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Adipokines, Insulin Resistance, and Coronary Artery Calcification

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Objectives
We evaluated the hypothesis that plasma levels of adiponectin and leptin are independently but oppositely associated with coronary artery calcification (CAC), a measure of subclinical atherosclerosis. In addition, we assessed which biomarkers of adiposity and insulin resistance are the strongest predictors of CAC beyond traditional risk factors, metabolic syndrome, and plasma C-reactive protein (CRP).

Background
Adipokines are fat-secreted biomolecules with pleiotropic actions that converge in diabetes and cardiovascular disease.

Methods
We examined the association of plasma adipocytokines with CAC in 860 asymptomatic, nondiabetic participants in the SIRCA (Study of Inherited Risk of Coronary Atherosclerosis).

Results
Plasma adiponectin and leptin levels had opposite and distinct associations with adiposity, insulin resistance, and inflammation. Plasma leptin was positively (top vs. bottom quartile) associated with higher CAC after adjustment for age, gender, traditional risk factors, and Framingham risk scores (tobit regression ratio 2.42 (95% confidence interval [CI]: 1.48 to 3.95; \( p = 0.002 \)) and further adjustment for metabolic syndrome and CRP (tobit regression ratio: 2.31; 95% CI: 1.36 to 3.94; \( p = 0.002 \)). In contrast, adiponectin levels were not associated with CAC. Comparative analyses suggested that levels of leptin, interleukin-6, and soluble tumor necrosis factor receptor-2, as well as the homeostasis model assessment of insulin resistance (HOMA-IR) index, predicted CAC scores, but only leptin and HOMA-IR provided value beyond risk factors, metabolic syndrome, and CRP.

Conclusions
In SIRCA, although both leptin and adiponectin levels were associated with metabolic and inflammatory markers, only leptin was a significant independent predictor of CAC. Of several metabolic markers, leptin and the HOMA-IR index had the most robust, independent associations with CAC. (J Am Coll Cardiol 2008;52:231–6)

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Adipokines are fat-secreted biomolecules with diverse signaling effects that modulate insulin resistance, hepatic lipoprotein production, and vascular inflammation (1). Two in particular, adiponectin and leptin, are almost exclusively fat derived and have antithetic actions in insulin resistance and in vascular signaling (2). Because of these properties, adiponectin and leptin have been proposed as biomarkers of adipose function that may add value in predicting cardiovascular disease (CVD) risk and provide targets for therapeutic interventions.

Levels of adiponectin, an insulin-sensitizing hormone with anti-inflammatory properties (3), are reduced in obesity, type 2 diabetes, and coronary artery disease (CAD) compared with controls (4–6). Indeed, several (7,8) but not all (9,10) epidemiologic studies suggest that reduced plasma adiponectin levels are independent predictors of CVD. Leptin, on the other hand, is a pleiotropic adipokine that modulates innate immune functions and vascular signaling in addition to its central role in regulation of appetite and energy expenditure (11). In contrast to adiponectin, leptin levels directly correlate with insulin resistance, obesity (12,13), and several CVD risk factors (14). Leptin levels have been associated with CVD beyond body mass index (BMI) in some (15–17) but not all studies (18).

We previously examined the association of plasma levels of C-reactive peptide (CRP), resistin, interleukin (IL)-6, and soluble tumor necrosis factor receptor-2 (sol-TNFR2),
as well as metabolic syndrome, with coronary artery calcification (CAC) in the SIRCA (Study of Inherited Risk of Coronary Atherosclerosis) (19–22). In this report, we examined the association of adiponectin and leptin with CVD risk factors and CAC in SIRCA and then compared the relative value of all measured biomarkers of adiposity and insulin resistance in predicting CAC scores beyond traditional risk factors, metabolic syndrome, and plasma CRP.

Methods

Study participants. SIRCA is a single-center, community-based, cross-sectional study of factors associated with CAC (21,23). Participants were healthy adults 30 to 75 years with family histories of premature CVD but without evidence of clinical CAD (defined as myocardial infarction, coronary revascularization, angiographic evidence of CAD, or ischemia seen on a cardiac stress test), diabetes, serum creatinine level >3.0 mg/dl, or elevated total cholesterol (>300 mg/dl). This report focuses on 860 unrelated, nondiabetic SIRCA participants.

Evaluated parameters. Study subjects were evaluated in a fasting state at the General Clinical Research Center at the Hospital of the University of Pennsylvania, Philadelphia (21,23). Plasma levels of adiponectin, leptin, resistin, and insulin (Linco, St. Charles, Missouri), as well as IL-6 and sol-TNFr2 (R&D Systems, Minneapolis, Minnesota), were measured by enzyme-linked immunosorbent assays. The CRP levels were assayed as described (21). The intra-assay and interassay coefficients of variance for pooled human plasma were 5.7% and 9.9% for adiponectin; 5.5% and 12.4% for leptin; 4.6% and 4.3% for resistin; 4.1% and 11.6% for insulin; 8.7% and 10.9% for IL-6; 5.3% and 12.1% for sol-TNFr2; and 8.0% and 8.3% for CRP, respectively. Framingham risk scores (FRS) were calculated as described by Wilson et al. (24). Participants were classified as having metabolic syndrome using the National Cholesterol Education Program (NCEP) definition (25). The homeostasis model assessment of insulin resistance (HOMA-IR) index = fasting glucose (mmol/l) × fasting insulin (μU/ml)/22.5 (26) was used as a measure of insulin resistance. Global Agatston CAC scores (27), measured by electron beam tomography (Imatron, San Francisco, California) were determined as previously described (21,23).

Statistical analysis. Data are reported as median with first and third quartiles (Q1 = 25th percentile, Q3 = 75th percentile) or mean ± standard deviation for continuous variables and as proportions for categoric variables. Spearman correlations of plasma adiponectin and leptin levels with other continuous variables are presented. Crude associations of adipokine levels with categoric variables were examined using the Kruskal-Wallis rank test. Tobit regression, using natural log (CAC+1) as the outcome, was used for the analysis of CAC data because of its marked right-skewed distribution and the presence of many zero scores (28). The tobit model is designed to assess the relationship between explanatory variables and a censored dependent variable at one end, at which many observations are clustered. We chose this modeling because CAC scores are censored at zero and the use of ordinary least-squares regression on such a nonnormal distribution would produce biased estimates and invalid inference. Tobit modeling has otherwise similar assumptions about error distributions as the linear regression model.

Because of potential gender differences in adipose associations with CVD, models are presented for each gender separately and combined when appropriate. The association between CAC and highest versus lowest quartile of adiponectin and leptin levels were assessed in incremental models including the variables age (age and age²), race, gender, family history of CAD, exercise (none vs. any), medications (aspirin, statins, angiotensin-converting enzyme inhibitors), FRS, metabolic syndrome, and CRP. Gender differences in the association of adipokines with CAC were assessed using the likelihood-ratio test (LRT). A priori BMI data were not included in the models because adipokines may be intermediate in the causal pathway between adiposity and subclinical atherosclerosis.

We used the likelihood-ratio test in nested models to assess the incremental value of each biomarker of adiposity and insulin resistance, adiponectin, leptin, resistin, IL-6, sol-TNFr2, HOMA-IR data (all included as log-transformed variables), and metabolic syndrome in predicting CAC scores beyond established risk factors. Statistical analyses were performed using Stata 9.0 software (Stata Corporation, College Station, Texas). A tobit regression model was fit in Stata (29) using the tobit command with the ll(0) option to indicate left censoring at a CAC score of 0.

Results

Characteristics of participants. As previously described (19,21), the SIRCA sample is predominantly Caucasian (Table 1). Plasma levels of adiponectin and leptin were significantly higher in women than in men (p < 0.001 for both). Almost 40% had CAC scores above the 70th percentile, consistent with accelerated atherosclerosis most likely related to recruitment strategy based on a family history of CVD.
Differential association of adiponectin and leptin with cardiovascular risk factors. Adiponectin and leptin correlated only modestly (and inversely) with each other, whereas associations with lipid, metabolic, and inflammatory variables were greater for both adipokines in women than men (Online Appendix Table A). Among all factors, adiponectin’s strongest (direct) correlation was with plasma levels of insulin resistance (HOMA-IR, IL-6, sol-TNFR2, and resistin with CAC in adjusted analyses (e.g., p = 0.10, 0.37, 0.68, and 0.18, respectively, for gender differences in age- and race-adjusted models). Therefore, except for adiponectin, results of these analyses are presented for both genders combined. The HOMA-IR index and plasma levels of leptin, IL-6, and sol-TNFR2, as well as the NCEP-defined metabolic syndrome (glucose cut point >110 mg/dl), provided significant improvements in the association with CAC beyond traditional risk factors and FRS (Table 3). After further adjustment for metabolic syndrome and CRP data, only HOMA-IR (LRT chi-square = 10.39, p < 0.01) and plasma leptin levels (LRT chi-square = 6.87, p < 0.01) significantly improved model prediction of CAC (Table 3).

### Discussion

Adiponectin and leptin are fat-secreted hormones with opposing actions on insulin resistance and vascular inflammation. Plasma leptin and adiponectin had opposite correlations with lipid, metabolic, and inflammatory risk factors, but we found that only plasma leptin levels were independently associated with CAC. Further, in a comparison of several metabolic biomarkers, leptin and the HOMA-IR index had the most robust associations with CAC scores beyond traditional risk factors, NCEP-defined metabolic syndrome, and plasma levels of CRP.

Leptin is an important negative regulator of body weight (11). Paradoxically, obesity is associated with increased plasma leptin levels, most likely because of resistance to its actions in the setting of increased production by adipose tissue (30). Leptin activates the endothelium and induces smooth muscle cell proliferation, and its receptors are expressed in atherosclerotic plaques (31). Recent studies suggest an association between plasma leptin levels and atherosclerotic CVD in humans, including angiographic CAD (32) and CVD events (33). In a case-control (n = 377) study nested within the WOSCOPS (West of Scotland Coronary Prevention Study), plasma leptin levels predicted CVD even after adjustments for traditional risk factors, BMI, and plasma CRP levels (15). However, in a nested case-control study from the Quebec Cardiovascular Study Cohort, plasma leptin levels were not related to CVD events (18).

Few data are available on the association between leptin and direct measures of atherosclerosis in humans. Van den Beld et al. (34) found no association between plasma leptin levels and carotid intima-media thickness (IMT) in 403 healthy elderly men, whereas Ciccone et al. (35) reported an
association of leptin with IMT in 126 healthy Italians. We previously reported that leptin levels were associated with CAC in a type 2 diabetic sample even after controlling for established risk factors, including CRP and measures of subclinical vascular disease (16). Recently Irribarren et al. (36) reported an association of plasma leptin levels with CAC in older women in the ADVANCE (Atherosclerotic Disease, Vascular Function and Genetic Epidemiology) study, but this association was not significant after controlling for metabolic risk factors and BMI data. In SIRCA, we found an association of plasma leptin with CAC even after controlling for metabolic syndrome and CRP.

Adiponectin has emerged as a unique fat-secreted hormone that regulates insulin sensitivity (37). Atheroprotective effects may be directed through inhibition of the nuclear factor kappa-B inflammatory pathway in vascular cells (38). Plasma factor kappa-B inflammatory pathway in vascular cells (38) of women, may be directed through inhibition of the nuclear factor kappa-B inflammatory pathway in vascular cells (38). Plasma mone that regulates insulin sensitivity (37).

### Table 2

**Association of Plasma Levels of Adiponectin and Leptin With Coronary Artery Calcification in Tobit Multivariable Models**

<table>
<thead>
<tr>
<th>Variables Adjusted for</th>
<th>Women Ratio (95% CI)</th>
<th>Men Ratio (95% CI)</th>
<th>Combined Ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adiponectin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basic model (i.e., age, gender, race)</td>
<td>0.60 (0.26–1.39)</td>
<td>1.36 (0.75–2.48)</td>
<td>*</td>
</tr>
<tr>
<td>Basic model, family history, exercise, medications, FRS</td>
<td>1.22 (0.54–2.78)</td>
<td>1.44 (0.79–2.62)</td>
<td>1.28 (0.79–2.07)</td>
</tr>
<tr>
<td>Basic model, family history, exercise, medications, metabolic syndrome</td>
<td>1.13 (0.48–2.69)</td>
<td>1.50 (0.82–2.75)</td>
<td>1.31 (0.80–2.14)</td>
</tr>
<tr>
<td>Basic model, family history, exercise, medications, FRS, metabolic syndrome</td>
<td>1.36 (0.58–3.19)</td>
<td>1.55 (0.85–2.84)</td>
<td>1.40 (0.86–2.29)</td>
</tr>
<tr>
<td>Above model plus CRP</td>
<td>1.82 (0.76–4.34)</td>
<td>1.51 (0.82–2.76)</td>
<td>1.47 (0.89–2.41)</td>
</tr>
<tr>
<td>Leptin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basic model (i.e., age, gender, race)</td>
<td>5.64 (2.37–13.40)</td>
<td>3.26 (1.81–5.87)</td>
<td>3.77 (2.31–6.14)</td>
</tr>
<tr>
<td>Basic model, family history, exercise, medications, FRS</td>
<td>3.15 (1.36–7.31)</td>
<td>2.21 (1.20–4.08)</td>
<td>2.42 (1.48–3.95)</td>
</tr>
<tr>
<td>Basic model, family history, exercise, medications, metabolic syndrome</td>
<td>3.45 (1.42–8.34)</td>
<td>2.25 (1.22–4.16)</td>
<td>2.54 (1.54–4.20)</td>
</tr>
<tr>
<td>Basic model, family history, exercise, medications, FRS, metabolic syndrome</td>
<td>3.06 (1.28–7.30)</td>
<td>2.11 (1.13–3.91)</td>
<td>2.30 (1.39–3.80)</td>
</tr>
<tr>
<td>Above model plus CRP</td>
<td>1.99 (0.79–4.99)</td>
<td>2.68 (1.40–5.15)</td>
<td>2.31 (1.36–3.94)</td>
</tr>
</tbody>
</table>

Results of tobit regression are presented as the ratio of increase in coronary artery calcification score for top versus bottom quartile of adipokine data. Medications included aspirin, statins, angiotensin-converting enzyme inhibitors. *p < 0.01 for gender interaction. CI = confidence interval; CRP = C-reactive protein; FRS = Framingham risk score.

levels are depressed in patients with CAD (40) and are associated with clinical CVD in patients with diabetes (7). A nested case-control study by Maahs et al. (41) suggested that low levels of plasma adiponectin predict short-term CAC progression, more so in nondiabetics. Several recent prospective studies of clinical CVD, however, have been negative. In a nested case-control study from the Strong Heart Study, there was no association with incident CAD events (10). Similarly, in the British Women’s Heart and Health Cohort Study, adiponectin levels were not associated with CVD (9). More recently, Sattar et al. (42) looked at 589 men with fatal and nonfatal CAD and 1,231 controls and found no difference in median adiponectin levels despite adiponectin associations with HDL and CRP. A 7-study meta-analysis by the same authors failed to demonstrate a consistent relationship of adiponectin with CAD events (42).

Despite correlations with lipids, metabolic factors, and insulin resistance, we also did not find an inverse association of adiponectin levels with CAD. The reasons for conflicting study findings are uncertain but may reflect differences in study design and populations as well as heterogeneous outcomes including subclinical atherosclerotic and different CVD outcomes. In fact, Steffes et al. (43) unexpectedly found a positive association of adiponectin with CAC in a study of more than 3,000 young adults age 33 to 45 years. Finally, several studies suggest that the high molecular weight adiponectin complex, but not the lower molecular weight hexamer, may be the active signaling molecule (44,45). Few epidemiologic studies, however, have assayed the different circulating forms of adiponectin.

We also determined which of several metabolic biomarkers predicted CAC scores beyond established clinical CVD risk factors. Leptin, HOMA-IR, and to a lesser extent IL-6 and sol-TNF2, provided incremental value beyond FRS, metabolic syndrome, and CRP. Our finding that HOMA-IR levels are associated with CAC beyond all other risk factors is consistent with most (22) but not all

### Table 3

**Incremental Value of Metabolic Syndrome, CRP, Adipocytokines, or HOMA-IR in Predicting Coronary Calcium Scores Beyond Established Risk Factors**

<table>
<thead>
<tr>
<th>Variable Added to Model</th>
<th>Likelihood Ratio Test (Chi-Square)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Added to Full Model*</td>
</tr>
<tr>
<td>Metabolic syndrome</td>
<td>6.78 †</td>
</tr>
<tr>
<td>CRP</td>
<td>2.05</td>
</tr>
<tr>
<td>Adiponectin</td>
<td>0.52</td>
</tr>
<tr>
<td>Adiponectin in men</td>
<td>3.56</td>
</tr>
<tr>
<td>Adiponectin in women</td>
<td>0.24</td>
</tr>
<tr>
<td>Resistin</td>
<td>1.69</td>
</tr>
<tr>
<td>Leptin</td>
<td>11.41†</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>14.83‡</td>
</tr>
<tr>
<td>IL-6</td>
<td>7.31 †</td>
</tr>
<tr>
<td>sol-TNF2</td>
<td>4.73§</td>
</tr>
</tbody>
</table>

*Full model includes age, gender, race, 10-year FRS, family history, exercise, and medications (aspirin, statin, angiotensin-converting enzyme inhibitors). †p < 0.01, ‡p < 0.001, §p < 0.05. Abbreviations as in Tables 1 and 2.
studies that found that hyperinsulinemia and insulin resistance indices were independently associated with atherosclerosis and CVD. The clinical application of insulin-based measures, however, is challenging given the lack of assay standardization and because of ultradian and circadian variation in circulating insulin.

**Study limitations.** This study has several limitations. The study sample is cross-sectional, and causal relationships cannot be determined from the results. Moreover, it is a study of a population consisting primarily of Caucasians with a family history of premature CVD who are otherwise deemed to be at low risk; therefore, the generalizability of our findings across other populations and ethnic groups is uncertain. In addition, CAC is not a direct measure of coronary atherosclerosis. In autopsy studies, however, CAC has been shown to be a quantitative estimate of coronary atherosclerosis (47). It has also been shown to be an independent predictor of CVD (48).

**Conclusions**

We found that plasma levels of leptin but not adiponectin were associated with CAC after controlling for traditional cardiovascular risk factors, metabolic syndrome, and CRP levels. Whether leptin signaling promotes human atherosclerotic CVD directly remains to be established. Finally, leptin levels and the HOMA-IR index had stronger associations with CAC scores than other adipocytokines in this asymptomatic sample. A systematic comparison of multiple adipocytokine and insulin resistance biomarkers across diverse clinical settings is warranted to establish which provide utility as metabolic biomarkers of clinical CVD.

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**REFERENCES**


Key Words: adiponectin • leptin • coronary artery calcification • atherosclerosis • inflammation.

APPENDIX

For a table on the Spearman correlations of plasma levels of adiponectin and leptin with cardiovascular risk factors, please see the online version of this article.