The Impact of Calcification on the Biomechanical Stability of Atherosclerotic Plaques

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Background—Increased biomechanical stresses in the fibrous cap of atherosclerotic plaques contribute to plaque rupture and, consequently, to thrombosis and myocardial infarction. Thin fibrous caps and large lipid pools are important determinants of increased plaque stresses. Although coronary calcification is associated with worse cardiovascular prognosis, the relationship between atheroma calcification and stresses is incompletely described.

Methods and Results—To test the hypothesis that calcification impacts biomechanical stresses in human atherosclerotic lesions, we studied 20 human coronary lesions with techniques that have previously been shown to predict plaque rupture locations accurately. Ten ruptured and 10 stable lesions derived from post mortem coronary arteries were studied using large-strain finite element analysis. Maximum stress was not correlated with percentage of calcification, but it was positively correlated with the percentage of lipid ($P=0.024$). When calcification was eliminated and replaced with fibrous plaque, stress changed insignificantly; the median increase in stress for all specimens was 0.1% (range, 0% to 8%; $P=0.85$). In contrast, stress decreased by a median of 26% (range, 1% to 78%; $P=0.02$) when lipid was replaced with fibrous plaque.

Conclusions—Calcification does not increase fibrous cap stress in typical ruptured or stable human coronary atherosclerotic lesions. In contrast to lipid pools, which dramatically increase stresses, calcification does not seem to decrease the mechanical stability of the coronary atheroma.

Key Words: atherosclerosis n plaque n stress n calcification n lipids

Disruption of the fibrous cap of the atheroma is a common cause of myocardial infarction. Thin fibrous caps and large lipid pools increase atherosclerotic fibrous cap stresses and are common features of ruptured lesions. Factors that may contribute to rupture include increased biomechanical stress within the fibrous cap and weakening of the fibrous cap matrix by proteolytic enzymes or inflammation.1

Although coronary artery calcification is associated with worse cardiovascular prognosis, the influence of calcification on biomechanical plaque stresses is unclear.2,3 It is possible that the stiff calcium deposits establish an adverse stress distribution, increasing the propensity to rupture. However, it is also possible that calcification is a marker for the extent of disease or for another process such as inflammation or infection; in such cases, calcification of an individual lesion would not necessarily impact the stability of that lesion.

Insight into the individual factors of plaque stability is important, because strategies to prevent plaque rupture may rely on identifying factors that contribute prominently to lesion stability. To evaluate the impact of calcification on plaque structural stability, we applied finite element analysis to human coronary atherosclerotic lesions. Finite element analysis allows the study of complex geometries and the determination of the impact of specific material properties on stress magnitudes and distribution.

In this study, maximum principal stresses were determined in stable and ruptured human coronary atherosclerotic lesions. Calcification in each specimen was then replaced with a fibrous plaque to determine the impact of calcification on biomechanical stresses. A similar analysis was performed by replacing the lipid pool in each lesion with fibrous plaque to compare the relative impacts of calcification and lipid. Both ruptured and stable specimens were used to include these plaque types in the study. A previous study evaluated the differences between ruptured and stable plaques4; the focus of the present study was evaluating the relative contributions of calcification and lipid.

Methods

Specimens

Ten ruptured and 10 stable post mortem specimens were obtained from the Armed Forces Institute of Pathology (Washington, DC).
These specimens were obtained from the coronary arteries (10 left anterior descending coronary arteries, 3 left circumflex arteries, 4 right coronary arteries, and 3 ramus intermedius arteries) of 19 men and 1 woman aged 35 to 86 years. Regions of artery wall, fibrous plaque, lipid deposits, and calcification were identified in a histological cross-section of each artery, making all reasonable effort to construct a model that mimicked the in vivo geometry. Fibrous plaque was defined as any uncalcified, nonlipid plaque. Although lesions were not selected to include particular plaque components, all lesions had at least one lipid or one calcification region. The images were digitized using OPTIMAS 6.5.

Material Properties

Finite element analysis was performed with ADINA version 7.3 on a computer workstation using isotropic, incompressible, Mooney-Rivlin materials undergoing large strains and displacements. Mooney-Rivlin materials can be described by 2 constants, $D_1$ and $D_2$, representing coefficients of the strain energy density function. $D_1$ is proportional to the elastic modulus at zero strain. This “rubber-like” material was chosen to model the strain stiffening behavior of biological materials.8

Arterial wall properties were taken from the artery calculations in a previous study, which yielded $D_1=2644.7$ Pa and $D_2=8386.6$. The parameters for plaque and calcification were determined using a curve fit to our previously published uniaxial test data of human atheroma.2 Representative stresses were converted to true stresses for this purpose. For calcification, $D_1=18804.5$ Pa and $D_2=20$; for fibrous plaque, $D_1=5105.3$ Pa and $D_2=13$. Lipid properties were adapted from studies of the mechanical properties of lipids typically found in the atheroma: for lipids, $D_1=50$ Pa and $D_2=0.5$.8

Structural Analysis

The principles of this analysis are similar to those previously used by our laboratory to study plaque stability.4,9 Finite element analysis divides a complex structure into small areas called elements for which the stress distribution can be more easily studied. Quadrilateral elements were generated to be 175 the arterial diameter in size, resulting in 3000 to 6000 plane strain elements per model. An internal luminal pressure of 14 600 Pa (110 mm Hg) was applied. Each lesion was analyzed 3 times. First, the lesion was processed as originally digitized; this was called the unaltered model. Then, the arterial elements were generated to be 175 the arterial diameter in size, and 1 woman aged 35 to 86 years. Regions of artery wall, fibrous plaque, lipid deposits, and calcification were identified in a histological cross-section of each artery, making all reasonable effort to construct a model that mimicked the in vivo geometry. Fibrous plaque was defined as any uncalcified, nonlipid plaque. Although lesions were not selected to include particular plaque components, all lesions had at least one lipid or one calcification region. The images were digitized using OPTIMAS 6.5.

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The percent area of calcification was calculated as follows:

$$\text{Ca} = 100 \times \frac{A_{Ca}}{A_{UL} - A_{EL}}$$

where $A_{Ca}$ indicated the area of calcification; $A_{UL}$ the area of the inner elastic lamina; and $A_{EL}$ the area of the unoccluded lumen. A similar calculation was performed to determine the percent area of lipid.

To gauge the amount of increase in internal stress, a percent change in stress was calculated. The percent change in stress when calcification is removed was given by the following equation:

$$\%\Delta = 100 \times \frac{\sigma_{nc} - \sigma_{s}}{\sigma_{s}}$$

where $\sigma_{s}$ is the maximum principal stress of the “no calcium” model, and $\sigma_{nc}$ is the maximum principal stress of the “no calcium” model. An analogous formula was used for the “no lipid” models.

Statistics

Statistical analysis was performed with SigmaStat (Jandel Corp). To compare the differences between 2 sets of data with normal distributions, such as maximum stress of ruptured lesions versus the maximum stress of stable lesions, an unpaired $t$ test was used. If normality tests failed, the Mann-Whitney Rank Sum test was used. $P<0.05$ was considered significant for concluding that the 2 sets of data had different means (t test) or medians (Mann-Whitney).

To test the hypothesis that the percent area of calcification correlated with increasing plaque stresses, 2 approaches were used. First, we tested for a correlation between the amount of plaque calcification and the maximum stress levels in that plaque using the nonparametric Spearman’s correlation test. Second, we used Spearman’s test to test for a correlation between the amount of calcification and the change in stress when calcification was replaced by fibrous plaque. $P<0.05$ was considered statistically significant. Each test was performed for the following 3 groups: the 10 ruptured lesions, the 10 stable lesions, and all 20 specimens combined. Analogous tests were performed for lipid as a comparison.

Results

Stresses in Atherosclerotic Lesions

Consistent with prior studies, ruptured lesions had higher maximum stresses ($P=0.038$), although they did not contain statistically significantly different amounts of lipid or calcification ($P=0.287$ for lipid, $P=0.362$ for calcification; Table).4 The major increases in stress occurred near lipid pools or bordering the lumen, which supports other, similar studies.9,10 The maximum stresses occurred in very small areas, with most of the fibrous plaque regions generally having stress magnitudes <20% of the maximum stresses (Figure 1). The amounts of lipid and calcification were not correlated ($P=0.172$ for stable lesions, $P=0.919$ for ruptured lesions, and $P=0.114$ for all 20 lesions).

One approach to explore the relationship of calcification to stress is to test the correlation between the amount of calcification and the maximum stresses across the set of specimens. No significant correlation was found between stress and the percent area of calcification in any of the 3 groups studied ($P=0.826$ for all specimens, $P=0.946$ for ruptured specimens, and $P=0.811$ for stable plaques; Figure 2). This result suggests that having more calcification does
not indicate the presence of higher stresses in the lesion. In contrast, there was a significant correlation between stress and the percent area of lipid ($P=0.024$; correlation coefficient, 0.5) for all of the specimens. This result indicates that the more lipid a plaque contains, the higher the maximum internal stresses. In the smaller subgroups of lesions (stable and ruptured), this relationship did not reach statistical significance.

**Impact of Calcification or Lipid on Stress**

Another approach to evaluate the contribution of calcification to plaque stress is to examine the changes in stress when the calcification is replaced with fibrous plaque. A similar consideration was performed for lipid. This approach is particularly amenable to finite element analysis, because all other factors, such as lesion geometry, can be kept constant. The median increase in maximum stress for all specimens when calcification was replaced with fibrous plaque was 0.1% (interquartile range, 0% to 3%; total range, 0% to 8%). This change was statistically insignificant ($P=0.85$). In contrast, stress significantly decreased by a median of 26% (interquartile range for decrease, 5% to 56%; total range, −1% to 78%) when lipid was replaced with fibrous plaque ($P=0.021$).

The correlation between an increase in stress when calcium was replaced with fibrous plaque and the percent area of calcification was statistically significant for the stable lesions ($P<0.001$; correlation coefficient, 0.848), the ruptured lesions ($P=0.025$; correlation coefficient, 0.68), and for all the lesions together ($P<0.001$; correlation coefficient, 0.77). Thus, large amounts of calcification, by virtue of bearing mechanical load, actually decrease stresses on the fibrous plaque to a modest degree. These findings suggest that calcification does not decrease plaque stability; in fact, removal of larger amounts of calcification may result in a less stable atheroma.

There was a significant negative correlation between the amount of lipid and the amount of stress change when the lipid was replaced with fibrous plaque and all 20 specimens were analyzed ($P=0.017$; correlation coefficient, −0.53; correlation coefficient, 0.53).
$P=0.126$ for stable specimens and $P=0.060$ for ruptured specimens; Figure 3). This result suggests that the more lipid a lesion contains, the larger the stress reduction that will occur on removal.

**Discussion**

Calcification is commonly found in atherosclerosis, and the presence and extent of calcification is associated with worse prognosis.\textsuperscript{11-13} However, the impact of calcification within a specific lesion is unclear. Calcification can clearly influence coronary artery interventions and is associated with fewer procedural successes and more extensive arterial dissection.\textsuperscript{14} However, the phenomenon of atherosclerotic plaque rupture is biomechanically very different from coronary interventions, because the typical strains in a coronary artery under physiological blood flow are quite small. Our findings suggest that coronary artery calcifications do not significantly affect the stability of the atheroma, in contrast to the significant reduction of stability associated with lipid. In fact, although removing the calcification led to a statistically insignificant change in stress, the stress was increased in all calcified models.

These results have potential implications for the evaluation of and interventions for coronary artery disease. Interventional therapies targeted to the calcification of specific lesions identified from scanning techniques such as electron beam
computed tomography may not be as useful as treating the systemic manifestations of atherosclerosis.\textsuperscript{2,15} In contrast, our data are consistent with the dramatic success of lipid-lowering therapies in the prevention of coronary events.\textsuperscript{16} Although lipid-lowering therapies may not replace the lipid regions with fibrous plaque in a way analogous to our models, decreasing the extent of lipid can dramatically lower internal stresses by eliminating the soft material that causes the fibrous cap to bear enormous stresses. In this regard, a technique such as intravascular ultrasound elastography holds promise because it can detect the presence of soft materials such as lipid.\textsuperscript{17}

If calcification modestly decreases fibrous plaque stresses, why is the detection of arterial calcification an important prognostic indicator? Rather than affecting the stability of a single atheroma, calcification may reflect the extent of disease or another systemic process. For example, if calcification occurs more commonly in the setting of particular types of inflammation, detection of calcification would be expected to segregate patients into a high-risk category. This concept is supported by a recent study of calcification in the aortic arch measured by chest radiogram in >110 000 patients followed for almost 30 years.\textsuperscript{18} In this study, aortic arch calcification was associated with coronary heart disease risk in both men and women. Thus, aortic arch calcification may reflect the general burden of disease or be a marker of more aggressive disease.

To facilitate structural analysis, the materials were assumed to be isotropic, incompressible, and uniform solids. A single pair of parameters was assumed for each material, despite reported variances for each class of material. The maximum stresses in such materials were then used as indicators of plaque stability. These stresses do not necessarily correspond to regions of actual rupture because in vivo materials have more complex characteristics than those used in this study. Assumptions of isotropy indicate that differences in radial and circumferential moduli are not taken into account. However, the purpose of the study was to test the hypotheses that the presence of calcification contributes to internal plaque stresses. Thus, idealization of material properties is reasonable because we examined relative stress changes, not exact stress magnitudes. In addition, a previous study showed that the location of rupture is closely related to an area of high stress in most cases, but that rupture may occur at the second or third highest stress region. This is possibly due to weaker fibrous caps at these regions. Finally, although macrophage infiltration is important in determining plaque material properties, there are currently no reliable methods of incorporating the effects of inflammation into finite element models.

Two-dimensional analysis of post-mortem specimens cannot account for all structural features of the atheroma in vivo. Geometrical problems and changes in maximum stresses of up to 20\% when the mesh was refined were unlikely to influence the conclusions because the analyses were performed by altering the material properties in the geometry of the same lesion. There are no shear stresses, torques, or time-varying forces acting on the lesion in the models. Although additional forces exist in vivo, estimation of stresses induced by a static pressure load alone has previously been useful for identifying high stresses in human lesions as well as rupture site locations. Other simulations of dynamic stress on similar specimens give almost identical results (results not shown). Also, typical levels of fluid shear stress (\textasciitilde 1 to 1.5 Pa) are insignificant compared with the computed wall stresses (typically \textasciitilde 10 000 Pa).

Our study design could not determine the relation of calcium to inflammation, nor could we predict fibrous cap strength in calcified lesions. Calcified lesions may, in fact, have lower rupture stresses due to microscopic defects caused by the calcium or due to differences in distribution. In addition, it is possible that specimens exist in which the calcification actually increases the internal plaque stresses. Because of the relatively small number of specimens used in

![Figure 3. Relationship of plaque components to changes in stress. A, Relationship between change in stress when calcification was replaced with fibrous plaque (ordinate) and percent area of calcification in unaltered specimen. A significant correlation was found (\(P\textless 0.001\)). B, Percent change in maximum stress after replacement of lipid with fibrous plaque (ordinate) versus percent area of lipid. This correlation was also significant (\(P=0.017\)). Linear regression lines are shown to illustrate trends.](http://circ.ahajournals.org/)

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this study, this possibility cannot be excluded. However, our results demonstrate that within a typical lesion, the presence of lesion lipid is much more important for biomechanical stability than calcium.

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