Brief Rapid Communications

C-Reactive Protein Is Associated With Subclinical Epicardial Coronary Calcification in Men and Women
The Framingham Heart Study

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Background—High C-reactive protein (CRP) levels are associated with an increased risk of cardiovascular events, even in apparently healthy individuals. It has not been established whether elevated CRP reflects an increased burden of subclinical coronary atherosclerosis.

Methods and Results—We studied a stratified random sample of 321 men and women (mean age 60 years) from the Framingham Heart Study who were free of clinically apparent cardiovascular disease. Subjects underwent electron-beam computed tomography to assess the number of coronary calcifications and the coronary artery calcification (CAC) Agatston score. Spearman correlation coefficients between CRP and CAC score were calculated and adjusted for age, age plus individual risk factors, and age plus the Framingham coronary heart disease risk score. For both sexes, CRP was significantly correlated with the Agatston score (age-adjusted Spearman correlation: 0.25 for men, 0.26 for women; both $P<0.01$). After adjustment for age and Framingham risk score, the correlation remained significant ($P=0.01$) for both sexes. Further adjustment for body mass index attenuated the correlation coefficient for women (0.14, $P=0.09$) but not for men (0.19, $P<0.05$).

Conclusions—High CRP levels are associated with increased coronary calcification. Among individuals with elevated CRP, subclinical atherosclerosis may contribute to an increased risk for future cardiovascular events. (*Circulation*. 2002;106:1189-1191.)

Key Words: coronary disease ■ inflammation ■ imaging

Prospective studies have shown that inflammation, manifested by elevated levels of C-reactive protein (CRP), is associated with an increased risk of cardiovascular events.[1] It is not known, however, why CRP levels are elevated in persons at risk for future events. CRP may be a marker of the presence and burden of existing atherosclerosis, or CRP itself may participate in the development of new atherosclerotic lesions.[2,3] Prior studies have failed to establish an association between CRP and subclinical atherosclerosis, which has led to speculation that elevated CRP levels may reflect an increased tendency for plaque rupture rather than a high atherosclerotic burden.[4,5]

This uncertainty underscores the need for a better understanding of the relationship between CRP and coronary atherosclerosis. One well-validated measure of coronary atherosclerosis is the coronary artery calcification (CAC) score provided by electron-beam computed tomography (EBCT).[6]

Prospective reports indicate that high CAC scores, like high CRP levels, may predict an increased risk of cardiac events.[7] In 2 prior studies of the relationship between CRP levels and CAC, however, no association was found.[4,5] Data from a general population free of apparent cardiovascular disease are lacking. The availability of CRP and CAC measures in a stratified sample from the Framingham Heart Study provides an opportunity to examine this relationship in a well-characterized, community-based cohort.

Methods

Subjects

The present investigation is based on a stratified sample of participants from the Framingham Offspring Study. The cohort was initially recruited in 1971 and consisted of 5124 persons aged 5 to 70 years.[8] Subjects have been examined approximately every 4 years. Cardiovascular risk factor information was collected as previously described.[8]

TABLE 1. Baseline Characteristics

	Men (n=167)	Women (n=154)
Age, y	59±9	61±9
Total cholesterol, mg/dL	203±38	216±36
High-density lipoprotein cholesterol, mg/dL	43±13	56±16
Systolic blood pressure, mm Hg	130±18	128±19
Smoker, %	16	12
Diabetes, %	13	8
Body mass index, kg/m²	29.1±4.2	27.6±6.2
Framingham risk score*	3.2±2.7	1.4±4.4
Median CRP, mg/L	1.5	1.5

Values are expressed as mean±SD unless otherwise indicated.
*Framingham CHD risk score, excluding age.

Of the first 3219 subjects attending the sixth examination cycle (1995 to 1998), we excluded from sampling 349 who had clinically apparent cardiovascular disease, 357 who lived outside New England, and 7 who were not between 35 and 84 years of age. The remaining 2506 subjects were stratified by sex, quartiles of age, and quintiles of Framingham coronary heart disease (CHD) risk score. Those with Framingham CHD risk scores in the first and second quintiles were classified as "low risk," those in the third and fourth quintiles as "medium risk," and those in the highest quintile as "high risk." Subjects were sampled randomly and equally from each stratum, and invited to undergo EBCT. Thirteen percent of eligible individuals contacted declined to participate; refusals were handled by randomly selecting another person from that stratum.

CRP Determination and EBCT Imaging

Blood specimens from the fifth examination cycle (1991 to 1995) were tested for CRP using an enzyme immunoassay (Hemagen Diagnostics, Inc) as previously reported.[9] EBCT scans were performed between 1998 and 1999 using an Imatron C-150 XP scanner (GE Medical Systems) in accordance with previously published protocols.[6,10] Each scan was assessed by a technologist and over-read by a single experienced radiologist (M.E.C.) who was blinded to clinical data. A CAC score was calculated using the method described by Agatston.[6] Reproducibility was assessed by having 20 scans reread in a blinded fashion ($r=0.97$ for replicate readings). Image noise in each scan was assessed by determining the SD of pixel numbers in a region of interest within the aorta.
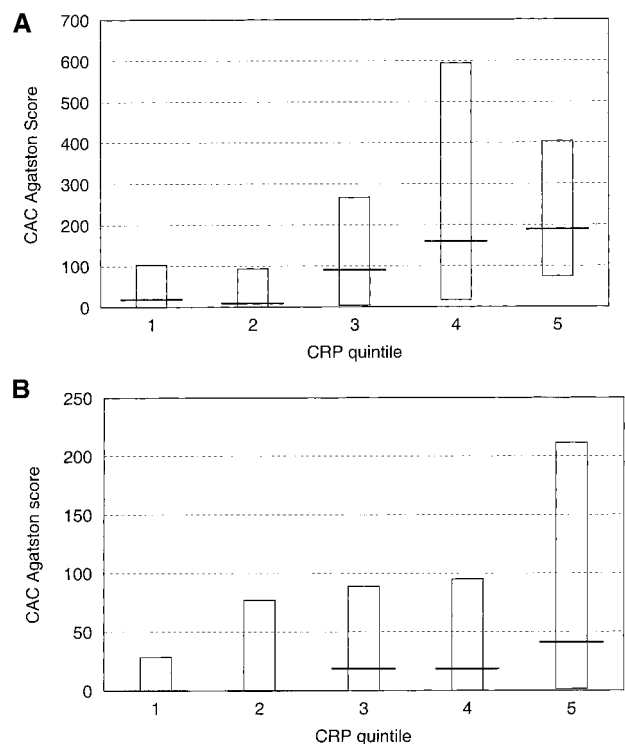
The examination and EBCT protocols were approved by the Institutional Review Boards of Boston Medical Center and Beth Israel Deaconess Medical Center, respectively.

Statistical Methods

We computed Spearman correlations for CRP and CAC score, with and without adjustment for age, age plus 1 risk factor, and age plus Framingham CHD risk score. This nonparametric approach was used because CRP and CAC had skewed distributions. The Framingham risk score was used as an index of the combined influence of individual risk factors.[11] Covariate data for risk factor adjustment were derived from the sixth examination cycle. A 2-sided probability value <0.05 was considered significant.

Results

EBCT imaging was performed on 327 participants, 321 of whom had CRP measured. Baseline characteristics are shown in Table 1. Quintile ranges for CRP were 0 to 0.04, 0.1 to 0.8, 0.9 to 2.3, 2.4 to 6.5, and 6.7 to 48.2 mg/L. At least 1 CAC lesion was found in 146 (87%) men and 97 (63%) women. Men had a median of 5 calcifications, and a median CAC score of 84. Corresponding values for women were 1 and 5, respectively.

CAC scores in men (A) and women (B) by CRP quintile. Medians (solid lines) and interquartile ranges (boxes) are displayed for CAC scores.

For both men (Figure 1A) and women (Figure 1B), median CAC scores increased with higher quintiles of CRP. Spearman correlation coefficients for the association between CRP and CAC score are shown in Table 2. There was a significant, positive correlation between CRP and CAC score after adjustment for age, age plus individual traditional risk factors, and age plus Framingham risk score. The results were similar when the number of calcifications was used instead of the CAC score (not shown).

We further adjusted CAC for body mass index (BMI) because of BMI's previously reported associations with CRP

TABLE 2. Correlation Between CRP and CAC Score

	Men (n=167)	Women (n=154)
Unadjusted	0.35*	0.34*
Adjusted for age	0.25*	0.26*
Adjusted for risk factors		
Age+systolic blood pressure	0.22*	0.24*
Age+TC:HDL ratio	0.23*	0.25*
Age+smoking	0.24*	0.25*
Age+diabetes	0.25*	0.23*
Age+BMI	0.22*	0.15‡
Adjusted for FRS		
Age+FRS	0.20*	0.20†
Age+FRS+BMI	0.19†	0.14‡

Spearman correlations between CRP and CAC score, after adjustment for the variables in the first column. FRS indicates Framingham risk score, excluding age; TC, total cholesterol; and HDL, high-density lipoprotein.

*$P≤0.01$, †$P<0.05$, ‡$P<0.10$.

and CAC.[12,13] Adjustment for BMI did not substantially affect the correlation in men, but the correlation between CRP and CAC score was reduced to 0.14 ($P=0.09$) in women. A similar pattern was observed when waist circumference was substituted for BMI (data not shown). Additional analyses adjusting for noise in addition to or instead of BMI produced similar results to analyses that adjusted for BMI alone.

Discussion

In this study cohort free of clinically apparent cardiovascular disease, we found that CRP levels were associated with CAC in both men and women, even after adjustment for age, individual traditional risk factors, and Framingham risk score.

Prior reports in 172 postmenopausal women[4] and 188 male Army personnel aged 40 to 45 years[5] did not demonstrate a positive association between CRP and CAC. Recently, Newman et al[14] observed a relationship between CAC and CRP in women but not men in an elderly cohort; however, adjustment for other cardiovascular disease risk factors was not reported.[14]

The present investigation involved subjects drawn from a well-characterized, community-based cohort free of cardiovascular disease and sampled to represent a broad spectrum of ages and cardiovascular risk. Consequently, the distributions of CRP and CAC score were broader than in the prior reports. It is possible that the negative findings in prior studies were related to their focus on smaller, relatively healthy populations with a low prevalence of coronary calcification.[4,5]

Adjustment for BMI attenuated the correlation between CRP and CAC in women but not in men. Increased adiposity may be one explanation for the association between CRP and CAC in women. Prior studies have found that BMI correlates with both CRP levels[12] and atherosclerosis measures.[13] Furthermore, the association between BMI and CRP is stronger in women than in men,[12] and weight loss has been linked to CRP reductions in women.[15] Although the underlying mechanisms have not been fully elucidated, adipocytes do express interleukin-6 and tumor necrosis factor-α, both of which stimulate CRP secretion.[16] The possibility that adjustment for BMI in women is "over-controlling," given the high correlation between CRP and BMI, should also be acknowledged.

Limitations

Because CRP levels were obtained 4 to 8 years before the EBCT, the association between CRP and CAC may have been modified by the progression of atherosclerosis in subjects with elevated CRP levels.[17] Whether CRP is a passive marker for processes involved in atherogenesis, or whether CRP itself participates in the formation of atherosclerotic plaques, has been an area of investigation.[2,3] The development of new atherosclerotic plaques in individuals with high CRP levels may explain why the association observed in our study was stronger than those of prior studies.

Image noise is correlated with BMI in EBCT scans ($r=0.8$ in our sample) and may confound coronary calcium readings in obese individuals. Analyses adjusting for noise in addition to BMI yielded similar results to analyses using BMI alone. Caution is needed when drawing conclusions about the relationship between obesity and coronary atherosclerosis solely on the basis of EBCT. Also, investigations using this modality should incorporate techniques to account for image noise in obese subjects.

Clinical Implications

Subjects with elevated CRP seem to have or develop a greater burden of subclinical coronary atherosclerosis. Both CRP and CAC have been used to predict risk for cardiovascular events.[1,7] Our findings raise the possibility that the prognostic data provided by these measures overlap. We hypothesize that an elevated burden of subclinical atherosclerosis contributes to an increased risk of cardiovascular events in individuals with high CRP levels. Further assessment of the comparative and additive value of these novel measures is warranted in carefully performed, prospective, population-based studies combining inflammatory markers and noninvasive atherosclerosis imaging.

References

1. Ridker PM. High-sensitivity C-reactive protein: potential adjunct for global risk assessment in the primary prevention of cardiovascular disease. *Circulation*. 2001;103:1813–1818.
2. Pasceri V, Willerson JT, Yeh ET. Direct proinflammatory effect of C-reactive protein on human endothelial cells. *Circulation*. 2000;102:2165–2168.
3. Lagrand WK, Visser CA, Hermens WT, et al. C-reactive protein as a cardiovascular risk factor: more than an epiphenomenon? *Circulation*. 1999;100:96–102.
4. Redberg RF, Rifai N, Gee L, et al. Lack of association of C-reactive protein and coronary calcium by electron beam computed tomography in postmenopausal women: implications for coronary artery disease screening. *J Am Coll Cardiol*. 2000;36:39–43.
5. Hunt ME, O'Malley PG, Vernalis MN, et al. C-reactive protein is not associated with the presence or extent of calcified subclinical atherosclerosis. *Am Heart J*. 2001;141:206–210.
6. Agatston AS, Janowitz WR, Hildner FJ, et al. Quantification of coronary artery calcium using ultrafast computed tomography. *J Am Coll Cardiol*. 1990;15:827–832.
7. Arad Y, Spadaro LA, Goodman K, et al. Prediction of coronary events with electron beam computed tomography. *J Am Coll Cardiol*. 2000;36:1253–1260.
8. Kannel WB, Feinleib M, McNamara PM, et al. An investigation of coronary heart disease in families: the Framingham Offspring Study. *Am J Epidemiol*. 1979;110:281–290.
9. Rost NS, Wolf PA, Kase CS, et al. Plasma concentration of C-reactive protein and risk of ischemic stroke and transient ischemic attack: the Framingham study. *Stroke*. 2001;32:2575–2579.
10. Achenbach S, Ropers D, Mohlenkamp S, et al. Variability of repeated coronary artery calcium measurements by electron beam tomography. *Am J Cardiol*. 2001;87:210–213.
11. Wilson PW, D'Agostino RB, Levy D, et al. Prediction of coronary heart disease using risk factor categories. *Circulation*. 1998;97:1837–1847.
12. Ford ES. Body mass index, diabetes, and C-reactive protein among U. S. adults. *Diabetes Care*. 1999;22:1971–1977.
13. Mahoney LT, Burns TL, Stanford W, et al. Usefulness of the Framingham risk score and body mass index to predict early coronary artery calcium in young adults (Muscatine Study). *Am J Cardiol*. 2001;88:509–515.
14. Newman AB, Naydeck BL, Sutton-Tyrrell K, et al. Coronary artery calcification in older adults to age 99: prevalence and risk factors. *Circulation*. 2001;104:2679–2684.
15. Tchernof AK, Nolan A, Sites CK, et al. Weight loss reduces C-reactive protein levels in obese postmenopausal women. *Circulation*. 2002;105:564–569.
16. Mohamed-Ali V, Pinkney JH, Coppack SW. Adipose tissue as an endocrine and paracrine organ. *Int J Obes Relat Metab Disord*. 1998;22:1145–1158.
17. Hashimoto H, Kitagawa K, Hougaku H, et al. C-reactive protein is an independent predictor of the rate of increase in early carotid atherosclerosis. *Circulation*. 2001;104:63–67.