**Objective**—Lower concentrations of adiponectin have been linked to subsequent risk of coronary heart disease in healthy individuals. Whether similar relationships exist for the development of systemic atherosclerosis, such as peripheral artery disease (PAD), is uncertain. We investigated the association between total adiponectin and risk of lower extremity PAD.

**Methods and Results**—We performed a prospective, nested case–control study among 18 225 male participants of the Health Professionals Follow-up Study who were free of diagnosed cardiovascular disease at the time of blood draw (1993–1995). During 14 years of follow-up, 143 men developed PAD. Using risk set sampling, controls were selected in a 3:1 ratio and matched on age, smoking status, fasting status, and date of blood draw (n=429). Median (interquartile range) adiponectin concentrations at baseline were lower among cases compared with controls (4.1 [3.2–5.5] versus 5.4 [3.8–7.5] µg/mL; P<0.001). A log-linear inverse association was evident over the full spectrum of adiponectin concentrations with PAD risk after controlling for baseline cardiovascular risk factors using restricted spline conditional logistic regression. Adiponectin was associated with a 42% lower risk of PAD per SD increase in natural log-transformed adiponectin (relative risk, 0.58; 95% confidence interval, 0.45–0.74) after adjustment for cardiovascular risk factors. The relative risk was attenuated (relative risk, 0.68; 95% confidence interval, 0.51–0.92) after further accounting for high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, C-reactive protein, and cystatin C. Additional adjustment for hemoglobin A1c, triglycerides, and γ-glutamyltransferase had little impact on this association (relative risk, 0.68; 95% confidence interval, 0.50–0.92).

**Conclusion**—Total adiponectin is inversely associated with risk of symptomatic lower extremity PAD in men.

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**Key Words:** adiponectin ■ atherosclerosis ■ biomarker ■ epidemiology ■ peripheral artery disease
with information on numerous risk factors of atherosclerosis and inflammation and over a decade of follow-up for incident PAD.

**Patients and Methods**

Materials and Methods are available in the online-only Supplement.

**Results**

Baseline characteristics of cases and controls are presented in Table 1. Although we matched cases to controls on current smoking status, cases had greater total pack-years of smoking and also were more likely to have a history of diabetes mellitus, hypertension, and hypercholesterolemia than controls. Cases had significantly lower concentrations of adiponectin and HDL cholesterol and higher levels of low-density lipoprotein (LDL) cholesterol, C-reactive protein (CRP), triglycerides, hemoglobin (Hb)A1c, creatinine, and cystatin C. Body mass index levels were virtually identical at baseline among case and controls.

We next examined the association between total adiponectin and specific cardiovascular risk factors among controls. Spearman age-adjusted partial correlation coefficients demonstrated positive associations between adiponectin and HDL (0.50, *P*<0.001), and LDL cholesterol (0.11, *P* = 0.03), and negative associations among adiponectin and triglycerides (−0.43, *P*<0.001), body mass index (−0.37, *P*<0.001), CRP (−0.23, *P*<0.001), HbA1c (−0.22, *P*<0.001), and γ-glutamyltransferase (−0.18, *P*<0.001); there were no significant associations with creatinine, cystatin C, estimated glomerular filtration rate, physical activity, or alcohol consumption (all *P*>0.29).

Adiponectin concentrations were also positively correlated with age (0.15, *P* = 0.001). Results were similar if cases and controls were combined with additional adjustment for case-control status.

Table 2 displays the relative risks (RRs) of PAD for total adiponectin as both categorical and continuous variables from conditional logistic regression. Subjects in the highest quartile of adiponectin had an 80% lower risk of PAD compared with the lowest quartile after adjustment for medical history and behavioral factors. Additional adjustment for HDL cholesterol, LDL cholesterol, CRP, and cystatin C attenuated the association, but it remained significantly inverse. In all multivariable models, we observed a consistent linear trend for lower risk with increasing quartiles of adiponectin (all *P* _trend_ <0.01). The adjusted restricted spline curve confirmed the log-linear inverse association over the full spectrum of

### Table 1. Baseline Characteristics of Men With Incident Lower Extremity Peripheral Artery Disease (Cases) and Matched Controls

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Cases (n=143)</th>
<th>Controls (n=429)</th>
<th><em>P</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>65.4 (8.1)</td>
<td>65.3 (8.1)</td>
<td>Matched</td>
</tr>
<tr>
<td>Smoking status, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current</td>
<td>32 (22)</td>
<td>90 (21)</td>
<td>Matched</td>
</tr>
<tr>
<td>Past</td>
<td>78 (55)</td>
<td>242 (56)</td>
<td></td>
</tr>
<tr>
<td>Never</td>
<td>23 (16)</td>
<td>82 (19)</td>
<td></td>
</tr>
<tr>
<td>Total adiponectin, µg/mL</td>
<td>4.1 (3.2–5.5)</td>
<td>5.4 (3.8–7.5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HDL cholesterol, mg/dL</td>
<td>41.7 (11.5)</td>
<td>48.5 (14.2)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LDL cholesterol, mg/dL</td>
<td>139.2 (34.8)</td>
<td>131.4 (33.3)</td>
<td>0.04</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>143 (105–195)</td>
<td>115 (80–165)</td>
<td>0.001</td>
</tr>
<tr>
<td>Hemoglobin A1c,* %</td>
<td>5.56 (5.34–5.96)</td>
<td>5.41 (5.24–5.59)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>C-reactive protein, mg/dL</td>
<td>2.24 (1.18–3.52)</td>
<td>1.18 (0.51–2.26)</td>
<td>0.02</td>
</tr>
<tr>
<td>Cystatin C, mg/dL</td>
<td>1.04 (0.91–1.22)</td>
<td>0.96 (0.86–1.07)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Creatinine, mg/dL</td>
<td>0.89 (0.80–1.02)</td>
<td>0.87 (0.78–0.96)</td>
<td>0.04</td>
</tr>
<tr>
<td>eGFR, mL-min⁻¹·1.73 m⁻² **</td>
<td>86.8 (74.3–96.4)</td>
<td>89.9 (82.3–96.3)</td>
<td>0.02</td>
</tr>
<tr>
<td>γ-Glutamyltransferase, U/L</td>
<td>27 (20–36)</td>
<td>23.0 (17–31)</td>
<td>0.15</td>
</tr>
<tr>
<td>Pack-years of smoking, y</td>
<td>26 (5–45)</td>
<td>18 (1–55)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>History of diabetes mellitus, n (%)</td>
<td>28 (20)</td>
<td>16 (4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>History of hypertension, n (%)</td>
<td>70 (49)</td>
<td>130 (30)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>History of hypercholesterolemia, n (%)</td>
<td>82 (57)</td>
<td>187 (44)</td>
<td>0.005</td>
</tr>
<tr>
<td>Parental history of myocardial infarction aged &lt;60 years, n (%)</td>
<td>22 (15)</td>
<td>44 (10)</td>
<td>0.09</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>25.8 (3.3)</td>
<td>25.6 (4.4)</td>
<td>0.48</td>
</tr>
<tr>
<td>Physical activity, MET-h/wk</td>
<td>22.7 (8.0–43.8)</td>
<td>27.4 (10.3–52.8)</td>
<td>0.003</td>
</tr>
<tr>
<td>Alcohol consumption, g/d</td>
<td>7.6 (0.9–17.8)</td>
<td>9.8 (1.8–20.3)</td>
<td>0.55</td>
</tr>
</tbody>
</table>

Data are expressed as mean (SD), median (interquartile range), or number (percentage). *P* values are derived from generalized linear mixed models for continuous variables and Cochran–Mantel–Haenszel tests for categorical variables controlling for matched sets. Age, smoking status, fasting status, and month of blood sampling were matching variables.

*eGFR indicates estimated glomerular filtration rate; HDL, high-density lipoprotein; LDL, low-density lipoprotein; and MET-h, metabolic equivalent task-hours.

*Data were missing for 1 case and 4 controls.

**eGFR was calculated according to the Chronic Kidney Disease Epidemiology Collaboration equation.
adiponectin concentrations with risk of PAD ($P_{\text{trend}}=0.009$; multivariable model 2; Figure).

We also examined the RR associated per each SD increment in continuous natural log-transformed total adiponectin in successive models, including potentially intermediary variables. The multivariable-adjusted RR was 0.58 (95% confidence interval [CI], 0.45–0.74; multivariable model 1), and modestly attenuated to 0.67 (95% CI, 0.51–0.89) after additionally accounting for both HDL and LDL cholesterol and to 0.68 (95% CI, 0.51–0.92) after also including CRP and cystatin C (multivariable model 2). Despite their correlations with adiponectin and associations with case–control status, adjustment for triglycerides, HbA1c, and γ-glutamyltransferase did not materially influence the association between adiponectin and PAD when added to the model (RR, 0.68; 95% CI, 0.50–0.92; multivariable model 3).

We performed several sensitivity analyses. We first stratified by HDL-cholesterol level because it was strongly correlated with adiponectin and attenuated the effect estimate for adiponectin to the greatest degree. The adjusted RRs for PAD per SD increment in adiponectin were 0.80 (95% CI, 0.32–2.03; n=76 cases) among men with HDL-cholesterol levels ≤40 mg/dL and 0.65 (95% CI, 0.39–1.06; n=67 cases), respectively, among men with levels >40 mg/dL. Similar results were obtained when cystatin C was substituted for estimated glomerular filtration rate, as gross measure of kidney function, or when subjects with imputed HbA1c were excluded.

We also repeated the analysis excluding 18 cases based only on a confirmed physician’s diagnosis (RR, 0.70; 95% CI, 0.51–0.95) or with prevalent diabetes mellitus (baseline history of type 2 diabetes mellitus or HbA1c ≥6.5%) (RR, 0.64; 95% CI, 0.45–0.93).

Finally, we found no significant interactions between total adiponectin and time to diagnosis after initial blood collection, predicted risk of PAD (based on multivariable model 2 without adiponectin), age, current smoking, and fasting status. We also did not observe an interaction with potential biochemical markers of PAD risk (all $P_{\text{interaction}}>0.10$).

### Meta-Analysis of Prospective Studies on Total Adiponectin and Risk of PAD
The previous prospective study reported an RR of 0.35 (95% CI, 0.17–0.74) when the lowest tertile of total adiponectin concentrations was compared with the highest. The RR comparing the first and third tertiles in this study was 0.28 (95% CI, 0.13–0.59). When tertile-specific estimates from the 2 studies

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**Table 2. Relative Risks (95% Confidence Intervals) of Lower Extremity Peripheral Artery Disease According to Total Adiponectin**

<table>
<thead>
<tr>
<th>Continuous Adiponectin (per Each SD Increase in ln-Transformed Variable)</th>
<th>Quartile of Adiponectin, Median (Range), µg/mL</th>
<th>$P_{\text{trend}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cases, n</td>
<td>143</td>
<td>53</td>
</tr>
<tr>
<td>Controls, n</td>
<td>429</td>
<td>90</td>
</tr>
<tr>
<td>Matching variables</td>
<td>0.58 (0.47–0.72)</td>
<td>1.00 (reference)</td>
</tr>
<tr>
<td>Multivariable model 1</td>
<td>0.58 (0.45–0.74)</td>
<td>1.00 (reference)</td>
</tr>
<tr>
<td>Multivariable model 2</td>
<td>0.68 (0.51–0.92)</td>
<td>1.00 (reference)</td>
</tr>
<tr>
<td>Multivariable model 3</td>
<td>0.68 (0.50–0.92)</td>
<td>1.00 (reference)</td>
</tr>
</tbody>
</table>

Relative risks are derived from conditional logistic regression models. Matching variables were age, smoking status, fasting status, and date of blood draw. $P_{\text{trend}}$ is calculated by treating the median of natural log-transformed adiponectin concentrations of each quartile as a continuous variable. Multivariable model 1 is adjusted for pack-years of smoking, history of type 2 diabetes mellitus, history of hypertension, history of hypercholesterolemia, parental history of myocardial infarction aged <60 years, body mass index, and physical activity. Multivariable model 2 is model 1 plus high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, C-reactive protein, and cystatin C. Multivariable model 3 is model 2 plus hemoglobin A1c, triglycerides, and γ-glutamyltransferase.

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**Figure.** Dose–response relationship between total adiponectin and relative risk of peripheral artery disease (PAD). The figure depicts the adjusted relative risk of PAD as a function of natural log-transformed plasma adiponectin concentrations, which were then back-transformed to the original values for ease of interpretation. Data are fitted by a spline conditional logistic regression model with 3 knots. Age, smoking status, fasting status, and date of blood sampling were matching variables. The model is adjusted for high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, C-reactive protein, cystatin C, pack-years of smoking, history of type 2 diabetes mellitus, history of hypertension, and history of hypercholesterolemia, parental history of myocardial infarction of those who aged <60 years, body mass index, and physical activity. The 95% confidence interval (CI) is indicated by the dashed lines.
were meta-analyzed, the RR across tertiles was 0.31 (95% CI, 0.13–0.49) with no evidence for heterogeneity among the findings of these studies ($\chi^2=0.14; P_{\text{heterogeneity}}=0.71; I^2=0.0\%$).

Discussion

In this nested case–control study, we found a strong inverse association between total adiponectin and risk of lower extremity PAD in otherwise healthy men. This association was apparent over the entire range of adiponectin concentrations and persisted even after controlling for traditional biochemical risk factors, such as HDL cholesterol, LDL cholesterol, CRP, and other established PAD risk factors, including cumulative lifelong smoking, hypertension, hypercholesterolemia, and diabetes mellitus. No interactions were observed between adiponectin and lipids, and markers of glycemic control, inflammation, or kidney function.

To our knowledge, only 1 prospective study has investigated the effect of adiponectin on risk of incident PAD. Similar to our findings in men, data from the Women’s Health Study showed a strong inverse association between total and high molecular weight adiponectin with risk of symptomatic PAD (defined as intermittent claudication or PAD revascularization) among 110 women with incident PAD and 230 controls. When tertile-specific estimates from that study and ours were meta-analyzed, we observed a 69% lower risk, again suggesting a particularly strong inverse association of adiponectin with PAD, even relative to other forms of CVD.

Several potential mechanisms could explain the lower risk of PAD associated with higher adiponectin concentrations. Adiponectin may suppress smooth muscle cell proliferation and foam cell formation of macrophages and inhibit monocytic cell adhesion to endothelial cells. It may suppress inflammatory pathways in endothelial cells through downregulation of the nuclear factor kappa B pathway, a key regulator in tumor necrosis factor-α and other cytokines. Adiponectin also reduces the development of atherosclerosis in mice prone to its development, whereas in humans, low adiponectin may predict coronary artery disease severity and progression. Also, adiponectin may lower the risk of atherosclerosis through its effect on insulin sensitivity, and subsequently lower risk of type 2 diabetes mellitus.

Although compelling experimental evidence suggests a protective role of adiponectin in atherosclerosis, prospective data on adiponectin and risk of forms of CVD other than PAD seem less consistent. There are several possible explanations for this discrepancy. First, heterogeneity in case definitions may play a role: combining atherosclerotic events inversely related to adiponectin with other cardiovascular outcomes less immediately related to atherosclerosis, such as heart failure and fatal CVD (including sudden cardiac death). Second, the discrepancy may relate to the paradoxically increased risk of all-cause and CVD mortality associated with adiponectin mostly observed among not only older adults and patients with prevalent heart failure, CVD, PAD, or on hemodialysis but also apparently healthy men free of coronary artery disease. Adiponectin has pleiotropic roles beyond its known insulin-sensitizing actions, including clearance of apoptotic cells. As a result, it may directly improve insulin sensitivity and endothelial function even as levels rise in response to ongoing processes that lead to cellular apoptosis and necrosis and presumably mortality. Regardless, in our population of otherwise healthy subjects, we found no indication that the association between adiponectin and PAD risk differed over our age range or over predicted probabilities of PAD, suggesting that adiponectin acts similarly on risk of PAD among individuals at lower and higher risk. Third, we cannot rule out a sex difference because the independent association between adiponectin and other forms of CVD so far has predominantly been demonstrated in men and not in women even in mixed-sex studies, although results from the Women’s Health Study make this possibility less likely.

The potentially confounding or modifying role of kidney disease in the association between adiponectin and risk of CVD has not been extensively investigated. Most previous studies of adiponectin and PAD have not included measures of kidney function. Impaired kidney function may increase circulating adiponectin levels, although we did not observe a correlation between adiponectin and estimates of kidney function. The association of adiponectin with risk of PAD did not differ by levels of creatinine, cystatin C, or estimated glomerular filtration rate in this study, although most participants had normal kidney function and only very few (<5%) would have met criteria for moderate renal insufficiency (estimated glomerular filtration rate <60 mL·min⁻¹·1.73 m⁻² according to the Chronic Kidney Disease Epidemiology Collaboration equation).

The association of adiponectin with PAD risk persisted after adjustment for HDL cholesterol, despite the strong positive correlation between the 2 variables at baseline. Given the attenuation of the association after HDL cholesterol adjustment noted by others and us, the effect of adiponectin on the vascular system may be mediated in part through HDL-cholesterol metabolism. Indeed, adiponectin may accelerate reverse cholesterol transport by increasing HDL assembly in the liver through increased expression and secretion of apolipoprotein A-I and ATP-binding cassette transporter 1 in the liver. Further, in macrophages, adiponectin leads to upregulation of the expression of ATP-binding cassette transporter 1 and increased HDL-mediated cholesterol efflux. Adiponectin may also have a direct role on HDL catabolism through apolipoprotein A-I metabolism.

Strengths of the current study are the variety of biochemical and traditional risk factors that we included: the use of risk set sampling to select controls, the prospective design, the long-term follow-up, the homogeneity of our study population, and the use of adjudicated events with a pronounced atherosclerotic origin. Some potential limitations merit consideration. First, we used symptomatic PAD as an end point. Subclinical or asymptomatic PAD, which potentially could have been detected by ABI screening, may have been missed, similar to asymptomatic coronary or cerebral atherosclerosis in studies on myocardial infarction or stroke. However, end points included in this analysis were confirmed by medical records, reducing the likelihood of false-positive cases albeit at the risk of false-negative noncases. Moreover, this definition encompasses a degree of severity that is of unequivocal importance to both patients and physicians. Second, we studied men of predominantly white descent, although we have no...
reason to assume that adiponectin might be of less importance in other ethnicities.\textsuperscript{46,47} Third, adiponectin concentrations, and included covariables alike, were only determined at baseline, although intradividual concentrations of adiponectin are reasonably stable over time,\textsuperscript{48} and changes over time would result in exposure misclassification and attenuate the results toward the null. Finally, our findings are observational and, despite the wide variety of potentially confounding factors we adjusted for, unmeasured or residual confounding may be present. However, it should also be noted that some of the biochemical variables we adjusted for may be in the causal pathway, which would underestimate the true relationship between adiponectin and risk of PAD.

In conclusion, we observed a strong linear inverse association between total adiponectin and risk of symptomatic lower extremity PAD in men free of manifest atherosclerotic disease. The association seemed to be independent of important biochemical or traditional clinical risk factors of CVD. These findings suggest a prominent role of adiponectin in the initiation and progression of atherosclerotic diseases, such as PAD. Future mechanistic studies and prospective studies with well-characterized individuals are warranted to better understand the role of adiponectin in atherosclerosis.

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Disclosures
None.

References
Lower extremity peripheral artery disease is a manifestation of systemic atherosclerosis that has received considerably less clinical and research attention than coronary or cerebrovascular disease. Although cholesterol and inflammatory risk factors are also strong predictors in this form of cardiovascular disease, peripheral artery disease may be less related to thrombosis or plaque rupture. This raises the possibility that factors with antiatherosclerotic and anti-inflammatory properties, like adiponectin, may be of particular importance in the pathogenesis of this predominantly atherosclerotic type of cardiovascular disease. To study this, we prospectively followed middle-aged male health professionals without existing cardiovascular disease. After traditional cardiovascular risk factors were taken into account, each SD increase in total adiponectin was associated with a 32% lower risk of symptomatic peripheral artery disease. Given the inconsistencies in independent associations between adiponectin and other forms of cardiovascular disease, this finding, if confirmed, suggests a more prominent role of adiponectin in the development of (peripheral) atherosclerosis.

Significance

Lower extremity peripheral artery disease is a manifestation of systemic atherosclerosis that has received considerably less clinical and research attention than coronary or cerebrovascular disease. Although cholesterol and inflammatory risk factors are also strong predictors in this form of cardiovascular disease, peripheral artery disease may be less related to thrombosis or plaque rupture. This raises the possibility that factors with antiatherosclerotic and anti-inflammatory properties, like adiponectin, may be of particular importance in the pathogenesis of this predominantly atherosclerotic type of cardiovascular disease. To study this, we prospectively followed middle-aged male health professionals without existing cardiovascular disease. After traditional cardiovascular risk factors were taken into account, each SD increase in total adiponectin was associated with a 32% lower risk of symptomatic peripheral artery disease. Given the inconsistencies in independent associations between adiponectin and other forms of cardiovascular disease, this finding, if confirmed, suggests a more prominent role of adiponectin in the development of (peripheral) atherosclerosis.
Total Adiponectin and Risk of Symptomatic Lower Extremity Peripheral Artery Disease in Men

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