Structure-Dependent Dynamic Mechanical Behavior of Fibrous Caps From Human Atherosclerotic Plaques

Richard T. Lee, MD; Alan J. Grodzinsky, PhD; Eliot H. Frank, PhD; Roger D. Kamm, PhD; and Frederick J. Schoen, MD, PhD

Background. Although thrombosis associated with a fissured atherosclerotic plaque is believed to be the most common cause of acute coronary syndromes, the underlying factors that trigger plaque rupture are currently unknown. However, the mechanical behavior of the plaque is probably of critical importance.

Methods and Results. To test the hypothesis that the mechanical properties of a plaque are dependent on its composition and, in particular, that the stiffness of fibrous caps changes within the range of frequencies carried by a physiological pressure wave, the stress–strain relation was studied in 27 fibrous caps and related to the underlying histological structure of the fibrous cap. Fibrous caps were obtained during 14 autopsies from the abdominal aorta and were classified by histological examination as cellular (n=7), hypocellular (n=9), or calcified (n=11). Hypocellular fibrous caps were 1–2 times stiffer than cellular caps (p<0.005), and calcified caps were 4–5 times stiffer than cellular caps (p<0.005). All 27 fibrous caps demonstrated an increase in stiffness with increasing frequencies of stress ranging from 0.05 to 10 Hz; the increase in stiffness was similar in all three histological classes.

Conclusions. We conclude that the stiffness of fibrous caps from human atherosclerotic plaques is related to the underlying histological structure and that the stiffness increases with frequency in the range of physiological heart rates. The protective benefit of β-adrenergic receptor blocking agents in coronary artery disease may, in part, be related to the frequency dependence of atherosclerotic plaque stiffness. (Circulation 1991;83:1764–1770)

Oclusive or mural thrombi that are associated with fissured atherosclerotic plaques are the most common cause of acute myocardial infarction, sudden cardiac death, and unstable angina.1,2 Angiographic, angioscopic, and postmortem pathological studies have confirmed that the transition from stable to unstable coronary artery disease is usually due to fracture of the most superficial layer of the atheroma, the fibrous cap.2–6 Despite major advances in the understanding of both the pathobiology and treatment of coronary atherosclerosis, the mechanism of acute fibrous cap injury is unknown.7

Understanding the distribution of mechanical stress in an atherosclerotic plaque is an important prerequisite to understanding plaque rupture. The stresses within the plaque are determined by the complex mechanical properties and variable mixture of plaque components. Recent studies that correlated pathological data with mathematical modeling suggest that the stiffness of the components of the plaque is an important determinant of the stress distribution and propensity toward fissuring of atherosclerotic plaques.8,9 However, detailed measurements of the mechanical properties of plaques have not been performed. The stiffness of many biological materials varies under conditions in which stress or strain varies with time, such as during a changing heart rate. The dynamic nature of these mechanical properties is of particular interest because β-adrenergic receptor blocking agents, which prevent surges in heart rate, may decrease the incidence of plaque rupture.7 This study was designed to examine the relation between the mechanical properties of fi-
brous caps from human atherosclerotic plaques and the underlying histological appearance by light microscopy and to examine the dynamic nature of these properties in the range of frequencies carried by a pressure wave at physiological heart rates.

**Methods**

Fibrous caps from human atherosclerotic plaques were obtained during routine autopsies at Brigham and Women's Hospital. Plaques were harvested only when the autopsy was performed within 12 hours of death. Twenty-seven plaques were dissected from the abdominal aorta of 14 otherwise unselected patients (age, 71 ± 9 years); in four patients, one plaque was obtained; in seven patients, two plaques were obtained; and in three patients, three plaques were obtained. Abdominal aortic plaques provided flat and large areas most amenable to accurate stress and strain measurements. Plaques selected fulfilled the following criteria: 1) were at least 5 mm from a vessel ostium, 2) contained no overlying thrombus, 3) were at least 9 mm in diameter, and 4) were visibly uncomplicated, with no surface fracture or visible irregularities. After the portion of the aorta with the plaque was cut away from the remainder of the aorta, the fibrous cap was dissected free of adventitia, media, and necrotic plaque components. A random section of each fibrous cap was removed and fixed in 10% neutral buffered formalin for histological studies.

Dynamic mechanical testing was performed with an ultrasensitive, servo-controlled mechanical spectrometer (Dynastat, IMASS, Hingham, Mass.). An acrylic cylindrical base was mounted onto the actuator of the spectrometer. The sample was bathed in room temperature normal saline and held between the base and a 7-mm diameter stainless steel platen that was connected to the spectrometer load cell (Figure 1). To simulate the radial stresses experienced by the arterial wall, an applied static load of 0.33 N produced a compressive stress normal to the surface of the plaque of −9.3 kPa (equivalent to a blood pressure of 70 mm Hg). A dynamic stress amplitude of 0.5 kPa with a varying frequency was superimposed about this mean after the tissue had been allowed to creep for 30 minutes (Figure 2); this testing protocol is similar to that used to test the dynamic compression properties of other tissues. The load cell and actuator displacement signals were continuously fed to a microcomputer system, which computed the amplitude and phase of the fundamental and the next three higher harmonics of each signal by means of a digital Fourier transform routine; the details of this data acquisition and analysis procedure have been described previously. Because the steel platen and actuator base are much stiffer than the specimens, the strains of the testing apparatus are assumed to be negligible. The nominal resolution of the load transducer and the displacement transducer was less than 1.0 g and 0.05 μm, respectively. The amplitude of the complex dynamic stiffness was defined and computed as the ratio of the stress to the displacement fundamental that was normalized to the specimen thickness, which was measured at the end of the experiment with the spectrometer. The thickness of the specimens was 1.1±0.2 mm. For all specimens, mechanical testing was completed within 4 hours of harvesting the sample.

Specimens for histological study were conventionally embedded in paraffin, cut in cross-section at 5–6 μm, and stained with hematoxylin and eosin for overall morphological analysis, Voerhoff-van Gieson’s stain (for elastin analysis), Masson’s trichrome stain (for collagen analysis), and von Kossa’s reagent (for calcium phosphate analysis). Specimens were prospectively classified as cellular, hypocellular, or calcified in a manner similar to that used by Kragel et al by a pathologist (F.J.S.) who had no knowledge of the results of the mechanical testing (Figure 3).

**Figure 1.** Schematic drawing of mechanical testing apparatus. Fibrous cap (Cap in NS) is bathed in normal saline and is 9 mm in diameter and approximately 1 mm thick. Cylindrical steel platen is 7 mm in diameter and is mounted on a load cell. Acrylic base is mounted on a servocontrolled actuator. Load and displacement signals are fed to a computer system that analyzes the signals by a digital Fourier transform routine (see "Methods").

**Figure 2.** Tracings showing dynamic stress (Load) imposed on a hypocellular specimen and resultant strain (Displacement) at low sinusoidal frequencies recorded on a chart recorder. Frequencies displayed are 0.05, 0.1, and 0.2 Hz.
Cellular fibrous caps consisted of smooth muscle cells or other cells admixed with collagen or elastic fibers. Hypocellular fibrous caps consisted of extracellular connective tissue matrix with only rare cells. Calcified fibrous caps had granular blue–purple deposits in hematoxylin and eosin–stained sections that stained black in sections stained by von Kossa’s reagent, as previously described. The data were analyzed by means of an analysis of variance with histological class as a between-groups factor and with frequency as a repeated measure. Only three frequencies (0.5, 1, and 2 Hz) were analyzed because these frequencies are nearest the dominant frequencies of physiological pressure waves. Patient identification was included as a random effect to account for possible correlations between values from the same patient. Post hoc tests were performed with Tukey’s Studentized range statistic to correct for multiple comparisons. In all statistical testing, a probability value less than 0.05 was considered to be significant.

Results

Seven fibrous caps were classified as cellular; nine were hypocellular, and 11 were calcified. The initial creep strains for cellular, hypocellular, and calcified specimens were 16.6±6.1%, 6.8±1.3%, and 1.2±0.2%, respectively. The amplitude and phase of the dynamic mechanical stiffness are reported in Figure 4. There was a highly significant relation between the mechanical properties of the fibrous cap as measured by dynamic stiffness and the composition as described by histological class (Table 1). Hypocellular caps were, on average, approximately 1–2 times stiffer than cellular caps, and calcified caps were 4–5 times stiffer than cellular caps. Calcified caps demonstrated a much wider variability in stiffness than cellular or hypocellular caps; however, 10 of 11 calcified caps had dynamic stiffness values higher than the mean values for hypocellular or cellular caps. All 27 fibrous caps tested demonstrated a change in stiffness throughout the frequency range used in this study. Between 0.5 and 1 Hz, the average increase in dynamic stiffness was 6.6±3.4% (range, 0.7–14.1%). Between 1 and 2 Hz, the average increase in dynamic stiffness was 6.6±2.6% (range, 2.4–11.7%).

The main effect of histological class was highly significant (F=12.97, p=0.001). The effect of frequency was also highly significant (F=17.91, p=0.0001). Because the dynamic stiffness of the calcified group exhibited a considerably larger standard deviation than the other two groups, the data were reanalyzed with a logarithmic transformation that had the effect of reducing the heterogeneity of variance across groups. This analysis yielded similar results with histological class remaining highly significant (F=17.21, p=0.001) and frequency also remaining highly significant (F=139, p<0.0001). Post hoc tests indicated that at each frequency the dynamic stiffness differed significantly (p<0.05) between each pair of histological classes. Moreover, orthogonal polynomial contrasts revealed a highly significant linear trend, with dynamic stiffness increasing as a function of frequency (p<0.0001), but they provided no evidence of a quadratic component (p=0.79) and no tendency for the linear trend to differ significantly across histological classes.

In 15 specimens, dynamic testing was immediately repeated; because results were nearly identical, only the first stiffness values are shown. The nonzero phase angle of the measured displacement with respect to the applied load reflects the viscoelastic behavior of the tissue in response to dynamic loads.
Discussion

Recent studies suggest that the natural history of human atherosclerotic plaques follows two distinct stages; first, a time-dependent gradual narrowing of the lumen, and second, an acutely unstable period that may lead to thrombosis and an unstable coronary syndrome.12-15 Although ruptured plaques may also be found incidentally in persons dying from noncoronary diseases, up to 95% of patients dying suddenly from ischemic heart disease will have one or more ruptured plaques.16 Plaque rupture is frequently followed by mural or occlusive luminal thrombosis or embolization, leading to myocardial ischemia.

Although Constantinides,17 in 1966, proposed that fissuring of coronary artery plaques leads to thrombosis and myocardial infarction, the mechanism of plaque rupture remains unclear. Recent clinical studies confirming a circadian variation in a number of acute cardiovascular syndromes suggest that the transition from a stable to unstable coronary syndrome is not a random process. A number of pathophysiological mechanisms may be related to this circadian variation. Platelet aggregability is increased and various components of the clotting system are more active in the morning, when acute cardiovascular syndromes are more common.18-21 In addition, there are morning troughs in the activity of the intrinsic fibrinolytic system.22,23 Thus, plaque rupture may actually be a random event, and the circadian variation may be related to the responses of the finely tuned balance between thrombosis and thrombolysis.

However, because sympathetic adrenergic activity also increases in the morning, leading to a surge in systolic and diastolic blood pressures and heart rate, mechanical factors may influence plaque rupture.7 The hypothesis that increases in heart rate predispose the plaque to rupture is indirectly supported by many studies demonstrating a protective benefit of β-adrenergic receptor blocking agents against myocardial infarction and sudden death. In the Multicenter Investigation of Limitation of Infarct Size (MILIS),24 Intravenous Streptokinase in Myocardial Infarction (ISAM),25 and Beta-Blocker Heart Attack Trial (BHAT)26 studies, β-adrenergic receptor blocking agents eliminated the morning increase in myocardial infarction. In addition, Hjalmarson et al27 recently reported that an increased heart rate was independently predictive of postinfarction mortality.

Mechanical Properties of Plaques

An understanding of plaque rupture will ultimately require an understanding of the mechanical properties of human atheromatous tissue. In this study, we describe the relation of histological structure to uniaxial unconfined compressive dynamic stiffness in fibrous caps from human atherosclerotic plaques. Hypocellular fibrous caps were approximately 1-2 times stiffer than cellular caps, and calcified caps were 4-5 times stiffer than cellular caps. The specimen-to-specimen variation in stiffness was much greater for calcified plaques, probably because of differing degrees of calcification throughout the specimen. In all 27 specimens, the dynamic stiffness increased throughout a range of frequencies similar to the frequencies contained in a pressure wave at physiological heart rates.

When a material is perfectly elastic and isotropic, its stiffness may be described in general by two moduli of elasticity (Young's modulus and Poisson's ratio) that will not vary with the frequency of stress or strain. However, biological materials are heterogeneous, have a high water content, and are not perfectly elastic.28 The frequency-dependent increase in stiffness of fibrous caps observed in this study is a reflection of the "viscoelastic" and "poroelastic" natures of these materials. The frequency-dependent stiffness of fibrous caps is particularly interesting because previous studies have demonstrated that the stiffness of normal vessel walls is relatively constant at frequencies higher than 1-2 Hz.29,30 Although an increase in stiffness amplitude would make the fibrous cap more resistant to deformation, this change in stiffness may lead to important shifts in stress distribution within the plaque. When one part of a structure becomes stiffer than other parts, regions of stress concentration develop. For example, Richard-son et al found that stress was concentrated near the edge of the fibrous cap when the cap was five times stiffer than the normal vessel wall.8 Therefore, an increase in stiffness in the fibrous cap caused by a surge in heart rate may lead to a further increase in stress near the junction of the cap with the more normal intima, where plaque fissuring often occurs.

The relation of this small increase in stiffness to the clinical protection provided by β-adrenergic receptor blockade remains hypothetical. The prevention of cardiac events among the small, but statistically significant, subset of patients treated by β-adrenergic receptor blocking agents may also be due to antiarrhythmic effects, the reduction in myocardial oxygen demand, or the reduction of other stresses on the plaque such as turbulence or shear stresses. Clearly, increases in heart rate and cardiac output alone do not cause plaque rupture because cardiac events induced by exercise testing are rare. However, frequency-dependent properties of plaque components could change the probability of plaque

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<th>Table 1. Dynamic Stiffness of Fibrous Caps</th>
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<td>Frequency (Hz)</td>
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Values are mean±SD.

By analysis of variance, the effects of histological class (p=0.001) and frequency (p=0.0001) are both highly significant.

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rupture, particularly if the mechanism of fracture was fatigue (see below).

Limitations
Like most biological materials, atherosclerotic plaques are mechanically and morphologically complex. The three-dimensional structure of the plaque suggests that mechanical properties such as stiffness vary with the direction of the imposed stress or strain. The uniaxial unconfined compression stiffness values described in this study are necessary but, most likely, insufficient to describe completely the mechanics of the plaque. To describe the stress–strain relation in the atherosclerotic plaque more completely, further

Figure 4. Plots of amplitude (in megapascals) and phase (in degrees) of dynamic stiffness vs. frequency (in Hertz) for seven cellular fibrous caps (panel A), for nine hypocellular fibrous caps (panel B), and for 11 calcified fibrous caps (panel C).
studies of plaque anisotropy will be important. For example, by measuring tensile circumferential moduli and fracture stresses, computer modeling of the plaque may be performed with much greater accuracy. Our preliminary studies suggest that human atherosclerotic plaque components are significantly stiffer in the circumferential and longitudinal directions than in the radial direction. In addition, the plaque may continuously change its structure; for example, foam cells may secrete enzymes that locally weaken the fibrous cap. The effects of these changes may also require consideration.

In this study, to obtain specimens that were as flat and as large as possible, we tested plaques from the abdominal aorta. Plaques from coronary stenoses may behave differently. However, the histological appearance of these fibrous caps was indistinguishable from those found in coronary arteries, and coronary artery plaques have such a small surface area that accurate mechanical testing is difficult. It is also possible that, given the complex structure of the fibrous cap, the stress fields under the steel platen are not uniform. Thus, the values reported here should be considered global averages for each specimen.

Relation to Plaque Fracture

The relation of uniaxial unconfined compression as performed in this study to plaque fracture should be considered. Radial compressive stress alone is extremely unlikely to lead to plaque rupture; we have been unable to produce evidence of fracture (such as a sudden change in strain) in human fibrous caps despite gradual increases in static compressive stress to more than 20 atm. The failure of these specimens to fracture despite uniaxial normal compressive stresses greater than those used at percutaneous coronary angioplasty is not surprising because the specimens in our testing apparatus are not under external circumferential or longitudinal tension as the plaque is during angioplasty. Indeed, when unconfined compression leads to fracture of a material, the fracture is generally due to lateral tensile strain caused by the axial strain of compression; the relation of axial to lateral strain is defined by Poisson’s ratio. Also, shear stress may play a role within the plaque, leading to failure as circumferential planes slide against each other. Collagenous structures tend to be more resistant to tensile stress than to shear stress, but the effect of shear stress may be decreased by proteoglycans, which accumulate in atherosclerotic lesions.

Fracture mechanics is a complex science. Although fracture must occur after a certain amount of deformation, the mechanism of fracture is quite different in different materials. Materials with very little plasticity (such as glass) undergo brittle fracture. Biological materials have a higher degree of plasticity and can undergo significant deformation before fracture; this behavior is called ductile fracture. In addition, many materials fracture after repeated loading of a magnitude smaller than that which would cause fracture with a single load; this mechanism, which may play an important role in plaque rupture, is called fatigue fracture.

In clarifying the mechanisms that lead to plaque fracture, it is a prerequisite that the nature of the distribution of stress in the plaque be understood. Given accurate mechanical testing data, estimation of these stresses is now possible with computer modeling under certain assumptions.8 This study reports dynamic parameters that will be essential to the task of understanding the mechanical behavior of atherosclerotic plaques. With recent developments in intravascular ultrasonography, the stress distribution in the atherosclerotic lesion may be estimated in vivo if ultrasonic tissue appearance corresponds to mechanical behavior. Ultimately, this approach may be more useful than routine angiography in predicting which plaques are most susceptible to future rupture and may also provide insight into how interventions change the natural history of acute cardiovascular syndromes.

References


KEY WORDS • atherosclerosis • myocardial infarction • coronary artery disease • plaque • fibrous cap
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