Vascular Biomarkers in the Prediction of Clinical Cardiovascular Disease
The Strong Heart Study
Mary J. Roman, Jorge R. Kizer, Lyle G. Best, Elisa T. Lee, Barbara V. Howard, Nawar M. Shara, Richard B. Devereux

Abstract—We compared the ability of separately measured intimal-medial thickness and atherosclerotic plaque to predict incident cardiovascular disease. American Indian men and women from the Strong Heart Study who were free of cardiovascular disease were evaluated with carotid ultrasound and cardiovascular disease risk factor assessment. End-diastolic intimal-medial thickness of the common carotid arteries was measured and averaged. Arterial mass (cross-sectional area) was calculated from intimal-medial thickness and end-diastolic diameter. Atherosclerosis was defined by focal plaque (discrete thickening \( \geq 50\% \) relative to the adjacent wall) and the number of carotid segments containing plaque (plaque score); 2441 participants (age 63±8 years) were followed-up for a mean of 7.7±2.8 years, during which time 495 experienced incident cardiovascular disease events. Time-to-event analyses were performed in groups stratified according to diabetes and hypertension status. Cardiovascular disease events were predicted by presence and extent of atherosclerosis in all groups; intima-medial thickness and arterial mass were only associated with outcomes when neither hypertension nor diabetes was present. Unequivocal evidence of atherosclerosis (plaque) and its extent (plaque score) are independently associated with incident cardiovascular disease events in individuals without preexisting cardiovascular disease regardless of diabetes and hypertension status. Hypertension-related increases in intima-media thickness and arterial mass appear to limit their use as measures of early or diffuse atherosclerosis and, hence, association with cardiovascular disease outcomes. These findings support the utility of separate assessment of focal atherosclerosis and intimal-medial thickness in epidemiological studies, trials, and risk stratification protocols. (Hypertension. 2012;59:29-35.)

Key Words: cardiovascular disease prognosis ■ carotid arteries ■ epidemiological methods
Hypertension was defined by Joint National Committee 7 criteria of the second and third of three consecutive readings was recorded. The mean brachial artery after 5 minutes of rest by trained personnel using an Omron 907 device (OMRON Healthcare, Kyoto, Japan). The mean of fasting plasma glucose ≥7.0 mmol/L (126 mg/dL) or by use of hypoglycemic treatment. Diabetes was defined by the American Diabetes Association criteria as fasting plasma glucose ≥10.0 mmol/L (181 mg/dL). Carotid cross-sectional area, a measure of vascular volume or mass, was calculated as previously described. Wall thickness and diameters were performed on several cycles and averaged. IMT of the far wall and end-diastolic diameter. All ultrasound findings to cardiovascular events were determined in Cox regression analyses adjusting for age, sex, body mass index, waist circumference, current smoking, nonhigh-density lipoprotein cholesterol, high-density lipoprotein cholesterol, triglycerides, and estimated glomerular filtration rate. The study population was additionally adjusted for fasting glucose and the nondiabetic groups are additionally adjusted for systolic blood pressure and the nondiabetic groups are additionally adjusted for fasting glucose. Two-tailed P < 0.05 was considered significant.

We tested for multiplicative interactions between vascular measures and sex, diabetes, and hypertension by including appropriate cross-product terms in Cox models. There was no significant effect-measure modification by sex for any of the 4 vascular measures (IMT, arterial mass, plaque, and plaque score) in relation to outcomes, but there was evidence of multiplicative interaction of diabetes status with both IMT (P = 0.021) and vascular mass (P = 0.001), and of hypertension status with both IMT (P = 0.001) and vascular mass (P = 0.001). Although similar effect-modification by diabetes or hypertension status was not observed for the presence (P = 0.424 and P = 0.714, respectively) or extent of atherosclerosis (P = 0.554 and P = 0.425, respectively), all analyses were stratified by diabetes status and by hypertension status. The C-statistic, which is equivalent to the area under the receiver-operating characteristic curve, was calculated as a measure of model discrimination. All analyses were performed with SPSS version 19 (SPSS, Chicago, IL) or STATA version 11 (Stata, College Station, TX).

Results

Population Characteristics and CVD Outcomes

A total of 2441 participants were free of prevalent clinical CVD at the time of examination. Mean age was 63 ± 8 years (range, 51–84 years); 65% were women and body mass index was 31.3 ± 6.6 kg/m². Hypertension was present in 52.2% of the population, of whom 69% were using antihypertensive medications. Use of lipid-lowering therapy was uncommon at the time of examination (2.4%). Diabetes was present in 47.6% of the population, and 27.4% were active smokers. Among the 2441 participants, 495 (20.3%) experienced initial fatal and nonfatal CVD events (101 myocardial infarction, 204 definite coronary heart disease, 92 stroke, 98 congestive heart failure) during a mean follow-up of 7.7 ± 2.8 years; 21.4% of initial events were fatal.

Traditional CVD risk factors, vascular biomarkers, and CVD outcomes are compared in Tables 1 and 2 stratified according to diabetes and hypertension status. Ages were higher and renal function was lower in the 2 groups with hypertension compared to the other 2 groups. Body mass index and waist circumference were higher and rates of smoking were lower in the groups with hypertension and/or diabetes compared to the normal group. Body mass index and waist circumference were also significantly higher in the 2 groups with diabetes compared to the group with hypertension alone. The proportion of men was lowest in those with both hypertension and diabetes. The 4 groups were compa-
Arterial mass was associated with outcomes in either model with incident CVD in both age-adjusted and sex-adjusted analyses. Results from the Cox models are presented in Table 3. All vascular imaging biomarkers were strongly associated with cardiovascular disease outcomes in all 4 groups. In secondary analyses, adding use of antihypertensive or glucose-lowering medications to multivariable analyses did not substantially alter results (data not shown).

In the entire population, the C-statistic for prediction of events by risk factors alone (0.700; 95% CI, 0.674–0.726) was significantly increased by addition of either plaque (C-statistic=0.714; 95% CI, 0.688–0.739; P=0.011) or plaque score (C-statistic=0.719; 95% CI, 0.694–0.744; P=0.001) to the model. In view of the significant effect modification observed for carotid IMT by hypertension and diabetes status, wherein carotid IMT was only predictive of outcome in the normal group, receiver-operator characteristic curve analyses using IMT were restricted to this group. Although addition of IMT substantially increased the magnitude of the C-statistic compared to the risk factor model alone (from 0.735 [95% CI, 0.678–0.791] to 0.748 [95% CI, 0.690–0.806], the change was not statistically significant (P=0.198).

To determine whether carotid IMT might be more predictive of incident stroke or coronary artery disease than plaque, we performed additional analyses in the entire group because

<table>
<thead>
<tr>
<th>Variable</th>
<th>No HTN or DM (n=734)</th>
<th>HTN Alone (n=545)</th>
<th>DM Alone (n=432)</th>
<th>HTN and DM (n=730)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>62.1±7.5</td>
<td>64.6±8.4*</td>
<td>61.9±6.9</td>
<td>63.2±7.2†</td>
</tr>
<tr>
<td>Male, %</td>
<td>39.2</td>
<td>38.0</td>
<td>32.2</td>
<td>29.5*</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>29.2±6.2</td>
<td>30.7±6.0*</td>
<td>32.1±6.8*</td>
<td>33.4±6.8*</td>
</tr>
<tr>
<td>Waist circumference, cm</td>
<td>100±15</td>
<td>104±14*</td>
<td>108±15*</td>
<td>111±15*</td>
</tr>
<tr>
<td>Current smoking, %</td>
<td>37.9</td>
<td>27.1*</td>
<td>27.3‡</td>
<td>17.2*</td>
</tr>
<tr>
<td>Systolic pressure, mm Hg</td>
<td>120±11</td>
<td>143±19*</td>
<td>121±11</td>
<td>139±21*</td>
</tr>
<tr>
<td>Fasting glucose, mg/dL</td>
<td>99±11</td>
<td>100±11</td>
<td>191±75*</td>
<td>184±67*</td>
</tr>
<tr>
<td>Non-HDL cholesterol, mg/dL</td>
<td>147±39</td>
<td>146±39</td>
<td>143±39</td>
<td>145±39</td>
</tr>
<tr>
<td>HDL cholesterol, mg/dL</td>
<td>45±14</td>
<td>46±15</td>
<td>39±10*</td>
<td>43±13*</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>127±74</td>
<td>140±86</td>
<td>167±132*</td>
<td>171±129*</td>
</tr>
<tr>
<td>eGFR, ml/min per 1.73 m²</td>
<td>86±16</td>
<td>79±22*</td>
<td>86±21</td>
<td>78±26*</td>
</tr>
</tbody>
</table>

**Table 1. Comparison of Demographic Variables and Cardiovascular Disease Risk Factors Stratified According to Diabetes and Hypertension Status**

<table>
<thead>
<tr>
<th>Variable</th>
<th>No HTN or DM (n=734)</th>
<th>HTN Alone (n=545)</th>
<th>DM Alone (n=432)</th>
<th>HTN and DM (n=730)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intimal-medial thickness, mm</td>
<td>0.72±0.14</td>
<td>0.75±0.15‡</td>
<td>0.75±0.16§</td>
<td>0.76±0.15*</td>
</tr>
<tr>
<td>Arterial mass, mm²</td>
<td>15.03±4.12</td>
<td>16.14±4.27*</td>
<td>16.10±4.49*</td>
<td>16.59±4.27*</td>
</tr>
<tr>
<td>Atherosclerotic plaque, %</td>
<td>57.2</td>
<td>66.4‡</td>
<td>66.4†</td>
<td>66.2‡</td>
</tr>
<tr>
<td>Plaque score</td>
<td>1.2±1.4</td>
<td>1.6±1.7*</td>
<td>1.4±1.4</td>
<td>1.6±1.7*</td>
</tr>
<tr>
<td>Incident CVD, n (%)</td>
<td>74 (10.1)</td>
<td>97 (17.8§)</td>
<td>110 (25.5)*</td>
<td>214 (29.3)*</td>
</tr>
</tbody>
</table>

**Table 2. Comparison of Vascular Biomarkers and Cardiovascular Disease Outcomes Stratified According to Diabetes and Hypertension Status**

CVD indicates cardiovascular disease; DM, diabetes mellitus; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; HTN, hypertension.

*P<0.001, †P<0.05, ‡P<0.005 vs no HTN or DM group.
of the relatively small number of separate events in the 4 subgroups. Multivariable analyses adjusting for the covariates listed in Table 3 as well as for the presence or absence of diabetes and hypertension indicated that IMT was not an independent predictor of either coronary heart disease or of stroke, whereas atherosclerotic plaque was a strong independent predictor of coronary heart disease (heart rate, 1.88; 95% CI, 1.35–2.61; \( P < 0.001 \)) and plaque score was an independent predictor of stroke (heart rate, 1.19; 95% CI, 1.04–1.35; \( P = 0.012 \)).

**Discussion**

Our study shows that the presence and extent of carotid plaque, direct manifestations of atherosclerosis, are strong predictors of incident CVD, independent of the effects of diabetes, hypertension, and other established risk factors. Importantly, our findings are adjusted for both traditional CVD risk factors as well as estimated glomerular filtration rate, the nonhypertensive groups are additionally adjusted for systolic blood pressure and the nondiabetic groups are additionally adjusted for fasting glucose.

Studies that have analyzed IMT and plaque separately generally show the greatest risk of future CVD events to be conferred by the presence of focal plaque. In one of the first such studies,7 2000 healthy subjects aged 30 to 70 years were followed-up for 6 years after baseline carotid and femoral ultrasound examination. Cardiovascular events occurred in 5.5% of those with increased IMT (diffuse thickening >1 mm) and in 18.4% of those with plaque (focal thickening of IMT >2 mm). Similarly, in 10 000 healthy individuals followed-up for 10 years in the Cafes-Caves Study, incident CVD occurred in 8.6% of those with increased IMT and in 39.3% of those with plaque.8 However, neither of these studies adjusted for CVD risk factors. In the Kuopio Ischemic Heart Disease Risk Factor Study of 2,181 middle-aged Finnish men, the 4-year risk of acute myocardial infarction was increased 2.1-fold in those with increased IMT (>1 mm) and 3.4-fold in those with nonobstructive plaque in comparison to those with normal carotid ultrasound studies;8 again, results were not adjusted for CVD risk factors.

Three recent analyses in population-based studies separately examined IMT and plaque in relation to outcomes and adjusted for CVD risk factors. Among 1249 participants aged 18 to 99 years in the San Daniele Study, a town in northeastern Italy, the relative risk of ischemic cerebrovascular events (ischemic stroke or transient ischemic attack) was 5.6 (95% CI, 3.2–10.1) for CCA IMT >1 mm and 10.4 (95% CI, 6.4–17.1) for ≥1 plaque compared to those with normal IMT and no plaque at baseline examination ≈12 years earlier.12 The analyses included those with prevalent CVD at baseline, and data were not available from the baseline evaluation to examine IMT as a continuous measure.

In the Atherosclerosis Risk in Communities Study, categories (<25th percentile, 25th to 75th percentile, >75th percentile) of averaged CCA, bifurcation, and internal carotid artery IMT and plaque were added to the Atherosclerosis Risk in Communities 10-year coronary risk score in 13 145 participants without prevalent CVD at baseline.13 The area under the receiver-operating characteristic curve significantly improved when plaque, but not IMT, was added to traditional risk factors in women. Although both plaque and IMT increased the area under the receiver-operating characteristic curve in men, the increase was greater for IMT than plaque.

### Table 3. Multivariable Cox Regression Models* of Relation of Vascular Biomarkers to Cardiovascular Outcome Stratified According to Diabetes and Hypertension Status

<table>
<thead>
<tr>
<th>Vascular Biomarker</th>
<th>No HTN or DM</th>
<th>HTN Alone</th>
<th>DM Alone</th>
<th>HTN and DM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IMT, per SD</td>
<td>HR (95% CI)</td>
<td>P</td>
<td>HR (95% CI)</td>
</tr>
<tr>
<td>Age-adjusted and sex-adjusted model</td>
<td>1.29 (1.05–1.59)</td>
<td>0.017</td>
<td>0.95 (0.78–1.16)</td>
<td>0.616</td>
</tr>
<tr>
<td>Multivariable model</td>
<td>1.26 (1.01–1.57)</td>
<td>0.041</td>
<td>0.96 (0.78–1.17)</td>
<td>0.681</td>
</tr>
<tr>
<td>Arterial mass, SD</td>
<td>1.42 (1.15–1.74)</td>
<td>0.001</td>
<td>1.16 (0.95–1.42)</td>
<td>0.142</td>
</tr>
<tr>
<td>Age-adjusted and sex-adjusted model</td>
<td>1.39 (1.11–1.73)</td>
<td>0.004</td>
<td>1.11 (0.90–1.36)</td>
<td>0.331</td>
</tr>
<tr>
<td>Multivariable model</td>
<td>2.53 (1.39–4.60)</td>
<td>0.002</td>
<td>1.79 (1.07–3.00)</td>
<td>0.026</td>
</tr>
<tr>
<td>Atherosclerotic plaque</td>
<td>2.26 (1.25–4.10)</td>
<td>0.007</td>
<td>1.74 (1.06–2.86)</td>
<td>0.030</td>
</tr>
<tr>
<td>Plaque score, per segment</td>
<td>1.42 (1.23–1.63)</td>
<td>&lt;0.001</td>
<td>1.21 (1.083–1.36)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age-adjusted and sex-adjusted model</td>
<td>1.41 (1.23–1.63)</td>
<td>&lt;0.001</td>
<td>1.17 (1.05–1.30)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

CI indicates confidence interval; DM, diabetes mellitus; HR, hazard ratio; HTN, hypertension; IMT, intimal-medial thickness; SD, standard deviation.

*Multivariable models are adjusted for age, sex, body mass index, waist circumference, current smoking, non-HDL cholesterol, HDL cholesterol, triglycerides, and estimated glomerular filtration rate. The nonhypertensive groups are additionally adjusted for systolic blood pressure and the nondiabetic groups are additionally adjusted for fasting glucose.
Because this study evaluated averaged IMT measured from areas that might include plaque in the IMT measurement, it is possible that the significance of IMT in relation to outcomes is overstated. The extent of atherosclerosis, i.e., the presence of plaques in multiple segments, was not examined in relation to outcome.

In 2965 members of the Framingham Offspring Study cohort followed-up for 7.2 years, mean IMT of the CCA, maximum IMT of the internal carotid artery, and plaque within the internal carotid artery (defined as IMT > 1.5 mm) were all associated with incident cardiovascular events. Only the internal carotid artery IMT and plaque resulted in an increase in the C-statistic generated by consideration of the Framingham risk score. The present study confirms this observation for both the presence and the extent of atherosclerosis. Similarly to the Framingham Offspring Study, the C-statistic was not significantly improved by addition of common carotid IMT in our study. However, our group of normal participants (no hypertension or diabetes) is relatively small and lacks sufficient power for assessment of the incremental discriminatory value of this measure. Larger studies of healthy individuals will be necessary to determine whether the increased magnitude of the C-statistic achieved by carotid IMT suggested here in fact represents genuine incremental discriminatory value of this measure. Larger studies of healthy individuals will be necessary to determine whether the increased magnitude of the C-statistic achieved by carotid IMT suggested here in fact represents genuine improvement in risk prediction.

An indirect validation of the superiority of carotid plaque over carotid IMT is provided by the substantially stronger relation of carotid plaque area to significant underlying coronary artery disease as evidenced by computed tomography angiogram. Similarly, right carotid artery plaque area was more strongly related to incident myocardial infarction and ischemic stroke than carotid IMT in the Tromsø Study. These results are of particular interest because IMT measurement could incorporate plaque thickness if plaque was present in the predefined area where IMT measurements were performed. Although plaque area derived from a 2-dimensional ultrasound study may not be a precise measure of atherosclerosis burden, these findings are similar to those using the semiquantitative plaque score in the present study. The Rotterdam Study found IMT and plaque (evaluated as none vs at least 3 plaques using a plaque score) to be equally predictive of myocardial infarction independent of traditional risk factors; the authors comment on the ease of plaque assessment compared to the precision required for IMT measurement. Furthermore, in the Rotterdam Study, increasing plaque score (0–6) increased the risk of stroke and, in a separate population of elderly men, plaque score independently predicted all-cause and cardiovascular mortality.

There is emerging interest in the concept of “vascular” as opposed to biological age as well as the optimal “vascular biomarker” to use as an adjunct to traditional risk stratification techniques. Although a recent review and a meta-analysis of published data indicated that carotid IMT is a predictor of cardiovascular events, the authors conceded that IMT measurements were not uniformly made in plaque-free area and that focal plaque may have been included in IMT measurement as a consequence of study protocol dictating site of measurement or requiring “maximum IMT.” The present study indicates that of vascular structural parameters, the presence and extent of direct evidence of atherosclerosis are more strongly associated with future risk of clinical CVD than is IMT. Interestingly, although carotid artery atherosclerosis is a manifestation of cerebrovascular disease, the majority of events predicted are attributable to coronary heart disease, underscoring both the systemic nature of atherosclerosis as well as the greater frequency of cardiac compared to cerebral manifestations.

There are several potential limitations to our study. In the Strong Heart Study, IMT was only measured in the distal CCA. Data from the Cardiovascular Health Study and the British Regional Heart Study suggest a stronger association of CCA IMT with prevalent stroke, whereas bifurcation or internal carotid artery IMT were more strongly related to prevalent myocardial infarction. However, adjusted relative risks for prediction of incident events were only marginally different between the CCA and internal carotid artery IMT in the Cardiovascular Health Study. A major advantage of CCA IMT is its higher measurement yield compared to other segments. In the Atherosclerosis Risk in Communities Study, IMT measurements were obtained from the CCA in 91.4%, from the bifurcation in 77.3%, and from the internal carotid artery in 48.6% of participants. A report on 1881 Rotterdam Study participants showed a similar trend in measurement yield: 96% in the CCA, 64% in the bifurcation, and 31% in the internal carotid artery. Measurement yield of CCA IMT in the current study was comparable to that in these earlier studies (96%). The present findings derive from a cohort of American Indians at high cardiometabolic risk, such that generalizability to other populations cannot be assumed. However, stratification and adjustment showed that the presence and extent of atherosclerosis were associated with incident CVD independent of diabetes and hypertension and other risk factors. Furthermore, the same traditional risk factors for cardiovascular disease in the general U.S. population have been shown to be operative in the Strong Heart Study population. Although one cannot necessarily extrapolate results from this generally obese and insulin-resistant population to nonobese noninsulin-resistant populations, our findings will be applicable to a greater proportion of the broader population if current trends in the increasing prevalence of obesity and diabetes continue unabated.

**Perspectives**

The present study shows that unequivocal evidence of atherosclerosis (plaque) and its extent (plaque score) are independently associated with first incident CVD events in individuals, regardless of diabetes and hypertension status. In contrast, IMT and arterial mass were only found to be associated with CVD outcome in the absence of diabetes and hypertension, likely attributable to hypertension-mediated vascular hypertrophy, lessening the likelihood that diffuse vessel wall thickening and lumen dilatation are manifestations of atherosclerosis. These findings highlight the value of plaque as a vascular biomarker and support the utility of separate assessment of focal atherosclerosis and IMT in epidemiological studies, as well as risk assessment protocols. Whether these direct measures of atherosclerosis afford...
improved risk prediction in selected subgroups warrants further study in larger samples.

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Disclosures
None.

References


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