Potential of Quantity of Coronary Artery Calcification to Identify New Risk Factors for Asymptomatic Atherosclerosis

Julie E. Maher,1 Jonathan A. Raz,2 Lawrence F. Bielak,1 Patrick F. Sheedy, II,3 Robert S. Schwartz,4 and Patricia A. Peyser1

The goals of this study of adults asymptomatic for coronary artery disease (CAD) were to examine the relations between established CAD risk factors and quantity of coronary artery calcification (CAC) in three arterial locations using generalized linear mixed models and to estimate the variability in quantity of CAC explained by established CAD risk factors and the variability due to noise or artifact in the measure. The community-based sample included 740 asymptomatic adults (378 women) aged 20–59 years without hypertension or diabetes. Participants were recruited from Rochester, Minnesota, between 1990 and 1994. Quantity of CAC in three arterial locations was detected noninvasively by electron beam computed tomography. Sex, arterial location, age, body size, blood pressure, lipid metabolism, and smoking were significantly (p < 0.05) associated with quantity of CAC. Age was more strongly associated with quantity of CAC in the left than in the right coronary or circumflex arterial locations (p < 0.005). In each sex, risk factors together explained less than 40% of the variability in quantity of CAC. Noise or artifact in the measure accounted for only a small proportion of unexplained variability. Future studies of new risk factors for artery-specific quantity of CAC and its progression could provide additional etiologic insights into the atherosclerotic process. Am J Epidemiol 1996; 144:943-53.

atherosclerosis; calcinosis; calcium; coronary disease; coronary vessels; models, statistical; risk factors; tomography, x-ray computed

Many comprehensive studies have identified risk factors for coronary artery disease (CAD). Yet, the total disease process is not fully understood (1), with 30–70 percent of CAD deaths not explained by established CAD risk factors (2, 3). A major limitation of previous studies of the epidemiology of CAD has been reliance on clinical endpoints, including angina, myocardial infarction, and sudden death, which misclassify many individuals with coronary atherosclerosis because they lack symptoms (4). A measure of asymptomatic coronary atherosclerosis would address this limitation and reduce the need for very large, lengthy cohort studies. A quantitative measure would provide additional statistical power to detect risk factor associations. Since location of coronary atherosclerosis is an indicator of prognosis (5), an artery-specific measure would be useful.

Coronary artery calcification (CAC) is part of the atherosclerotic process (6, 7) and predicts future CAD morbidity and mortality in asymptomatic (8, 9) and symptomatic (10) adults. Not every atherosclerotic plaque has calcification, but if calcification is present in the epicardial arteries it is almost always part of an atherosclerotic plaque (11). Calcification may appear in preatheromas and most often appears in fibroatheromas (12). Research in the molecular biology of atherosclerosis suggests that calcification is a well-organized and active process similar to bone formation (6, 7). As reviewed (6, 7), the mineral found in CAC, hydroxyapatite, is the same as in bone, several proteins that regulate bone formation are present in arteries, and a cell with the ability to calcify in vitro has been isolated from arteries. Calcification may represent the body's attempt to stabilize plaque (7); however, the interface between calcium and other tissue may create a high-stress site prone to rupture (6).
Electron beam computed tomography (CT) can non-invasively detect, locate, and quantify CAC. In vitro, electron beam CT’s measure of quantity of CAC is positively associated with degree of stenosis (13, 14) and with the amount of plaque (13, 15). In vivo, electron beam CT-detected CAC is a sensitive marker for angiographically defined CAD in all epicardial arteries combined (16–23) and in individual epicardial arteries (20), and its measure of quantity of CAC is strongly associated with maximal stenosis in all epicardial arteries combined (21, 24, 25).

The goals of this study of adults asymptomatic for CAD were to examine the relations between established CAD risk factors and quantity of CAC in three arterial locations using generalized linear mixed models (26–30) and to estimate the variability in quantity of CAC explained by established CAD risk factors and the variability due to noise or artifact in the measure.

**MATERIALS AND METHODS**

**Sample**

The Rochester Family Heart Study is a community-based study of the genetic epidemiology of CAD and essential hypertension in Rochester, Minnesota (31). In January 1984, a letter describing the purpose of the Rochester Family Heart Study and a questionnaire were sent to 5,270 households with at least two children enrolled in Rochester schools. On the questionnaire, the head of each household provided demographic information on each individual in the household. No information was collected on risk factors or disease status. On the basis of the questionnaire data, 1,653 households were ranked by the amount of information they could potentially yield about the relations between risk factor variability, gene variability, and risk for CAD and hypertension. Households were recruited in rank order. All of the schoolchildren’s available great-grandparents, grandparents, parents, siblings, and children at least age 5 years at the time of participation were studied. Between December 1, 1984 and August 1, 1991, 601 households containing 3,974 individuals in 580 pedigrees participated in the Rochester Family Heart Study.

For this study, individuals were recruited between December 1, 1990 and April 1, 1994 from almost 2,000 Rochester Family Heart Study participants aged 20–59 years. A total of 882 individuals were contacted. Eleven of these individuals were ineligible due to pregnancy, lactation, or prior heart surgery, and one additional individual had Alzheimer’s disease. The participation rate of those eligible was 92 percent (800/870). Each of the 800 participants received a physical examination, blood draw, and electron beam CT examination during a visit to the Mayo Clinic. Medications used within 1 month prior to the physical examination were recorded, and medical records were reviewed. The Mayo Clinic Institutional Review Board approved the study protocol. After study procedures were explained to participants, they gave written, informed consent.

Sixty evaluated participants were excluded from this study. Two participants had unusable electron beam CT data, and the additional 58 participants were excluded on the basis of information in the medical records. These included 22 participants considered asymptomatic for CAD because of previous angiography or radionuclide heart scan, 30 asymptomatic individuals with a previous diagnosis of hypertension, four asymptomatic individuals with diabetes mellitus, and two asymptomatic individuals with both hypertension and diabetes mellitus. Only one hypertensive individual was not on medication. The final sample included 740 participants (378 women) approximately uniformly distributed across ages 20–59 years. There were 736 white Americans, one Native American, one Latin American, and two Asian Americans.

**Risk factor measures**

Measures of age, body size, blood pressure, lipid metabolism, and cigarette smoking were studied as possible predictors of quantity of CAC because they are established risk factors for CAD (32). Age at examination (in years) was calculated using an individual’s birth date. After participants removed shoes and outer clothing, height and weight were measured using a wall stadiometer and a beam balance. Body mass index (BMI) (weight (kg)/height$^2$ (m$^2$)) was used as a measure for body size. Systolic blood pressure was measured in the right arm with a random-zero sphygmomanometer (Hawkslet & Sons, Ltd., West Sussex, England) at the Korotkoff phase I sound. Three measures, taken at least 2 minutes apart, were averaged. Systolic pressure was used because no other blood pressure measure is a better predictor of cardiovascular endpoints (33). Standard enzymatic methods were used to measure plasma total cholesterol (31). After precipitation of lipoproteins containing apolipoprotein B, high density lipoprotein cholesterol (HDL cholesterol) was measured (31). The estimated coefficients of variation were less than 1.2 percent for total cholesterol and less than 5.6 percent for HDL cholesterol. Cholesterol/HDL cholesterol ratio was used as a measure of lipid metabolism. Smoking status was defined, on the basis of self-report, as having a history of cigarette smoking (i.e., ever having been a cigarette smoker) or not.
Electron beam CT examination

In the beginning of the study, each participant received one scan run with an electron beam CT scanner (Imatron C-100, Imatron Incorporated, South San Francisco, California). In January 1993, the protocol was modified to include a second scan run several minutes after the first. The second scan run, which was performed for half of the sample (191 of 378 women and 180 of 362 men), was added to the protocol to assess reproducibility of lesions and within-participant variability in CAC (19). Each scan run consisted of 40 contiguous, 3-mm thick transverse tomograms obtained from the level of the right branch of the pulmonary artery to the apex of the heart. Scan time per tomographic level was 100 msec. Electrocardiographic triggering was used to obtain tomograms at the same phase in the cardiac cycle for each participant (either at 80 percent of the RR interval or 600 msec past the QRS complex). No iodine contrast agent was used, and the radiation dose of a scan run to the skin at the back was 10 mGy (1 rad). A radiologic technologist scored each examination. A radiologist inspected the technical quality of each tomogram, evaluated the scoring, and interpreted the findings of the examination.

A technologist scored an examination by placing a region of interest around each hyperattenuating focus of CAC in each tomographic level. For these analyses, a focus of CAC was defined as an area of at least two adjacent pixels (0.52 mm² under field of view of 26; 0.69 mm² under field of view of 30) with a CT number above 130 Hounsfield units located within each epicardial artery. Calcific area (in mm²) within each region of interest was calculated using vendor-supplied software (Imatron Incorporated). Since interobserver and intraobserver reliabilities are high (34), only one technologist scored a scan run. Quantity of CAC in the left anterior descending, left main, right coronary, and circumflex arteries was defined as the sum of all calcific areas in the given epicardial artery. Because it is difficult to identify consistently the left main artery (34), the quantity of CAC in the left main and the left anterior descending arteries was summed and referred to as the left arterial location.

As yet, there is no uniformly agreed upon focus size to define CAC. Use of a smaller focus size to define CAC leads to higher sensitivity and lower specificity for detecting angiographically defined CAD and to lower repeatability of specific lesions (19). For these analyses, foci of at least two adjacent pixels were included for the following reasons. These investigations concern quantity of CAC rather than presence or absence of CAC. Since over a quarter of foci this size are repeatable (19), some may represent CAC rather than noise or artifact. The statistical variability in quantity of CAC due to noise and artifact was estimated using the two electron beam CT scan runs available for half of the sample.

Statistical methods

All analyses except tests of sex differences in the risk factors and quantity of CAC were performed separately for men and women because frequency of CAD events, profiles of CAD risk factors, and the correlation among risk factors vary by sex (35, 36). The 0.05 level of significance was used.

Sex differences in the risk factors were tested using $t$ tests and a chi-square test. Associations among the risk factors were assessed by Pearson correlation coefficients and $t$ tests.

Sex differences in quantity of CAC were evaluated using the Mann-Whitney $U$ test. Differences among the arterial locations in quantity of CAC were evaluated using a Wilcoxon matched-pairs signed-ranks test applied to log-transformed quantity of CAC ($\ln(\text{quantity of CAC} + 1)$). For a participant with two electron beam CT scan runs, only the first scan run was used for these tests.

Other studies relating risk factors to quantity of CAC have used linear regression with log-transformed quantity of CAC ($\ln(\text{quantity of CAC} + 1)$) in the whole heart from a single electron beam CT scan run as the outcome variable (24, 37, 38). Linear regression's assumptions of normality, homoscedasticity, and independence (39) would have been violated in this study. The sex-specific distributions of quantity of CAC in specific arterial locations, as well as the whole heart, contained high relative frequencies of zeros and were highly skewed, even after log-transformation. No monotonic transformation will make the data more nearly normal, since the mode is at the minimum value of zero and any monotonic transform will have the mode at the minimum value. The variance of quantity of CAC increased with age and other CAD risk factors, as did the variance of other quantitative measures of atherosclerosis in previous studies (40, 41). Quantity of CAC was not independent among arterial locations within the same participant or between the dual scan runs on the same participant. Ignoring the violation of these three assumptions would have led to inefficient estimators and biased inference (42).

The limitations of linear regression were overcome by analyzing the relation between quantity of CAC in each arterial location and risk factors using generalized linear mixed models (26–30) (see appendix 1). The models were fit using generalized estimating equations for participant-specific models. In this approach, the logarithm of the expected quantity of CAC...
was modeled as a linear function of the covariates, and the variance of quantity of CAC was modeled proportional to the expected quantity of CAC and an additional dispersion parameter. The distribution of quantity of CAC was otherwise unspecified. These models were "mixed" since they included both random participant by arterial location interaction effects and fixed effects of the risk factors and arterial locations. The random effects accounted for the lack of independence among measures from the three arterial locations on the same participant, as well as between measures of CAC quantity from the dual electron beam CT scan runs. Furthermore, the model allowed us to include in the same analysis participants with one electron beam CT scan run and those with dual electron beam CT scan runs, without averaging the two scan runs. To obtain estimates of adjusted associations, a full model was fit that included fixed effects of the five risk factors and the three arterial locations and random participant by arterial location interaction effects. Also eligible for inclusion in the model were terms representing the quadratic effect of age and the risk factor by arterial location interactions; these terms were omitted if the regression coefficients were not significant at the 0.05 level, where significance was assessed using approximate F tests (26, 27, 29). The fit of the model was evaluated using Anscombe residual plots (43), and the proportion of variability in quantity of CAC explained by the fixed effects was estimated using the concordance correlation coefficient (26, 29, 44). After the full model was fitted, the average within-participant variance was computed as a percentage of the average total residual variance in each arterial location. This ratio allowed us to estimate the proportion of statistical variability in quantity of CAC that was due to noise and artifact.

Because some participants from this study were from the same pedigree as other participant(s), the final sex-specific models were refit with only one randomly selected participant per pedigree in women (n = 292) and men (n = 276). The inferences were essentially the same, suggesting that the correlation among participants within pedigrees was not unduly influencing the model results.

The unadjusted associations between each risk factor and the quantity of CAC were also assessed. These unadjusted associations were defined by fitting models that contained a single risk factor, dummy variables for the three arterial locations, and the random effects. Estimates of the adjusted and unadjusted associations between CAC and a particular covariate were computed as the multiplicative increase due to a one standard deviation increase in a continuous covariate (such as age or BMI) or a change in status in a dichotomous covariate (such as smoking or a dummy variable representing a particular arterial location). The 95 percent confidence intervals were computed for the multiplicative effects based on approximate standard errors (26, 27, 29).

RESULTS

The men in the sample had significantly higher mean systolic pressure and cholesterol/HDL cholesterol ratio than did the women (p < 0.001), but the women and men did not significantly differ in the other risk factors (table 1). All continuous risk factors were positively correlated with one another in both women and men (p < 0.05) (data not shown). The most highly correlated continuous risk factors were systolic pressure and BMI in women (r = 0.33, p < 0.001), and cholesterol/HDL cholesterol ratio and BMI in men (r = 0.37, p < 0.001). Having a history of smoking was significantly associated with higher mean cholesterol/HDL cholesterol ratio (p < 0.05) in both sexes and also with higher mean BMI (p = 0.008) and mean age (p = 0.005) in men.

Descriptive statistics for quantity of CAC in each arterial location by 10-year age group and sex are presented in table 2. When the data were pooled over

<table>
<thead>
<tr>
<th>Risk factor (units)</th>
<th>Women (n = 378)</th>
<th>Men (n = 362)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>40.9 (10.9) 20.2-59.6</td>
<td>40.1 (12.1) 20.0-59.7</td>
</tr>
<tr>
<td>BMI† (kg/m²)</td>
<td>25.5 (5.2) 15.8-50.1</td>
<td>26.2 (3.8) 18.0-39.3</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)*</td>
<td>111.7 (12.5) 77.3-161.3</td>
<td>114.6 (10.8) 83.0-174.0</td>
</tr>
<tr>
<td>Cholesterol/HDL cholesterol*†,‡</td>
<td>4.0 (1.4) 1.3-11.4</td>
<td>5.2 (1.8) 2.2-13.4</td>
</tr>
<tr>
<td>History of smoking (%)</td>
<td>41.3</td>
<td>47.5</td>
</tr>
</tbody>
</table>

* p < 0.001 for t test of equality of means between women and men.
† SD, standard deviation; BMI, body mass index; HDL, high density lipoprotein cholesterol.
‡ Five observations in women and two observations in men are deleted because of missing values.

Table 1. Sex-specific coronary artery disease risk factors, Rochester, Minnesota, 1990–1994

TABLE 2. Descriptive statistics for quantity of CAC*,† in mm² in each arterial location by age in 378 women and 362 men, Rochester, Minnesota, 1990-1994

<table>
<thead>
<tr>
<th>Arterial location</th>
<th>Age (years)</th>
<th>20-29</th>
<th>30-39</th>
<th>40-49</th>
<th>50-59</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
</tr>
<tr>
<td>Left</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Women (Maximum)‡</td>
<td>0.07 (0.39)</td>
<td>0.26 (1.18)</td>
<td>0.73 (3.73)</td>
<td>2.74 (10.20)</td>
<td>1.02 (5.72)</td>
<td></td>
</tr>
<tr>
<td>Men (Maximum)</td>
<td>0.23 (0.85)</td>
<td>1.12 (3.14)</td>
<td>8.17 (22.69)</td>
<td>20.05 (42.68)</td>
<td>7.95 (26.25)</td>
<td></td>
</tr>
<tr>
<td>Right coronary</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Women (Maximum)</td>
<td>0.13 (0.49)</td>
<td>0.63 (1.16)</td>
<td>0.35 (1.10)</td>
<td>1.50 (7.27)</td>
<td>0.65 (3.81)</td>
<td></td>
</tr>
<tr>
<td>Men (Maximum)</td>
<td>0.39 (1.19)</td>
<td>0.74 (1.38)</td>
<td>4.11 (14.14)</td>
<td>9.74 (38.60)</td>
<td>4.01 (21.42)</td>
<td></td>
</tr>
<tr>
<td>Circumflex</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Women (Maximum)</td>
<td>0.03 (0.14)</td>
<td>0.13 (0.65)</td>
<td>0.26 (1.03)</td>
<td>0.39 (1.22)</td>
<td>0.22 (0.91)</td>
<td></td>
</tr>
<tr>
<td>Men (Maximum)</td>
<td>0.21 (0.71)</td>
<td>0.39 (1.49)</td>
<td>2.22 (7.14)</td>
<td>3.38 (12.71)</td>
<td>1.68 (7.69)</td>
<td></td>
</tr>
</tbody>
</table>

* CAC, coronary artery calcification; SD, standard deviation.
† A focus of CAC was defined as an area of at least two adjacent pixels (0.52 mm² under field of view of 26; 0.69 mm² under field of view of 30) with a computed tomographic number above 130 Hounsfield units located within each epicardial artery. For a participant with two electron beam computed tomographic scan runs, the first scan run was used.
‡ Maximum, the maximum quantity of CAC. The minimum quantity of CAC was zero in all age groups for each sex and arterial location.

In the age groups, men had significantly (p < 0.001) more CAC than did women in each arterial location. In women, quantity of CAC was significantly higher in the left (p = 0.004) and right coronary (p < 0.001) arterial locations than in the circumflex arterial location but did not differ significantly between the left and the right coronary arterial locations. In men, quantity of CAC was significantly higher in the left (p < 0.001) and right coronary (p < 0.001) arterial locations than in the circumflex arterial location and was significantly (p = 0.002) higher in the left arterial location than in the right coronary arterial location.

Each of the risk factors considered alone (table 3) was positively associated with quantity of CAC in women and men (p < 0.05). In the full models containing all risk factors, the quadratic effect of age was not significant in either women or men and was excluded. Significant age by arterial location interactions (p < 0.005) were present in women and men. There was some evidence for cholesterol/HDL cholesterol ratio by arterial location interactions in women; however, collinearity between the age and cholesterol/HDL cholesterol ratio by arterial location interactions made interpretation of this model difficult. Therefore, results are reported for the model with only the age by arterial location interactions.


<table>
<thead>
<tr>
<th>Trait</th>
<th>Women</th>
<th></th>
<th></th>
<th>Men</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>95% CI†</td>
<td></td>
<td></td>
<td>95% CI†</td>
</tr>
<tr>
<td>Age</td>
<td>2.26</td>
<td>1.68-3.05</td>
<td>4.27</td>
<td>3.40-5.36</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI‡</td>
<td>2.60</td>
<td>2.39-3.26</td>
<td>1.36</td>
<td>1.08-1.72</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic blood pressure</td>
<td>2.27</td>
<td>1.70-3.02</td>
<td>1.46</td>
<td>1.15-1.85</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cholesterol/HDL cholesterol†,§</td>
<td>1.98</td>
<td>1.56-2.53</td>
<td>2.07</td>
<td>1.64-2.60</td>
<td></td>
<td></td>
</tr>
<tr>
<td>History of smoking</td>
<td>2.05</td>
<td>1.03-4.05</td>
<td>3.25</td>
<td>1.62-6.55</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Multiplicative increase in quantity of coronary artery calcification is for a 1 standard deviation increase in age (measured in years), in body mass index (measured in kg/m²), in systolic blood pressure (measured in mmHg), in cholesterol/HDL cholesterol ratio, or for having a history of smoking.
† CI, confidence interval; BMI, body mass index; HDL cholesterol, high density lipoprotein cholesterol.
‡ 95% CI for multiplicative increase in quantity of coronary artery calcification.
§ Five observations in women and two observations in men are deleted because of missing values.
In the final model for women (table 4), age, BMI, and cholesterol/HDL cholesterol ratio were positively associated with quantity of CAC \( (p < 0.05) \). The age effect was greater for the left arterial location than for the right coronary \( (p < 0.001) \) or circumflex \( (p < 0.001) \) arterial locations. Although the 95 percent confidence interval for the age effect for the right coronary arterial location included one, it did not differ significantly from the age effect for the circumflex arterial location. These risk factors and arterial locations explained approximately 38 percent of the variability in quantity of CAC.

For men, the final model (table 5) indicated that age, cholesterol/HDL cholesterol ratio, and having a history of smoking were positively associated with quantity of CAC \( (p < 0.05) \). Consistent with the findings for women, the effect of age was greater for the left arterial location than for the right coronary \( (p = 0.002) \) and circumflex \( (p < 0.001) \) arterial locations, and the effect of age did not differ significantly between the right coronary and circumflex arterial locations. These risk factors and arterial locations explained approximately 9 percent of the variability in quantity of CAC.

For the sex-specific final models, the predicted values for each arterial location across age were calculated for nonsmokers with mean BMI, cholesterol/HDL cholesterol ratio, and systolic pressure. For example, in men, the predicted value in the left arterial location for a nonsmoker with mean values for each of the risk factors but with age 1 standard deviation above the mean would be equal to \( 1.75 \times 5.13 \) or 8.98 mm\(^2\) (table 5). To illustrate the modeled, artery-specific association between quantity of CAC and age in women and men, the artery-specific plots of ln(quantity of CAC + 1) versus age were overlaid with the corresponding lines for ln(predicted value + 1) (figures 1 and 2). These figures also demonstrate that the variance of quantity of CAC increased with age.

The estimated statistical variability in quantity of CAC due to noise and artifact (i.e., average within-participant variance as a percentage of the average total residual variance) was low in each arterial location in both women and men. In women, these estimated percentages were one in the left, four in the right coronary, and 19 in the circumflex arterial locations. The corresponding estimates in men were one in both the left and right coronary arterial locations and two in the circumflex arterial location.

**DISCUSSION**

This study of 740 asymptomatic participants aged 20–59 years demonstrated that quantity of CAC varied significantly by sex, other established CAD risk factors, and arterial location. In each sex, much of the variability in quantity of CAC remained unexplained by the risk factors and arterial locations, with very little of the unexplained variability due to noise or artifact in the measure.

**Associations with risk factors**

Other studies have investigated associations between CAD risk factors and quantity of CAC in the epicardial arteries combined, rather than in individual

**TABLE 4. Estimated mean quantity of CAC* and adjusted associations for the final model in 378 women†, Rochester, Minnesota, 1990–1994**

<table>
<thead>
<tr>
<th>Trait</th>
<th>Mean quantity of CAC ‡</th>
<th>Multiplicative increase§</th>
<th>95% CI†, ¶</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left arterial location</td>
<td>0.29</td>
<td></td>
<td>0.14–0.60</td>
</tr>
<tr>
<td>Right coronary arterial location</td>
<td>0.23</td>
<td></td>
<td>0.16–0.32</td>
</tr>
<tr>
<td>Circumflex arterial location</td>
<td>0.09</td>
<td></td>
<td>0.06–0.13</td>
</tr>
<tr>
<td>Age:left arterial location#</td>
<td>5.07</td>
<td></td>
<td>3.00–8.59</td>
</tr>
<tr>
<td>Age:right coronary arterial location#</td>
<td>1.26</td>
<td></td>
<td>0.87–1.81</td>
</tr>
<tr>
<td>Age:circumflex arterial location#</td>
<td>1.65</td>
<td></td>
<td>1.20–2.25</td>
</tr>
<tr>
<td>BMI*</td>
<td>2.36</td>
<td></td>
<td>1.89–2.94</td>
</tr>
<tr>
<td>Systolic blood pressure</td>
<td>1.25</td>
<td></td>
<td>0.87–1.81</td>
</tr>
<tr>
<td>Cholesterol/HDL cholesterol* ratio</td>
<td>1.22</td>
<td></td>
<td>1.01–1.48</td>
</tr>
<tr>
<td>History of smoking</td>
<td>1.21</td>
<td></td>
<td>0.75–1.94</td>
</tr>
</tbody>
</table>

* CAC, coronary artery calcification; CI, confidence interval; BMI, body mass index; HDL cholesterol, high density lipoprotein cholesterol.
† Five observations are deleted because of missing values for cholesterol/HDL cholesterol ratio.
‡ Mean quantity of CAC (mm\(^2\)) in each arterial location calculated for nonsmokers with the mean values for each of the other risk factors.
§ Multiplicative increase in quantity of CAC is for a 1 standard deviation increase in age (measured in years), BMI (measured in kg/m\(^2\)), systolic blood pressure (measured in mmHg), cholesterol/HDL cholesterol ratio, or for having a history of smoking.
¶ 95% CI for mean quantity of CAC in each arterial location or multiplicative increase in quantity of CAC.
# Estimate is for the age effect at the specified arterial location.
TABLE 5. Estimated mean quantity of CAC* and adjusted associations for the final model in 362 men†, Rochester, Minnesota, 1990–1994

<table>
<thead>
<tr>
<th>Trait</th>
<th>Mean quantity of CAC‡</th>
<th>Multiplicative Increase§</th>
<th>95% CI†, ‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left arterial location</td>
<td>1.75</td>
<td>1.20–2.54</td>
<td></td>
</tr>
<tr>
<td>Right coronary arterial location</td>
<td>1.59</td>
<td>0.99–2.56</td>
<td></td>
</tr>
<tr>
<td>Circumflex arterial location</td>
<td>0.61</td>
<td>0.37–1.02</td>
<td></td>
</tr>
<tr>
<td>Age: left arterial location#</td>
<td>5.13</td>
<td>4.05–6.51</td>
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</tr>
<tr>
<td>Age: right coronary arterial location#</td>
<td>2.53</td>
<td>1.55–4.14</td>
<td></td>
</tr>
<tr>
<td>Age: circumflex arterial location#</td>
<td>2.44</td>
<td>1.61–3.69</td>
<td></td>
</tr>
<tr>
<td>BMI*</td>
<td>1.13</td>
<td>0.90–1.44</td>
<td></td>
</tr>
<tr>
<td>Systolic blood pressure</td>
<td>1.20</td>
<td>0.95–1.53</td>
<td></td>
</tr>
<tr>
<td>Cholesterol/HDL cholesterol* ratio</td>
<td>1.74</td>
<td>1.42–2.14</td>
<td></td>
</tr>
<tr>
<td>History of smoking</td>
<td>1.72</td>
<td>1.05–2.82</td>
<td></td>
</tr>
</tbody>
</table>

* CAC, coronary artery calcification; CI, confidence interval; BMI, body mass index; HDL cholesterol, high density lipoprotein cholesterol.
† Two observations are deleted because of missing values for cholesterol/HDL cholesterol ratio.
‡ Mean quantity of CAC (mm²) in each arterial location calculated for nonsmokers with the mean values for each of the other risk factors.
§ Multiplicative increase in quantity of CAC is for a 1 standard deviation increase in age (measured in years), in BMI (measured in kg/m²), in systolic blood pressure (measured in mmHg), in cholesterol/HDL cholesterol ratio, or for having a history of smoking.
# 95% CI for mean quantity of CAC in each arterial location or multiplicative increase in quantity of CAC.
† Estimate is for the age effect at the specified arterial location.

arterial locations, among adults asymptomatic for CAD. In a study of 111 hypercholesterolemic men, only age and triglycerides were positively associated ($p < 0.05$) with quantity of CAC based on stepwise linear regression (37). Wong et al. (38) studied self-reported risk factors in 675 men and 190 women who were self- or physician-referred. In stepwise linear regression analyses, age, male sex, hypertension, diabetes, hypercholesterolemia, obesity, and history of smoking were positively associated ($p < 0.10$) with quantity of CAC. Recently, Simon et al. (45) studied 618 men at risk for CAD. Quantity of CAC was categorized into four grades by using arbitrary cutpoints. A logistic regression model was fit with age, systolic pressure, cholesterol/HDL cholesterol ratio, current smoking, diabetes, and electrocardiogram-left ventricular hypertrophy as predictors. In this model, age, systolic pressure, and cholesterol/HDL cholesterol ratio were positively associated ($p < 0.05$) with higher amount of CAC. None of the above studies gave an estimated $R$-square.

This study found that men had significantly more CAC in each arterial location than did women. Arterial location, age, BMI, systolic pressure, cholesterol/HDL cholesterol ratio, and having a history of smoking were each significantly associated with quantity of CAC in both men and women. When the CAD risk factors were considered simultaneously, age, BMI, and cholesterol/HDL cholesterol ratio were significantly associated with quantity of CAC in women, and age, cholesterol/HDL cholesterol ratio, and having a history of smoking were significantly associated with quantity of CAC in men. In both sexes, age was more strongly associated with quantity of CAC in the left arterial location than in the other locations.

Unexplained variability and reproducibility

More than 60 percent of variability in quantity of CAC in women and 90 percent of the variability in quantity of CAC in men remained unexplained by the risk factors and arterial locations. This parallels findings from other studies that 30–70 percent of CAD deaths are not explained by established risk factors (2, 3) and that approximately 75 percent of the variability in coronary raised lesions is not explained by established risk factors (46). In this study, the risk factors and arterial locations explained more of the variability in quantity of CAC in women (38 percent) than in men (9 percent); this difference may be due to women having less variability in quantity of CAC than do men in this sample.

FIGURE 1. Ln(quantity of coronary artery calcification + 1) in three arterial locations on first examination versus age in 378 women with the line for the ln(predicted value + 1) overlaid, Rochester, Minnesota, 1990–1994. Plots for the (a) left, (b) right coronary, and (c) circumflex arterial locations are presented. For a participant with two electron CT scan runs, quantity of coronary artery calcification is based on the first scan run. The predicted values were calculated at each age for nonsmokers with the mean values for each of the other risk factors.

FIGURE 2. Ln(quantity of coronary artery calcification + 1) in three arterial locations on first examination versus age in 362 men with the line for the ln(predicted value + 1) overlaid, Rochester, Minnesota, 1990–1994. Plots for the (a) left, (b) right coronary, and (c) circumflex arterial locations are presented. For a participant with two electron CT scan runs, quantity of coronary artery calcification is based on the first scan run. The predicted values were calculated at each age for nonsmokers with the mean values for each of the other risk factors.
Very little unexplained variability was due to measurement error in quantity of CAC. Of the unexplained variability, the average percentage due to within-participant variability was low (1-2 percent in men and 1-19 percent in women) in each arterial location, which is consistent with the high reproducibility previously found for all epicardial arteries combined (19, 47, 48) and for individual epicardial arteries (48). The circumflex arterial location in women had the highest percentage of within-participant variability (19 percent) because there was very little CAC in the circumflex arterial location and small absolute differences in quantity of CAC between the dual scan runs for an individual created large relative differences. The estimates of within-participant variability based on the dual scan runs suggest that most of the unexplained variability in quantity of CAC was not due to noise or artifact in the CAC measure.

Electron beam CT measures of CAC compared with other noninvasive measures

Electron beam CT measures of CAC provide a noninvasive measure of coronary atherosclerosis for large epidemiologic studies with advantages over fluoroscopy, treadmill testing, and ultrasonography of the carotid arteries. While fluoroscopy provides noninvasive measures of CAC, it is less sensitive than electron beam CT at detecting angiographically defined CAD (17), has much higher radiation exposure, and does not provide more than gross quantification. Treadmill testing is even less sensitive than fluoroscopy (49) and is not artery specific. Ultrasonography of the carotid arteries provides a quantitative measure of disease, intima media thickening, but it is only weakly correlated with extent and severity of CAD (50). Electron beam CT is the only sensitive, relatively low-cost, noninvasive technique that identifies individuals with coronary atherosclerosis and provides an artery-specific, quantitative measure.

Study limitations

This study used quantity of CAC as a measure for coronary atherosclerosis, but not all atherosclerotic plaque is calcified and not all calcified plaque may be detected with electron beam CT (51). Thus, participants in this study may have atherosclerosis that was not detected. Several studies have investigated the relation between CAC and other indicators of atherosclerosis. In vitro, quantity of CAC is highly correlated with amount of atherosclerotic plaque in an artery: The estimated correlation between square root-transformed quantity of CAC and square root-transformed amount of plaque is 0.90 (15). Among individuals under age 60 years who underwent angiography, log-transformed quantity of CAC is a stronger predictor of maximal stenosis (estimated R-square, 55 percent) than CAD risk factors (estimated R-square, 25 percent) (24). In the same patients, the estimated sensitivity of CAC (using the definition in this study of at least 2 adjacent pixels) for identifying at least 50 percent stenosis at angiography in all epicardial arteries combined is high (100 percent), and the corresponding specificity is low (34 percent) (19). Since atherosclerosis exists without stenosis (52), the low specificity of CAC at this definition could be due, in part, to the presence of CAC in nonobstructive lesions (22).

The findings of this study are limited to asymptomatic white Americans aged 20-59 years who do not have hypertension or diabetes.

Conclusions

Established CAD risk factors were significantly associated with quantity of CAC. Age was more strongly associated with quantity of CAC in the left than in the right coronary or circumflex arterial locations. In each sex, much of the variability in quantity of CAC remained unexplained by established factors, which parallels findings from studies of CAD mortality (2, 3) and coronary raised lesions (46). From evaluation of dual scan data, noise or artifact in the measure accounted for only a small proportion of the unexplained variability.

Unexplained variability is most likely due to risk factors that were not considered in this study. Future studies of new risk factors, as yet perhaps unidentified, for artery-specific quantity of CAC and its progression could provide additional etiologic insights into the atherosclerotic process. Plasma homocysteine, serum carotenoids, and fibrinolytic parameters are potential risk factors for CAC emerging from other studies of atherosclerosis (53-56). In addition, factors that play a role in osteogenesis, including dietary vitamin D and calcium supplements, are potential risk factors for CAC (6). Since CAC is determined, in part, by genetic factors in inbred strains of mice (57), studies are also focusing on how genes are related to artery-specific CAC in humans (58). Genes expressed during bone formation are obvious candidates (6, 7). Other candidates include genes acting through established pathways of CAD risk such as lipid metabolism (59, 60) and through novel pathways related to vascular tone, growth factors, or cellular adhesion (61). In addition to identifying other risk factors, electron beam CT can be used to measure changes in quantity of CAC over time (62). The generalized linear mixed models used in this study can be extended to investigate artery-specific
changes over time. By providing a quantitative measure for artery-specific coronary atherosclerosis in individuals who would be considered free of CAD based on symptoms, quantity of CAC has the potential to increase our understanding of the atherosclerotic process.

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REFERENCES


APPENDIX 1

The form of the generalized linear mixed models used in this study is presented here. Define $y_{ijk}$ to be the quantity of CAC measured for participant $i$ in arterial location $j$ ($j = 1, 2, 3$) for scan run $k$ ($k = 1, 2$), $a_{ij}$ to be the random participant by arterial location interaction effect, $a_i$ to be the effect of arterial location $j$, $x_{ij}$ to be the values of the $p$ risk factors for participant $i$, and $\beta_1, \ldots, \beta_p$ to be the fixed effects of the risk factors. In addition, let $E(y_{ijk}|a_{ij}) = \mu_{ijk}$, which is the expected quantity of CAC for participant $i$ in arterial location $j$, and let $\sigma^2$ be the within-participant error variance. The generalized linear mixed models used in this study had the form:

$$\log E(y_{ijk}|a_{ij}) = \alpha_j + \beta_1 x_{ij} + \ldots + \beta_p x_{ip} + a_{ij},$$

$$\text{Var}(y_{ijk}|a_{ij}) = E(y_{ijk}|a_{ij})\sigma^2.$$  

It is assumed that $a_{1i}, a_{2i}, a_{3i}$ have mean zero, unknown variances $\sigma_{a1}, \sigma_{a2}, \sigma_{a3}$ and correlations $\rho_{12}, \rho_{13}, \rho_{23}$, that $a_{1i}, a_{2i}, a_{3i}$ are independent of $a_{i1}, a_{i2}, a_{i3}$ for $i \neq i'$ (i.e., the participants are independent), and that, conditional on the random participant by arterial location interaction effects, the quantity of CAC measures for a participant are independent (i.e., the variability not accounted for by arterial locations, risk factors, and participants is due to noise or artifact in the measure).