Determination of layer-specific mechanical properties of human coronary arteries with nonatherosclerotic intimal thickening and related constitutive modeling

Gerhard A. Holzapfel,1,2 Gerhard Sommer,2 Christian T. Gasser,2 and Peter Regitnig3

1Computational Biomechanics, Graz University of Technology; 2School of Engineering Sciences, Royal Institute of Technology, Stockholm, Sweden; and 3 Institute of Pathology, Medical University Graz, Graz, Austria

Submitted 7 September 2004; accepted in final form 21 May 2005

Holzapfel, Gerhard A., Gerhard Sommer, Christian T. Gasser, and Peter Regitnig. Determination of layer-specific mechanical properties of human coronary arteries with nonatherosclerotic intimal thickening and related constitutive modeling. Am J Physiol Heart Circ Physiol 289: H2048–H2058, 2005. First published July 8, 2005; doi:10.1152/ajpheart.00934.2004.—At autopsy, 13 nonstenotic human left anterior descending coronary arteries [71.5 ± 7.3 (mean ± SD) yr old] were harvested, and related anamnesis was documented. Preconditioned prepared strips (n = 78) of segments from the midregion of the left anterior descending coronary artery from the individual layers in axial and circumferential directions were subjected to cyclic quasi-static uniaxial tension tests, and ultimate tensile stresses and stretches were documented. The ratio of outer diameter to total wall thickness was 0.189 ± 0.014; ratios of adventitia, media, and intima thickness to total wall thickness were 0.4 ± 0.03, 0.36 ± 0.03, and 0.27 ± 0.02, respectively; axial in situ stretch of 1.044 ± 0.06 decreased with age. Stress-stretch responses for the individual tissues showed pronounced mechanical heterogeneity. The intima is the stiffest layer over the whole deformation domain, whereas the media in the longitudinal direction is the softest. All specimens exhibited small hysteresis and anisotropic and strong nonlinear behavior in both loading directions. The media and intima showed similar ultimate tensile stresses, which are on average three times smaller than ultimate tensile stresses in the adventitia (1,430 ± 604 kPa circumferential and 1,300 ± 692 kPa longitudinal). The ultimate tensile stretches are similar for all tissue layers. A recently proposed constitutive model was extended and used to represent the deformation behavior for each tissue type over the entire loading range. The study showed the need to model nonstenotic human coronary arteries with nonatherosclerotic intimal thickening as a composite structure composed of three solid mechanically relevant layers with different mechanical properties. The intima showed significant thickness, load-bearing capacity, and mechanical strength compared with the media and adventitia.

human left anterior descending coronary artery; elasticity; material model; mechanical properties; ultimate tensile strength

One central aim in cardiovascular solid mechanics is the investigation of the mechanobiological behavior of arteries in health and disease, which may better explain their function on the basis of their structure and mechanics, i.e., vital information for clinical treatments of artery diseases, for designs of vascular implants such as stents and grafts, and for tissue engineering. For example, 657,000 percutaneous transluminal coronary angioplasty (PTCA) procedures were performed in the United States in 2002 (1). To improve our understanding of the mechanisms involved in PTCA procedures and stent designs, it is fundamental to better explore the mechanical properties and role of the separate arterial layers of coronary arterial walls and to develop efficient computational models.

Most of the studies have focused on the mechanical properties of animal coronary arteries (3, 11, 22, 25, 26, 33, 42). In addition to a collagenous adventitia and a medial layer (consisting of smooth muscle, collagen, and some elastin), a coronary arterial wall may consist of a complex intimal layer that develops rapidly in early years and continues to grow gradually throughout life (41). This process is not observed in animals. The intima is a prominent layer with a distinctive organization of layered groups of collagen fibers that condense into a few separate alignment families (4). Another fundamental difference from animals is that the intima of aged human specimens exhibits considerable mechanical strength (37). The axial pre-stretch is much less for aged human arteries than for animal arteries; it can even be <1.0; i.e., the vessel then elongates axially on excision (37). Hence, the constitutive parameters of animal arteries are of limited value for the development of, for example, appropriate stents and vascular grafts or tissue-engineered substitutes that are functional. In particular, from the mechanical point of view, grafts should be designed with material properties nearly identical to those of the host vessel, because only then is there no compliance mismatch at the anastomosis.

Only a few in vitro data on the mechanical properties of human coronary arteries are available in the literature (2, 6, 10, 14, 31, 34, 40a); three-dimensional constitutive models that are able to describe the typical nonlinear and anisotropic material response and that are of practical use for finite-element methods are rare. Because human coronary arteries with nonatherosclerotic intimal thickening comprise three (thick-walled) layers, a better understanding of their mechanical function at the tissue level requires a layer-specific experimental approach and the design of constitutive descriptions that can be implemented efficiently in numerical computations. It appears that Vito and Demiray (42) were the first to document separate mechanical response data for the media and adventitia from canine aorta, which they obtained from uniaxial strip tests in the axial and circumferential directions. Later, Richardson and Keeny (34) performed mechanical measurements during uniaxial tests of intima segments from the region between the left circumflex and left anterior descending (LAD) coronary arteries. There is considerable uncertainty about the mechanical properties and
role of the adventitia, media, and intima (including failure). To the authors’ knowledge, a systematic study of the layer-specific mechanical response of human coronary arteries is not available.

With the long-term goals of improving our understanding of the mechanisms involved in PTCa procedures and improving stent designs, we explore the underlying mechanics of the adventitia, media, and intima of human coronary arteries with nonatherosclerotic intimal thickening. Because LAD coronary arteries are of central importance in heart and circulatory pathophysiology [restenosis after stenting is more common in the LAD than at coronary locations (13, 23)], the present work focuses on examination of this arterial segment. One specific aim is the experimental investigation of the passive mechanical properties of arterial strips from each of the three layers of the midregions of human LAD coronary arteries, and the mechanical role of the intima attracts particular attention. Another goal is the determination of three-dimensional constitutive models for the description of the mechanical response of each of the tissues.

**MATERIALS AND METHODS**

**Specimens.** Thirteen hearts, from 3 women and 10 men [71.5 ± 7.3 (SD) y, range 54 – 80], were harvested within 24 h of death. The axial in situ length of an LAD coronary artery was determined by measuring the distance between two superficial surgical knots made of surgical fibers and prepared by a pathologist. The ex situ length was measured after 1 h of equilibration of the excised artery in 0.9% physiological saline solution at 37 ± 0.1°C. Then the axial in situ stretch, defined as the ratio of in situ segment length to ex situ segment length, was computed.

Information about the anamnesis of the coronary arteries is summarized in Table 1. Only straight segments without palpable circumcised wall hardening were used. An artery was rejected if, during preparation, 1) the straight segment showed an atherosclerotic plaque or 2) eccentric intimal thickening was macroscopically visible (40). Therefore, a total of 27 hearts were investigated, and 14 were rejected.

The segments were harvested from the location between the first and the second diagonal branch, which is part of the mid-LAD coronary artery [segment I according to the Coronary Artery Surgery Study (28)]. The use of autopsy material from human subjects was approved by the Ethics Committee of the Medical University Graz.

**Layer preparation.** Because our own preliminary studies revealed that, for aged human coronary arteries, the intima is a prominent layer of considerable thickness and mechanical strength, we aimed to anatomically separate the heterogeneous arterial wall structure into three layers. Preliminary tests on aged human coronary arteries indicated that it was impossible to obtain intact (leak-free) tubes of the adventitia, media, and intima for inflation and extension experiments. In recent work (39), however, we succeeded in separating the adventitia from the media-intima layer of aged human femoral arteries, which are much larger in diameter than coronary arteries and lack multiple branches.

After the harvested arteries were pruned from the remaining adipose and connective tissues, they were cut along the axial direction to obtain flat rectangular sheets. From each sheet, neighboring strips of axial orientation (the long side of the strip is aligned with the axial direction) and circumferential orientation of the arterial wall were cut out with a sharp knife (for prepared strip samples, see Fig. 3 in Ref. 19). The rectangular form of the samples was verified repeatedly during the preparation using a ruler. Sample length, width, and thickness were measured by means of a video extensometer.

For anatomic separation of the arterial sample into its three layers, we tried to find suitable locations at which the layer separation process can easily be initiated. Then, by separating the layers and carefully disconnecting the interconnective tissue with a scalpel, which was sometimes necessary, the arterial layers could be separated. According to the authors’ experience, this technique is much more difficult or may even be impossible for arteries of young experimental animals. However, the layer preparation process was not always successful, because subsequent macroscopic inspection of the histological images established that the result was not the desired single arterial layer (intima, media, or adventitia). If this was the case, the experimental data were not used, but data from a neighboring strip were obtained if the layer separation was successful. Hence, a total of 55 entire arterial strips were used from the 13 LAD coronary arteries, and 78 (i.e., 6 × 13) separated strips were used for the study.

Pieces of sandpaper were mounted at the ends of the prepared strip samples with superadhesive gel to facilitate a defined clamping in the tensile testing machine and to prevent slippage during testing. Finally, two black straw chips were glued transversely in parallel onto the middle part of the samples to act as gauge markers for the axial deformation measurements (for representative tissue samples, see Fig. 4 in Ref. 19). The strip samples were allowed to equilibrate for 30–60 min before testing in a calcium-free 0.9% physiological saline solution at 37 ± 0.1°C.

**Mechanical testing.** Uniaxial tensile tests with bidimensional measurements of coronary strip specimens were performed on a computer-controlled, screw-driven high-precision tensile testing machine adapted for small biological specimens (μ-Strain ME 30-1, Messphysik, computer-controlled, screw-driven high-precision tensile testing machine adapted for small biological specimens (μ-Strain ME 30-1, Messphysik,

Table 1. Anamnesis

<table>
<thead>
<tr>
<th>Specimen No.</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>V</th>
<th>VI</th>
<th>VII</th>
<th>VIII</th>
<th>IX</th>
<th>X</th>
<th>XI</th>
<th>XII</th>
<th>XIII</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>80</td>
<td>73</td>
<td>80</td>
<td>64</td>
<td>73</td>
<td>75</td>
<td>73</td>
<td>75</td>
<td>72</td>
<td>77</td>
<td>69</td>
<td>64</td>
<td>54</td>
</tr>
<tr>
<td>Gender</td>
<td>F</td>
<td>M</td>
<td>F</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>M</td>
</tr>
<tr>
<td>Primary disease</td>
<td>PT</td>
<td>HC</td>
<td>PN</td>
<td>CK</td>
<td>PC</td>
<td>PN</td>
<td>CC</td>
<td>PC</td>
<td>LC</td>
<td>DE</td>
<td>HT</td>
<td>SA</td>
<td>BC</td>
</tr>
<tr>
<td>Cause of death</td>
<td>PAE</td>
<td>GHD</td>
<td>GHD</td>
<td>UR</td>
<td>IL</td>
<td>GHD</td>
<td>MOF</td>
<td>GHD</td>
<td>PN</td>
<td>PN</td>
<td>AP</td>
<td>AP</td>
<td>GHD</td>
</tr>
</tbody>
</table>

Atherosclerosis

<table>
<thead>
<tr>
<th></th>
<th>Aorta</th>
<th>Coronary arteries</th>
<th>Cerebral arteries</th>
<th>Renal arteries</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
</tbody>
</table>

M, male; F, female; AP, apoplexy; BC, bronchus carcinoma; CC, colon carcinoma; CK, polycystic kidney disease; DE, dementia; GHD, global heart dilation; HC, hydrocephalus internus with tetraparesis; HT, hypertension; IL, ileus; LC, liver carcinoma; MOF, multiple organ failure; PAE, pulmonary artery embolism; PC, pancreas carcinoma; PN, pneumonia; PT, phlebothrombosis of the leg; SA, salmonellosis; UR, uremia. Assessment of atherosclerosis is based on autopsy reports: ✓, medium- or high-grade atherosclerosis; -, no or low-grade atherosclerosis.

AJP-Heart Circ Physiol • VOL 289 • NOVEMBER 2005 • www.ajpheart.org
Fürstenfeld, Austria). The specimens were investigated in a Perspex container filled with 0.9% physiological saline solution maintained at 37 ± 0.1°C by a heater-circulation unit (Ecoline E 200, Lauda, Lauda-Königshofen, Germany), and the tensile force was measured with a 25-N class 1 strain-gauge load cell (model F1/25N, AEP converter). The upper and lower crossheads of the testing machine are moved in opposite directions so that the gauge region of the samples is always in the same field of view. A crosshead stroke resolution of 0.04 μm and a minimum load resolution of 1 mN of the 25-N load cell is specified by the manufacturer. Digital control of the electric drive of the machine, as well as data acquisition of the crosshead position and applied load, was performed by an external digital controller (EDC 25/90W, DOLI, Munich, Germany), especially designed for screw-driven tensile testing machines. Gauge length and width were measured optically using a PC-based (CPU 586) video extensometer (model ME 46-350, Messphysik), utilizing a full-image charge-coupled device camera that allows automatic gauge mark and edge recognition (19). The corresponding deformation data were averaged with respect to the measuring zone and sent to the data-processing unit in real time.

Before testing was done, preconditioning was achieved by executing five loading and unloading cycles at a constant crosshead speed of 1 mm/min for each test to obtain repeatable stress-strain curves. Thereafter, the sample underwent a cyclic quasi-static uniaxial extension test, with continuous recording of tensile force and gauge length and width at the same crosshead speed of 1 mm/min. After tensile testing was completed, the sample thickness was measured. Finally, for a determination of the ultimate tensile stress and associated stretch, the sample was reclamped into the testing machine, and the strain was increased with the same crosshead speed of 1 mm/min until fracture occurred. Because the width (and thickness) of the samples inside and outside the gauge region was similar, the fracture occasionally occurred outside the gauge region of the sample close to one of the clamps, so another sample was prepared and tested if enough material was available.

After each test, the strip samples were inserted into a 4% buffered formaldehyde solution (pH 7.4) for fixation and further histological evaluation.

**Histology.** The separated tissues were embedded in paraffin and sectioned at 3 μm, and consecutive sections were stained with hematoxylin and eosin and elastica Van Gieson. Histological investigations were accomplished to confirm primarily correct layer separation. The membrana elastica interna and the membrana elastica externa, both seen in hematoxylin-and-eosin- and elastica Van Gieson-stained sections, served as structural components to ensure the correct separation of the arterial layers. Additionally, histological sections were used to assess the histostructural homogeneity of the different layers, particularly the intimas, i.e., without circumscribed atherosclerotic plaques. With this additional histological investigation, all specimens were classified as appropriate, as described above.

**Analysis and data fitting.** Arterial tissue subject to loading exhibits strong nonlinearity (with a typical exponential stiffening effect at higher loads) and anisotropy, and it also undergoes finite strains. Thus it is necessary to derive a formulation of the material behavior within the realm of nonlinear continuum mechanics (16, 30).

Each arterial sample is regarded as incompressible (5), which requires that \(\lambda_1 \lambda_2 \lambda_3 = 1\), where \(\lambda_1\), \(\lambda_2\), and \(\lambda_3\) are the principal stretches of the deformation (when there is no shear) associated with the radial, circumferential, and axial directions. To model the general mechanical characteristics of the arterial tissues, we use a strain-energy function \(\Psi\) (per unit volume), which extends a recently proposed multilayer model for arterial walls (17, 18, 20):

\[
\psi = \mu(I_1 - 3) + \frac{k_1}{k_2} \left(\exp[k_2((1 - \rho)(I_1 - 3)^2 + \rho(I_4 - 1)^2)] - 1\right) \tag{1}
\]

where \(I_4 = \lambda_1^4 + \lambda_2^4 + (\lambda_3 \lambda_4)^{-2}\) and \(I_4 > 1\) are invariants (16), \(k_2 > 0\) and \(\rho \in [0,1]\) are dimensionless parameters, and \(\mu > 0\) and \(k_1 > 0\) are stresslike parameters. Equation 1 describes the strain energy stored in a composite reinforced by two families of (collagen) fibers that are arranged in symmetrical spirals, here assumed to be mechanically equivalent. For the specific loading state (no shear loads) and with the assumption that all the fibers are embedded in the tangential surface of the tissue (no components in the radial direction), \(I_4\) in Eq. 1 can then simply be expressed as

\[
I_4 = \lambda_2^2 \cos^2 \varphi + \lambda_3^2 \sin^2 \varphi \tag{2}
\]

The anisotropic term in Eq. 1 contributes to \(\Psi\) only when \(I_4 > 1\) (see discussion in Ref. 18). The parameter \(\varphi\) in Eq. 2 is the angle between the fiber reinforcement (orthotropy) and the circumferential direction in the individual layers, which, therefore, acts as a geometrical parameter. Because the structural orientation of the individual layers was not investigated, \(\varphi\) is used as a phenomenological variable. The function Eq. 1, with Eq. 2, is sufficiently general to capture the typical features of each arterial response. It ensures mechanically and mathematically reliable behavior in the sense discussed in Refs. 17 and 18. Because each layer responds with similar mechanical characteristics, we use subsequently the same form of strain-energy function (but a different set of material parameters) for each layer.

The five parameters \((\mu, k_1, k_2, \varphi, p)\) in Eqs. 1 and 2 are obtained by means of the standard nonlinear Levenberg-Marquardt algorithm. We minimize the “objective function”

\[
\chi^2 = \sum_{i=1}^{n} \left[ w_1 (\sigma_{\text{test}} - \sigma_{\text{fit}})^2 + w_2 (\varepsilon_{\text{test}} - \varepsilon_{\text{fit}})^2 \right] \tag{3}
\]

where \(n\) is the number of data points, \(w_1\) and \(w_2\) are weighting factors, and

\[
\sigma_{\text{fit}} = \left( \frac{\partial \varepsilon}{\partial \lambda_i} \right)_{\text{fit}} \tag{4}
\]

are the Cauchy stresses in the circumferential and axial directions of the artery predicted by the function \(\Psi\) for the \(i\)th data record. The associated experimental Cauchy stresses, \(\sigma_{\text{test}}\) and \(\sigma_{\text{fit}}\), are calculated directly from the original data as \(\sigma_{\text{test}} = \frac{\Delta \varepsilon_{\text{test}}}{\Delta \lambda_{\text{test}}} / \lambda_{\text{test}}\), where \(\sigma_{\text{tens}}\) represents the Cauchy stress in the circumferential or axial direction and \(\lambda_{\text{tens}} = \frac{\zeta}{L}\) for the associated stretch ratio, with gauge lengths \(L\) and \(L\) measured in the loaded and unloaded configurations, respectively. The current tensile force is denoted by \(f\), and \(A\) is the cross-sectional area of the specimen in the unloaded configuration.

For the fitting process, we have considered data points only on the loading branches. This is justified by the small hystereses between loading and unloading branches for all arteries investigated (see below).

As a measure for the “goodness of fit,” we use the root mean square error measure \(\varepsilon\), which is based on the value of \(\chi^2\) of a certain constitutive model and is defined as

\[
\varepsilon = \left[ \frac{\chi^2}{n - q} \right]^{1/2} \tag{5}
\]

where \(n\) is the number of data points and \(q\) is the number of parameters of the strain-energy function, which in our case is five; hence, \(n - q\) is the number of degrees of freedom.

The value \(\varepsilon_{\text{fit}}\) is the sum of all Cauchy stresses for each data point divided by the number of all data points.
RESULTS

Anamnesis. Table 1 shows the anamnesis of the patients (specimens I–XIII) in terms of age, gender, primary disease, and cause of death. Furthermore, the condition of the aortic, coronary, cerebral, and renal arteries with regard to atherosclerosis, from the postmortem report, is provided. Because a correlation between the anamnesis of a patient and the mechanical properties of arteries is evident (15), it seems important to obtain a complete picture of the arterial mechanics of aged patients (19, 38).

Geometry and axial in situ stretch. The outer diameter of the mid-LAD (middle segments) coronary arteries from 13 hearts is 4.5 ± 0.3 (SD) mm. Length, width, and thickness of the total wall for the 55 samples are as follows: 7.21 ± 1.21, 2.81 ± 0.38, and 0.87 ± 0.23 (SD) mm, respectively; length-to-width ratio is 2.61 ± 0.70. It is most likely that this length-to-width ratio implies the desired homogeneous stress-strain state within the gauge range (Saint-Venant’s principle). The ratio of total wall thickness to outer diameter of all specimens (I–XIII) is 0.189 ± 0.014 (mean ± SD), and the average wall thickness computed from the number of samples for each segment was related to the respective outer diameter. Thickness for each arterial layer, i.e., adventitia, media, and intima, for all specimens (I–XIII, n = 26 for each layer) is 0.34 ± 0.08, 0.32 ± 0.11, and 0.24 ± 0.17 (SD) mm, respectively. The ratios of adventitia, media, and intima thickness to total wall thickness are 0.40 ± 0.03, 0.36 ± 0.03, and 0.27 ± 0.02 (mean ± SD), respectively.

Axial in situ stretch is 1.044 ± 0.06 (mean ± SD). Statistical analysis was performed to test for significant correlations between age and axial in situ stretch. For this, Pearson’s correlation coefficient (r) was calculated, and P was determined on the basis of Student’s t distribution. P < 0.05 was considered significant. There were significant negative correlations between age and axial in situ stretch (r = −0.80, P = 0.017). This suggests that axial in situ stretches of the human LAD coronary artery decrease with age.

Tissue preconditioning and stress-stretch behavior. Figure 1 shows a representative plot of the preconditioning behavior of an intima strip sample with 10 load cycles. The hysteresis loops shift during repeating loading-unloading cycles toward a larger deformation and converge to a certain deformation with smaller hysteresis. We know that the number of preconditioning cycles depends on the origin of the specimen (e.g., species, localization, type, and age), and in the literature up to 15 preconditioning cycles are used (22). For preconditioning of the present specimens, five loading-unloading cycles were sufficient.

The stress-stretch plots in Figs. 2–4 show the mechanical responses, i.e., Cauchy stress (σ) vs. stretch (λ), of the different arterial tissues in the circumferential and longitudinal directions for specimens I–XIII. All three tissue types exhibit a pronounced anisotropic and nonlinear mechanical response with remarkably small hysteresis over finite strains.

To verify whether the tissue properties change between death and mechanical testing, we correlated that time period with the stretch at 40 kPa (Cauchy) stress for all tested media strip samples. We computed r = −0.426, P = 0.147 (for circumferential samples) and r = 0.064, P = 0.870 (for longitudinal samples), where P < 0.05 was considered significant. Hence, these data indicate insignificant correlation. Additionally, Pearson’s correlation coefficients computed for the intima and adventitia samples also indicate insignificant correlation. This result was expected, because intima and adventitia
titia samples are bradytrophic tissues for which the proteolytic activity is delayed for several days after death.

Ultimate tensile stresses and related stretches. The ultimate tensile stresses of the coronary arterial tissues, e.g., \( \sigma_{\text{ult}} \), were computed from the experimentally determined fracture forces according to

\[ \sigma_{\text{ult}} = \frac{f_f}{WT} \lambda_{\text{ult}} \]  

where \( f_f \) is the fracture tensile force and \( W \) and \( T \) are the width and the thickness of the strip sample in the unloaded configuration, respectively. The ultimate stretch ratio \( (\lambda_{\text{ult}}) \) was computed as \( l/L \), where \( l \) and \( L \) are the measured gauge lengths in the loaded (state at fracture) and unloaded configurations, respectively. The average ultimate tensile stresses \( (\bar{\sigma}_{\text{ult}}) \) and the associated ultimate stretches \( (\lambda_{\text{ult}}) \) for the individual tissues from two separate orientations, which fractured in the gauge region, are indicated in Table 2 for 40 samples.

Histology. Histological analyses of the specimens showed homogeneous diffuse intimal hyperplasia (Fig. 5), which is the result of a nonatherosclerotic process (40): intimal cells (mainly myofibroblasts) proliferate concentrically and lead to an increase of extracellular matrix (containing mainly collagen fibers) and thickening of the intima to restore baseline wall stress. In the present study, the mean thickness of the intima was 27% of the total wall thickness. The occurrence of the elastic lamellae in the media was not easily discernable.

Constitutive modeling. The constitutive parameters \( \mu, k_1, k_2, \varphi, \) and \( \rho \) for the individual tissue layers of all 13 specimens are summarized in Table 3. Additionally, the error measures, mean values, and related standard deviations are stated.

Figure 6 shows a comparative study between the circumferential and longitudinal stress-stretch responses of samples obtained from the three arterial layers (specimen IX). In particular, a comparison between experimental data, with numerical results obtained from the strain-energy function (Eq. 1), is provided. Figure 7 summarizes the stress-stretch model results by using mean constitutive parameters from Table 3.

DISCUSSION

One important issue in cardiovascular solid mechanics is the determination of the mechanical properties of human arterial walls with nonatherosclerotic intimal thickening under various loading conditions. Clearly, aged human arterial walls are heterogeneous three-layered composites with layer-specific histological features and mechanical functions. There is a need to better characterize the underlying mechanics of the individ-
ual tissue types, in particular, LAD coronary arteries. This issue is of clinical importance in relation to morbidity and mortality and, also, of crucial interest to the stent-producing industry.

**Geometry and axial in situ stretch.** A few studies report diameter and thickness of the human LAD coronary artery; however, sometimes the location is not documented. On the basis of human LAD coronary arteries from four men (63.5 ± 7.9 (SD) yr), Canham et al. (4) reported outer diameters for proximal (4.30 ± 0.47 mm) and distal (2.5 ± 0.37 mm) LAD coronary arteries. They used histological sections from which measurements were obtained. According to Canham et al., the outer diameter decreases significantly from the proximal to the distal region, and the intimal layer shows decreasing dominance for the more-distal segment but remains the dominant layer compared with the media and adventitia. Kang et al. (21) report a luminal diameter of 2.9 ± 0.2 mm (15 mid-LAD coronary artery segments) by using quantitative coronary angiography (47.4 ± 2.5 yr, 9 men and 6 women), which coincides very well with the values obtained in our study. In addition to atherosclerotic LAD coronary arteries, Gradus-Pizlo et al. (12) measured the geometry of mid-LAD coronary arteries from five patients with normal coronary angiograms (mean age 57 yr, range 48–77) by high-frequency transthoracic echocardiography. From the images, the average outer diameter (4.0 mm) and wall thickness (1.0 mm) were measured (average wall thickness-to-diameter ratio = 0.25). Richardson and Keeny (34) reported thicknesses of intima samples of human coronary arteries in the range of 0.15–0.35 mm. Lu et al. (25) presented morphometric data for porcine coronary arteries (right coronary artery and LAD coronary artery), documenting a wall thickness-to-luminal diameter ratio of 0.05 at a transmural pressure of 100 mmHg. The mean intima-to-media-to-adventitia thickness ratio for the human mid-LAD segments is 27:36:40; in aged human iliac arteries, this ratio is 13:56:31 (38).

No data concerning the axial in situ stretches of human and animal coronary arteries can be found in the literature. Axial in situ stretches are, however, of fundamental importance for the computation of in situ stress states. In the present study, the axial in situ stretches was determined to be 1.044 ± 0.06 (mean ± SD), which is an average of ~5% prestretch with respect to the load-free length. For iliac arteries, Schulze-Bauer et al. (38) measured an axial in situ stretch of 1.071 ± 0.087 (10 external iliac arteries from 52- to 87-year-old humans). However, Fung et al. (9) reported axial in situ stretches of 1.5 for rabbit iliac arteries.

**Layer-specific stress-stretch behavior.** The different arterial tissues exhibit a marked mechanical heterogeneity (Figs. 2–4). In the light of these data, it is clear that a single-layer approach for characterizing the mechanical response of human coronary arteries and for performing local stress analyses is inappropriate. In addition, pronounced nonlinear, inelastic, and anisotropic mechanical responses over finite strains for all arterial tissues are observed. The adventitia samples and the samples of the intima tested in the longitudinal direction show the most pronounced nonlinearity. All tissues tested indicate initially compliant, but then stiffer, behavior over finite stretches in both orientations. This finding justifies the model assumption of a rather soft matrix material, into which the collagenous fibers are embedded. By “stiffness,” we shall mean subsequently the ratio of the Cauchy stress to the associated extension ratio, i.e., stretch −1. Hence, a small gradient indicates less stiffness (specimen VI in Fig. 4A), whereas a larger gradient indicates more stiffness (specimen IX in Fig. 4A). Another common mechanical characteristic is a relatively small hysteresis, although coronary arteries are of the muscular type. Small hysteresis indicates how energy dissipation in the material during load cycles.

Next, we discuss briefly the major mechanical features of the individual tissues. The stress-stretch curves for the adventitia indicate quite a large dispersion among the individual samples, and a clear anisotropic behavior is evident (Fig. 2). The adventitia samples oriented in the circumferential direction stiffen at a much higher stretch than those tested in the longitudinal direction. The dispersion of the stress-stretch curves is much smaller for the media than for the adventitia samples (Fig. 3). Also, the media shows a clearly pronounced anisotropic behavior, with the mechanical response of strips in the longitudinal direction tending to be softer than that of strips in the circumferential direction. The dispersion of the intimal stress-stretch curves is much smaller for samples tested in the longitudinal direction than in the circumferential direction (Fig. 4). Interestingly, adventitia and intima samples tested in the longitudinal direction (Figs. 2B and 4B, respectively) exhibit a tendency to be stiffer than corresponding samples in the circumferential direction, whereas for the media samples the opposite is the case. Significant anisotropy in human coronary intima, with the same characteristics as those shown in the present study, has also been documented (34). The apparent anisotropy, in particular for the adventitia and the intima, may be caused by the organization of the collagen fibers, which are almost entirely responsible for the resistance to stretch in the high loading domain (35).

On the basis of the experimental data, the media seem to be the softest layer over the whole deformation domain, and the intima and adventitia exhibit considerable stiffness, particularly in the high loading domain.
Ultimate tensile stresses and related stretches. The ultimate tensile stresses of the media and intima are very similar, and there appears to be no systematic difference between the longitudinal and circumferential directions. For the intima samples, this finding is analogous to that reported previously (34), where stress at fracture varied over 133–490 kPa. In our study, the strip samples from the adventitia, however, show, on average, about three times higher ultimate tensile stresses than samples from the related media and intima (Table 2). The ultimate tensile stretches are similar for all layers. Similar experiments on strip samples of human descending aortas were performed by Mohan and Melvin (27). Their results on ultimate tensile stresses and stretches are comparable with those obtained from the adventitial samples in our study, although Mohan and Melvin tested nonseparated strips.

Constitutive model. Dictated by the anisotropic and nonlinear mechanical response of the arterial tissue, an extended version of a previously published constitutive model was used (17, 18, 20). The model involves a small number of material parameters, so that a least-squares procedure avoids problems of nonuniqueness associated with their sensitivity to small changes in the data (8, 17).

The average stiffness of the tissues in the low loading domain (at which the noncollagenous matrix material is mainly active) is lowest for the media and highest for the intima: 1.27 ± 0.63 and 27.90 ± 10.59 kPa, respectively (Table 3). The average stiffness of the individual tissue in the high loading domain, dominated by the recruitment of collagenous fibers, is governed by the parameters $k_1$ and $k_2$, which are also lowest for the media ($k_1 = 21.60 ± 7.12$ kPa and $k_2 = 8.21 ± 3.27$ kPa) and highest for the intima ($k_1 = 263.66 ± 490.95$ kPa and $k_2 = 170.88 ± 125.47$ kPa). This indicates that, also in the high loading domain, the intima shows significant stiffness.

The dimensionless parameter $\rho \in [0,1]$ ranges between 0 and 1. If $\rho = 0$, the second term of Eq. 1 reduces to an isotropic (rubberlike) model, similar to that proposed in Ref. 7. If $\rho = 1$, the second term in Eq. 1 reduces to an anisotropic model proposed in Ref. 17. Hence, $\rho$ is a measure of anisotropy. In the present study, the mean values of $\rho$ are similar for the adventitia and intima ($0.55 ± 0.18$ and $0.51 ± 0.14$, respectively) and smaller for the media ($0.25 ± 0.09$), which indicate less anisotropic behavior than for the other two tissues.

The error measure $\epsilon$ is very close to zero for all arterial layers. This indicates a very good correlation between the model and the experimental data for all arterial layers and specimens (Fig. 6). This supports the use of the constitutive model (Eq. 1) employed. By taking the mean values of the five constitutive parameters for each arterial layer (Table 3), we obtain a kind of “standard model,” which reflects the mean of all mechanical data obtained from the human mid-LAD coronary artery specimens. Although the variances among individual specimens are large, the different mechanical characteristics of the individual tissues become clear. The standard model responses in the form of stress-stretch curves are illustrated in Fig. 7, which may serve as a constitutive basis for addressing more complex boundary-value problems.

Implications for vascular physiology and mechanobiology. Our experimental data attempt to emphasize the heterogeneous properties of the arterial wall and the importance of modeling a mid-LAD coronary artery with nonatherosclerotic intimal

Fig. 5. Photomicrographs of 3-μm-thick hematoxylin-and-eosin-stained sections from wall layers in longitudinal direction stemming from the gauge section after anatomic separation and mechanical testing. A: intima. Note homogeneous thickening due to diffuse intimal fibrous hyperplasia. B: media. Note mainly short-appearing nuclei and muscular fibers, indicating longitudinal direction of the artery; separation of some smooth muscle cells may have occurred during testing. C: adventitia. Note tendency to separate because of loose collagen fibers in the outer part. Original magnification ×200.
thickening as a structure composed of three solid mechanically relevant layers with layer-specific constitutive behavior. This is particularly relevant if stress-strain distributions through the wall thickness are of interest. This study reveals that the intimal samples of the human vessel segments demonstrate remarkable thickness, load-bearing capacities, and mechanical strengths. The intima may therefore contribute significantly to vascular physiology. In addition, ultimate tensile stress data suggest that at higher levels of pressure the adventitia changes to a stiff “jacketlike” tube that prevents the artery from overstretch and rupture (39).

The exciting challenge of vascular mechanobiology (and mechanopathology) is to explore the correlation between biological processes such as growth, remodeling, and disease and the mechanical environment of the different vascular tissues expressed in terms of quantities such as stresses and strains. Hence, constitutive models for the individual vascular tissues with biomechanical, mathematical, and computational efficacy are needed. Many of the arterial wall models are based on a (single-layer) homogeneous wall structure and, therefore, on the assumption that the mechanical properties do not change through the wall thickness. Increased insight into the mechanical properties and geometrical dimensions of the individual vascular tissues, in particular, from human coronary arteries, is also needed for applications in biomedical engineering. For example, we know that the dimensions of stent struts, which are in contact with the intimal surface of the arterial wall, have a strong effect on the stress concentration in the intima and in the clinical outcome after stenting (20, 32); stent struts may lead to intimal laceration and endothelial cell denudation (24, 36), which is a local effect. Hence, in that specific case, knowledge of the mechanical properties of the intima is important.

### Table 3. Constitutive parameters and error measure for adventitia, media, and intima of specimens I–XIII

<table>
<thead>
<tr>
<th>Specimen No.</th>
<th>μ (kPa)</th>
<th>k₁ (kPa)</th>
<th>k₂ (dimensionless)</th>
<th>φ (degrees)</th>
<th>ρ (dimensionless)</th>
<th>ε (dimensionless error measure)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adventitia</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>4.04</td>
<td>32.50</td>
<td>103.63</td>
<td>72.7</td>
<td>0.65</td>
<td>0.043</td>
</tr>
<tr>
<td>II</td>
<td>2.41</td>
<td>13.78</td>
<td>81.02</td>
<td>67.6</td>
<td>0.75</td>
<td>0.056</td>
</tr>
<tr>
<td>III</td>
<td>4.91</td>
<td>67.23</td>
<td>49.47</td>
<td>61.3</td>
<td>0.50</td>
<td>0.026</td>
</tr>
<tr>
<td>IV</td>
<td>16.41</td>
<td>8.42</td>
<td>37.16</td>
<td>75.7</td>
<td>0.95</td>
<td>0.070</td>
</tr>
<tr>
<td>V</td>
<td>9.65</td>
<td>82.06</td>
<td>144.98</td>
<td>53.4</td>
<td>0.40</td>
<td>0.028</td>
</tr>
<tr>
<td>VI</td>
<td>7.92</td>
<td>25.36</td>
<td>67.85</td>
<td>70.3</td>
<td>0.70</td>
<td>0.072</td>
</tr>
<tr>
<td>VII</td>
<td>7.07</td>
<td>23.86</td>
<td>7.01</td>
<td>74.5</td>
<td>0.55</td>
<td>0.080</td>
</tr>
<tr>
<td>VIII</td>
<td>2.25</td>
<td>37.06</td>
<td>48.36</td>
<td>65.8</td>
<td>0.40</td>
<td>0.046</td>
</tr>
<tr>
<td>IX</td>
<td>6.16</td>
<td>32.89</td>
<td>167.31</td>
<td>75.3</td>
<td>0.30</td>
<td>0.045</td>
</tr>
<tr>
<td>X</td>
<td>14.93</td>
<td>119.25</td>
<td>213.81</td>
<td>53.4</td>
<td>0.60</td>
<td>0.074</td>
</tr>
<tr>
<td>XI</td>
<td>12.71</td>
<td>35.14</td>
<td>84.65</td>
<td>69.3</td>
<td>0.60</td>
<td>0.063</td>
</tr>
<tr>
<td>XII</td>
<td>3.13</td>
<td>1.72</td>
<td>67.92</td>
<td>55.3</td>
<td>0.40</td>
<td>0.085</td>
</tr>
<tr>
<td>XIII</td>
<td>6.69</td>
<td>6.60</td>
<td>32.18</td>
<td>76.2</td>
<td>0.35</td>
<td>0.075</td>
</tr>
<tr>
<td>Mean±SD</td>
<td>7.56±4.66</td>
<td>38.57±32.53</td>
<td>85.03±58.94</td>
<td>67.0±8.5</td>
<td>0.55±0.18</td>
<td>0.059±0.020</td>
</tr>
<tr>
<td>Media</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>0.94</td>
<td>13.28</td>
<td>21.0</td>
<td>0.25</td>
<td>0.024</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>1.09</td>
<td>25.77</td>
<td>5.93</td>
<td>25.8</td>
<td>0.30</td>
<td>0.063</td>
</tr>
<tr>
<td>III</td>
<td>1.79</td>
<td>21.01</td>
<td>5.77</td>
<td>23.0</td>
<td>0.20</td>
<td>0.023</td>
</tr>
<tr>
<td>IV</td>
<td>1.63</td>
<td>26.40</td>
<td>9.57</td>
<td>26.6</td>
<td>0.40</td>
<td>0.037</td>
</tr>
<tr>
<td>V</td>
<td>2.54</td>
<td>16.67</td>
<td>13.85</td>
<td>17.0</td>
<td>0.20</td>
<td>0.078</td>
</tr>
<tr>
<td>VI</td>
<td>0.73</td>
<td>30.61</td>
<td>5.36</td>
<td>18.7</td>
<td>0.30</td>
<td>0.036</td>
</tr>
<tr>
<td>VII</td>
<td>0.93</td>
<td>24.91</td>
<td>7.52</td>
<td>12.3</td>
<td>0.15</td>
<td>0.053</td>
</tr>
<tr>
<td>VIII</td>
<td>0.32</td>
<td>27.95</td>
<td>5.43</td>
<td>10.9</td>
<td>0.09</td>
<td>0.034</td>
</tr>
<tr>
<td>IX</td>
<td>2.31</td>
<td>8.45</td>
<td>12.84</td>
<td>24.9</td>
<td>0.30</td>
<td>0.065</td>
</tr>
<tr>
<td>X</td>
<td>0.85</td>
<td>31.17</td>
<td>2.57</td>
<td>16.4</td>
<td>0.10</td>
<td>0.023</td>
</tr>
<tr>
<td>XI</td>
<td>1.21</td>
<td>12.98</td>
<td>8.41</td>
<td>29.7</td>
<td>0.30</td>
<td>0.046</td>
</tr>
<tr>
<td>XII</td>
<td>0.96</td>
<td>21.54</td>
<td>7.77</td>
<td>20.5</td>
<td>0.15</td>
<td>0.015</td>
</tr>
<tr>
<td>XIII</td>
<td>1.19</td>
<td>20.10</td>
<td>10.87</td>
<td>22.2</td>
<td>0.40</td>
<td>0.082</td>
</tr>
<tr>
<td>Mean±SD</td>
<td>1.27±0.63</td>
<td>21.60±7.12</td>
<td>8.21±3.27</td>
<td>20.61±5.5</td>
<td>0.25±0.09</td>
<td>0.045±0.022</td>
</tr>
<tr>
<td>Intima</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>28.62</td>
<td>124.01</td>
<td>180.43</td>
<td>69.5</td>
<td>0.55</td>
<td>0.054</td>
</tr>
<tr>
<td>II</td>
<td>42.43</td>
<td>264.32</td>
<td>286.97</td>
<td>53.7</td>
<td>0.70</td>
<td>0.067</td>
</tr>
<tr>
<td>III</td>
<td>25.30</td>
<td>234.48</td>
<td>176.34</td>
<td>57.8</td>
<td>0.50</td>
<td>0.075</td>
</tr>
<tr>
<td>IV</td>
<td>53.95</td>
<td>1860.70</td>
<td>454.42</td>
<td>46.8</td>
<td>0.40</td>
<td>0.043</td>
</tr>
<tr>
<td>V</td>
<td>24.75</td>
<td>369.53</td>
<td>343.14</td>
<td>39.9</td>
<td>0.70</td>
<td>0.057</td>
</tr>
<tr>
<td>VI</td>
<td>15.93</td>
<td>35.45</td>
<td>42.66</td>
<td>71.3</td>
<td>0.72</td>
<td>0.107</td>
</tr>
<tr>
<td>VII</td>
<td>34.66</td>
<td>54.80</td>
<td>92.74</td>
<td>72.3</td>
<td>0.35</td>
<td>0.157</td>
</tr>
<tr>
<td>VIII</td>
<td>26.17</td>
<td>60.10</td>
<td>110.34</td>
<td>74.2</td>
<td>0.35</td>
<td>0.070</td>
</tr>
<tr>
<td>IX</td>
<td>20.30</td>
<td>182.59</td>
<td>228.58</td>
<td>13.1</td>
<td>0.40</td>
<td>0.039</td>
</tr>
<tr>
<td>X</td>
<td>27.47</td>
<td>32.42</td>
<td>72.38</td>
<td>66.0</td>
<td>0.40</td>
<td>0.056</td>
</tr>
<tr>
<td>XI</td>
<td>26.16</td>
<td>56.26</td>
<td>77.80</td>
<td>66.3</td>
<td>0.50</td>
<td>0.066</td>
</tr>
<tr>
<td>XII</td>
<td>16.50</td>
<td>80.22</td>
<td>73.72</td>
<td>78.2</td>
<td>0.65</td>
<td>0.041</td>
</tr>
<tr>
<td>XIII</td>
<td>20.39</td>
<td>72.75</td>
<td>81.94</td>
<td>74.7</td>
<td>0.45</td>
<td>0.035</td>
</tr>
<tr>
<td>mean±SD</td>
<td>27.90±10.59</td>
<td>263.66±490.95</td>
<td>170.88±125.47</td>
<td>60.3±18.2</td>
<td>0.51±0.14</td>
<td>0.067±0.033</td>
</tr>
</tbody>
</table>

μ (kPa), k₁ (kPa), k₂ (dimensionless), φ (degrees), and ρ (dimensionless) are from Eqs. 1 and 2, and ε (dimensionless error measure) is from Eq. 5.
Limitations and open problems. Determination of the passive mechanical properties of arterial walls is based not only on the distribution and orientation of tissue elements (layers) but also on their coupling, which was not investigated here. Hence, continued research is required to identify the related mechanics of tissue interconnection. In addition, a relatively small number of tissue samples were investigated, so a meaningful correlation between biomechanical properties of the different arterial tissues and related anamnesis could not be quantified.

Another limitation of the study is that the structural integrity at the lateral edges of the strips is disturbed. For example, collagenous fibers that are cut off during preparation may retract spontaneously, which can lead to alterations in the tensile response. Although the strips tested in the axial and circumferential directions were always taken from neighboring locations, they are different samples with probably more-or-less different composition and mechanical properties. Because we were only able to prepare strip samples for uniaxial extension tests, the data do not cover the whole physiological domain. The design of a new microtester that can measure the biaxial properties of small specimens may help extend the present data. However, it is theoretically impossible to characterize the three-dimensional response of anisotropic elastic materials by planar biaxial tests alone. In this context, biaxial tests (or, equivalently, extension-inflation tests) have little if any advantage over separate uniaxial tests at different orientations. Composition of the specimens may also vary throughout their dimension. The assumption of homogeneity, in particular for the adventitia, may result in an underestimate of stress values for the inner part of the adventitia, because the outer parts, in which the collagenous fibers are less tightly packed (Fig. 5C), are likely to be less load bearing. Similarly, to consider the individual tissues as incompressible materials may also lead to an underestimate of stress values, because a stress-induced fluid extrusion, in particular from the outer part of the adventitia, as documented in Fig. 5C, would be associated with smaller cross sections and, thus, higher stress values.

In addition, a small number of samples fractured within the gauge section, although a second sample could sometimes be prepared and tested. Dumbbell-shaped samples might increase the success rate of fracture tests. Because fiber angles can...
directly enter the constitutive formulation, it would also be valuable to obtain information about the concentration and three-dimensional structural arrangements of collagen and (medial) smooth muscle components in the different tissues. More research is needed to characterize this important aspect of vascular physiology. The present study has focused on the heterogeneity of the arterial wall of human LAD coronary arteries with nonatherosclerotic intimal thickening, although we did not account for residual stretches. When considering the marked differences in the mechanical properties of the three arterial tissues, one may anticipate layer-specific residual stresses that ensure balanced stress distributions in the loaded configuration.

Despite strong legal and institutional restrictions on the use of human material, high interspecimen variation, and irregularity of specimens due to atherosclerosis, investigators of vascular solid mechanics should consider the use of human arteries for at least two reasons: 1) they allow an anatomic separation into their tissue components and, therefore, investigation of the underlying mechanics of the individual tissue types, and 2) exploration of the mechanics of human arteries from aged patients is most likely to have direct and significant clinical implications, because several cardiovascular diseases occur later in life and are treated by vascular implants. Although aged human arteries represent only a specific group of vessels, they seem to provide a promising basis for significant advances in the field of layer-specific vascular physiology and mechanobiology.

ACKNOWLEDGMENTS

The authors are indebted to the late C. A. J. Schulze-Bauer, who helped to initiate the early part of the study. The authors are grateful to L. Windholz (Graz University of Technology) for excellent support and valuable discussions and to P. Lanz and F. Schmidt for contributions to layer preparations and experimental tests.

GRANTS

Financial support for this research was provided by the Austrian Science Foundation under START-Award Y74-TEC and by the Fonds zur Fortsetzung experimental tests.

REFERENCES


4. Canham PB, Finlay HM, Dixon JG, Boughner DR, and Chen A. Mechanical properties of vessels with nonatherosclerotic intimal thickening, although we did not account for residual stretches. When considering the marked differences in the mechanical properties of the three arterial tissues, one may anticipate layer-specific residual stresses that ensure balanced stress distributions in the loaded configuration.


AJP-Heart Circ Physiol • VOL 289 • NOVEMBER 2005 • www.ajpheart.org