C-Reactive Protein and Coronary Artery Calcification
The Study of Inherited Risk of Coronary Atherosclerosis (SIRCA)

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Objective—Circulating levels of C-reactive protein (CRP) predict cardiovascular events. In contrast, an association between CRP and direct measures of atherosclerosis has not been established clearly. In the largest study to date, we examined the association of plasma CRP with coronary artery calcification (CAC) in 914 asymptomatic subjects in the Study of Inherited Risk of Coronary Atherosclerosis (SIRCA).

Methods and Results—In age-adjusted, cross-sectional analysis, there was a weak association between plasma CRP levels and CAC in women (odds ratio [OR] for ordinal regression, 1.1 [1.04 to 1.17] per 1.0 mg/L increase in CRP; P = 0.005) but not in men. The association between CRP and CAC in women remained significant after adjusting for traditional risk factors (OR, 1.08 [1.00 to 1.14]; P = 0.048) but was lost after further adjustment for body mass index (BMI) (OR, 1.02 [0.94 to 1.08]; P = 0.7).

Conclusions—In SIRCA, CRP was not associated with CAC in men, and a weak association in women was lost after adjustment for BMI. The relation between CRP and clinical events might not be related to atherosclerotic burden. Measures of inflammation, such as CRP, and indices of atherosclerosis, such as CAC, are likely to provide distinct information regarding cardiovascular risk. (Arterioscler Thromb Vasc Biol. 2003;23:1851-1856.)

Key Words: atherosclerosis ■ coronary calcification ■ inflammation ■ risk factors ■ electron beam CT

Several lines of evidence suggest that inflammation plays a major role in the development of atherosclerosis and its clinical manifestations.1 In prospective epidemiologic studies, plasma levels of inflammatory markers, particularly C-reactive protein (CRP), predict myocardial infarction and cardiovascular death.2–6 However, CRP is associated with many established risk factors, including obesity and insulin resistance,7–10 and the relation between CRP and coronary artery disease (CAD) is attenuated after adjustment for risk factors in some11 but not other1–6 studies. The extent to which CRP levels predict clinical events depends on the relation of CRP to the burden of underlying atherosclerosis or the milieu leading to plaque rupture and thrombosis and is unknown.

Given that CRP levels predict clinical events, it is of substantial interest to dissect the pathophysiology of this relation. In contrast to clinical events, an independent association between CRP levels and coronary artery12–17 or carotid13–18,19–20 atherosclerosis has not been established clearly. Coronary artery calcification (CAC), measured by electron beam tomography (EBT), might be useful in identifying novel risk factors for coronary atherosclerosis in asymptomatic subjects. The amount of CAC at EBT is correlated with the burden of atherosclerosis at both autopsy and coronary angiography,21,22 and preliminary studies suggest that CAC is a predictor of clinical CAD events in both symptomatic23 and asymptomatic24,25 subjects. Studies of CAC might permit differentiation of factors associated with coronary atherosclerosis from those related to plaque rupture or thrombosis.

Small studies of CRP and CAC in healthy subjects have produced conflicting results.13–17 Whereas some found no association between CRP and CAC,13,15,16 others have reported a weak relation that was either sex dependent14,17 or lost after adjusting for other risk factors, particularly obesity.17 It is unclear whether these conflicting reports reflect the limitations of study design and analysis or real differences in the pathophysiology of CAC, a measure of coronary atherosclerotic burden, and elevated CRP, a marker of inflammation. Recent data from the South Bay Heart Watch Study support the concept that CAC scores and plasma CRP levels might provide independent and complementary information regarding the risk of cardiovascular events.26

In the largest study to date, we applied a variety of multivariable approaches suited to the analysis of CAC data27–29 to determine whether plasma CRP levels predicted CAC scores in the Study of Inherited Risk of Coronary Atherosclerosis (SIRCA) and whether the association between CRP and CAC was influenced by sex and measures of obesity, as suggested by recent reports.14,17

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Methods

Research Subjects
SIRCA is a cross-sectional study of factors associated with CAC in asymptomatic subjects recruited on the basis of a family history of premature CAD. Study design and initial findings have been published. Subjects were eligible for SIRCA if they were free of clinical CAD, were men aged 30 to 65 or women aged 35 to 70, and had a family history of premature CAD. Exclusion criteria limited the influence of major, traditional CAD risk factors (known diabetes, total cholesterol >300 mg/dL, cigarette smoking >1 pack per day, or blood pressure >160/100 mm Hg) to facilitate the discovery of novel influences on CAC. The University of Pennsylvania Institutional Review Board approved the study protocol. A total of 1237 subjects, including siblings, gave informed consent and were enrolled in SIRCA. This report focuses on unrelated subjects (n=914).

Evaluated Parameters
Study subjects were evaluated at the General Clinical Research Center after a 12-hour, overnight fast. A detailed description of evaluated parameters is provided online (see Appendix A at http://www.ahajournals.org). Plasma samples were assayed for CRP with an ultrahigh-sensitivity latex turbidimetric immunoassay (Wako Pure Chemical Industries, Ltd) on a commercially available system (Cobas Fara II). Interassay variability for low- and high-concentration internal standards was 8.0% and 8.3%, respectively. Global CAC scores were determined as described by using customized software (Imatron), according to the method of Agatston et al from 40 continuous, 3-mm-thick computed tomograms collected on an EBCT scanner (Imatron).

Statistical Analysis
Data are reported as median and range, mean ± SD if continuous, and as proportions if categorical. Analyses were performed for men and women separately because previous reports confirmed the sex-dependent association between risk factors, including CRP, and CAC. Plasma CRP levels were divided into 3 categories (<1, 1 to <3, and ≥3 mg/L). The median CAC score across CRP categories was compared with the Kruskal-Wallis test and Wilcoxon non-zero, continuous data (eg, CAC scores). Tobit conditional regression applies linear regression to ordinal logistic regression1.29 with the CAC outcome expressed in ordinal categories. Tobit conditional regression assumes linear regression to approximate the presence of no, mild, moderate, and severe coronary artery calcification.

Three multivariable models were used in all CAC analytic approaches for men and women separately. The first contained CRP, age, and age2. The second contained CRP, age, age2, traditional risk factors, and potential confounding factors (see online Appendix B). The third model included body mass index (BMI) in addition to CRP and all other risk factors to assess whether body mass influenced the association between CRP and CAC. Results were similar whether CRP levels were included as a continuous or as a categorical variable (≤1, 1 to <3, and ≥3 mg/L). The significance of interaction terms between sex and plasma CRP was determined with the likelihood-ratio test. Results of logistic regression and logistic modeling (CAC on a logarithmic scale) are presented as the ratio of CAC score for a 1.0 mg/L increase in plasma CRP level. The results of logistic regression are presented as the odds ratio of being in a higher CAC category for a similar increase in CRP. The proportional-odds assumption of ordinal regression was satisfied for CRP in all models. Statistical analyses were performed with STATA 8.0 software (STATA Corp).

Results
Baseline Characteristics of SIRCA Subjects
Table 1 shows demographic characteristics, CRP levels, and CAC scores in the SIRCA sample (n=914). The sample was largely white (95%). Women were older than men, and the prevalences of cigarette smoking, hypertension, and diabetes were low, as expected from recruitment criteria. A high prevalence of subclinical atherosclerosis as determined by CAC scores reflected recruitment based on family history of CAD (Table 1).

Association of CRP With Traditional Risk Factors
Factors that were associated with CRP in fully adjusted, multivariable linear regression are shown in Table 2. In women, BMI, triglycerides, glucose, and hormone replacement therapy (HRT) were positive predictors of CRP levels, whereas exercise, statin use, and aspirin use were negatively associated with CRP. In men, BMI, LDL cholesterol, and cigarette smoking were positive predictors, whereas HDL cholesterol was negatively associated with CRP. Although HRT was associated with higher CRP levels in women (1.3 [0 to 16.8] vs 2.7 [0.1 to 14.0] mg/dL; P<0.001), female sex was associated with CRP even after controlling for HRT use.
in women (P=0.006). Thus, HRT alone did not account for the higher CRP levels in women.

**Association of CRP With CAC**

Median CAC scores increased across ordinal CRP categories in women (Kruskal-Wallis $\chi^2=22.5$, $P<0.001$; $\chi^2$ for trend $=24.6$, $P<0.001$) but not in men ($\chi^2=2.5$, $P=0.29$; $\chi^2$ for trend $=1.32$, $P<0.05$; Figure). Table 3 shows the adjusted association between plasma CRP (per 1 mg/L increase) and CAC as determined with different analytic approaches for women and men. Age-adjusted analysis for women showed a weak but significant association between CRP and CAC (Table 3A). CRP levels remained significant predictors of CAC scores in women after adjusting for traditional risk factors, excluding BMI, by tobit regression, ordinal regression, and logistic regression of CAC=0. However, the association between CRP and CAC was lost completely in women after further adjustment for BMI (Table 3, top) or waist circumference (data not shown). No significant association was found between CRP and CAC in men in any multivariable model (Table 3, bottom).

**Discussion**

CAC, measured at EBT, might be useful for identifying novel risk factors and exploring the relation of risk factors with coronary atherosclerosis. We have examined the association between plasma CRP and CAC in a large sample of asymptomatic subjects specifically recruited to identify factors associated with coronary atherosclerosis. We found a weak association between CRP and CAC in women but not in men. However, this association was lost after adjustment for BMI or waist circumference.

Inflammation is believed to play a major role in the initiation and progression of atherosclerosis and in the development of its clinical complications. Plasma levels of CRP predict future myocardial infarction and cardiovascular death. Although the relation between CRP and clinical events is attenuated after adjustment for risk factors, most studies show a significant association of CRP with clinical events in analyses adjusted for confounding risk factors, including BMI and the metabolic syndrome. However, it remained unclear whether CRP is still predictive after adjustment for insulin sensitivity and adipose biomarkers, such as leptin and adiponectin.

The risk of a clinical coronary event reflects the burden of underlying coronary atherosclerosis, factors that lead to plaque rupture, and factors that promote thrombus formation. Results of studies that relate CRP levels to carotid atherosclerosis have been conflicting. Positive associations have been reported in smokers. However, larger, population-based studies have found a weak or no association after adjustment for other risk factors. The prospective, population-based Bruneck study found that an association between CRP and carotid atherosclerosis was conditional on cigarette smoking and chronic infections. In a study of 3173 offspring in the Framingham cohort, Wang and colleagues found a positive association in women but not in men.

Several small studies that examined CRP and CAC in asymptomatic subjects have produced conflicting results. Redberg et al found no association between quintiles of CRP and ordinal CAC categories in a cross-sectional study of 172 asymptomatic, postmenopausal women. CRP levels were similar in cases (CAC>0) and controls (CAC=0) and were not associated with CAC in a multivariable analysis of 188 men nested within the larger Prospective Army Coronary Calcium Study. Bielak and colleagues found that high plasma CRP levels (>2.4 mg/L) were not associated with CAC scores >80th percentile in 228 subjects in the Epidemiology of Coronary Artery Calcification Study. Newman et al examined risk factors for CAC in 614 older adults and found an association between plasma CRP levels...
and quartiles of CAC in unadjusted ordinal-regression analysis in women but not in men. A stratified analysis of 321 men and women from the Framingham Heart Study showed significant age-adjusted Spearman correlations between plasma CRP levels and CAC scores, even after adjusting for age and Framingham risk score. However, adjustment for BMI markedly attenuated this relation, with complete loss of the association in women. Conflicting findings in these studies might reflect small sample sizes, differences in sample demographics, limitations in analysis, or lack of a true association between CRP and the burden of atherosclerosis.

Our investigation differs from previous studies in that our sample was specifically recruited to have a low prevalence of traditional risk factors, other than family history of CAD. This strategy was used to enrich the sample for novel risk factors in a population with increased cardiovascular risk. The reasons for the lack of association between CRP and CAC, in contrast to a more consistent association between CRP and clinical events, are unclear. However, this finding supports the concept that CRP levels might not be related to atherosclerosis per se, distinct from being a marker of plaque rupture and thrombosis. Therefore, CRP might not be useful in identifying the underlying mechanisms of atherosclerosis initiation or progression or in patient populations with premature development of atherosclerosis. Recently, Park et al found that CAC scores and plasma CRP levels provided incremental information regarding the risk of clinical cardiovascular events in 967 asymptomatic, nondiabetic subjects in the South Bay Heart Watch Study who were followed up for 6.4 ± 1.3 years. Thus, asymptomatic subjects might benefit from risk stratification based on CRP levels and CAC, because they appear to reflect different mechanisms that lead to clinical events.

Body mass accounted in large part for the association between CRP and CAC in women. The complex relation of obesity and inflammation with atherosclerosis and clinical CAD might vary by sex. Furthermore, sex-related differences in body fat might not be reflected in standard estimates such as BMI or waist circumference. Whether measures of adipose tissue hormonal activity that directly link adipose tissue to inflammatory signaling (eg, interleukin-6, tumor necrosis factor-α, adiponectin, and leptin) will be superior markers of atherosclerotic cardiovascular disease than CRP remains to be determined.

This study has several limitations. First, the SIRCA sample was not population based and largely comprised white subjects. Findings might thus not be generalizable, particularly to minority groups in whom CAC scores might be lower than in whites. Despite this, sample recruitment was specifically designed to provide unique information about novel risk factors in a population with increased cardiovascular risk. There is controversy regarding the type of atherosclerotic plaques detected at EBT and the utility of CAC in predicting clinical events. However, CAC exhibits a strong correlation with both histopathologic and angiographic measures of atherosclerosis, and prospective data support its ability to predict future events. We used a commercial assay of CRP, and it is theoretically possible that a lack of sensitivity in the lower range might have biased the results toward the null. However, few subjects had CRP levels of 10 mg/dL or greater, and CRP levels in our study were similar to those in other asymptomatic samples. The results of this cross-sectional study need to be confirmed in population-based, prospective studies of diverse measures of atherosclerosis progression.

Conclusions

We found a weak association between CRP and CAC in women but not in men, but this association was completely lost after adjustment for measures of adiposity. The relation
between CRP and clinical events might not be related to atherosclerotic burden. Measures of inflammation, such as CRP, and indices of the burden of atherosclerosis, such as CAC, might provide independent but complementary information regarding cardiovascular risk.

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