Association of Insulin Resistance and Inflammation With Peripheral Arterial Disease

The National Health and Nutrition Examination Survey, 1999 to 2004

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Background—Although the role of inflammation in the pathophysiology of peripheral arterial disease (PAD) is well established, the contribution of insulin resistance (IR) to PAD is less clear. We hypothesized that IR is associated with PAD and that the presence of IR would influence the association between C-reactive protein (CRP) and PAD, an association established predominantly in healthy individuals.

Methods and Results—We analyzed data from 3242 adults in the National Health and Nutrition Examination Survey (NHANES) 1999 to 2004 who underwent measurement of ankle brachial index, CRP, and fasting glucose and insulin, enabling calculation of homeostasis model of IR (HOMA-IR). Odds ratios (ORs) and 95% CIs were estimated by logistic regression. The mean prevalence of PAD (defined as an ankle brachial index \( \leq 0.9 \)) was 5.5% (SE, 0.47%). HOMA-IR was independently associated with PAD (OR, 2.06; 95% CI, 1.1 to 4.0; \( P = 0.03 \) for quartile 4, \( P \) for trend across quartiles \( = 0.047 \)) after adjustment for age, gender, race/ethnicity, hypertension, hyperlipidemia, smoking, body mass index, chronic kidney disease, and CRP. Elevated CRP (\( \geq 3 \) mg/L) also was strongly associated with PAD (OR, 2.2; 95% CI, 1.3 to 3.6; \( P = 0.003 \) versus CRP \( < 1 \) mg/L). Stratifying subjects on the basis of median HOMA-IR, we found that CRP \( \geq 3 \) mg/L was no longer significantly associated with PAD in subjects with IR (OR, 1.3; 95% CI, 0.8 to 2.1; \( P = 0.3 \), \( P \) for interaction \( = 0.08 \)).

Conclusions—These findings demonstrate that IR is strongly and independently associated with PAD. Furthermore, IR modifies the association of inflammation with PAD. These data establish a role of IR in PAD and highlight the relative importance of inflammation in patients with and without IR. (Circulation. 2008;118:33-41.)

Key Words: inflammation ■ insulin resistance ■ peripheral vascular disease

Both insulin resistance and inflammation have been implicated in the development of atherosclerosis. The contribution of inflammation to atherogenesis has been demonstrated in both cellular and molecular investigations and confirmed in epidemiological studies demonstrating an association of C-reactive protein (CRP; an inflammatory marker) with increased cardiovascular risk.\(^1\)–\(^3\) Similarly, insulin resistance, increasingly appreciated as an important component of atherogenesis, is associated with clinical atherosclerosis in epidemiological studies and predicts future cardiovascular events.\(^4\)–\(^8\)

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Peripheral arterial disease (PAD) is an important manifestation of systemic atherosclerosis affecting an estimated 10 million Americans and is associated with significant limb morbidity and cardiovascular mortality.\(^10\) The role of insulin resistance in PAD is not well established, although several lines of evidence support a linkage. Insulin resistance contributes significantly to the development of diabetes mellitus, a known risk factor for PAD,\(^10\) and studies have shown that incident PAD may be associated with the metabolic syndrome\(^11\)–\(^12\) and glucose intolerance,\(^13\) both manifestations of insulin resistance. No prior study has evaluated the relationship between PAD and a direct measurement of insulin resistance. The homeostasis model of insulin resistance (HOMA-IR) is a simple measure of insulin resistance derived from fasting glucose and insulin values that correlates well with insulin sensitivity derived from the glucose clamp technique, the gold standard measure of insulin sensitivity.\(^14\) HOMA-IR has been shown to correlate with cardiovascular disease\(^6\)–\(^8\) and cerebrovascular disease,\(^15\)–\(^18\) but the relationship between HOMA-IR and PAD has not previously been established.

In contrast to the limited data on insulin resistance and PAD, several studies have shown that inflammatory markers,
including CRP, are directly associated with PAD,\textsuperscript{19,20} predict the initial development of PAD,\textsuperscript{21,22} and portend adverse outcomes in patients with established PAD.\textsuperscript{23,24} However, the importance of CRP as a predictor of PAD and cardiovascular disease has been established predominantly in healthy individuals.\textsuperscript{1,21} In subjects with diabetes or other established cardiovascular risk factors, the association between CRP and vascular disease is less clear. Indeed, conflicting data exist, with several studies demonstrating a significant attenuation of the association between CRP and myocardial infarction or stroke in subjects with diabetes,\textsuperscript{25,26} although the data are not uniform.\textsuperscript{27} With these inconsistent findings and the accumulating evidence supporting the close interrelationship of inflammation and insulin resistance,\textsuperscript{28–30} we hypothesized that the presence of insulin resistance might modify the association between CRP and PAD. Accordingly, we used the National Health and Nutrition Examination Survey (NHANES) from 1999 to 2004 to evaluate the association between HOMA-IR, a direct measure of insulin resistance, and PAD and to determine the influence of insulin resistance on the association of inflammation and PAD.

Methods

The NHANES is a series of surveys of the noninstitutionalized civilian population in the United States. Sampling is performed in a complex, stratified, multistage manner to provide nationally representative data in an effort to assess the health and nutritional status of adults and children in the United States. The 1999 to 2004 NHANES was reviewed and approved by the National Center for Health Statistics Institutional Review Board. Informed consent was obtained from all subjects.

Ankle Brachial Index Measurements

Beginning in 1999, adults $\geq 40$ years of age were asked to participate in a lower-extremity examination, including the ankle brachial index (ABI), a diagnostic test for PAD with excellent performance characteristics (79% to 95% sensitivity and 95% to 100% specificity).\textsuperscript{10} Systolic pressure was measured in the supine position in the right arm (brachial artery) and in the posterior tibial artery of both ankles with an 8-MHz Doppler probe. Blood pressures were measured twice at each site in participants 40 to 59 years of age and only once for participants $\geq 60$ years of age. The ABI was calculated by dividing the systolic blood pressure in the ankle by the systolic blood pressure in the arm. We assigned a diagnosis of PAD if either leg had an ABI $\leq 0.90$. Patients with ABI values $>1.40$ were excluded because these values may be falsely elevated as a result of severe vascular calcification.

Laboratory Methods

Standard automated biochemical analysis was used to determine nonfasting serum glucose (Beckman Synchrone LX20, Beckman Coulter, Fullerton, Calif), and fasting glucose was measured with the enzyme hexokinase method. Fasting insulin was determined with the 2-site immunoenzymometric assay ( Tosoh AIA-PACK IRI, Tosoh Bioscience Inc, Grove City, Ohio) in 2003 to 2004. For earlier years (1999 to 2002), the Pharmacia insulin radioimmunoassay kit was used for insulin measurement (Pharmacia Diagnostics AB, Uppsala, Sweden). Because these methods yield slightly different measurements, insulin values were converted as recommended in the analytic guidelines.\textsuperscript{33} High-sensitivity CRP was measured by latex-enhanced enzyme hexokinase method. Fasting insulin was determined with the Coulter, Fullerton, Calif), and fasting glucose was measured with the Beckman Synchron LX20, Beckman Coulter, Fullerton, Calif). Changes in insulin and glucose values from the 1999 and 2000 examinations were corrected as suggested in the laboratory documentation files. Fasting lipids were analyzed with the Hitachi 704 Analyzer (Roche Diagnostics, Basel, Switzerland). HOMA-IR was calculated as follows: $(\text{fasting glucose (mmol/L)} \times \text{fasting insulin (}\mu\text{mol/L})) / 22.5$.\textsuperscript{32}

Covariates

Race/ethnicity, gender, and age were assessed by self-report. Race/ethnicity was categorized as non-Hispanic white, non-Hispanic black, Mexican American, or other. Subjects were considered to have hypertension if they reported a physician diagnosis of hypertension, if they reported taking prescription medications for hypertension, or if systolic blood pressure was $\geq 140$ mm Hg and/or diastolic blood pressure was $\geq 90$ mm Hg. These blood pressures were taken separately from the ABI examination and measured with a mercury sphygmomanometer. A diagnosis of hypercholesterolemia was assigned if the subject reported a physician diagnosis of hypercholesterolemia or reported taking prescription medications for hypercholesterolemia or if the total cholesterol level was $\geq 6.21$ mmol/L (240 mg/dL). Subjects were considered to have diabetes mellitus if the subject reported a physician diagnosis of diabetes, if the subject reported taking prescription medications for diabetes (either insulin or oral agents), if nonfasting plasma glucose was $\geq 11.1$ mmol/L (200 mg/dL), or if fasting plasma glucose was $\geq 7$ mmol/L (126 mg/dL). Active smokers were determined from self-report (a positive answer to the question, “Do you now smoke cigarettes?”). Those who did not meet these criteria were considered former smokers if they answered yes to the question, “Have you smoked at least 100 cigarettes in your life?” and never smokers if they had never smoked in their lifetime. Body mass index (BMI) was calculated as weight (kg) divided by height (m$^2$) (n = 3198). We used the Modification of Diet in Renal Disease Study equation to estimate glomerular filtration rate (GFR) from serum creatinine (SCr): GFR (mL · min$^{-1}$ · 1.73 m$^2$) = $175 \times (SCr)^{-1.154} \times (\text{age})^{-0.203} \times (0.742 \text{ if female}) \times (1.210 \text{ if black})$. Subjects with an estimated GFR $<60$ mL · min$^{-1}$ · 1.73 m$^2$ were classified as having chronic kidney disease. Diagnosis of congestive heart failure, angina, heart attack, and stroke was made by self-report. Any cardiovascular disease was defined as prevalent myocardial infarction, heart failure, or stroke on the basis of a subject’s affirmative response to the question, “Has a doctor or other health professional ever told you that you have had a [heart attack/congestive heart failure/stroke]?” Subjects were categorized as having the metabolic syndrome on the basis of the National Cholesterol Education Program criteria if they had 3 of the 5 following criteria: high-density lipoprotein cholesterol (HDL-C) $<1.3$ mmol/L (50 mg/dL) (men) and $<1.5$ mmol/L (50 mg/dL) (women), waist circumference $>102$ cm (men) or $>88$ cm (women), triglycerides $>1.7$ mmol/L (150 mg/dL), fasting glucose $>5.6$ mmol/L (100 mg/dL), and elevated blood pressure (systolic $>130$ mm Hg or diastolic $>8$ mm Hg).

Derivation of Sample Population

Our analysis combined data from the NHANES 1999 to 2004 examinations. ABI measurements were available for 7571 subjects in NHANES 1999 to 2004, of which 113 were excluded on the basis of ABI $>1.40$. One half of these participants, a representative sub-sample of the US population, were asked to attend a morning session during which fasting blood work was collected. Only individuals who had fasted at least 8 to 24 hours were assigned a nonzero fasting weight and thus were included in our sample population.\textsuperscript{31} This left 3499 with glucose and insulin values enabling calculation of HOMA-IR. We excluded subjects who were taking insulin (n = 80) given the limitations of HOMA-IR measurements in this subpopulation.\textsuperscript{34} After the exclusion of subjects who did not have complete information available on other covariates of interest, the remaining 3242 subjects constituted the sample population for this analysis.

Statistical Methods

NHANES uses a complex, multistage, probability sampling design to select participants representative of the civilian, noninstitutional-
ized US population. Given that our analysis was limited to the representative subsample of individuals with fasting blood work, we used the fasting sample weights to account for the sampling design. Subjects were included in our analysis only if they had fasted a minimum of 8 hours and had been assigned a nonzero fasting weight. We generated a combined 6-year fasting weight variable by assigning two thirds of the 4-year weight for 1999 to 2002 if the subject participated during 1999 to 2002 or assigning one third of the 2-year weight for 1999 to 2002 if the subject participated during 2003 to 2004.

Analyses were performed with SAS version 9.1 (SAS Institute, Inc, Cary, NC) callable SUDAAN version 9.01 (Research Triangle Institute, Research Triangle Park, NC) to account for the complex sample design. Age- and gender-adjusted baseline subject characteristics are reported as the weighted mean and SE or the weighted percentile and SE. Categorical variables were compared by use of the χ2 test or logistic regression. Comparisons of mean values across groups and correlations between continuous variables were achieved by linear regression. Odds ratios (ORs) and 95% CIs were estimated by logistic regression. We divided HOMA-IR values into quartiles and examined the presence of a linear trend across quartiles. We further evaluated the odds of PAD in each quartile compared with the reference group, HOMA-IR quartile 1. In a multivariable model, we then adjusted for age, gender, race/ethnicity, hypertension, hyperlipidemia, smoking, BMI, chronic kidney disease, and CRP. A value of P<0.05 was considered statistically significant except for interaction terms, for which a value of P<0.10 was considered statistically significant.

To assess whether the presence of insulin resistance influenced the relationship between CRP and PAD, we stratified the population by median HOMA-IR level to indicate the presence or absence of insulin resistance and reevaluated the relationship between CRP and PAD in these 2 categories. Given the lack of established threshold values of HOMA-IR to indicate insulin resistance, we used the median HOMA-IR level as a threshold value in our analysis. A formal test for interaction also was performed to evaluate the impact of insulin resistance on the association between CRP and PAD.

The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

Results

Of the 3242 subjects in our sample population, 256 participants had an ABI ≤0.90, for an overall unadjusted weighted prevalence (mean) of PAD of 5.5% (SE, 0.47%). Age- and gender-adjusted baseline characteristics of participants with and without PAD are summarized in Table 1. Subjects with PAD had a significantly higher mean age (66.4 years [SE, 1.1 years] versus 55.5 years [SE, 0.3 years]; P<0.00001) and a higher prevalence of hypertension, coronary heart disease, and chronic kidney disease. An elevated CRP (>3 mg/L) was noted in a significantly higher proportion of PAD subjects (54.6% [SE, 5.0%] versus 40.3% [SE, 1.0%]), and mean CRP levels were significantly higher in PAD subjects (7.5 mg/dL [SE, 1.3 mg/dL] versus 4.4 mg/dL [0.2 mg/dL]; P=0.02). There were no significant differences in gender distribution or BMI.

Association of HOMA-IR and PAD

Age- and gender-adjusted baseline characteristics of subjects in each HOMA-IR quartile are shown in Table 2. Subjects in the higher HOMA-IR quartiles were less likely to be female or of non-Hispanic white race. Cardiovascular risk factors, including hypertension, hyperlipidemia, and diabetes, were significantly more prevalent in increasing HOMA-IR quartiles. A history of smoking (current or former) and the presence of chronic kidney disease were not different in the groups. HOMA-IR correlated significantly with several baseline variables, including BMI (r=0.45, P<0.0001), high-density lipoprotein (r=−0.32, P<0.0001), triglycerides (r=0.22, P<0.0001), and CRP (r=0.12, P=0.0002). There was no correlation between HOMA-IR and LDL (r=0.03, P=0.05) or total cholesterol (r=0.01, P=NS).

Age- and gender-adjusted PAD prevalence estimates increased in a graded fashion in increasing HOMA-IR quartiles: 5.0% (SE, 0.8%) in quartile 1, 5.4% (SE, 0.9%) in quartile 2, 6.1% (SE, 0.7%) in quartile 3, and 7.8% (SE, 1.1%) in quartile 4 (P=0.037, χ2 test) (Figure 1). Compared with subjects in the lowest HOMA-IR quartile, subjects in increasing quartiles had a graded increase in odds of PAD: OR=1.24 (95% CI, 0.8 to 2.0) for quartile 2, OR=1.53 (95% CI, 0.96 to 2.4) for quartile 3, and

<table>
<thead>
<tr>
<th>Table 1. Age- and Gender-Adjusted Subject Characteristics</th>
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<tbody>
<tr>
<td>PAD Present</td>
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<tr>
<td>n</td>
</tr>
<tr>
<td>Male gender, %</td>
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<tr>
<td>Age group, %</td>
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<td>40–49 y</td>
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<tr>
<td>60–69 y</td>
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<td>≥70 y</td>
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<tr>
<td>Race/ethnicity, %</td>
</tr>
<tr>
<td>White</td>
</tr>
<tr>
<td>Black</td>
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<tr>
<td>Mexican American</td>
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<tr>
<td>Other</td>
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<tr>
<td>Smoking status, %</td>
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<tr>
<td>Never</td>
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<td>Former</td>
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<tr>
<td>Current</td>
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<tr>
<td>Hypertension, %</td>
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<td>Diabetes, %</td>
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<td>Coronary heart disease, %</td>
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<td>Stroke, %</td>
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<tr>
<td>Chronic kidney disease, %</td>
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<tr>
<td>BMI, mean, kg/m²</td>
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<tr>
<td>HOMA-IR, mean</td>
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<tr>
<td>CRP &gt;3 mg/L, %</td>
</tr>
</tbody>
</table>

The χ2 test was used for statistical comparisons for gender, age, and race/ethnicity. All remaining prevalence estimates and mean values were adjusted for age and gender (except for gender, age, and race/ethnicity). For categorical variables, data are shown as weighted percent (SE), and logistic regression adjusting for age and gender was used for statistical comparison. For continuous variables, data are shown as mean (SE), and statistical comparisons are achieved by linear regression with adjustment for age and gender. HOMA-IR was log transformed for comparison.
In a multivariable model adjusting for age, gender, and race/ethnicity (Table 3, model 1), HOMA-IR remained associated with PAD with increasing odds of PAD across HOMA-IR quartiles: OR = 1.19 (95% CI, 0.8 to 1.9) for quartile 2, OR = 1.4 (95% CI, 0.9 to 2.3) for quartile 3, and OR = 1.83 (95% CI, 1.2 to 2.9) for quartile 4 compared with subjects in HOMA-IR quartile 1 (P for trend = 0.015). In further analyses also adjusting for traditional atherosclerotic risk factors (hypertension, hyperlipidemia, and smoking) and BMI (Table 3, model 2), the linear association between HOMA-IR quartiles and PAD persisted (P for trend = 0.039), and quartile 4 had a 2-fold increased odds of PAD (OR, 2.07; 95% CI, 1.1 to 3.9) compared with quartile 1. Finally, the magnitude and direction of the association between HOMA-IR and PAD persisted after additional adjustment for nontraditional factors related to PAD, including chronic kidney disease and CRP (P for trend = 0.047), and subjects in quartile 4 had an OR of 2.06 (95% CI, 1.1 to 4.0) compared with those in quartile 1 (Table 3, model 3). Additional adjustment for diabetes resulted in a marginal attenuation of the association of insulin resistance with PAD (P for trend = 0.058), although subjects in the highest HOMA-IR quartile continued to have an 2-fold increase in the odds of PAD (OR, 1.96; 95% CI, 1.03 to 3.8). After adjustment for hemoglobin A1c as a measure of recent glycemic control instead of diabetes, the relationship remained statistically significant (P for trend = 0.047) (Table 3).

These findings were unchanged when HOMA-IR was used as a continuous variable (after log transformation to improve normality). Using a multivariable model accounting for age, gender, race/ethnicity, hypertension, hyperlipidemia, smoking, BMI, chronic kidney disease, and CRP, we found that a 1-unit increase in log-HOMA conferred a 33% increased odds of PAD (OR, 1.33; 95% CI, 1.03 to 1.70; P = 0.029). This relationship persisted even after additional adjustment for diabetes (OR, 1.30; 95% CI, 1.0 to 1.67; P = 0.047) or hemoglobin A1c (OR, 1.36; 95% CI, 1.02 to 1.8; P = 0.036).

Given the recognized relationship between insulin resistance and diabetes, a well-established risk factor for PAD, we repeated our analyses excluding subjects with diabetes to evaluate the association of HOMA-IR with PAD independently of the effect of diabetes. We excluded all subjects with diabetes (n = 485, including those with self-report of diabetes, those taking insulin or oral agents, and subjects with a fasting blood glucose level >126 mg/dL or nonfasting level >200 mg/dL), leaving 2757 subjects...
remaining for analysis. In this nondiabetic sample population, there remained a graded increase in PAD prevalence across increasing HOMA-IR quartiles, with crude estimates of 3.8% (SE, 0.69%), 4.7% (SE, 0.94%), 5.7% (SE, 0.79%), and 6.1% (SE, 1.38%) across the 4 quartiles, respectively ($P$=0.037 by $\chi^2$ test). In univariate logistic regression analysis, a 1-quartile increase in HOMA-IR was associated with an increased odds of PAD compared with a CRP $<1$ mg/L (OR, 1.18; 95% CI, 0.98 to 1.42). Additionally, compared with subjects in the lowest HOMA-IR quartile (in an unadjusted model), subjects in increasing quartiles maintained a graded increase in odds of PAD: OR = 1.24 (95% CI, 0.8 to 1.97) for quartile 2, OR = 1.52 (95% CI, 0.92 to 2.5) for quartile 3, and OR = 1.62 (95% CI, 0.9 to 2.9) for quartile 4. As expected, exclusion of nearly 500 individuals from our sample population attenuated the associations between HOMA-IR and PAD; nonetheless, a graded increase in PAD prevalence and graded increases in the odds of PAD by increasing HOMA-IR quartiles were still observed.

### Table 3. Multivariable Logistic Regression Analysis of the Association of HOMA-IR and PAD

<table>
<thead>
<tr>
<th>Quartile 2</th>
<th>Quartile 3</th>
<th>Quartile 4</th>
<th>$P$ for Trend</th>
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<tbody>
<tr>
<td>OR</td>
<td>95% CI</td>
<td>OR</td>
<td>95% CI</td>
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<tr>
<td>Unadjusted</td>
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<tr>
<td>Model 1</td>
<td>1.20</td>
<td>0.8–2.0</td>
<td>1.40</td>
</tr>
<tr>
<td>Model 2</td>
<td>1.39</td>
<td>0.8–2.3</td>
<td>1.59</td>
</tr>
<tr>
<td>Model 3</td>
<td>1.35</td>
<td>0.8–2.3</td>
<td>1.57</td>
</tr>
<tr>
<td>Model 3+diabetes</td>
<td>1.34</td>
<td>0.8–2.3</td>
<td>1.55</td>
</tr>
<tr>
<td>Model 3+HbA1c</td>
<td>1.36</td>
<td>0.8–2.3</td>
<td>1.59</td>
</tr>
</tbody>
</table>

HbA1c indicates glycosylated hemoglobin. ORs and 95% CIs were estimated by logistic regression with quartile 1 as the reference population. Data also were analyzed for the presence of a linear trend across quartiles. Model 1 adjusts for age, gender, and race/ethnicity; model 2 also adjusts for hypertension, hyperlipidemia, smoking, and BMI; model 3 additionally adjusts for chronic kidney disease and CRP.
We examined the interrelationships between inflammation, insulin resistance, and PAD in this nationally representative sample population and found that insulin resistance, indicated by HOMA-IR, is strongly associated with PAD and that the presence of insulin resistance attenuates the association between CRP and PAD. Furthermore, this is the first study to illustrate the association of PAD and a direct measure of insulin resistance, HOMA-IR, a simple method that uses plasma insulin and glucose in a single fasting blood sample. HOMA-IR has previously been reported only in relation to cardiovascular and cerebrovascular disease. Indeed, we found a roughly 25% increased odds of PAD for each 1-quartile increase in HOMA-IR, a finding that remained consistent despite adjustment for typical atherosclerotic risk factors, factors related to insulin resistance (ie, BMI), and glycemic control (hemoglobin A1c). In addition, although the trend across quartiles was marginally no longer statistically significant after adjustment for diabetes, the point estimates were essentially unchanged, and subjects in the highest HOMA-IR quartile continued to have a nearly 2-fold increased odds of PAD. Using HOMA-IR as a continuous variable, we found a strong relationship between PAD and insulin resistance that persisted even after additional adjustment for diabetes or hemoglobin A1c. These data suggest that insulin resistance may have an association with PAD along the entire spectrum of insulin resistance and distinct from the impact of diabetes. Even after the exclusion of subjects with diabetes, there were graded increases in PAD prevalence with increasing HOMA-IR quartiles, although the associations between HOMA-IR and PAD were no longer statistically significant. Our data support prior observations that PAD is associated with the metabolic syndrome and glucose intolerance, both surrogate markers of insulin resistance.

In contrast to the limited data on the association of insulin resistance and PAD, the link between inflammation and PAD has been well established. Our demonstration of a strong association between CRP and PAD is consistent with prior epidemiological evidence linking inflammation with atherosclerosis in the coronary and peripheral arterial beds. However, much of the information on inflammation and atherosclerosis has been generated in healthy individuals. Less clear is whether this relationship persists in individuals with established cardiovascular risk factors such as diabetes. Sakkinen et al26 studied the relationship between CRP and myocardial infarction over a 20-year period, and although they found a positive relationship between CRP and myocardial infarction in the overall population, the association was abolished in individuals with diabetes. The same group also showed no significant relationship between CRP and stroke among patients with diabetes or hypertension.25 Prospective data from the Strong Heart Study in an American Indian population found no predictive value of CRP for incident cardiovascular events in diabetic individuals.28 In contrast, the Women’s Health Study2 found that CRP added prognostic information regarding cardiovascular events even in subjects with the metabolic syndrome, and a study by Schulze et al27 found a strong relationship between CRP and cardiovascular events among men with diabetes. However, none of these studies focused specifically on PAD, and none directly examined the role of insulin resistance.

Given the conflicting data on the relationship of CRP and vascular disease among subjects with diabetes and metabolic syndrome, we explored the possibility that insulin resistance in particular might influence the relationship between CRP and PAD. Indeed, our analysis found a strong association between CRP and PAD among individuals who were insulin sensitive. However, this association was no longer evident in subjects with insulin resistance. The attenuation of the relationship between CRP and PAD in the presence of insulin resistance may be explained in part by the close interrelationship of inflammation and insulin resistance in vascular disease. Extensive experimental data demonstrate that inflammation leads to impaired insulin metabolic signaling, resulting in insulin resistance. Insulin resistance may in turn exacerbate inflammation via increased cytokine and adipokine expression (including tumor necrosis factor alpha-6, interleukin-6, leptin, monocyte chemoattractant protein-1, plasminogen activator inhibitor-1, and others), elevation of free fatty acid levels, and impaired endothelial nitric oxide synthase activity. The reciprocal interaction of inflam-
mation and insulin resistance creates a cycle of further increasing cardiovascular risk.

These findings also support the notion that atherogenesis results from the complex interplay of multiple atherosclerotic risk factors, each of variable influence in different vascular beds. In individuals with few risk factors, inflammation may have a relatively large contribution to the development of atherosclerosis. In contrast, in the presence of a potent cardiovascular risk factor, insulin resistance, the relative contribution of inflammation to the pathogenesis of atherosclerosis may be diminished. In our study, subjects with insulin resistance but without inflammation (CRP <1 mg/L) already had a high prevalence of PAD (5.5%) compared with those who were insulin sensitive and without inflammation (2.2%). Among insulin-resistant subjects, the presence of inflammation then resulted in only a marginal increase in PAD prevalence (to 6.9% in the highest CRP category) that was not statistically significant. Whether these data imply that insulin resistance reduces the association of inflammation and PAD by a distinct effect on vascular function or simply by increasing inflammation, they highlight the complexity of the interaction of these risk factors in PAD and suggest that the role of inflammation in PAD may be modified in individuals with insulin resistance.

Additionally, although there are common risk factors for the development of vascular disease, the impact of specific risk factors on the development of disease is not the same in the peripheral vascular bed as in the coronary or cerebrovascular circulations.11 Indeed, cigarette smoking, one of the strongest risk factors for the development of PAD, was shown in the Edinburgh Artery Study42 to have a greater impact on PAD (OR, 1.8 to 5.6 for the risk of PAD in smokers versus nonsmokers) than on coronary artery disease (OR, 1.1 to 1.6). In contrast, hypertension and hyperlipidemia, potent risk factors for coronary atherosclerosis, appear to have less impact on the development of PAD, with a 10-mg/dL increase in total cholesterol conferring only a 10% increased risk of PAD.41 These findings suggest that individual risk factors may have variable impact on different vascular beds, and as such, a focused analysis of the impact of insulin resistance on PAD is warranted and may shed light on the differential pathophysiology of PAD compared with CAD and cerebrovascular disease.

These pathophysiological differences may explain in part the differences observed between our results and the findings of the Women’s Health Study2 and a study by Schulze et al,27 which suggest that CRP adds prognostic information regarding incident cardiovascular disease in subjects with the metabolic syndrome2 or diabetes27 at baseline. Our results also stand in contrast to a cross-sectional study from Vu et al43 in a smaller NHANES cohort suggesting that CRP remains associated with PAD even in patients with metabolic syndrome or diabetes, although not in those with established cardiovascular disease. The discrepancy is explained in part by differences in statistical methodology (the authors used the group with low CRP and no established disease as the reference population for all comparisons) and use of metabolic syndrome as opposed to a direct measure of insulin resistance.

These data are derived from a nationally representative cohort and therefore are generalizable to the US adult population. The ABI method has been demonstrated to have excellent sensitivity and specificity for the diagnosis of PAD.10 Despite these strengths, potential limitations of the present study merit consideration. In NHANES, the ABI was calculated from the blood pressure in only 1 arm, raising the potential for misclassification by not excluding the possibility of subclavian stenosis. Additionally, our analysis was limited to the subset of participants with ABI measurements who had a fasting blood sample drawn, reducing our sample size and potentially limiting power. However, our final sample population was nonetheless substantial, consisting of >3200 individuals in whom there were 256 cases of PAD. We also are limited by the lack of established threshold values of HOMA-IR for indicating the presence or absence of insulin resistance. Prior studies have used quartiles8 or quintiles,9 and given the lack established guidelines, we elected to use standard median and quartile cut points in our analysis. Additionally, there are notable differences in clinical variables by HOMA-IR quartile; even with multivariable models, there may be confounding that may not be adequately accounted for in our statistical models. Finally, the cross-sectional nature of our study does not allow us to draw conclusions regarding causality, and prospective studies are required to clarify any temporal relationship and to delineate the true causal relationships between inflammation, insulin resistance, and PAD.

Conclusions

Insulin resistance is strongly and independently associated with PAD. The presence of insulin resistance attenuates the association of inflammation with PAD. These data establish a role of insulin resistance in PAD and suggest that future studies are warranted to better understand the complex interplay of inflammation and insulin resistance in peripheral arterial disease.

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Disclosures

None.

References

3. Tracy RP, Lemaitre RN, Psaty BM, Ives DG, Evans RW, Cushman M, Meilahn EN, Kuller LH. Relationship of C-reactive protein to risk of


Peripheral arterial disease (PAD) is an important manifestation of systemic atherosclerosis. Systemic inflammation and insulin resistance are closely linked pathological states, and each contributes to the development of atherosclerosis. However, the specific association of insulin resistance with PAD and the influence of insulin resistance on the relationship between inflammation and PAD have not been established. Using data from the National Health and Nutrition Examination Survey 1999 to 2004, we found that insulin resistance, as measured by the homeostasis model of insulin resistance, is strongly associated with PAD independently of known cardiovascular risk factors. Furthermore, we found that the presence of insulin resistance blunts the association between inflammation, as measured by C-reactive protein, and PAD. These findings suggest a direct link between PAD and insulin resistance along its entire spectrum and highlight the complex interplay of insulin resistance and inflammation in atherosclerosis.
Association of Insulin Resistance and Inflammation With Peripheral Arterial Disease: The National Health and Nutrition Examination Survey, 1999 to 2004
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