>
<td>22% (13/58)</td>
<td>0.84 (0.32–2.15)</td>
<td>0.71</td>
</tr>
<tr>
<td>Quartile IV (N = 65)</td>
<td>26% (17/65)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Participants with no history of diabetes mellitus (N = 671)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quartiles I (N = 166)</td>
<td>16% (26/166)</td>
<td>2.30 (1.30–4.07)</td>
<td>0.004</td>
</tr>
<tr>
<td>Quartile IV (N = 159)</td>
<td>32% (51/159)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

† Adjusted for age, sex, race, systolic blood pressure, serum creatinine, BMI, total cholesterol, HDL-cholesterol, non-HDL cholesterol, triglycerides, serum insulin, fasting blood glucose, glycosylated hemoglobin, history of diabetes, and use of diabetes medications.
with diabetes, therefore limiting our ability to detect a significant association between resistin and ischemia in this subgroup. However, the absolute rate of inducible ischemia was notably higher in non-diabetics with elevated resistin levels (32%) than it was in diabetics in the same resistin quartile (26%). These findings raise the possibility that resistin may be on the causative pathway to inducible ischemia via a mechanism involving insulin resistance. In diabetics, perhaps there is a ceiling effect where insulin resistance is already so high that resistin cannot make it worse. Alternatively, resistin may be displaced by some other mediator of insulin resistance that is present in diabetics.

Lastly, the use of diabetes medications may indirectly target resistin and its downstream effects. Most notably, some previous studies have found the diabetes medication rosiglitazone, a transcription regulator peroxisome proliferator-activated receptor (PPARγ) agonist, to decrease resistin expression [3,35,36]. However, a small prospective double-blinded randomized study of rosiglitazone versus placebo did not find a significant difference in serum resistin levels following 12 weeks of medication use [37]. Furthermore, rosiglitazone was only recently approved by the Federal Drug Administration in 1999, so at the time of study enrollment in 2000–02, rosiglitazone was not widely used by participants in our study for diabetes and unlikely to have contributed significantly to our findings.

Strengths of this study include its relatively large sample size and the careful measurement of a wide range of potential confounding variables. However, several limitations should be considered when interpreting our results. First, most of our participants were older men, all with stable CHD. Therefore, we cannot generalize our findings to women, younger populations, healthy populations without prevalent coronary disease, or patients who had recent acute coronary events. Second, the resistin assays had a variation of 20%. However, we assayed all samples in duplicate and used the average of the two samples as our measurement of serum resistin. Furthermore, any measurement variation that were to occur would bias the results toward null. Lastly, we cannot determine the direction of association or establish causality in our observations of resistin and cardiac function due to the cross-sectional design of this study. Therefore, mechanisms underlying these findings must be explored in future studies.

In summary, we found that higher levels of serum resistin were associated with poor exercise capacity and inducible cardiac ischemia among patients with stable CHD. This association appeared to be most significantly explained by greater inflammation in patients with high resistin levels, although metabolic factors and insulin resistance also contributed to this association. Likely a component of multiple complex pathways, resistin warrants further study as a possible mediator of coronary heart disease.

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