Mechanical Properties of Model Atherosclerotic Lesion Lipid Pools

Howard M. Loree, Barbara J. Tobias, Lorna J. Gibson, Roger D. Kamm, Donald M. Small, Richard T. Lee

Abstract Structural analysis of atherosclerotic coronary arteries has suggested that stress concentrations are associated with plaque rupture and that these stress concentrations are critically dependent on the geometry and mechanical properties of the fibrous cap and lipid pool. Recent clinical trials of lipid-lowering therapy have shown a significant reduction in cardiac events associated with plaque rupture perhaps because of the changing composition of subintimal lipid pools. To test the hypothesis that changes in lipid composition can change the mechanical properties of lipid pools, we measured the dynamic shear moduli of combinations of cholesterol monohydrate crystals, phospholipids, and triglycerides similar to those found in atherosclerotic lesions. Increasing the cholesterol monohydrate concentration from 0% to 50% increased the real component of the dynamic shear modulus (storage modulus or stiffness) by 4.5 times at a frequency of 1 Hz (P<.001). All specimens demonstrated an increase in stiffness with increasing frequencies of stress ranging from 0.1 to 3 Hz. We conclude that the stiffness of model atherosclerotic plaque lipid pools is related to the concentration of cholesterol monohydrate crystals. Because the relative concentration of cholesterol monohydrate increases during early regression of experimental atherosclerosis, the resultant stiffening of the lipid pool may reduce stresses in plaque caps. However, the magnitude of the contribution of changing lipid stiffness to the reduction of cardiac events seen in clinical studies is unclear. (Arterioscler Thromb. 1994;14:230-234.)

Key Words • atherosclerosis • myocardial infarction • coronary disease • biomechanics

Many episodes of acute myocardial ischemia are caused by fracture of the surface of an atherosclerotic lesion. This catastrophic biomechanical event appears to occur more frequently near regions of high circumferential tensile and shear stresses that are located close to "soft" lipid pools. Structural analysis of model atherosclerotic vessels has suggested that these "stress concentration regions" are critically dependent on the geometry of the fibrous cap and lipid pool but not on the percent reduction in lumen area. Recently, two clinical trials of lipid-lowering therapy have raised interesting questions about the role of subintimal structure in the pathogenesis of acute ischemia. In one study, 120 men at high risk for cardiac events were studied with quantitative angiography after 2.5 years of lipid-lowering therapy. The degree of change in luminal diameter attributed to lipid-lowering therapy was small, yet a surprisingly significant reduction in clinical events was seen in the lipid-lowering group. In the second study, the St Thomas' Atherosclerosis Regression Study (STARS), lipid lowering in 90 men for 59 months was also beneficial as assessed by angiographic measurements; although the angiographic change was small, a significant reduction in cardiac events was again seen. Although the positive angiographic measurements in the relatively short treatment periods of these two studies are encouraging, these studies raise questions regarding the mechanisms of the benefits of cholesterol-lowering therapy. Possible explanations that are not mutually exclusive include the larger lumen (and thus need for a larger thrombus to occlude the lumen) and improved endothelial function. Another possible explanation is a physicochemical change in the atherosclerotic lesion. The mechanical properties of the lipid pool are critical in determining the distribution of stress in the lesion, and animal experiments have demonstrated that the atherosclerotic regression process leads to changes in the composition of the lipid pool. The lipid pool comprises water, phospholipids, cholesterol monohydrate, and cholesterol esters, and up to 60% of the dry weight of the atheromatous plaque is lipid. Cholesterol monohydrate exists as platelike crystals, whereas cholesterol esters are largely present as oils. Early in the atherosclerotic regression process, cholesterol esters appear to be reduced in part by hydrolysis to yield cholesterol monohydrate; after prolonged periods both cholesterol esters and cholesterol monohydrate decline, and the lesion actually becomes smaller and more protein rich. The biomechanical effect of this change in lipid pool composition may be important. The conversion of the liquid cholesterol esters to crystalline cholesterol monohydrate may have the effect of "stiffening" the lipid pool. This would improve the structural stability of the lesion and possibly decrease the probability that the fibrous cap would fracture. To examine the hypothesis that changes in lipid components can change the me-
Composition of Four Lipid Test Specimens

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Lipid Component, Weight %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cholesterol Monohydrate</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td>3</td>
<td>25</td>
</tr>
<tr>
<td>4</td>
<td>50</td>
</tr>
</tbody>
</table>

Specimens were aqueous mixtures of 40% lipid and 60% water by weight.

Mechanical properties of lipid pools, we evaluated the mechanical properties of lipid combinations similar to those seen in atherosclerotic lesions. Because lipids have properties of both fluids and solids, the viscoelastic properties, which are functions of loading frequency, were assessed.

Methods

Specimens

The specimens in this study were mixtures of purified lipid components as described in the Table. Compositions of cholesterol monohydrate, phospholipids, and liquid triglycerides were chosen to model the range of lipid compositions found in atherosclerotic lesions. Triglycerides were considered a reasonable substitute for cholesterol esters, since the physicochemical properties of both classes of compounds (particularly their viscosities in the liquid state) are similar. Specimens were mixed thoroughly, packed in N₂, and stored for 1 week at 18°C before testing.

Mechanical Testing Apparatus

The dynamic shear modulus of the model lipid pools was measured as a representative parameter of stiffness. Mechanical testing was performed with a torsion rheometer (dynamic mechanical thermal analyzer, Polymer Laboratories, Amherst, Mass.). A schematic of the apparatus is shown in Fig 1. The apparatus has two parallel plates of radius R that hold the test sample of thickness L. The top plate is oscillated in the circumferential direction by a torsion drive coil. The lower plate does not rotate but can be raised or lowered by a stepper motor drive. The top plate is supported by an air bearing, which is rigid with virtually zero friction. This bearing incorporates a transducer to measure the normal force on the plates resulting from expansion of the sample during shear. A computer controls the torsion drive coil and monitors the movement of the top plate by means of an angular-displacement transducer, maintaining a prescribed amplitude and frequency. The electrical current through the torsion drive coil is directly related to the torque through the top plate. The computer uses the known current through the torsion coil and the measured angular displacement to calculate the amplitude ratio and phase difference between the required torque and the imposed rotational displacement by a direct quadrature technique. In this method, the computer uses the signal from the normal-force transducer to maintain a zero normal force by adjusting the bottom plate height. Thus, the sample thickness may change slightly during the experiment.

Testing Protocol

All shear experiments were conducted at a temperature of 37°C. The first stage of the experiment was a strain sweep to determine the linear range of rotational strain over which the shear modulus was independent of imposed rotational strain. The second stage of the experiment was a frequency sweep from 0.1 to 3.0 Hz. These frequency sweeps were conducted at the following peak strains within the linear range: specimen 1, 1.138%; specimen 2, 0.980%; specimen 3, 1.047%; and specimen 4, 1.174%. At least three frequency sweeps were performed for each specimen. For each specimen type, the same sample was used for the strain sweep and the three frequency sweeps, with the sample thickness reset to the initial value before each frequency sweep. The following initial sample thicknesses were used: specimen 1, 0.322 mm; specimen 2, 0.374 mm; specimen 3, 0.700 mm; and specimen 4, 1.053 mm. The plate radius was 15 mm. Different thicknesses were chosen for each specimen to provide an acceptable signal-to-noise ratio for torque measurements at the peak shear strains described above (approximately 1.0%). Sample thickness changed by less than 5% during each frequency sweep.

Data Analysis

The shear strain γ at any point on the top of the cylindrical sample is described by

\[ \gamma = \gamma_o = \phi \frac{I_o}{r} \]

where r is the radial coordinate of the point, ϕ the torsion angle, and L the thickness of the sample, using a cylindrical coordinate system (r-θ-z). Note that ϕ is assumed to be a small angle such that \( \phi = \sin \phi \).

From the theory of elasticity, the shear stress τ at any point on the top of the sample is given by

\[ \tau = \tau_o = \gamma G \]

where G is the shear modulus of the sample. Note that the sample is assumed to be a linear isotropic solid, i.e., having a shear modulus that is uniform throughout the specimen, independent of orientation, and independent of applied shear stress.

For an imposed torsion angle, there will be resulting shear stresses in the sample that vary linearly with radius. The measured twisting moment M is determined by the distribution of shear stresses integrated across the top of the sample cross section with area A. M is described by

\[ M = \int_A r \tau_o dr dA = \phi G I_o / L \]

where I_o is the polar moment of inertia and equal to πR^4/2 for the cylindrical sample.

The imposed oscillatory torsion angle can be represented by

\[ \phi = \phi_o \sin \omega t \text{ or } \phi = \phi_o e^{i \omega t} \]
where \( \phi_0 \) and \( \omega \) are the amplitude and frequency, respectively, of the torsion angle and \( i \) is equal to \( \sqrt{-1} \). The measured twisting moment can be represented by

\[
M = M_0 \sin (\omega t + \delta) \quad \text{or} \quad M = M_0 e^{i(\omega t + \delta)}
\]

where \( M_0 \) is the amplitude of the twisting moment and \( \delta \) is the phase difference between \( \gamma \) and \( M \). Note that the apparatus is calibrated to subtract the moment due to acceleration of the servo and top plate. Inertial effects within the sample are neglected. At any frequency, the dynamic shear modulus \( G \) for the cylindrical lipid sample may be calculated using the expression

\[
G = \frac{L M_0}{\pi R^4 \phi_0} e^\delta
\]

which may be rewritten in complex form as

\[
G = G' + iG'' = (2LM_0/\pi R^4 \phi_0)(\cos \delta + i \sin \delta)
\]

with the real component

\[
G' = (2LM_0/\pi R^4 \phi_0) \cos \delta
\]

and the imaginary component

\[
G'' = (2LM_0/\pi R^4 \phi_0) \sin \delta
\]

\( G' \) is in phase with the strain and is known as the storage modulus because it determines the energy stored in the sample due to the imposed strain. \( G'' \) lags behind the strain by 90° and is called the loss modulus because it determines the energy dissipation associated with the imposed strain. Note that for a newtonian liquid, \( G' = 0 \) and \( G'' = \eta_0 \), where \( \eta \) is the newtonian steady-flow viscosity.

The data were analyzed by ANOVA with cholesterol monohydrate concentration as a between-groups factor. Post hoc tests were performed between classes with Student's \( t \) test. A value of \( P<.05 \) was considered statistically significant in all testing. Results are presented as mean±SD.

**Results**

The storage and loss moduli of the lipid specimens are reported in Figs 2 through 4. Fig 2 illustrates the storage moduli data as a function of cholesterol monohydrate concentration and the frequency of imposed rotational shear strain. There was a highly significant relation between the mechanical properties of the lipid specimen as measured by storage modulus and the composition as described by cholesterol monohydrate concentration (\( F=600.9, \ P<.001 \)). Increasing the cholesterol monohydrate concentration from 0% to 50% increased the storage modulus by 4.5 times at a frequency of 1 Hz. The storage moduli for the four specimens at 1 Hz were as follows: specimen 1, 53.1±6.5 Pa; specimen 2, 32.3±4.0 Pa; specimen 3, 81.1±3.4 Pa; and specimen 4, 293.9±18.9 Pa (Fig 3). Post hoc testing showed that the storage modulus differed significantly (\( P<.05 \)) between each pair of specimens at a frequency of 1 Hz.

All four lipid specimens demonstrated an increase in storage modulus with increasing frequency throughout the frequency range tested. Between 0.1 and 1 Hz, the average increases in storage modulus for the four specimens were as follows: specimen 1, 9.9±8.4%; specimen 2, 27.4±10.2%; specimen 3, 25.5±4.7%; and specimen 4, 23.5±2.3% (range for all specimens, 4.0% to 33.7%). Between 1 and 3 Hz, the average increases in storage modulus for the four specimens were as follows: specimen 1, 15.1±12.7%; specimen 2, 24.6±15.9%; specimen 3, 27.5±4.9%; and specimen 4, 10.6±2.5% (range for all specimens, 0.5% to 55.9%).

Fig 4 illustrates the loss moduli data as a function of cholesterol monohydrate concentration and frequency of imposed rotational shear strain. There was also a highly significant relation between the mechanical properties of the lipid specimen as measured by loss modulus and the composition as described by cholesterol monohydrate concentration (\( F=1333, \ P<.001 \)). The loss moduli for the four specimens at 1 Hz were as follows: specimen 1, 55.0±1.9 Pa; specimen 2, 82.4±2.4 Pa;...
viscoelastic material. By increasing the concentration of
response to imposed shear strain) and its loss modulus
strain). The storage modulus for newtonian liquids is
negligible. Since lipids are viscoelastic, ie, they have the
properties of both solids and liquids, both the storage
and loss moduli are important. In this study, however,
that the biophysical properties of the lipid pool influ-
ence the stability of the atherosclerotic lesion, so
changes in these properties could lead to changes in the
probability that a lesion will rupture and cause acute thrombosis.

The dynamic shear modulus of a material is repre-
sented by its storage modulus (in-phase, or elastic,
component of dynamic shear modulus) vs frequency (FREQ) for four lipid
specimens tested at 37°C and a maximum shear strain of
approximately 1%. Data are from multiple runs with the same
specimen 3, 61.6±1.9 Pa; and specimen 4, 205.0±6.4 Pa.
Post hoc tests showed that the loss modulus differed significantly (P<.05) between each pair of specimens at
a frequency of 1 Hz. For specimens 2, 3, and 4, the loss
modulus increased with increasing frequency, whereas
in specimen 1 the opposite effect was observed.

**Discussion**

It has been observed that lipid-rich lesions are more
likely to rupture and cause myocardial infarction than
are lipid-poor lesions. Computer analyses of the
distribution of stress in coronary atherosclerotic
plaques have suggested that rupture of the plaque
occurs in regions of stress concentration due to the large
difference in stiffness between the fibrous plaque cap
and the underlying lipid pool. Therefore, it is likely
that the biophysical properties of the lipid pool influence
the stability of the atherosclerotic lesion, so
changes in these properties could lead to changes in the
probability that a lesion will rupture and cause acute thrombosis.

The dynamic shear modulus of a material is repre-
sented by its storage modulus (in-phase, or elastic,
response to imposed shear strain) and its loss modulus
(out-of-phase, or dissipative, response to imposed shear
strain). The storage modulus for newtonian liquids is
negligible, whereas for elastic solids the loss modulus is
negligible. Since lipids are viscoelastic, ie, they have the
properties of both solids and liquids, both the storage
and loss moduli are important. In this study, however,
the storage modulus was considered a measure of lipid
stiffness, since it reflects the elastic-solid character of a
viscoelastic material. By increasing the concentration of
cholesterol monohydrate crystals from 0% to 50%, the
storage modulus increased by 4.5 times at a physiological
frequency of 1 Hz. Over this same range of chole-
sterol concentration, the loss modulus increased by 3.7
times at 1 Hz. The behavior of the 10% cholesterol
sample was unusual, in that its storage modulus was less
than that of the 0% cholesterol sample and its loss
modulus greater than that of the 25% cholesterol
sample. It is unclear whether these results reflect a
nonlinear trend in dynamic shear modulus with increasing
cholesterol concentration or perhaps structural
changes in the 10% cholesterol sample that occurred
during the strain sweep stage of the experiment, such as
polymer degradation due to viscous heating.

In the first 6 months of experimental atherosclerosis
regression, cholesterol esters are hydrolyzed to chole-
sterol monohydrate, which precipitates readily to form
plateletlike crystals. It is possible that the decrease in
liquid cholesterol ester and increase in cholesterol
monohydrate crystals increase the stiffness of the lipid
pool, thereby increasing the stability of the entire lesion.
However, it is important to note that despite the
statistically significant increase in the storage compo-
nent of dynamic shear modulus with increasing chole-
sterol monohydrate concentration as found in this study,
the lipid pool is still several orders of magnitude less
stiff than the fibrous plaque. Thus, this change in the
biomechanical properties of the lipid pool may be
insufficient to change the stability of the lesion. Alter-
native hypotheses for the reduction in clinical events
seen in recent studies of cholesterol-lowering therapy
include the increases in lumen size and the potential
recovery of endothelial function. It should be noted that
angiographic lumen size is a poor predictor of which
lesions will rupture and cause infarction, so the
small increases in lumen size may not explain the
reduction in events. The potential benefits of cholesterol-
lowering therapy on endothelial function in humans
are currently being evaluated.

It is difficult to estimate the magnitude of reduction
of stress in the lesion caused by an increase in lipid-pool
dynamic stiffness as measured in this study. Previous
finite-element analyses of stress in atherosclerotic le-
sions incorporated static, linear, elastic-solid material
parameters. Development of a finite-element model
of atherosclerotic lesions that includes dynamic behav-
ior would be necessary to evaluate the effects of chang-
ing lipid-pool dynamic stiffness. In a pilot static, nonlin-
ear, elastic finite-element analysis of four lesions from a
previous study, the static Young's modulus of the lipid
pool was calculated from the dynamic shear modulus
measured in this study, with the assumption that the
lipid pool is isotropic and incompressible (H.M. Loree,
PhD, et al, unpublished observations). In this pilot
analysis, circumferential stresses were not significantly
changed by an increase in lipid-pool stiffness of the
magnitude observed in the present study. Thus, the
potential relation of lipid stiffness to the reduction in
cardiac events seen in lipid-lowering trials should be
considered speculative.

**Limitations**

Several limitations in this study should be considered.
The composition of lipids in atherosclerotic plaques is
quite variable, with a range of cholesterol monohydrate
content from only a few percent to more than 50%. The composition of lipid specimens tested is different from those found in atherosclerotic lesions because triglycerides were used instead of cholesterol esters. This substitution was necessary because of the high cost and limited availability of purified cholesterol esters. Lipid triglycerides are considered a reasonable substitute for cholesterol esters, since the physicochemical properties of both classes of compounds, particularly their physical interactions with cholesterol and phospholipids, and their viscosities in the liquid state are similar. The viscosity range for liquid triglycerides and liquid cholesterol esters in the range of body temperature is about 0.3 to 3 Poise (1 Poise = 0.1 kg/[m • s] = 0.1 Pa/s). In addition, atherosclerotic plaques are complex and contain modified leukocytes, cellular debris, and varying amounts of calcium. This study attempted to isolate the effects of only one component of this complex structure; however, since lipids often constitute up to 60% of the dry weight of the plaque, this study represents a logical first step in the attempt to model the lipid composition of the plaque.

It is important to note that the shear moduli measured in this study do not completely describe the mechanical behavior of the lipid pool. For example, it is not possible to derive a Young’s modulus from the shear modulus unless the material is isotropic and linearly elastic and Poisson’s ratio for the material is known. At the small amplitudes of strain used in this study, the assumption of linearity was reasonable. However, it is likely that the lipid pool of the plaque is not isotropic, since the cholesterol monohydrate crystals may appear to have a strong orientation. Thus, the measurements in this study must be considered an approximation, since isotropy was assumed. The relative importance of different lipid material properties to the clinical events of plaque rupture is unclear.

In the absence of reproducible animal models of atherosclerotic plaque rupture or methods of identifying the lipid-pool composition in patients, it is difficult to estimate the clinical impact of changing the stiffness of the lipid pool. However, it is clear that the material properties of plaque components play an important role in determining rupture locations. Understanding ways to improve lesion stability, such as increasing the strength of the fibrous cap or increasing the stiffness of the lipid pool, could lead to strategies to prevent acute plaque rupture.

Acknowledgments

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References


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