Biaxial elastic material properties of porcine coronary media and adventitia

Aditya Pandit, Xiao Lu, Chong Wang, and Ghassan S. Kassab

Department of Biomedical Engineering, University of California, Irvine, California

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Pandit, Aditya, Xiao Lu, Chong Wang, and Ghassan S. Kassab. Biaxial elastic material properties of porcine coronary media and adventitia. Am J Physiol Heart Circ Physiol 288: H2581–H2587, 2005; doi:10.1152/ajpheart.00648.2004.—The importance of mechanical stresses and strains has become well recognized in vascular physiology and pathology. To compute the stress and strain on the various components of the vessel wall, we must know the constitutive equations for the different layers of the vessel wall. The objective of the present study is to determine the constitutive equation of the coronary artery treated as a two-layer composite: intima-media and adventitial layers. Twelve hearts were obtained from a local slaughterhouse, and the right coronary artery and left anterior descending artery were dissected free from the myocardium. The vessel wall was initially mechanically tested biaxially (inflation and axial extension) as a whole (intact wall) and subsequently as intima-media or adventitial layer. A Fung-type exponential strain energy function was used to curve fit the experimental data for the intact wall and individual layers for the right coronary artery and left anterior descending artery. Two methods were used for the determination of material constants, including the Marquardt-Levenberg nonlinear least squares method and the genetic algorithm method. Our results show that there were no statistically significant differences in the material constants obtained from the two methods and that either set of elastic constants results in good fit of the data. Furthermore, at an in vivo value of axial stretch ratio, we find that the stiffness is as follows: intima-media > intact > adventitia. These results underscore the composite nature of coronary arteries with different material properties in each layer. The present results are necessary for analysis of coronary artery mechanics and to provide a fundamental understanding of vessel physiology.

The significance of stress and strain in vascular cell biology and pathology has become well recognized (11). A large number of problems in vascular physiology and pathophysiology depend on the stresses and strains in the cellular and extracellular matrix of the vessel wall. It is possible to measure the strains but not the stresses. The determination of the stresses requires a precise understanding of the mechanical properties of the tissue; i.e., we must know the constitutive equations for the different layers of the vessel wall.

A common feature of blood vessels is the layered construction of their walls, which consist of intimal, medial, and adventitial layers. Each layer has a zero-stress-resultant and zero-stress-moment state and a set of material properties. If we consider these layers as elastic shells, then these states of zero-stress-resultants and zero-stress-moments can be referred to as the “states of zero stress” (11). The entire wall is a composite material made up by these layers with different elastic constants and zero-stress states. The passive stress and strain distribution in the whole wall can be computed from the elasticity and zero-stress states of the individual layers.

There are currently three views on the stress distribution in the vessel wall: 1) the classic view where the vessel wall is considered as made of homogeneous material and that the no-load state is equal to the zero-stress state (see review in Ref. 3); 2) the 1980s view where the vessel wall is considered as homogeneous, the state of zero-stress-resultant and zero-stress-moments has been measured and has been found to be quite different from the no-load state (10, 28); 3) the 1990s and present view where the vessel wall is made of several layers each of which has its own elasticity constants and its own state of zero-stress-resultants and zero-stress-moments (8, 16, 24, 25, 29, 30).

In the present study, we wanted to pursue the latest view for the coronary arteries. We treat the vessel wall as a two-layered structure consisting of the intima-media (endothelial cells and vascular smooth muscles, including elastin and some collagen) and adventitia layer (collagen, fibroblasts, and elastin). The vessel wall was initially mechanically tested as a whole and subsequently as the intima-media or adventitial layer. Two experiments were done for each layer, which included inflation and axial stretch. An exponential strain energy function was used to curve fit the experimental data on the intact wall and individual layers for the porcine right coronary artery (RCA) and left anterior descending (LAD) artery. Two methods were explored for the determination of material constants: the classic Marquardt-Levenberg (12, 21) and a more recent method using genetic algorithm (6, 26). The major conclusion of the present study is that the coronary artery is a composite material of which the intima- medial and adventitial layers have different material properties. These features are important to understand coronary vasa vasorum, baroreceptors, atherogenesis, restenosis, stenting, and many other clinically relevant issues.

MATERIALS AND METHODS

Experimental Measurements

Biaxial test: intact vessel. The triaxial (inflation, extension, and torsion) testing of coronary arteries has been recently described by Kassab’s group (20). Although the experimental protocol included torsion, we limited the present analysis to inflation and axial extension at zero torsion. Briefly, 12 hearts from Danish Landrace-Yorkshire pigs were obtained from a local slaughterhouse. The hearts used for testing of whole wall and media had a mean weight of 463 ± 57 g (n = 7), whereas the hearts used for testing of adventitia weighed 245 ± 32 g (n = 5). The RCA and LAD artery were dissected and tested biaxially within 2–3 h of heart harvest. The arterial specimen was mounted on the cannula horizontally on both ends in an organ bath at room temperature (22°C). The organ bath chamber was designed to maintain the specimen immersed in a physiological Krebs solution. The cannulas were made to pass through the holes on the side walls of the chamber, and then a circulative pump was used to
maintain the volume of the solution in the chamber. One side of the cannula was connected with a pressure transducer (Summit Disposable Pressure Transducer, Baxter Healthcare; error of \( \pm 2\% \)) through a Y tube. A pressure regulator was used to control the luminal pressure in the arterial specimen. The cannula was mounted on a linear stage (Daedal Positioning Table, type 40206 LNMS D L2 C2 M1, Parker Hannifin) where the motor for linear motion (type SDL 603, Parker Hannifin) drives the stage and records the axial force by a load cell (Sensotec model; Sensotec error at 0.15–0.25% at full scale). The load cell was calibrated with a series of weights, and a linear relation (correlation coefficient of 1.000) in the range of 0–2.45 N was confirmed. The axial stretch ratio \( (\lambda_a) \) was varied from 1.1 to 1.4 in increments of 0.1. The transmural pressure was varied from 20 to 140 mmHg in increments of 20 mmHg at every \( \lambda_a \) using a Ca\(^{2+}\)-free Krebs solution containing EGTA (2.6 \( \mu \)M) to prevent vessel tone. Microbeads (stainless steel, 60–100 \( \mu \)m in diameter) were dispersed on the outer surface of the segment, which were videotaped during testing to measure displacement and outer diameter.

**Biomechanical principles.** The artery is considered to be a two-layer cylinder. Because the intima is relatively thin in the normal vessel, the intima-media is considered as the inner layer and the layer cylinder. If the radii of the interface of the media and adventitia and the outer boundary of the adventitia are denoted by \( r_m \) and \( r_o \), respectively, the two components of the mean stresses were computed according to Eqs. 3 and 4 using the respective radii and wall thicknesses. Similarly, the strain was computed with the respective circumference as given by Eq. 1. Finally, the wall thickness of each individual layer was similarly determined by Eq. 2 with the appropriate loaded radius and no-load wall area.

**Equations 1–4** were also applied individually to each separate layer. If the radii of the interface of the media and adventitia and the outer boundary of the adventitia are denoted by \( r_m \) and \( r_o \), respectively, the two components of the mean stresses were computed according to Eqs. 3 and 4 using the respective radii and wall thicknesses. Similarly, the strain was computed with the respective circumference as given by Eq. 1. Finally, the wall thickness of each individual layer was similarly determined by Eq. 2 with the appropriate loaded radius and no-load wall area.

**Theoretical Formulation**

**Biomechanical principles.** The artery is considered to be a two-layer cylinder. Because the intima is relatively thin in the normal artery, the intima-media is considered as the inner layer and the adventitia as the outer layer. The circumferential deformation of the entire artery or its individual layers may be described by the midwall circumferential Lagrangian Green’s strain, which is defined as follows:

\[
E_{th} = \frac{1}{2} [\lambda_a^2 - 1] \tag{1a}
\]

where \( \lambda_a \) is the midwall stretch ratio \( (\lambda_a = c(C)) \), \( c \) refers to the midwall circumference of the vessel in the loaded state, and \( C \) refers to the corresponding midwall circumference in the zero-stress state. Similarly, the axial Green strain is given by:

\[
E_{a} = \frac{1}{\lambda_a} \tag{1b}
\]

where \( \lambda_a \) is the local axial stretch ratio defined as the change in axial length between loaded and no-load state.

The midwall circumference in the loaded state was computed from the average of inner and outer radius. The inner radius \( (r_i) \) of the vessel can be computed from the incompressibility condition for a cylindrical vessel as:

\[
r_i = \sqrt{r_o^2 - \frac{A_0}{\pi \lambda_a}} \tag{2}
\]

where \( r_o \) and \( A_0 \) are outer radii at the loaded state and the wall area in the no-load state, respectively. Because all the quantities on the right-hand side of Eq. 2 are measured, the loaded inner radius can be computed. The wall thickness \( h \) was computed as \( h = r_o - r_i \).

The mean second-Piola Kirchhoff stresses in the circumferential \( (S_{th}) \) and axial \( (S_a) \) directions are given by:

\[
S_{th} = \frac{Pr_i}{h \lambda_a^2} \tag{3}
\]

and

\[
S_a = \frac{1}{\lambda_a^2} \left[ \frac{F}{\pi (r_o^2 - r_i^2)} + \frac{Pr_i^2}{h(r_o + r_i)} \right] \tag{4}
\]

where \( F \) and \( h \) are the axial force and wall thickness, respectively, and \( P \) is pressure.

**Strain Energy Function**

A well-known approach to elasticity of bodies capable of finite deformation is to postulate the form of an elastic potential or strain energy function (14). Following the arguments made by Fung (11), we use the following form of strain energy function:

\[
p_0 W^{(2)} = C (\exp Q - 1) \tag{5a}
\]

\[
Q = a_1 (E_{ih} - E_{0h}) + a_2 (E_{ih}^2 - E_{0h}^2) + 2a_4 (E_{ih} E_{0h} - E_{0ih} E_{0h}) \tag{5b}
\]

where \( C, a_1, a_2, \text{ and } a_4 \) are constants and asterisked quantities are strains corresponding to a reference pair of stresses at the homeostatic state (80 mmHg physiological pressure and \( \lambda_a = 1.4 \) axial stretch). Here the superscript “2” over \( p_0 W^{(2)} \) signifies that this is a two-dimensional approximation, treating the arterial wall as a membrane and ignoring the radial stress. The symbol \( W \) represents the strain energy per unit mass of the material, and \( p_0 \) is the mass density at zero stress. \( C \) has the units of stress (force/area); \( a_i, a_2 \text{ and } a_4 \) are dimensionless constants. Although Eq. 5 applies either to the loading or the unloading curve with different set of constants, we shall focus on the former. The differentiation of the strain energy equation leads to the stress-strain relationship as:

\[
S_{ij} = \frac{\partial (p_0 W)}{\partial E_{ij}}, \quad (i, j = 0, z) \tag{6}
\]

In these formulas, \((0, z)\) is a set of local right-handed cylindrical coordinates with an origin lying on the neutral surface of the blood
vessel wall, the axis \( \theta \) pointing in the circumferential direction, and \( z \) in the axial direction. The strains are finite and referred to the zero-stress state; \( E_{zz} \) and \( E_{\theta \theta} \) are normal strains, and \( \epsilon_{i\theta} = \epsilon_{\theta i} \) are shear strains taken as zero because of the axisymmetric loading conditions. The subscripts \( i \) and \( j \) range over 1, 2; with 1 referring to \( \theta \); 2 referring to \( z \). When Eqs. 5 and 6 are applied to intima-media, every symbol should have a superscript (im). Similarly, adventitial layer should have a superscript (ad). Note that the quadratic form of \( Q \) is written for two dimensions in the spirit of the theory of thin shells in classic mechanics. The blood vessel material is incompressible (2, 4). In two dimensions, however, it is not incompressible (5).

**Determination of Elastic Constants**

If we combine Eqs. 5 and 6, we obtain a constitutive relation that relates the circumferential and axial stresses to strains as:

\[
S_{\theta \theta} = \frac{C}{2} \exp \left[ a_1 (E_{\theta \theta} - E_{\theta \theta}^0) + a_2 (E_{zz}^2 - E_{zz}^0) \right] + 2a_3 (E_{\theta \theta} E_{zz} - E_{\theta \theta}^0 E_{zz}^0) / (2a_1 E_{\theta \theta} + 2a_2 E_{zz}) \tag{7a}
\]

and

\[
S_{zz} = \frac{C}{2} \exp \left[ a_1 (E_{\theta \theta} - E_{\theta \theta}^0) + a_2 (E_{zz}^2 - E_{zz}^0) \right] + 2a_3 (E_{\theta \theta} E_{zz} - E_{\theta \theta}^0 E_{zz}^0) / (2a_1 E_{\theta \theta} + 2a_2 E_{zz}) \tag{7b}
\]

The goal of an algorithm to determine the material constants \( C, a_1, a_2, \) and \( a_3 \) is to minimize the square of the difference between theoretical (proposed function, Eq. 7) and experimental values of circumferential \( (S_{\theta \theta}) \) and axial, \( S_{zz} \), stresses as:

\[
\text{Error} = \sum_{i=1}^{N} \left( \frac{C}{2} \exp \left[ a_1 (E_{\theta \theta}^i - E_{\theta \theta}^0) + a_2 (E_{zz}^i - E_{zz}^0) + 2a_3 (E_{\theta \theta} E_{zz} - E_{\theta \theta}^0 E_{zz}^0) \right] / (2a_1 E_{\theta \theta} + 2a_2 E_{zz}) \right) - (S_{\theta \theta}^i) \right)^2 + \sum_{i=1}^{N} \left[ \frac{C}{2} \exp \left[ a_1 (E_{\theta \theta}^i - E_{\theta \theta}^0) + a_2 (E_{zz}^i - E_{zz}^0) + 2a_3 (E_{\theta \theta} E_{zz} - E_{\theta \theta}^0 E_{zz}^0) \right] / (2a_1 E_{\theta \theta} + 2a_2 E_{zz}) \right] - (S_{zz}^i) \right]
\]

where \( N \) represents the total number of experimental points used to determine the material constants of each curve. Two approaches were used to minimize the error expressed by Eq. 8 as outlined below.

**Marquardt-Levenberg Method**

In this approach, the determination of the material constants of the strain energy function was carried out using Mathematica, which utilizes the Marquardt-Levenberg (M-L) method for nonlinear optimization. The optimized cost function is expressed by Eq. 8. Initially all four parameters \( (C, a_1, a_2, \) and \( a_3 \) were evaluated. The value of \( a_3 \) was then fixed at the predetermined mean for the intact wall, and each respective layer and the three parameters \( (C, a_1, \) and \( a_2 \) were reevaluated.

**Genetic Algorithm Method**

The genetic algorithm (GA) is a stochastic global search that attempts to mimic biological evolution. GA operates on a population of potential solutions applying the principle of survival of the fittest to produce the best approximation to a solution. At each generation, a new set of approximations is created by the process of selecting individuals according to their level of fitness in the problem domain and breeding them together using operators in analogy to biological genetics. This process leads to the evolution of populations of individuals that are better suited to their environment than their parents.
similar to natural selection. The objective is to minimize the cost function in Eq. 8 based on a probabilistic rather than numerical approach (6, 26).

Briefly, we developed a simple code to implement the GA using the MATLAB Genetic Algorithm Toolbox (27). A number of parameters were selected, including the size of the population, probability of crossover, and mutation; scale for mutation and Tournament probability; initial guess values for lower and upper limit of crossover, and mutation; and number of generations. The error function (Eq. 8) was evaluated based on the initial parameters. These values were stored in an array, and the search was initiated for the best “individuals” or material constants. We set an elitism and recombinant individual as per Tournament algorithm or Roulette Algorithm based on the probability of subsequent tournament algorithm. We selected two parents based on Tournament Algorithm and roulette principle; i.e., the lower the value, the better chance. The selection of children from the two parents was made using crossover, mutations, and elitism. This process was repeated a number of times, and the fitness value was computed at each cycle. The converged values represented a minima for Eq. 8. We then fixed $a_4$ and redetermined the values of $C$, $a_1$, and $a_2$ by using the GA.

Statistical Analysis

The “goodness” of fit was determined by a correlation coefficient $R^2$ for the relation between calculated and experimental values. A $P$ value <0.05 indicated that the differences between RCA and LAD artery and their respective layers were statistically significant.

RESULTS

The circumferential stress-strain relation for the intact RCA at an axial stretch ratio of 1.4 is shown in Fig. 1 during loading and unloading of pressure. All subsequent data and analysis will focus on the loading curve. The mean circumferential stress-strain relations averaged over all the data for the intact wall, media, and adventitia are shown in Fig. 2. $A$, $B$, and $C$, respectively, for the LAD artery at various axial stretch ratios. The experimental stress-strain data from all hearts were fitted to Eqs. 5 and 6 using M-L nonlinear least squares fit and GA method. The initial fit of the data involved the determination of four material elastic constants; i.e., $C$, $a_1$, $a_2$, and $a_4$. Once $a_4$ was determined for the entire set of data (intact wall, media, or adventitia), the mean value was fixed, and the remaining constants were reevaluated. A different mean value of $a_4$ was used for each respective group (LAD intact wall, media, and adventitia; RCA intact wall and media). The mean value of $a_4$ for the intact LAD was 0.15 ± 0.05 and that of RCA was 0.15 ± 0.05. Similarly, the mean $a_4$ for the medial layers for LAD and RCA were 0.34 ± 0.12 and 0.32 ± 0.22, respectively. The value corresponding to the adventitia of LAD artery was 0.77 ± 0.60. Similar values were obtained initially for the GA and were subsequently used in the M-L method to determine the remaining three constants.

Table 1 summarizes the elastic constants for seven intact LAD arteries for the GA methods. Also listed are values of circumferential and axial strains at homeostatic values (80 mmHg, $a_x = 1.4$). These seven LAD arteries had their adventitia dissected away and their media tested. The material constants for those hearts are listed in Table 2. Five other LAD vessels were tested for their adventitia once their media was dissected away, and their material constants are shown in Table 3.

The RCA of five hearts were tested first as a whole (Table 4) followed by dissection of adventitia and hence tested the media as shown in Table 5. For the intact vessels, all constant were statistically similar.

Table 6 shows a summary of $P$ values for the comparison of intact wall and individual layers. The comparisons are made for both the M-L and GA methods. Finally, a comparison of the product of constants (i.e., $C \cdot a_1$, $C \cdot a_2$, and $a_1 \cdot a_2$) is also presented. A $P$ value of <0.05 was considered as statistically significant.

We compared the intact and media of RCA and LAD arteries with and without a constitutive model. For a model-dependent

Table 1. Material constants of strain energy function obtained from experimental stress-strain data of intact LAD artery

<table>
<thead>
<tr>
<th>Animal No.</th>
<th>$C$, kPa</th>
<th>$a_1$</th>
<th>$a_2$</th>
<th>$E_{ss}$</th>
<th>$E_{zz}$</th>
<th>$R^2$ for $S_1$</th>
<th>$R^2$ for $S_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart 1</td>
<td>30.7</td>
<td>1.4</td>
<td>2.3</td>
<td>0.82</td>
<td>0.9</td>
<td>0.99</td>
<td>0.98</td>
</tr>
<tr>
<td>Heart 2</td>
<td>38.4</td>
<td>1.5</td>
<td>2.4</td>
<td>0.71</td>
<td>0.51</td>
<td>0.98</td>
<td>0.92</td>
</tr>
<tr>
<td>Heart 3</td>
<td>34.7</td>
<td>2.6</td>
<td>1.1</td>
<td>0.47</td>
<td>0.47</td>
<td>0.99</td>
<td>0.98</td>
</tr>
<tr>
<td>Heart 4</td>
<td>41.3</td>
<td>1.5</td>
<td>1.7</td>
<td>0.67</td>
<td>0.98</td>
<td>0.97</td>
<td>0.98</td>
</tr>
<tr>
<td>Heart 5</td>
<td>35.2</td>
<td>1.0</td>
<td>1.8</td>
<td>0.93</td>
<td>0.49</td>
<td>0.98</td>
<td>0.98</td>
</tr>
<tr>
<td>Heart 6</td>
<td>69.3</td>
<td>0.7</td>
<td>1.5</td>
<td>0.91</td>
<td>0.49</td>
<td>0.98</td>
<td>0.98</td>
</tr>
<tr>
<td>Heart 7</td>
<td>55.9</td>
<td>1.5</td>
<td>1.1</td>
<td>0.56</td>
<td>0.47</td>
<td>0.98</td>
<td>0.98</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>43.6 ± 13.9</td>
<td>1.4 ± 0.6</td>
<td>1.7 ± 0.5</td>
<td>0.72 ± 0.17</td>
<td>0.49 ± 0.02</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$C$, $a_1$, $a_2$, are elastic constants. $E_{ss}$ and $E_{zz}$ are zero and normal strains, respectively. $S_1$ and $S_2$ are axial and circumferential stress, respectively. The value of $a_4$ was fixed at 0.15 for all animals. LAD, left anterior descending artery.

The value of $a_4$ was fixed at 0.34 for all animals.

Table 2. Material constants of strain energy function obtained from experimental stress-strain data of medial layer of LAD artery

<table>
<thead>
<tr>
<th>Animal No.</th>
<th>$C$, kPa</th>
<th>$a_1$</th>
<th>$a_2$</th>
<th>$E_{ss}$</th>
<th>$E_{zz}$</th>
<th>$R^2$ for $S_1$</th>
<th>$R^2$ for $S_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart 1</td>
<td>48.0</td>
<td>4.1</td>
<td>1.6</td>
<td>0.56</td>
<td>0.50</td>
<td>0.98</td>
<td>0.98</td>
</tr>
<tr>
<td>Heart 2</td>
<td>32.3</td>
<td>1.5</td>
<td>1.3</td>
<td>0.78</td>
<td>0.49</td>
<td>0.98</td>
<td>0.98</td>
</tr>
<tr>
<td>Heart 3</td>
<td>40.1</td>
<td>7.5</td>
<td>1.9</td>
<td>0.47</td>
<td>0.48</td>
<td>0.98</td>
<td>0.98</td>
</tr>
<tr>
<td>Heart 4</td>
<td>31.7</td>
<td>6.3</td>
<td>1.9</td>
<td>0.37</td>
<td>0.48</td>
<td>0.97</td>
<td>0.98</td>
</tr>
<tr>
<td>Heart 5</td>
<td>29.2</td>
<td>3.9</td>
<td>2.1</td>
<td>0.64</td>
<td>0.47</td>
<td>0.94</td>
<td>0.98</td>
</tr>
<tr>
<td>Heart 6</td>
<td>55.0</td>
<td>5.0</td>
<td>2.5</td>
<td>0.42</td>
<td>0.50</td>
<td>0.99</td>
<td>0.98</td>
</tr>
<tr>
<td>Heart 7</td>
<td>45.3</td>
<td>3.8</td>
<td>1.6</td>
<td>0.64</td>
<td>0.47</td>
<td>0.98</td>
<td>0.98</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>40.2 ± 9.7</td>
<td>4.6 ± 1.9</td>
<td>1.8 ± 0.4</td>
<td>0.52 ± 0.15</td>
<td>0.49 ± 0.01</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The value of $a_4$ was fixed at 0.34 for all animals.
Table 3. Material constants of strain energy function obtained from experimental stress-strain data of adventitial layer of LAD artery

<table>
<thead>
<tr>
<th>Animal No.</th>
<th>C, kPa</th>
<th>$a_1$</th>
<th>$a_2$</th>
<th>$E_{xx}$</th>
<th>$E_{yy}$</th>
<th>$R^2$ for $S_x$</th>
<th>$R^2$ for $S_y$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart 8</td>
<td>53.2</td>
<td>1.0</td>
<td>4.1</td>
<td>0.91</td>
<td>0.49</td>
<td>0.97</td>
<td>0.97</td>
</tr>
<tr>
<td>Heart 9</td>
<td>63.9</td>
<td>1.6</td>
<td>1.1</td>
<td>0.96</td>
<td>0.48</td>
<td>0.96</td>
<td>0.97</td>
</tr>
<tr>
<td>Heart 10</td>
<td>78.3</td>
<td>1.7</td>
<td>1.0</td>
<td>0.71</td>
<td>0.48</td>
<td>0.96</td>
<td>0.98</td>
</tr>
<tr>
<td>Heart 11</td>
<td>24.4</td>
<td>2.3</td>
<td>2.5</td>
<td>0.80</td>
<td>0.48</td>
<td>0.98</td>
<td>0.99</td>
</tr>
<tr>
<td>Heart 12</td>
<td>34.7</td>
<td>2.4</td>
<td>2.6</td>
<td>0.75</td>
<td>0.48</td>
<td>0.95</td>
<td>0.97</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>30.8 ± 21.7</td>
<td>1.8 ± 0.6</td>
<td>2.3 ± 1.3</td>
<td>0.82 ± 0.10</td>
<td>0.48 ± 0.0</td>
<td>0.97</td>
<td>0.97</td>
</tr>
</tbody>
</table>

The value of $a_4$ was fixed at 0.77 for all animals.

comparison, the RCA and LAD for both intact wall and media were considered. We found no statistically significant differences between RCA and LAD artery for any of the material constants ($C$, $a_1$, $a_2$, and $a_4$) for the intact wall or media. We also examined the differences in the circumferential stress-strain relation for the various axial stretch ratios independent of a constitutive model. Again, there were no significant differences in the model-independent stress-strain data for any axial stretch.

**DISCUSSION**

**Constitutive Equation of Blood Vessels**

The constitutive equation of the blood vessel wall was a much-debated subject in earlier years (see review in Ref. 11). Later, the following observation by Fung (11) was generally accepted: Soft tissues have a special kind of viscoelasticity where the percentage loss of energy by hysteresis per cycle in a cyclic loading and unloading process is only a few percent, and this percentage does not vary more than a factor of two over a frequency range of five decades. However, at any particular frequency, it is not easy to predict the exact value of the loss per cycle. Fung (11) and others showed that this is consistent with a model of viscoelasticity with a continuous relaxation spectrum. In cyclic loading and unloading, the stress-strain relationship is unique in the loading stroke and is also unique in the unloading stroke (although the two are somewhat different). Fung called such a material “pseudoelastic,” a term that is now widely used.

Alternative forms for the strain energy function abound (1, 11), especially the polynomial form of Patel et al. (23) and the logarithmic form by Hayashi (15). But the exponential form $E_5$ is accepted widely, mainly because it is the only form that we know how to invert; i.e., to derive analytically the tensorial strain-stress relationship from the stress-strain relationship by a method proposed by Fung (9). Many validation experiments have been done (11). Shear has been studied in the coronary arteries by Lu et al. (19), but it is zero if the deformation is axisymmetric as in the present study. More recently, Holzapfel et al. (16) introduced a strain energy function for three-dimensional, two-layer artery previously used in mechanics of fiber-reinforced composites. The three-dimensional model has the advantages of a few material parameters (five in each layer) and is partly based on the histological fiber structure. As a microstructural model, the constitutive relation remains to be validated histologically. As a phenomenological model, it appears promising for the characterization of three-dimensional, anisotropic, and nonlinear mechanical properties of arteries.

**Parameter Estimation**

Determination of constitutive equations is an inverse problem. We assume a form of the constitutive relation, and based on computed values of stress and strain from loading and deformation experiments, we determine the material properties that give satisfactory agreement between the theorized form and the experimental data. It should be noted, however, that there are inherent difficulties with nonlinear least squares procedures in that large differences in the values of the material constants may result in very small differences in the data. This is a well-known fact about class of nonlinear “inverse” problems (18). The difficulty is that the error function given by Eq. 8 has many relative minima. A simple solution to this difficulty was presented by Zeng et al. (31). Their approach involved the initial determination of the four constants followed by fixing one of the constants, namely $a_4$, which reduces the degree of freedom by one and makes the determination of the remaining three constants rather stable. The choice of fixing $a_4$ stems from the observation that variation of this parameter has the smallest effect on the curve fit.

**Comparison of M-L and GA Methods**

There are several significant differences between the traditional nonlinear least squares method (e.g., M-L) and the GA that deserve mention. These differences include 1) GA
searches a population of points in parallel, not a single point; 2) GA does not require derivative information or other auxiliary knowledge; only the objective function and the corresponding fitness levels influence the directions of search; 3) GA uses probabilistic transition rules, not deterministic ones; 4) GA works on an encoding of the parameter set rather than the parameter set itself (except where real-valued individuals are used); and 5) traditional methods may miss a local minimum depending on the optimization method employed. It is also important to note that the GA provides a number of potential solutions to a given problem, and the choice of final solution is left to the user. In cases where a particular problem does not have one individual solution (for example, a family of Pareto-optimal solutions), the GA is then potentially useful for identifying these alternative solutions simultaneously. In the present study, we did not find any statistically significant differences between the two methods of parameter estimation; hence, we gained confidence that the parameters estimated minimize the error between the proposed theoretical function and experimental data.

Convexity of Strain Energy Function

Although we did not put any restrictions on the choice of material constants, we verified that the material constants do not violate the physical requirements of hyperelasticity; i.e., the condition that the material must be stable under loading (22). Accordingly, we confirmed that the second derivative of strain energy function with respect to strain is positive definite.

Hence, the proposed strain energy functions are convex, which ensures physically meaningful mechanical behavior. This is easy to verify because of the quadratic form of $Q$ in Eq. 5. Indeed, it can be shown that $Eq. 5$ is locally convex if $a_1 > 0$, $a_2 > 0$ and $a_1a_2 > a_4^2$ for $C > 0$ (16).

A Two-Layer Model of Coronary Artery

The coefficient $C$ dictates the stress scale, the product $C\cdot a_1$ dictates the nonlinear rate of change in the circumferential stress with respect to strain, the product $C\cdot a_2$ has the same meaning in the axial direction, and $a_4$ indicates the interaction between terms in both circumferential and axial directions. For the RCA, for example, $C\cdot a_1$ is significantly smaller for the intact wall than the media for both M-L and GA methods ($P = 0.01$ for both methods) as shown in Table 6. For the LAD artery, the parameter $C\cdot a_1$ was found to be significantly smaller for the intact wall compared with the intima-media ($P = 0.022$ for both methods).

An inspection of Fig. 2, A–C, shows that the vessel stiffens circumferentially (leftward shift) with increase in axial stretch ratio. This behavior has been previously reported (e.g., 7, 17). Furthermore, at in vivo axial stretch ratio of 1.4, the slope of the circumferential stress-strain relation is larger for the intima-media layer than the intact wall, which is larger than the adventitial layer. This is consistent with our previous data on the incremental homeostatic elastic moduli: i.e., intima-media > intact > adventitia (19).

Limitations of Proposed Model

There are two approaches for the description of the biomechanics of vessel wall. In one approach, the vessel wall is regarded as a three-dimensional incompressible material. A second approach relies on the axisymmetric, thin-walled assumptions and regards the wall as a two-dimensional compressible membrane structure. The latter approach yields the mean stresses over the wall thickness in the circumferential and axial direction. Such a two-dimensional approach cannot describe the behavior of a thick-walled cylindrical tube under, for instance, inflation and torsion. The proposed form is limited to a membrane description that involves the mean quantities of stresses.

Significance of Study and Future Direction

Despite the recognition of the significance of stress and strain in gene expression, cell biology, cell adhesion, and cell differentiation and proliferation, we are still unable to determine the internal stresses and strains because we do not know the constitutive equations for the different layers of the blood
vessel wall. Most of the past determinations of the constitutive equation have treated the vessel wall as a homogeneous material. In the past two decades, advances in vascular mechanics have resulted from theory and experiment that demand greater detail. The separation of a vessel wall into several layers is a step toward the next level in the hierarchy of structure. Each level has a continuum model, and different levels have different models of continua. Clearly, the layered concept is not the end, and successively lower levels can be explored. Idealization of the coronary artery as composed of two layers each of homogenous materials may serve as a beginning for further analysis. More detailed modeling should be done if greater details are required; i.e., the modeling of the intima-media layer as composed of elastin and collagen fibers and muscle cells. The next logical step is to determine the three-dimensional constitutive equations; i.e., the stress-strain-history relations, of the individual layers that take into account the circumferential, axial, and radial stresses and strains and their respective shear components. Furthermore, the active smooth muscle stress must also be considered in future models to understand important clinical issues such as coronary vasospasm.

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