High-Molecular-Weight and Total Adiponectin Levels and Incident Symptomatic Peripheral Artery Disease in Women
A Prospective Investigation

Deborah Y. Ho, BA; Nancy R. Cook, ScD; Kathryn A. Britton, MD, MPH; Eunjung Kim, MSc; Mark A. Creager, MD; Paul M Ridker, MD, MPH; Aruna D. Pradhan, MD, MPH

Background—Adiponectin is linked to reduced diabetes risk and may be antiatherogenic, yet clinical data show no consistent relationship with incident cardiovascular events, especially among women. To our knowledge, no prior prospective studies have evaluated adiponectin, including high-molecular-weight (HMW) adiponectin, and incident peripheral artery disease (PAD).

Methods and Results—We evaluated the relationship of total adiponectin, HMW adiponectin, and the HMW-to-total adiponectin ratio with incident symptomatic PAD in a prospective, nested case-control study conducted within the Women’s Health Study (n = 1,101 cases, n = 2,300 controls, frequency matched in strata defined by 5-year age categories, smoking, fasting status, and follow-up time; median cohort follow-up = 13.2 years). Baseline median levels of HMW and total adiponectin were significantly lower in women developing PAD than in those remaining event free (HMW: 3.3 versus 3.8 g/mL, P = 0.0005; total: 5.6 versus 7.4 g/mL, P < 0.0001). The ratio did not differ significantly between groups. Age-adjusted PAD odds ratios (95% confidence intervals) across tertiles were 1.0, 0.66 (0.39–1.13), and 0.40 (0.22–0.74) for HMW and 1.0, 0.74 (0.43–1.25), and 0.35 (0.18–0.65) for total adiponectin (P trend = 0.004 and 0.001, respectively). Results were similar after adjustment for traditional cardiovascular risk factors, use of postmenopausal hormone therapy, high-sensitivity C-reactive protein, soluble intercellular adhesion molecule-1, leptin, hemoglobin A1c, and fasting insulin (adjusted odds ratio and 95% confidence interval for HMW: 1.0, 0.62 [0.29–1.34], 0.30 [0.12–0.74]; total: 1.0, 0.46 [0.22–1.00], 0.30 [0.12–0.76]; P trend = 0.01 for both).

Conclusions—Total and HMW adiponectin are inversely associated with incident PAD among initially healthy women. These prospective data support a protective role for this adipokine in peripheral atherosclerosis development. (Circulation. 2011;124:00-00.)

Key Words: adiponectin ■ biomarker ■ epidemiology ■ peripheral artery disease ■ women
myocardial infarction, stroke, or heart failure) and the variable inclusion of subjects with preexisting CVD.

Clinical Perspective on p ●●●

The disease-specific association of adiponectin with incident peripheral artery disease (PAD) has not been evaluated previously, with no published prospective data currently available. In 2 cross-sectional studies of individuals with diagnosed PAD, lower adiponectin was associated with lower ankle-brachial index (ABI) and reduced exercise performance, indicating more severe disease.20,21 Because low adiponectin is closely linked to insulin resistance and type 2 diabetes mellitus, 2 potent risk factors for PAD, we evaluated the association of baseline plasma levels of HMW, total, and the HMW-to-total adiponectin ratio with incident symptomatic PAD (intermittent claudication and peripheral artery revascularization) in the Women’s Health Study (WHS), a large cohort of relatively healthy middle-aged and older American women followed for a median of 13.2 years. Furthermore, we assessed whether potential risk associations may be explained by the presence of underlying subclinical inflammation as measured by high-sensitivity C-reactive protein (hsCRP) and soluble intercellular adhesion molecule-1 (sICAM-1).

Methods

Study Population

We designed a prospective, nested case-control study involving participants in the WHS, a randomized clinical trial evaluating low-dose aspirin and vitamin E in the primary prevention of CVD and cancer. The WHS study population, design, and clinical trial results have been described previously.22,23 In brief, between November 1992 and July 1995, a total of 39 876 female health professionals aged ≥45 years without prior cancer or CVD (including myocardial infarction, stroke, and coronary and peripheral arterial revascularization) were enrolled and randomized into the study. Among women enrolled, 28 345 (71%) provided baseline blood specimens, which were centrifuged and stored in liquid nitrogen study. Among women enrolled, 28 345 (71%) provided baseline blood specimens, which were centrifuged and stored in liquid nitrogen until analysis. Mailed questionnaires collected baseline and blood samples, which were centrifuged and stored in liquid nitrogen study. Among women enrolled, 28 345 (71%) provided baseline blood specimens, which were centrifuged and stored in liquid nitrogen until analysis. Mailed questionnaires collected baseline and follow-up information every 6 months during the first year and every 12 months thereafter. The median cohort follow-up at the time of case-control sampling was 13.2 years.

The study was approved by an institutional review board, and all subjects provided informed consent.

Outcome Ascertainment

Participants were surveyed annually for the occurrence of a number of incident health events including symptomatic PAD, defined as new report of intermittent claudication or PAD revascularization (surgery or catheter-based interventions). Case confirmation occurred through telephone interview conducted by a cardiovascular physician every 1 to 2 years during the conduct of the study. A confirmation of intermittent claudication was made after physician confirmation of intermittent claudication was made after physician diagnosis.24 In addition, we obtained medical records to assess the concordance of reported symptoms with diagnostic testing when available. Reports of peripheral arterial surgery or peripheral angioplasty were confirmed after review of operative notes or procedural reports, respectively. Cases were thus validated on the basis of response to the claudication questionnaire and medical record documentation of diagnostic procedures or vascular intervention. As of November 23, 2007, among 28 345 subjects providing baseline blood specimens, there were 556 self-reported PAD events; of these, 117 were confirmed through the aforementioned methods. Venous disease, lower-extremity arthritis, lumbar disc disease, and peripheral neuropathy were the main causes of nonischemic leg pain in confirmed events. Only individuals with confirmed events were considered in the present analysis.

Case-Control Selection and Matching

Control subjects (n=234) were frequency matched to the 117 WHS participants who developed confirmed PAD in a 2:1 ratio by strata defined according to 5-year age categories, smoking status (current, former, or never smoked), fasting status of submitted blood specimens, and follow-up time. Fasting was defined as ≥8 hours since last meal before sample collection; 75% of samples in this analysis were fasting. Of the 117 case subjects included in the initial sample for laboratory analysis, 2 subjects with unavailable adiponectin level and 5 with confirmed prerrandomization PAD events were excluded. As a result, some strata had ≥2 control subjects for each case subject but were retained in the analysis because of the frequency-matched design. Reassessment of each matched stratum also revealed ≥4 control subjects who no longer had matched case subjects; these individuals were therefore removed from the analysis. Thus, the final study sample comprised 110 case and 230 control subjects.

Laboratory Analysis

Baseline plasma samples from case and controls subjects were thawed and assayed. Samples had not been thawed previously for other studies. Total and HMW adiponectin levels were measured separately with the use of a sandwich enzyme-linked immunosorbent assay method (ALPCO Diagnostics, Salem, NH). The day-to-day variabilities of the assay for total adiponectin at 9.1 and 3.9 µg/mL and HMW adiponectin at 4.8 and 1.17 µg/mL were 9.8%, 10.2%, 11.4%, and 12.6%, respectively. The percent recoveries of total and HMW adiponectin after 1 freeze/thaw cycle reported by the assay manufacturer are 99% and 97%, respectively. Insulin was measured with an electrochemiluminescence immunoassay (Roche Diagnostics, Indianapolis, IN) with a cross-reactivity of <0.05% with proinsulin. Leptin was measured by an ultrasensitive enzymatically amplified 2-step sandwich immunoassay (R&D Systems, Minneapolis, MN). High-density lipoprotein (HDL), hsCRP, sICAM-1, and hemoglobin A1c (HbA1c) were measured as described previously.25

Statistical Analysis

We used repeated-measures analysis (SAS PROC mixed) to evaluate differences in means, the van Elteren test for differences in medians, and a matched χ² statistic to assess for differences in proportions, and a matched χ² statistic to assess for frequency matching in the study design. Continuous measures are presented as means or medians on the basis of normality of their distribution. Categorical data are represented as percentages. Age-adjusted Spearman partial correlation coefficients were calculated among control subjects to assess the associations between HMW adiponectin, total adiponectin, the HMW-to-total adiponectin ratio, body mass index (BMI), fasting insulin, HbA1c, and the subclinical markers of inflammation sICAM-1 and hsCRP.

We then assessed the relationship between HMW adiponectin, total adiponectin, and the HMW-to-total adiponectin ratio and incident PAD after dividing the sample into tertiles on the basis of the control distribution. Conditional logistic regression models were then constructed to estimate tertile-specific odds ratios and 95% confidence intervals, with the lowest tertile used as the referent group. Median values were used to test for a linear trend in increasing tertiles. Because adiponectin (and leptin) levels are not significantly altered by short-term (<48 hours) fasting,26,27 the primary analyses were not restricted to fasting subjects until adjustment for fasting insulin.

Initial models adjusted for age and the randomized treatment arms of the original WHS and accounted for matched factors of 5-year age categories, smoking, follow-up time, and fasting status (model 1).
Model 2 additionally adjusted for race, traditional CVD risk factors, and use of postmenopausal hormone therapy. Subsequent models additionally adjusted for markers of subclinical inflammation including hsCRP, sICAM-1, and leptin (model 3); HbA1c and fasting insulin (model 4); and HDL in place of history of high cholesterol (model 5). HDL was chosen because prior data in this cohort have demonstrated HDL to be the lipid marker most strongly associated with PAD risk.28 Sensitivity analyses were performed restricting the analysis to nondiabetic subjects (no baseline diagnosed diabetes mellitus and baseline HbA1c <6.5%). Model fit was compared by the likelihood ratio χ² statistic, with a higher value indicating superior fit.

Conditional logistic regression was also used to investigate the joint association of inflammatory markers (hsCRP and sICAM-1) and HMW or total adiponectin with future PAD. The primary sample was divided into above- and below-median groups for hsCRP and sICAM-1 and tertiles for total and HMW adiponectin. For this analysis, the adjustment variables used in model 2 were chosen to reflect inclusion of traditional cardiovascular risk factors. Those with low HMW or total adiponectin and above-median inflammatory marker level were made the referent group.

Statistical analyses were performed with the use of SAS version 9.2 (SAS Institute, Cary, NC). All confidence intervals are 2-tailed and calculated at a P=0.05 level of significance.

Results
Baseline characteristics of women who developed symptomatic PAD (case subjects) and those remaining free of symptomatic PAD (control subjects) are shown in Table 1. The majority of participants in this study were non-Hispanic whites. As might be expected, individuals who experienced PAD events were significantly more likely to have hypertension, hypercholesterolemia, or diabetes mellitus at baseline. Notably, the proportion of participants reporting family history of myocardial infarction did not differ significantly between groups. Baseline prevalences of other characteristics, including BMI, premenopausal and postmenopausal status with or without use of hormone therapy, exercise frequency, and alcohol use were also comparable.

Both HMW and total adiponectin were significantly decreased in case subjects despite a similar mean BMI (P<0.001 for both biomarkers). The ratio of HMW to total adiponectin, however, was not significantly different between groups, with the average ratio being 0.53 in this study population (P=0.69). High-sensitivity CRP and sICAM-1, previously shown to be independent predictors of peripheral atherosclerosis in this cohort,28 were significantly increased in cases, as were HbA1c and fasting insulin levels. Leptin levels were comparable between the 2 groups.

Spearman partial correlation coefficients adjusted for age are shown in Table 2 (restricted to control subjects). HMW and total adiponectin were very highly correlated (correlation coefficient=0.95, P<0.0001). Both were moderately inversely correlated with BMI, hsCRP, leptin, and fasting insulin but not with sICAM-1. The HMW-to-total adiponectin ratio was strongly correlated with HMW adiponectin, modestly correlated with total adiponectin (correlation coefficients=0.61 and 0.36 respectively; P<0.0001 for both), mildly inversely correlated with BMI, and not correlated with the remaining biomarkers.

Table 3 shows the crude and multivariable-adjusted estimated odds ratios for increasing tertiles of HMW adiponectin, total adiponectin, and HMW-to-total adiponectin ratio. In analyses matched on 5-year age groups, smoking, fasting status, and follow-up time, with additional adjustment for continuous age and the WHS treatment arms, increasing tertiles of both HMW and total adiponectin were inversely related to the risk of PAD (odds ratio for increasing tertiles: 1.0, 0.66, 0.40 for HMW; 1.0, 0.74, 0.35 for total; \( P_{\text{trend}} = 0.004 \) and 0.001, respectively). Further adjustment for traditional cardiovascular risk factors (model 2) did not materially alter these results, and there was no major impact of additional adjustment for markers of subclinical inflammation, sICAM-1, hsCRP, and leptin (model 3). Results were also similar with adjustment for HbA1c and fasting insulin in the subgroup of women providing fasting samples (model 4). There was no impact of substituting HDL for history of hypercholesterolemia (model 5). To assess whether our results might have been unduly influenced by inclusion of baseline diabetic patients, we conducted sensitivity analyses in which subjects with either baseline diabetes mellitus or HbA1c ≥6.5% were excluded (n=103 cases, n=226 controls remaining). In these analyses, results of every model were essentially identical (data not shown). Additional analyses explored the potential effect of adjustment for pack-years of smoking, renal dysfunction via estimated creatinine clearance, and type of hormone therapy used (estrogen only versus estrogen and progesterone) by adding these variables individually to model 2. Results were essentially unchanged (data not shown) and demonstrated a consistent risk decline with increasing tertiles of both HMW and total adiponectin.

In contrast, the HMW-to-total adiponectin ratio was not associated with future PAD (model 2 adjusted odds ratio for increasing tertiles: 1.0, 0.91, 1.02; \( P_{\text{trend}} = 0.96 \)). Given previously reported strong associations of the ratio with insulin resistance and incident type 2 diabetes mellitus,29,30 we further explored potential explanations for this lack of association in our data for PAD. Figure 1 shows a scatterplot of HMW and total adiponectin levels in control subjects, with HMW adiponectin plotted on the left vertical axis, total adiponectin on the horizontal axis, and tertiles of total adiponectin indicated by shading in gray. Data points are closely clustered around the regression line, reflecting the high correlation between the 2 variables. The HMW-to-total adiponectin ratio for each point is also represented on the figure as triangles, with values indicated by the right vertical axis. There is little variability of the ratio across tertiles of total adiponectin within this population, with values predominantly distributed between 0.4 and 0.6. Thus, although our analyses indicate that high HMW and total adiponectin are individually related to lower incidence of PAD, their strong correlation and largely fixed ratio may explain a lack of ability of the ratio to delineate risk in this study.

Comparison of the model likelihood ratio χ² statistic, a global measure of model fit with larger values indicating superior fit, showed that the model with total adiponectin had the highest value (16.8; df=5), followed by HMW adiponectin (14.1; df=5). There was no significant improvement in fit with addition of HMW adiponectin to a model with total adiponectin (likelihood ratio test statistic for nested models=0.35; \( P=0.16 \)) and similarly no evidence of improved fit with addition of total adiponectin to a model with HMW adiponectin alone (likelihood ratio test statistic for nested models=3.06; \( P=0.78 \)).
We then sought to investigate potential joint effects of adiponectin when combined with levels of hsCRP and sICAM-1 (Figure 2). Low adiponectin and high inflammatory marker levels were chosen as the high-risk referent group. A risk gradient was evident according to baseline adiponectin (total and HMW) irrespective of inflammatory biomarker level (all 4 interaction $P$ values $>0.5$). In all comparisons, the lowest risk of future PAD was seen in the group with high adiponectin and low inflammatory marker levels, and, conversely, the highest risk was seen in those with low adiponectin and high inflammatory marker levels. The effect was particularly pronounced in comparisons of adiponectin with sICAM-1, which was also the biomarker least correlated with adiponectin (Table 2).

**Discussion**

To our knowledge, this is the first prospective investigation of adiponectin with incident PAD. Baseline HMW and total adiponectin levels were found to be inversely associated with future symptomatic PAD in women independent of traditional cardiovascular risk factors, fasting insulin levels, and evidence of subclinical inflammation. Our findings were

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Cases (n=110)</th>
<th>Controls (n=230)</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>59.0 (7.7)</td>
<td>58.7 (7.5)</td>
<td>0.75</td>
</tr>
<tr>
<td>White, n (%)</td>
<td>106 (98.2)</td>
<td>214 (95.1)</td>
<td>0.18</td>
</tr>
<tr>
<td>Body mass index, kg/m$^2$</td>
<td>25.6 (4.4)</td>
<td>25.3 (4.5)</td>
<td>0.45</td>
</tr>
<tr>
<td>Smoker, n (%)</td>
<td>Current</td>
<td>49 (44.6)</td>
<td>106 (46.1)</td>
</tr>
<tr>
<td></td>
<td>Past</td>
<td>41 (37.3)</td>
<td>82 (35.7)</td>
</tr>
<tr>
<td></td>
<td>Never</td>
<td>20 (18.2)</td>
<td>42 (18.3)</td>
</tr>
<tr>
<td>Diabetes mellitus, n (%)</td>
<td>7 (6.4)</td>
<td>3 (1.3)</td>
<td>0.01</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>44 (40.0)</td>
<td>64 (27.8)</td>
<td>0.02</td>
</tr>
<tr>
<td>Hypercholesterolemia, n (%)</td>
<td>50 (45.5)</td>
<td>79 (34.4)</td>
<td>0.05</td>
</tr>
<tr>
<td>Family history of MI, n (%)</td>
<td>17 (15.5)</td>
<td>31 (13.5)</td>
<td>0.63</td>
</tr>
<tr>
<td>Postmenopausal, n (%)</td>
<td>Yes with current HT</td>
<td>30 (27.3)</td>
<td>92 (40.0)</td>
</tr>
<tr>
<td></td>
<td>Yes without current HT</td>
<td>44 (40.0)</td>
<td>81 (35.2)</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>18 (16.4)</td>
<td>27 (11.7)</td>
</tr>
<tr>
<td></td>
<td>Unknown</td>
<td>18 (16.4)</td>
<td>30 (13.0)</td>
</tr>
<tr>
<td>Exercise at least once a week, n (%)</td>
<td>45 (40.9)</td>
<td>91 (39.6)</td>
<td>0.84</td>
</tr>
<tr>
<td>Alcohol use, n (%)</td>
<td>Rare/never</td>
<td>51 (46.4)</td>
<td>90 (39.1)</td>
</tr>
<tr>
<td></td>
<td>1–3/mo</td>
<td>11 (10.0)</td>
<td>23 (10.0)</td>
</tr>
<tr>
<td></td>
<td>1–6/wk</td>
<td>34 (30.9)</td>
<td>78 (33.9)</td>
</tr>
<tr>
<td></td>
<td>$\geq$1/d</td>
<td>14 (12.7)</td>
<td>39 (17.0)</td>
</tr>
<tr>
<td>Biomarker level</td>
<td>Adiponectin, $\mu$g/mL</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>HMW</td>
<td>3.3 (0.5–12.3)</td>
<td>3.8 (0.4–16.7)</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>5.6 (1.5–23.7)</td>
<td>7.4 (1.6–27.4)</td>
</tr>
<tr>
<td></td>
<td>HMW-to-total adiponectin ratio</td>
<td>0.53 (0.1)</td>
<td>0.53 (0.1)</td>
</tr>
<tr>
<td></td>
<td>Leptin, ng/mL</td>
<td>19.8 (4.2–74.2)</td>
<td>19.4 (1.6–85.9)</td>
</tr>
<tr>
<td></td>
<td>C-reactive protein, mg/L</td>
<td>3.1 (0.1–48.4)</td>
<td>2.2 (0.1–52.9)</td>
</tr>
<tr>
<td></td>
<td>sICAM-1, ng/mL</td>
<td>418.5 (181.9–785.0)</td>
<td>370.1 (158.5–835.6)</td>
</tr>
<tr>
<td></td>
<td>Hemoglobin A$_{1c}$, %</td>
<td>5.1 (4.5–11.8)</td>
<td>5.0 (4.3–9.0)</td>
</tr>
<tr>
<td></td>
<td>Fasting insulin, $\mu$IU/mL</td>
<td>7.9 (1.7–50.9)</td>
<td>6.6 (1.9–93.3)</td>
</tr>
</tbody>
</table>

*Matching factor. Five-year age groups were also a matching factor but not age as a continuous variable, which is presented above.
†Restricted to individuals with fasting blood draws (n=83 cases, n=172 matched controls).
robust in analyses limited to nondiabetic subjects (by history and HbA1c <6.5%), and we found a persistent effect irrespective of baseline levels of hsCRP or sICAM-1. We also found no association for the ratio of HMW to total adiponec-tin, likely because of the high correlation of the 2 variables in this population of healthy women.

Although experimental data support antiatherosclerotic properties of adiponectin, epidemiological studies of adi-

Table 2. Spearman Partial Correlation Coefficients Among Controls, With Adjustment for Age

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>HMW-to-Total Adiponectin Ratio</th>
<th>Body Mass Index</th>
<th>hsCRP</th>
<th>sICAM-1</th>
<th>Leptin</th>
<th>Fasting Insulin</th>
<th>Hemoglobin A1c</th>
</tr>
</thead>
<tbody>
<tr>
<td>HMW</td>
<td>0.95*</td>
<td>0.61*</td>
<td>−0.40*</td>
<td>−0.34*</td>
<td>−0.07</td>
<td>−0.33*</td>
<td>−0.41*</td>
<td>−0.19†</td>
</tr>
<tr>
<td>Total</td>
<td>0.36*</td>
<td>−0.41*</td>
<td>−0.36*</td>
<td>−0.11</td>
<td>−0.38*</td>
<td>−0.47*</td>
<td>−0.18†</td>
<td>−0.11</td>
</tr>
<tr>
<td>Ratio</td>
<td>−0.19†</td>
<td>−0.12</td>
<td>0.06</td>
<td>−0.06</td>
<td>−0.06</td>
<td>−0.06</td>
<td>−0.11</td>
<td></td>
</tr>
</tbody>
</table>

HMW indicates high-molecular-weight; hsCRP, high-sensitivity C-reactive protein; and sICAM-1, soluble intercellular adhesion molecule-1.

*P<0.0001.
†P<0.05.

Table 3. Odds Ratios for Incident Peripheral Artery Disease by Increasing Tertiles of HMW, Total, and HMW-to-Total Adiponectin Ratio

<table>
<thead>
<tr>
<th>Odds Ratios by Tertile (95% CI)</th>
<th>Tertile 1 (Lowest)</th>
<th>Tertile 2</th>
<th>Tertile 3</th>
<th>P trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>HMW adiponectin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median, μg/mL</td>
<td>1.8</td>
<td>3.8</td>
<td>6.9</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>&lt;2.91</td>
<td>2.91–5.00</td>
<td>&gt;5.00</td>
<td></td>
</tr>
<tr>
<td>No. of cases</td>
<td>51</td>
<td>35</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>Model 1</td>
<td>1.0</td>
<td>0.66 (0.39–1.13)</td>
<td>0.40 (0.22–0.74)</td>
<td>0.004</td>
</tr>
<tr>
<td>Model 2</td>
<td>1.0</td>
<td>0.61 (0.33–1.12)</td>
<td>0.41 (0.20–0.85)</td>
<td>0.02</td>
</tr>
<tr>
<td>Model 3</td>
<td>1.0</td>
<td>0.57 (0.31–1.07)</td>
<td>0.39 (0.19–0.81)</td>
<td>0.01</td>
</tr>
<tr>
<td>Model 4*</td>
<td>1.0</td>
<td>0.62 (0.29–1.34)</td>
<td>0.30 (0.12–0.74)</td>
<td>0.01</td>
</tr>
<tr>
<td>Model 5*</td>
<td>1.0</td>
<td>0.64 (0.29–1.39)</td>
<td>0.32 (0.12–0.85)</td>
<td>0.02</td>
</tr>
<tr>
<td>Total adiponectin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median, μg/mL</td>
<td>4.1</td>
<td>7.3</td>
<td>12.0</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>&lt;5.44</td>
<td>5.44–9.67</td>
<td>&gt;9.67</td>
<td></td>
</tr>
<tr>
<td>No. of cases</td>
<td>51</td>
<td>39</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Model 1</td>
<td>1.0</td>
<td>0.74 (0.43–1.25)</td>
<td>0.35 (0.18–0.65)</td>
<td>0.001</td>
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<tr>
<td>Model 2</td>
<td>1.0</td>
<td>0.68 (0.37–1.26)</td>
<td>0.37 (0.18–0.76)</td>
<td>0.007</td>
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<tr>
<td>Model 3</td>
<td>1.0</td>
<td>0.66 (0.35–1.24)</td>
<td>0.35 (0.17–0.74)</td>
<td>0.006</td>
</tr>
<tr>
<td>Model 4*</td>
<td>1.0</td>
<td>0.46 (0.22–1.00)</td>
<td>0.30 (0.12–0.76)</td>
<td>0.01</td>
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<tr>
<td>Model 5*</td>
<td>1.0</td>
<td>0.48 (0.22–1.05)</td>
<td>0.33 (0.13–0.87)</td>
<td>0.03</td>
</tr>
<tr>
<td>HMW-to-total adiponectin ratio</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>0.43</td>
<td>0.52</td>
<td>0.62</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>≤0.48</td>
<td>0.48–0.57</td>
<td>&gt;0.57</td>
<td></td>
</tr>
<tr>
<td>No. of cases</td>
<td>42</td>
<td>31</td>
<td>37</td>
<td></td>
</tr>
<tr>
<td>Model 1</td>
<td>1.0</td>
<td>0.76 (0.43–1.33)</td>
<td>0.87 (0.50–1.53)</td>
<td>0.62</td>
</tr>
<tr>
<td>Model 2</td>
<td>1.0</td>
<td>0.91 (0.49–1.68)</td>
<td>1.02 (0.55–1.86)</td>
<td>0.96</td>
</tr>
<tr>
<td>Model 3</td>
<td>1.0</td>
<td>0.88 (0.47–1.64)</td>
<td>0.87 (0.47–1.63)</td>
<td>0.67</td>
</tr>
<tr>
<td>Model 4*</td>
<td>1.0</td>
<td>1.05 (0.49–2.25)</td>
<td>0.83 (0.39–1.74)</td>
<td>0.60</td>
</tr>
<tr>
<td>Model 5*</td>
<td>1.0</td>
<td>1.09 (0.51–2.36)</td>
<td>0.91 (0.42–1.95)</td>
<td>0.79</td>
</tr>
</tbody>
</table>

Median calculations include cases plus controls. Ranges have been rounded for ease of interpretation. HMW indicates high-molecular-weight; CI, confidence interval. Models are as follows: model 1 = matched on 5-year age categories, smoking, and fasting status, adjusted for age and Women’s Health Study treatment arms (vitamin E and low-dose aspirin); model 2 = model 1 + race (white or nonwhite), body mass index (linear continuous), diabetes mellitus (yes or no), hypertension (≥140/90 mm Hg, yes or no) hyperlipidemia (≥240 mg/dL, yes or no), family history of myocardial infarction (yes, no, or unknown), and use of hormone therapy (yes or no); model 3 = model 2 + high-sensitivity C-reactive protein, soluble intercellular adhesion molecule-1, and leptin; model 4 = model 3 + hemoglobin A1c and fasting insulin; model 5 = model 4 with high-density lipoprotein in place of history of high cholesterol.

*Restricted to individuals with fasting blood draws (n=83 cases, n=172 matched controls).
Adiponectin and incident CVD have yielded conflicting results. These inconsistencies may relate to a number of factors including (1) limited assessment of HMW adiponectin; (2) use of diverse CVD end points with potentially distinct pathophysiology, including combinations of nonfatal myocardial infarction, heart failure, or stroke; and, importantly, (3) variable inclusion of subjects with preexisting atherosclerotic disease. In patients with extant CVD, elevated adiponectin may result from compensatory upregulation, adiponectin resistance, and/or pathological weight loss.\textsuperscript{19} All possibilities supported by data demonstrating a positive association between adiponectin and adverse outcomes in this group.\textsuperscript{13,14,19,31,32} Similarly, in advanced PAD (PAD hospitalization or post-surgery), elevated total adiponectin was recently shown to portend reduced survival\textsuperscript{33,34}: Dieplinger et al.\textsuperscript{33} showed a 3% increase in all-cause mortality per 1-μg/mL increase in baseline total adiponectin (risk ratio 1.03 [95% confidence interval, 1.00–1.05]; \(P=0.03\)). In contrast, among subjects with milder forms of symptomatic PAD, elevated levels are associated with higher ABI and improved exercise performance.\textsuperscript{20,21} Thus, the observed associations may differ during periods of early- versus late-stage clinical disease.

In the present study, among a relatively homogeneous population of women without prior diagnosed CVD, we found a strong association between elevated adiponectin and reduced incidence of symptomatic PAD. Our findings suggest that adiponectin may be biologically relevant to pathogenesis of this disease. Although the main underlying mechanism in peripheral as in coronary atherosclerosis is atheroma initiation with progressive vascular occlusion and attendant reduc-

\begin{figure}
\centering
\includegraphics[width=\linewidth]{figure1}
\caption{Relationship between high-molecular-weight (HMW) adiponectin, total adiponectin, and HMW-to-total adiponectin ratio. Closed circles indicate values of total adiponectin plotted against HMW adiponectin (left vertical axis) for each control subject. Values cluster tightly around the regression line. Tertiles of total adiponectin are denoted by shading. Triangles indicate the HMW-to-total ratio (right vertical axis) for each individual.}
\end{figure}

\begin{figure}
\centering
\includegraphics[width=\linewidth]{figure2}
\caption{Odds ratios for incident peripheral artery disease by tertiles of high-molecular-weight (HMW) and total adiponectin and below- and above-median levels of high-sensitivity C-reactive protein (hsCRP) and soluble intercellular adhesion molecule-1 (sICAM-1). Data shown are multivariable-adjusted odds ratios estimated by conditional logistic regression, adjusted for age, Women’s Health Study treatment arms, race, body mass index, diabetes mellitus, hypertension, hyperlipidemia, family history of myocardial infarction, and use of hormone therapy (model 2). The referent is the high-risk group (odds ratio = 1.0) with low adiponectin and above-median inflammatory marker level. Med indicates medium.}
\end{figure}
tion in blood flow, the severity of PAD symptoms does not correlate well with the degree of hemodynamic obstruction, and revascularization does not completely normalize exercise performance. In this regard, mitochondrial dysfunction, impaired oxidative capacity, and enhanced oxidative stress are downstream features of altered skeletal muscle metabolism that correlate with functional limitation in patients with PAD and that may be directly modulated by adiponectin. Of the 2 known adiponectin receptors, the adiponectin receptor 1 is abundant in skeletal muscle and liver, whereas adiponectin receptor 2 is predominantly expressed in liver. It has been demonstrated recently that adiponectin binding to adiponectin receptor 1 induces calcium-dependent mitochondrial biogenesis. Furthermore, skeletal muscle–specific disruption of adiponectin receptor 1 in mice in vivo produces a phenotype characterized by impaired oxidative capacity, decreased mitochondrial DNA content, and, importantly, reduced exercise performance compared with controls. Thus, interestingly, beyond direct antiatherogenic effects on plaque development, these experimental data coupled with our prospective findings support a secondary mechanism by which adiponectin may contribute to the symptomatic expression of this disease.

Our study found no association between the baseline HMW-to-total adiponectin ratio and future PAD, although prior data linking the ratio to insulin resistance and incident type 2 diabetes mellitus are strong. Total adiponectin levels and the relative proportions of HMW adiponectin differ between men and women, with women having generally higher total and HMW levels as well as higher ratios. For instance, in the Atherosclerosis Risk in Community Study, in which the same quantitative assay was used, after adjustment for age and ethnicity, the mean levels of HMW adiponectin were 3.16 and 1.75 \( \mu \text{g/mL} \) with HMW-to-total adiponectin ratios of 0.44 and 0.37 in women and men, respectively (both \( P \leq 0.001 \)). The mechanism underlying this gender difference is unclear, although testosterone may selectively inhibit HMW adiponectin secretion. Some have suggested that the estrogen-androgen balance, and thus variation, not only by gender but menopausal status may also be important for regulating adiponectin multimer distribution. Although we could not assess the impact of sex hormone levels, in this population of relatively healthy women variation in the HMW-to-total adiponectin ratio was not associated with PAD risk.

Strengths of the present study include the prospective design, large sample size, long-term follow-up, and homogeneity of our study population, which may reduce confounding. However, several potential limitations should be considered. First, the WHS included mainly white women who were healthy at baseline, and thus our conclusions may not be generalizable to other groups. Second, because our study is observational, residual unmeasured confounding may be present. However, we were able to adjust for a broad range of established and emerging cardiovascular risk factors and found no material difference in our results. Third, the use of symptomatic PAD as the primary end point by definition excludes subclinical disease that might otherwise have been detected with the use of ABI or abnormal pulse examination; however, we believe that our data are not only mechanistically relevant but also clinically important because claudication and ischemia requiring limb revascularization are the principal clinical manifestations of PAD. Importantly, each case included in this analysis was confirmed through rigorous methods with the use of a validated claudication questionnaire, cardiovascular physician interview, and medical record review. It should be noted that the sensitivity of the self-administered Edinburgh Claudication Questionnaire for ABI-diagnosed PAD is reported to be 29%, although with a high specificity of 90%. In the present study, the Edinburgh Claudication Questionnaire was administered by a physician to subjects with a reported prior clinical diagnosis, and thus the accuracy is expected to be somewhat higher, although data are not available in this regard. In addition, our focus on symptomatic disease may have reduced the likelihood of end point misclassification, as would the characteristics of our study population comprised of health professionals. Whether or not women with PAD are more likely to have atypical leg symptoms or be more often asymptomatic is controversial, especially because some studies suggest that a lower (less stringent) ABI cutoff may be more appropriate in women. Regardless, misclassification of women with atypical PAD symptoms as noncases in our study would, if anything, have tended to bias our results toward the null.

Despite strong preclinical data supporting antiatherosclerotic properties of adiponectin, previous epidemiological studies have been somewhat inconsistent. Our findings indicate a strong inverse association between both HMW and total adiponectin and the development of symptomatic peripheral atherosclerosis in women. Our data require confirmation, yet a biological role is not only plausible but raises the intriguing possibility that therapeutic modulation of adiponectin, as already possible by use of certain currently available antidiabetic, antihypertensive agents and nutritional agents, may hold promise in prevention and treatment of this disease.

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Disclosures
Dr Creager is a consultant relevant to PAD for Astra-Zeneca, Genzyme, Merck, NormOxys, and Provasculon. Dr Ridker is listed as a coinventor on patents held by the Brigham and Women’s Hospital that relate to the use of inflammatory biomarkers in CVD.

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Symptomatic peripheral arterial disease in women: nontraditional bio-


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**CLINICAL PERSPECTIVE**

Lower-extremity peripheral artery disease (PAD) is a manifestation of atherosclerosis that has received considerably less clinical and research attention than coronary or cerebrovascular disease. PAD shares many risk factors with other cardiovascular diseases, including smoking, diabetes mellitus, hypertension, and hyperlipidemia; however, less is known about how PAD differs from atherosclerosis of other vascular territories. Studies of biomarkers and future disease risk can improve our ability to detect patients at heightened risk and our understanding of disease pathogenesis and, by extension, may identify potential novel modalities for treatment. Adiponectin is secreted from adipose tissue and is known to be inversely correlated with future diabetes mellitus risk. It may also be antiatherogenic. This study is the first to examine the relationship between adiponectin and PAD as a specific vascular end point. A large population of initially healthy women aged ≥45 years without existing cardiovascular disease was studied. After traditional cardiovascular risk factors were taken into account, women with high-molecular-weight or total adiponectin levels in the highest tertile had a 59% (high-molecular-weight) or 63% (total) reduced risk for future symptomatic PAD (intermittent claudication or lower-extremity revascularization) compared with women with levels in the lowest tertile. Given the lack of a consistently demonstrated relationship between adiponectin and other cardiovascular end points, this striking result, if confirmed, suggests a unique relationship of adiponectin in PAD development that may reflect a more prominent role of adipokines in peripheral atherosclerosis.
High-Molecular-Weight and Total Adiponectin Levels and Incident Symptomatic Peripheral Artery Disease in Women: A Prospective Investigation
Deborah Y. Ho, Nancy R. Cook, Kathryn A. Britton, Eunjung Kim, Mark A. Creager, Paul M Ridker and Aruna D. Pradhan

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