Biaxial mechanical properties of intact and layer-dissected human carotid arteries at physiological and supraphysiological loadings

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Sommer G, Regitnig P, Költlinger L, Holzapfel GA. Biaxial mechanical properties of intact and layer-dissected human carotid arteries at physiological and supraphysiological loadings. Am J Physiol Heart Circ Physiol 298: H898–H912, 2010. First published December 24, 2009; doi:10.1152/ajpheart.00378.2009.—Specimens of intact wall tubes of human common carotid arteries (CCA), internal carotid arteries (ICA) (n = 11, age 77.6 yr, SD 6.3), and related adventitia and media-intima tubes are mechanically examined. Cyclic, quasi-static extension-inflation tests at different axial stretches are performed on preconditioned tube specimens. Stress-free configurations show significant stress releases in the circumferential direction of the intact CCA and ICA walls and in the axial directions of the intact CCA walls and the CCA and ICA adventitias. All investigated tissues exhibit strong nonlinear, pseudelastic mechanical behavior with small hysteresis. The “inversion” feature, where the pressure/axial stretch relationship becomes a vertical line, is found only for intact walls. Axial “inversion stretches” are 1.15 (SD 0.06) for CCA and 1.14 (SD 0.06) for ICA, and related external axial forces are 0.43 N (SD 0.15) and 0.30 N (SD 0.22), respectively. Significant negative correlations between age and axial inversion stretches for CCA (r = −0.67, P = 0.03) and ICA (r = −0.29, P = 0.04) are identified. Adventitias are very compliant at low pressures, but change into stiff tubes at high pressures. The burst pressure of the adventitia is beyond 250 kPa. A relatively low burst pressure of ~60 kPa is found in the media-intima tubes, in which the pressure/circumferential stretch relationships are almost independent of the axial stretches. Stress analyses indicate a high degree of material anisotropy for all investigated tissues. High circumferential and axial stresses occur in the media-intima tubes at physiological conditions. The obtained data are intended to serve for an improvement of constitutive laws, determination of constitutive parameters, and enhancing our knowledge of the mechanical functions of arteries and their associated layers in specific pathophysiological and clinical problems, such as hypertension and angioplasty with stenting.

arterial wall mechanics; human carotid artery; biaxial testing; residual stress; biomechanics

CARDIOVASCULAR DISEASES are frequently treated by balloon angioplasty, a mechanical procedure during which a vessel is subject to loads far beyond the physiological domain. It is also known that the mechanical environment and properties of arteries play an important role in the origin and progression of vascular diseases. Therefore, detailed knowledge of their mechanical behavior is essential for the improvement of (non)surgical procedures and for the development of prosthetic materials and tissue equivalents.

Carotid arteries, including the common carotid (CCA) and the internal carotid arteries (ICA), are of particular biomedical and clinical interest, since they are prone to atherosclerosis and frequently they undergo treatments [angioplasty and stenting or carotid endarterectomy (CEA)] to prevent stroke. Carotid stenosis or the narrowing of one of the major arteries that carry blood to the brain can cause stroke (9), a leading cause of serious, long-term disability, and the third leading cause of death in the United States (36). A standard treatment consists of removing the narrowing by procedures such as carotid angioplasty with stenting (CAS) and CEA, where CAS (with embolic capture device) is increasingly used to treat carotid lesions, with success rates similar to CEA surgery, while carotid angioplasty without stenting is losing its importance.

The CCA is probably the most studied and best understood artery due to the ease in which long specimens, free of branches, may be excised (22). Investigations of ICAs are rare, even though plaque formation, and, consequently, CAS or CEA, occur in the CCA, the ICA, and in the carotid bifurcation. Moreover, most studies available in the literature deal with carotid arteries stemming from animals (see, e.g., Refs. 2, 5, 6, 10, 13, 14, 28, 34, 49). To the authors’ knowledge, there are only a few in vitro tube studies of human CCA (17, 25) and no in vitro tube studies on human ICA yet available in the literature. Since an artery is a heterogeneous structured composite consisting of three layers with different (visco)elastic properties, a thorough understanding of the behavior requires experimental data on the multiaxial biomechanical response of each layer. In particular, there are no in vitro tube studies yet available dealing with the layer-specific mechanical response of human CCAs or ICAs. In addition, there is growing experimental evidence that the adventitia plays an important role in various vascular processes, such as atherosclerosis, hypertensive remodeling, and restenosis after balloon angioplasty (see, e.g., Ref. 41). Most biomechanical studies used zero-pressure conditions (no-load state) as reference for the analysis, although, 25 yr ago, Vaishnav and Vossoughi (45) identified that the load-free configuration of an artery is not a stress-free state. In documented finite-element models of the human carotid bifurcation, mechanical properties of the ICA are assumed to be the same as for the CCA (see, e.g., Refs. 12 and 35). Accurate mechanical properties of the ICA will definitely improve the model prediction and extend the applications of such models.

Therefore, the aim of this study is to systematically determine the biomechanical behavior of human CCAs and ICAs and, subsequently, the adventitial and media-intima (MI) composite layers by means of extension-inflation tests at different axial stretches. Moreover, the occurring deformations (termed
as residual stretches) of the adventitia and MI tubes after dissection of the adventitia tube from the MI tube are determined. The stress-free configurations of the intact wall, the adventitia, and the MI composite in terms of the curvatures are also determined.

**MATERIALS AND METHODS**

**Material and Specimen Preparations**

Eleven human CCAs and 10 human ICAs were harvested from 11 human subjects (77.6 ± 6.3 yr, mean ± SD, ranging from 67 to 86 yr, 4 women and 7 men). Information about the anamnesis of the carotid arteries investigated is summarized in Table 1. Only straight segments without palpable circumscribed wall hardening were used. An artery was rejected during preparation if the straight segment showed atherosclerotic plaque formations or if eccentric intimal thickening was macroscopically visible. For the present study, the use of autopsy material from human subjects was approved by the Ethics Committee of Medical University Graz. According to the American Heart Association statistics, angioplasty interventions are mostly performed in elderly people, aged 65 yr and older (36). Thus the arteries were required to be aged (around 65± yr) and of low/medium grade atherosclerosis (see anamnesis in Table 1).

Immediately after excision, the carotid bifurcation were frozen and stored at −80°C. Before mechanical testing, the frozen samples were slowly thawed at 4°C and then prepared at room temperature (20°C). All tests were conducted for one sample within 24 h after defrosting, and all samples were tested within 14 days after excision. The cryopreservation was applied to neglect the influence of the active muscular response. Recent studies have shown that cryopreservation does not modify the biomechanical properties of the CCA (elastic arteries) (1, 4, 39).

Two straight arterial segments, one segment of ~50 mm in length from the CCA and one from the ICA with the same length, were cut out from the carotid bifurcation, depicted as segments A-B-C-D in Fig. 1. Two rings were cut from each segment. For the CCA, a ring with ~3 mm in height was cut proximal from the bifurcation for thickness measurement (segment D in Fig. 1), and a ring with ~1.5 mm in height was cut distal from the bifurcation for the determination of the intact wall and the layer-specific stress-free configurations (see Test Protocol below) (i.e., segments A and B in Fig. 1) and also for thickness measurements (segment B in Fig. 1). The same procedure was applied for the ICA segment, but mirrored at the bifurcation. The axial deformation measurements during tube testing, two gage markers (black-colored straw chips) representing the gage length were glued pointwise with cyanoacrylate adhesive gel in parallel on the middle part of the arterial segments. Both were orientated transverse to the vessel axis and had a separation distance of ~5 mm. These segments were cannulated at both ends with specially designed tube connectors matching the vessel diameter and inserted in the testing machine. For a detailed description of the experimental setup, see Ref. 43.

**Test Protocol**

**Intact wall testing.** Extension-inflation tests with continuous recording of transmural pressure (p), axial force (F), outer diameter, and gage length of the arterial segments were performed at transmural pressures ranging from 0 to 33.3 kPa (250 mmHg) at several axial stretches, ranging from 1.0 to 1.3 in increments of 0.05. Since the mechanical behavior of arteries is temperature sensitive (16, 23), specimens were allowed to equilibrate for ~10 min in a 37°C, calcium-free 0.9% NaCl physiological saline solution after the cannulated arterial segments were inserted in the testing machine. During inflation, the axial force was held constant by the testing machine, since preliminary isometric tests (arterial segments were forced to keep their lengths) led to buckling of the intact walls and the MI tubes. Therefore, the testing machine was controlling the axial stretch of the tube specimens. Buckling of the adventitia tube specimens during inflation up to a transmural pressure of ~150 kPa was not observed.

**Table 1. Anamnensis, death-to-removal lapse, and intramural hyperplasia-to-medial hypertrophy portion ratio**

<table>
<thead>
<tr>
<th>Donor</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>
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<td>80</td>
<td>76</td>
<td>83</td>
<td>67</td>
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<td>F</td>
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<td>M</td>
<td>F</td>
<td>M</td>
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<td>Primary disease</td>
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<td>BLC</td>
<td>TAA</td>
<td>CS</td>
<td>NA</td>
<td>FP</td>
<td>HI</td>
<td>DM</td>
<td>RF</td>
<td>BRC</td>
<td>CS</td>
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<td>MET</td>
<td>AR</td>
<td>MI</td>
<td>PAE</td>
<td>GHD</td>
<td>BH</td>
<td>BI</td>
<td>MI</td>
<td>AML</td>
<td>MI</td>
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<tr>
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<td>24</td>
<td>26</td>
<td>15</td>
<td>18</td>
<td>23</td>
<td>6</td>
<td>23</td>
<td>15</td>
<td>13</td>
<td>23</td>
</tr>
<tr>
<td>I/M, %</td>
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<td>10:90</td>
<td>30:70</td>
<td>25:75</td>
<td>20:80</td>
<td>25:75</td>
<td>40:60</td>
<td>5:95</td>
<td>N</td>
<td>N</td>
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<td>Aorta</td>
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<td>M</td>
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<td>H</td>
<td>H</td>
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<td>Coronary arteries</td>
<td>M</td>
<td>M</td>
<td>H</td>
<td>H</td>
<td>M</td>
<td>L</td>
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<td>M</td>
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<tr>
<td>Cerebral arteries</td>
<td>M</td>
<td>–</td>
<td>M</td>
<td>L</td>
<td>L</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>–</td>
<td>M</td>
<td></td>
</tr>
<tr>
<td>Renal arteries</td>
<td>L</td>
<td>L</td>
<td>H</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>M</td>
<td>L</td>
<td>H</td>
<td></td>
</tr>
</tbody>
</table>

I-XI, donor numbers; I/M, intramural hyperplasia-to-medial hypertrophy portion ratio; F, female; M, male; MEI, media infarction/stroke; BLC, bladder carcinoma; TAA, thoracic aortic aneurysm; CS, coronary sclerosis; NA, nicotine abuse; FP, fibrinous pericarditis; HI, head injury; DM, diabetes mellitus; RF, renal failure; BRC, breast carcinoma; ICP, intracranial pressure; MET, metastasis; AR, aneurysm rupture; MI, myocardial infarction; PAE, pulmonary artery embolism; GHD, global heart dilation; BH, brain hemorrhage; BI, brain infarction; AML, acute myeloid leukemia. Assessment of atherosclerosis is based on autopsy reports (–, no atherosclerosis; L, low grade; M, medium grade; H, high grade); N, not investigated.
At each increment of axial stretch, the intact wall was preconditioned axially and circumferentially; first, axially by five axial stretch cycles, ranging from the initial condition (0% axial stretch) to the desired axial stretch, and held at the desired axial stretch; and then circumferentially by five inflation-deflation cycles, ranging from 0 to 33.3 kPa (250 mmHg). Subsequently, the specimen was inflated and deflated one more time to obtain the “measuring cycle.” The axial stretching during preconditioning was performed quasi-statically (1 mm/min). The inflation and deflation during the preconditioning cycles and the measuring cycle were also performed quasi-statically at 16.7 kPa/min. The unloaded (referential) outer diameter was measured after the preconditioning and before the measurement cycle at 0-kPa transmural pressure and 0% axial stretch (\(\lambda_z = 1.0\)). If the axial load exceeded 2 N for the CCA or 1 N for the ICA at high axial stretches (e.g., \(\lambda_z = 1.25\) or \(\lambda_z = 1.3\)), the associated tests were not performed to avoid damage to the tissue. These axial loads correspond to an axial first Piola-Kirchhoff stress of 100 kPa in the samples, with the smallest outer radius \(R\) and thickness \(H\) values (CCA: \(R = 3.7\) mm, \(H = 1\) mm; ICA: \(R = 2.5\) mm, \(H = 0.8\) mm) stemming from preliminary tests. An axial first Piola-Kirchhoff stress of 100 kPa was assumed to cause no damage to the investigated tissue (21). After the tests, the specimen was taken out of the testing machine and submerged in a 37°C PSS tissue bath.

Layer separation procedure and residual stretches. After ~3-min equilibration in 37°C PSS and before layer separation, a picture considering a gauge for the residual stretch determination was taken. The adventitia could be carefully pulled off in a “turtleneck” fashion from the underlying MI tissue by carefully disconnecting the interconnective fibers (i.e., external elastic membrane fibers and collagen fibers) with a scalpel. For a representative macroscopic picture of a CCA during dissection, see Fig. 2A. This separation was feasible with the aid of a low-magnification light microscope \((\times 10)\) and surgical instruments (28, 32, 41). The difference in color and texture between the medial and the adventitial layers was used to separate them from one another. The mesh of collagenous fibers in the adventitia is white-red colored (red due to vasa vasorum), while the mesh of the elastin and muscle fibers in the media appears to be ivory colored. Furthermore, the media is much denser than the adventitia. During the separation, the tissue was periodically moistened with PSS. In general, this separation is “atraumatic” due to the weak adherence of the media to the adventitia (43). Turtleneck dissection was easier to perform for elderly arteries than for younger ones. This might be an evidence of stronger adherence of the adventitia to the media in younger arteries. Consequently, dissection of young arteries might be more afflicted with tissue damage during dissection. After separation, the adventitia retracted immediately in the axial direction, which indicates residual stretches (see Fig. 2B).

After ~5 min equilibration in 37°C PSS, additional scaled pictures were taken from the adventitia and the MI tube. The axial residual stretches of the adventitia and MI composite tube were computed as the ratio of the intact wall tube length and the adventitia tube length, and the MI composite tube length, respectively. These lengths were determined photogrammetrically from the pictures taken (see Fig. 2B). Analogously, the circumferential residual stretches were computed as the ratio of the outer intact wall diameter and the outer adventitia tube diameter, and the outer MI tube diameter, respectively. These diameters were determined by means of the videointensometer from the load-free tubes inserted in the testing machine.

Adventitia and MI tube testing. The procedure and protocol used for the adventitia and MI tube were performed in an analogous manner with respect to the intact wall. With exceptions that the extension-inflation tests of adventitia tubes were performed in two loading domains, named as “the physiological domain,” with transmural pressures ranging from 0 to 33.3 kPa, and “the high-pressure domain,” ranging from 0 to 100 kPa. Furthermore, the axial stretch ranged from 1.0 to 1.3 in increments of 0.05. Since preliminary tests showed that the MI tube can burst at transmural pressures of ~60 kPa, the MI tube was only pressurized from 0 to 20 kPa.

Determination of the stress-free configuration. Two rings with ~15 mm in height were taken for the determination of the layer-specific residual stresses: one cut from the proximal end of CCA (the very left segments A and B in Fig. 1), and one from the distal end of ICA (the very right segments A and B in Fig. 1). A ring with ~3 mm in height (segment B in Fig. 1) and, additionally, an “axial strip” \((i.e., a strip whose long sides were aligned with the vessel axis)\) (segment A in Fig. 1) were cut out from these 15 mm rings and glued pointwise with cyanoacrylate adhesive on small plastic tubes \((\phi 7\) mm), which served as supports in the tissue bath. For a detailed description of the method used to determine the curvatures of the individual tissue components, the reader is referred to Ref. 19.

As a result, a set of pictures was obtained that contained information on the initial and final geometries of the nonseparated specimens.

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Fig. 2. A: dissection of the intact artery in a “turtleneck” fashion into an adventitia tube and a media-intima tube. B: the axial retraction of the adventitia tube and the small elongation of the media-intima tube indicate residual stretches within the intact artery wall. C: representative circumferential and axial strips of the intact wall 16 h after a radial cut. D: the associated adventitia and media-intima, 6 h after “layer-dissection.”
and, subsequently, of the isolated “layer-specific” human carotid tissue strips (see Fig. 2, C and D). From these pictures, the curvatures of the individual tissue components were determined. The opening angle in the axial strips is not a characteristic quantity for residual deformations, since the angle depends on the strip length, and, furthermore, the cut specimens rarely took on the form of circular arcs (19).

**Thickness measurements.** For the intact wall thickness determination, the rings with 3 mm in height from the distal and proximal ends of the CCA and ICA were used (ring segments B and D in Fig. 1). Scaled pictures of the rings cut were taken after ~5 min immersed in PSS at 37°C. From these scaled pictures, the intact wall thickness was measured photogrammetrically at four positions of each ring, averaged regarding the four positions and then averaged regarding the two rings stemming from the same segment. Additionally, the rings were radially cut to get a strip. The average thickness of these strips was measured by means of a videoextensometer to compare it with the photometrical method. For a detailed description of the videoextensometer thickness measurement method, see Refs. 20 and 43. Adventitial and MI wall thicknesses were also determined by means of the videoextensometer thickness measurement method.

**Histology.** Histological investigations were performed to confirm correct and, as much as possible, atraumatic separation of the adventitia from the MI composite. Therefore, after the experiments, the gage regions of the adventitia and the MI tubes. Therefore, the residual stretches were added to the corresponding measured stretches $\lambda_0$ and $\lambda_c$.

**Statistics**

All data are given as means (SD). Statistical analyses were performed to test for significant correlations between age and 1) MI thickness, 2) axial inversion stretch, 3) external axial inversion force, 4) circumferential, 5) axial stretches (at “physiological conditions,” i.e., at $13.3 \text{kPa}$ and at $\lambda_2 = 1.05$ to $\lambda_2 = 1.10$), 6) intimal hyperplasia/media hypertrophy, and 7) elastin-collagen-muscle fraction. We assumed an average axial in vivo prestretch of CCA and ICA in the composite of the human neck to be between $\lambda_2 = 1.05$ and $\lambda_2 = 1.10$. Furthermore, we tested for significant correlations between time that elapsed between death and removal of the arteries and their mechanical properties at physiological pressure. Pearson’s correlation coefficients were calculated, and $P$ values were determined based on Student’s $t$-distribution. $P < 0.05$ was considered to be significant. Statistical analyses were performed using the OriginLab ORIGIN program package, release 7.5.
RESULTS

Geometry and Kinematics

The kinematics of the intact wall are described by the load-free geometries, outer radius $R_W$, wall thickness $H_W$, and gage length $Z_W$, and mapped into the deformed configuration with the related geometries $r_W$, $h_W$, and $z_W$, respectively (see Fig. 4). The kinematics of the individual layers are described by two subdeformations. First, the kinematics of the considered layers, e.g., the adventitial layer, are determined by the deformation that occurs between the separated load-free adventitia tube (with $R_A$, $Z_A$, $H_A$) and the load-free configuration of the intact wall. Thereby, the resulted deformed configuration of the adventitia is given by the related dimensions $r_A$, $h_A$, $z_A$. Second, axial stretching and pressurizing the intact wall leads to the deformed configuration of the adventitia ($r_A$, $z_A$, $h_A$). Note that the load-free configuration of the adventitia is not stress free due to residual stresses within the layer (19). The kinematics for the MI tube are performed in an analogous manner (see Fig. 4).

We found significant axial residual stretches after the dissection of the adventitia from the MI tube, as summarized in Table 2. Remarkably, the circumferential stretch of the adventitias decreased $-1\%$ after 100 kPa of pressurization and 30% of axial stretch in the CCA and ICA, which means that high-pressure loading of the separated adventitias caused almost no remaining stretch. By using the same loading conditions, the axial stretch of the adventitias increased $-3\%$ for the CCA, while there was no remaining axial stretch for the ICA. The stretches of the adventitia under loaded condition result as $r_A = r_W$, $z_A = z_W$, $h_A = h_W$.

The load-free outer radii and thicknesses of the intact walls ($R_W$, $H_W$), the separated load-free adventitia ($R_A$, $H_A$), and the MI tubes ($R_{MI}$, $H_{MI}$) for the CCA and the ICA are summarized in Table 2.

Table 2. Residual stretches and geometries of all investigated intact walls, separated adventitia, and media-intima tube specimens of the CCA and the ICA

<table>
<thead>
<tr>
<th>Artery</th>
<th>Layer</th>
<th>Circumferential Residual Stretch</th>
<th>Axial Residual Stretch</th>
<th>$R$, mm</th>
<th>$H$, mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCA</td>
<td>Intact wall</td>
<td>1.04 (0.06)</td>
<td>1.08 (0.05)</td>
<td>4.15 (0.31)</td>
<td>1.17 (0.16)</td>
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<td></td>
<td>Adventitia</td>
<td>1.00 (0.02)</td>
<td>0.98 (0.01)</td>
<td>3.61 (0.13)</td>
<td>0.70 (0.13)</td>
</tr>
<tr>
<td></td>
<td>Media-intima</td>
<td>1.04 (0.05)</td>
<td>1.09 (0.06)</td>
<td>2.67 (0.09)</td>
<td>0.86 (0.06)</td>
</tr>
<tr>
<td>ICA</td>
<td>Intact wall</td>
<td>1.00 (0.03)</td>
<td>0.98 (0.01)</td>
<td>2.15 (0.15)</td>
<td>0.53 (0.10)</td>
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<tr>
<td></td>
<td>Adventitia</td>
<td>1.00 (0.03)</td>
<td>0.98 (0.01)</td>
<td>2.15 (0.15)</td>
<td>0.53 (0.10)</td>
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</table>

Values are means (SD). CCA, common carotid artery; ICA, internal carotid artery. Circumferential residual stretches were calculated as $r_A/R_A$ for the adventitia and $p_{ad}/R_{MI}$ for the media-intima tube. Axial residual stretches were calculated as $z_A/Z_A$ for the adventitia and $p_{ad}/Z_{MI}$ for the media-intima tube. $R$ and $H$ are the outer radii and the thicknesses of the load-free intact wall ($R_W$, $H_W$), the separated load-free adventitias ($R_A$, $H_A$), and the media-intima tubes ($R_{MI}$, $H_{MI}$), respectively. For abbreviations, see Fig. 4 and RESULTS.
2. No significant correlation between age and MI thickness was found for the CCA and ICA.

Stress-free Configuration

Mean values and standard deviations (mean and SD) of the curvatures of the intact walls $W$, i.e., the arterial rings (before the radial cut and after 16 h), of the axial intact wall strips (immediately after excision and after 16 h), and of the adventitia $A$ and composite MI strips (immediately after dissection and after 6 h) of the CCA and the ICA are summarized in Table 3. The configurations of the circumferential and the axial strips after 16 and 6 h, respectively, are assumed to be stress free. For the case that histology confirmed correct layer separation, the curvatures of the adventitia and the MI composite strips are also documented. Approximately 80% of the adventitia specimens and all of the MI specimens were confirmed to be correctly separated and included in the subsequent analysis. In general, the curvature values of the circumferential oriented strips were always positive, whereas the axially oriented strips showed mainly negative curvature values. The intact walls in the circumferential direction show a significant decrease of the curvature after radial cutting of the rings of the CCA ($P = 0.003$) and ICA ($P = 0.002$), which indicated a release of residual stresses, whereas the circumferential-oriented adventitia of the CCA ($P = 0.079$) and ICA ($P = 0.746$), as well as the circumferential-oriented MI composite strips of the CCA ($P = 0.149$) and ICA ($P = 0.598$), showed no significant change 6 h after dissection. Moreover, all strips oriented in the axial direction of the CCA and ICA showed no significant change 16 and 6 h after dissection, as evident in Table 3. This indicates a fast residual stress release in the axial-oriented specimens. Significant stress release in the axial direction is observed in the intact walls of the CCA and the adventitia of the CCA and ICA, if the initial curvature was assumed to be 0 (i.e., straight cylinder of the tube specimen).

Load Deformation Behavior

Representative mechanical responses of a preconditioned intact wall, adventitia, and MI obtained from a CCA and an ICA are shown in the Figs. 5–10.

Intact wall. For the intact wall, the circumferential and axial stretches resulting from the applied pressure (0–33.3 kPa) were plotted for axial stretches ranging from 1.0 to 1.2 for the CCA and 1.0 to 1.25 for the ICA. The curves exhibit nearly elastic behavior (small hysteresis). Typically for soft collagenous tissue, the load deformation behavior is highly nonlinear, with pronounced stiffening at higher pressures. During cyclic preconditioning, there were only minimal changes in the mechanical response, indicated by the small shift between the initial loading cycles (indicated by dotted curves) and the final cycles (solid curves). For reasons of clarity, this is only shown for curves with the highest axial stretches in Figs. 5–10. Moreover, preconditioning was typically finished after only three to four inflation-deflation cycles. We investigated whether the axial stretch has any influence on the preconditioning behavior of the tissues. Remarkably, we found significant linear correlations between the axial stretches (0, 5, 10, 15%, etc.) and the “stretch softening” (stretch difference of the initial and the final preconditioning cycle) during preconditioning in the circumferential direction ($r = 0.817$, $P = 0.047$) for the CCA and ($r = 0.883$, $P = 0.047$) for the ICA. Thus, with increasing axial stretch, the CCAs in the circumferential direction experienced an increasing stretch softening during preconditioning, whereas the ICAs experienced an increasing “stretch hardening” with increasing axial stretch during preconditioning. No significant linear correlations were observed between the axial stretches (0, 5, 10, 15%, etc.) and the preconditioning behavior in the axial direction.

Another remarkable feature can be seen in the axial stretch-pressure plots (Figs. 5B and 8B). Up to a certain axial stretch, the slopes of the curves are positive and then they become slightly negative (i.e., the vessel contracts axially with increasing pressure). Thus, at a particular axial stretch (i.e., the “inversion stretch” with a related external axial force), the length of the specimen is independent of the pressure. The inversion stretches are not indicated in Figs. 5B and 8B, since their exact values are not known, but, for the particular specimen in Figs. 5B and 8B, they are ~1.18 for the CCA and 1.15 for the ICA. On average, we found axial inversion stretches of 1.15 (SD 0.06) for the CCAs ($n = 10$) and 1.14 (SD 0.06) for the ICAs ($n = 8$), and associated average external axial forces were determined to be 0.43 N (SD 0.15) for the CCA and 0.30 N (SD 0.22) for the ICA, respectively.

To show the patient-patient variability, the pressure-circumferential stretch behavior of all investigated intact wall specimens of CCA and ICA at their inversion stretch are plotted in Fig. 11. No correlations between age (and anamnesis) and pressure-circumferential stretch behavior can be seen. Donors I and V show a pronounced different circumferential stiffness

### Table 3. Curvatures of the intact wall $W$, the adventitia $A$, and the media-intima composite MI

<table>
<thead>
<tr>
<th>Artery</th>
<th>Circumferential After excision/before cut</th>
<th>Axial After excision/before cut</th>
<th>Circumferential After 16 h</th>
<th>Axial After 16 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intact wall $W$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CCA</td>
<td>0.381 (0.062)</td>
<td>−0.103 (0.096)</td>
<td>0.232 (0.072)</td>
<td>−0.110 (0.077)</td>
</tr>
<tr>
<td>ICA</td>
<td>0.562 (0.168)</td>
<td>−0.050 (0.110)</td>
<td>0.179 (0.128)</td>
<td>−0.052 (0.113)</td>
</tr>
<tr>
<td>Adventitia $A$</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CCA</td>
<td>0.185 (0.062)</td>
<td>−0.142 (0.074)</td>
<td>0.239 (0.038)</td>
<td>−0.125 (0.060)</td>
</tr>
<tr>
<td>ICA</td>
<td>0.112 (0.060)</td>
<td>−0.136 (0.093)</td>
<td>0.093 (0.050)</td>
<td>−0.132 (0.091)</td>
</tr>
<tr>
<td>Media-intima composite MI</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CCA</td>
<td>0.158 (0.055)</td>
<td>−0.025 (0.069)</td>
<td>0.169 (0.047)</td>
<td>−0.014 (0.108)</td>
</tr>
<tr>
<td>ICA</td>
<td>0.162 (0.072)</td>
<td>−0.052 (0.068)</td>
<td>0.143 (0.036)</td>
<td>−0.053 (0.075)</td>
</tr>
</tbody>
</table>

Values are means (SD) in mm$^{-1}$.
in the left and right neck side, whereas that difference is small for donor II. A pronounced different behavior between the CCA and ICA is observed for donor IX, but not for donors VI and VIII. Donor VII, who died of a brain hemorrhage, showed no statistically different behavior in the ICA, and, furthermore, the data from donor VIII, who had diabetes, were not statistically stiffer.

Adventitia. The mechanical response of the adventitia for the CCA and ICA is only shown for the high-pressure domain (see Figs. 6 and 9), since the mechanical behavior in the low-pressure domain was very similar, and, therefore, captured by the high-pressure behavior. Briefly, the adventitia in the low-pressure domain exhibit nearly elastic behavior with small hysteresis, nonlinearity, and pronounced stiffening at higher pressures. Remarkably, even in the high-pressure domain (up to 100 kPa), the adventitia showed nearly elastic behavior, with only small hysteresis. Furthermore, no signs of material damage were observed during high-pressure loadings. Interestingly, at high pressures (>33.3 kPa), the slopes of the curves in the axial pressure-stretch plots (Figs. 6B and 9B) seem to be nearly linear and independent of the axial stretch applied, whereas, at low pressures (<10 kPa), the axial distensibility is rapidly decreasing with increasing axial stretch. Additionally, some specimens were inflated up to a transmural pressure of 250 kPa (2.5 bar) to observe ultimate tensile strength properties of the adventitia tissue. At pressures up to 250 kPa, no rupture of the adventitia tube occurred. For that transmural pressure, we computed the circumferential stress ($\sigma_{00} = 4.2$ MPa) and the axial stress ($\sigma_{zz} = 1.6$ MPa) at a circumferential and axial stretch of $\lambda_0 = 1.32$ and $\lambda_z = 1.07$, respectively (Eqs. 1–3 were used). Remarkably, this led to the same characteristic behavior as experiments performed with 100 kPa. In particular, the circumferential and axial pressure-stretch plots of the 250-kPa cycle showed nearly elastic behavior with no observable dam-
age signs, i.e., the ultimate tensile stresses of the investigated adventitia were beyond the stresses computed above. The “inversion” feature was not observed for the adventitia tubes of CCA and ICA. Significant linear correlation between axial stretch and preconditioning behavior was not observed for the adventitia tubes.

MI. The MI (composite) tubes of the CCA and ICA showed mechanical behaviors similar to the intact walls (similar shapes of the curves, nearly elastic behavior, nonlinearity, and small hysteresis). However, in contrast to the intact wall, the MI tube in the axial direction showed no inversion feature (i.e., no vertical line), not even at high axial stretches of 1.25. In addition, the mechanical response expressed through the circumferential stretch-pressure relation seems to be (almost) independent of the axial stretch (see, e.g., Figs. 7 and 10A). Whereas the axial stretch-pressure behavior of the MI showed an increase in stiffening with increasing axial stretch similar to the intact wall. Moreover, the MI tubes of the CCA and the ICA showed similar behavior, only the ICA MI tubes seem to be consistently stiffer than the associated CCA MI tubes (see, e.g., the representative Figs. 7 and 10). At preliminary MI tube tests, it happened that the MI tube ruptured at pressures of ~60 kPa. For this pressure and an assumed axial in situ stretch of 1.1, we computed the circumferential and axial stress in the MI tube as $\sigma_\theta = 0.6 \text{ MPa}$ and $\sigma_z = 0.3 \text{ MPa}$, respectively. Significant linear correlations between axial stretch and preconditioning behavior were not observed for the MI tubes.

The intact wall and the adventitia tubes showed a decrease in circumferential stretches with increasing axial stretches, which indicates the “cross talk” between both directions, which was not shown for the MI tubes. Moreover, a “0% control” cycle (shown in Figs. 5–10), which was performed after the highest axial stretch test, almost resembled the initial 0% curve.

**Stress Analysis**

By means of Eq. 3, average circumferential and axial Cauchy stresses of the investigated tissues were computed for the following 13 pressure values (for the MI, only the first 10
values were computed: 1.5, 2, 3, 6.7, 9, 10, 13.3, 15, 18, 20, 22, 26.6, and 30 kPa, at different axial stretches (0–20%). Figure 12 shows representative plots of the intact wall of the CCA.

In general, circumferential and axial stresses increase with increasing axial stretch, but that is more pronounced in the axial direction. Since we used the Laplace law (1), we observed an almost linear relationship between the pressure and the stress, and at 0% axial stretch, a twice as high circumferential stress than the related axial stress. However, for the intact wall, we found circumferential-to-axial stress slope ratios for the adventitia and the MI composite of the CCA higher than two (2.8 for the intact wall, 5.1 for the adventitia, and 2.4 for the MI composite) (see Table 4), which shows more compliance in the axial direction and, therefore, suggests anisotropic behavior of these tubes. The adventitia of the ICA showed with 3.7 a relatively high stress slope ratio as well and, hence, a highly anisotropic behavior. Surprisingly, the intact wall and the MI composite of the ICA showed ratios close to two (2.2 and 2.1, respectively), which would suggest isotropic behavior. By an increase of the axial stretch up to 20% (for the MI only up to 10%), the circumferential-to-axial stress slope ratio decreases to ~2 for all tissues from the CCA and ICA. Only the ICA intact wall ratio showed a slight increase from 2.2 to 2.3.

The stresses in the intact wall and the MI composite are similar in the CCA and ICA for both directions, whereas the stresses in the adventitia of the CCA in the circumferential direction are significantly higher than the associated stresses in the ICA. The axial stresses of the CCA and ICA adventitias were not significantly different.

To study the influence of the residual stretches on the stress states in the adventitia tubes and the MI tubes, additional stress calculations were performed with residual stretches omitted. For an illustration of the influence of the residual stretches on the circumferential and axial stresses of the investigated tubes (W, A, MI) of CCA and ICA, stresses [mean (SD)] for the
mean physiological blood pressure of 13.3 kPa and for different axial stretches ranging from 0 to 20% are given in Table 5. In particular, at low axial stretches (0 and 5%), the CCA adventitia show a slight increase in the mean circumferential stresses, if residual stretches were considered. At 10% axial stretch, the residual stretches show no influence on the circumferential stresses, and, at higher axial stretches (15 and 20%), the circumferential stresses start to decrease. At a particular point, which we call the reverse point, the influence of both the residual stretches and the axial stretches on the circumferential stresses reverses. Before this point, the circumferential stresses were slightly increased, and afterwards they were slightly decreased, if residual stretches are considered. The mean axial stresses of the CCA adventitia are for all axial stretches slightly increased, if residual stretches are considered. The ICA adventitia showed a behavior similar to the CCA adventitia, but the reverse point is located at an axial stretch of 15–20%. The stresses in the MI tubes always decrease slightly, if residual stretches are considered. Interestingly, there seems to be no influence from the axial stretch and the orientations (circumferential or axial) on the stresses in the MI composite. In all cases, the above-mentioned residual stretch-dependent stress analysis were not significant due to the high SD observed in the tissues. However, some trend-setting assessments can be made.

**Histological Investigations**

The histological investigations confirmed that the adventitia was properly removed. The MI tube showed one to four ruptured elastic lamellae on the outer surface, but the integrity of the remaining MI composite (intact intima, intact elastic lamellae) was confirmed.

Microphotographs of representative sections are shown in Fig. 3. The elastin, collagen, and smooth muscle fractions in the intact wall, and the portion of intimal hyperplasia and medial hypertrophy (I/M portion ratio) responsible for the MI thickening are stated in Table 1. On average, we found an elastin-collagen-muscle fraction of 31:59:10 (SD 6:8:3) in the intact walls. The average I/M portion ratio was determined to be 22:78 (SD 11:11).
We found significant negative correlations between age and axial inversion stretches for the intact wall of the CCA \((r = -0.67, P = 0.03)\) and for the intact wall of the ICA \((r = -0.29, \ P = 0.04)\). Interestingly, no significant correlations were found between age and external axial inversion forces for the intact wall of the CCA \((r = 0.26, I = 0.46)\) and for the ICA \((r = 0.45, I = 0.26)\). No significant correlations between age and circumferential or axial stretches at physiological conditions were found for the intact wall, the adventitia, or the MI composite in the CCA and the ICA. No significant increase of intimal hyperplasia with age \((P < 0.05)\) was found. Moreover, no significant increase of medial hypertrophy with age \((P < 0.05)\) were found. Moreover, no significant decrease of elastin \((P < 0.05)\) and no significant decrease of collagen content with age \((P < 0.05)\) were found. Finally, no significant correlations were found between duration of death and external axial inversion forces for the intact wall of the ICA \((P < 0.05)\) and for the intact wall of the ICA \((P < 0.05)\) were found.

### DISCUSSION

An important area in vessel biomechanics is the determination of the mechanical loads and the associated deformations and stresses that occur in the arterial wall. Most studies on vessel wall mechanics investigate animal arterial tissues and consider arterial walls as single-layer homogeneous structures. However, in the understanding of the relation between artery mechanics and age, sex, and anamnesis, mechanical data originating from human tissues are more informative than those from animal tissues. Moreover, arterial walls are heterogenous three-layered composites with substantially different histological, physiological, and biomechanical features. Thus a better understanding of their mechanical functions requires layer-specific data.

### Geometry

The average ratios of outer diameter to wall thickness (DTR) at a transmural pressure of 0 kPa were 7.1 for the intact wall of the CCA and 6.2 for the intact wall of the ICA. This corresponds well to 8.9 for human CCAs obtained from angio-

### Table 4. Slopes of the Cauchy stress-transmural pressure relations at different axial stretches \((\lambda_z = 1.0, 1.05, 1.1, 1.15, 1.2)\) of the layers W, A, and MI of the CCA and ICA in the circumferential and axial directions

<table>
<thead>
<tr>
<th>Orientation</th>
<th>Artery</th>
<th>Layer</th>
<th>(\lambda_z = 1.0)</th>
<th>(\lambda_z = 1.05)</th>
<th>(\lambda_z = 1.1)</th>
<th>(\lambda_z = 1.15)</th>
<th>(\lambda_z = 1.2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Circumferential</td>
<td>CCA</td>
<td>W</td>
<td>3.93 (0.40)</td>
<td>4.26 (0.61)</td>
<td>4.59 (0.70)</td>
<td>5.02 (0.99)</td>
<td>5.43 (0.25)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A</td>
<td>10.56 (0.28)</td>
<td>11.13 (2.41)</td>
<td>11.14 (2.35)</td>
<td>10.94 (3.89)</td>
<td>13.28 (1.81)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MI</td>
<td>10.08 (2.14)</td>
<td>10.12 (1.93)</td>
<td>10.55 (1.85)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ICA</td>
<td>W</td>
<td>4.32 (1.07)</td>
<td>4.45 (0.89)</td>
<td>4.49 (0.95)</td>
<td>4.30 (0.52)</td>
<td>4.36 (0.65)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A</td>
<td>6.06 (0.96)</td>
<td>6.62 (1.26)</td>
<td>6.85 (1.28)</td>
<td>7.77 (1.40)</td>
<td>8.49 (1.29)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MI</td>
<td>10.63 (0.72)</td>
<td>10.86 (0.73)</td>
<td>10.98 (0.74)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Axial</td>
<td>CCA</td>
<td>W</td>
<td>1.39 (0.55)</td>
<td>1.33 (0.30)</td>
<td>1.68 (0.47)</td>
<td>1.66 (0.47)</td>
<td>3.15 (0.10)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A</td>
<td>2.09 (0.37)</td>
<td>3.34 (0.48)</td>
<td>5.02 (0.77)</td>
<td>5.45 (0.24)</td>
<td>6.41 (1.23)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MI</td>
<td>4.26 (0.97)</td>
<td>4.82 (0.25)</td>
<td>5.07 (1.04)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>ICA</td>
<td>W</td>
<td>1.97 (0.53)</td>
<td>1.81 (0.54)</td>
<td>1.82 (0.44)</td>
<td>1.63 (0.26)</td>
<td>1.86 (0.18)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A</td>
<td>1.64 (1.26)</td>
<td>2.34 (1.10)</td>
<td>2.63 (0.90)</td>
<td>3.39 (1.36)</td>
<td>3.98 (1.23)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MI</td>
<td>5.06 (0.35)</td>
<td>5.18 (0.35)</td>
<td>5.24 (0.35)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are means (SD). \(\lambda_z\). Axial stretches.

### Statistics

We found significant negative correlations between age and circumferential mechanical parameters. No significant correlations were found between age and external axial inversion forces for the intact wall of the CCA \((r = -0.29, I = 0.04)\) and for the ICA \((r = 0.45, I = 0.26)\). No significant correlations between age and axial stretches at physiological conditions were found for the intact wall, the adventitia, or the MI composite in the CCA and the ICA.}

### Table 5. Circumferential \(\sigma_{090}\) and axial stresses \(\sigma_{z}\) of the CCAs and ICAs at mean physiological pressure \(P = 13.3\ kPa (100\ mmHg)\) and at different axial stretches \((\lambda_z = 1.0, 1.05, 1.1, 1.15, 1.2)\) in the W, A, Aw, MI, and MIw

<table>
<thead>
<tr>
<th>(\lambda_z)</th>
<th>W</th>
<th>A</th>
<th>Aw</th>
<th>MI</th>
<th>MIw</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CCA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.0</td>
<td>49.0 (5.8)</td>
<td>135.0 (4.3)</td>
<td>142.3 (3.1)</td>
<td>120.9 (24.0)</td>
<td>119.2 (26.6)</td>
</tr>
<tr>
<td>1.05</td>
<td>52.5 (7.2)</td>
<td>140.5 (24.1)</td>
<td>142.4 (29.8)</td>
<td>125.2 (22.1)</td>
<td>123.3 (24.8)</td>
</tr>
<tr>
<td>1.1</td>
<td>55.3 (8.5)</td>
<td>140.2 (24.2)</td>
<td>140.2 (28.8)</td>
<td>128.6 (20.6)</td>
<td>126.7 (23.5)</td>
</tr>
<tr>
<td>1.15</td>
<td>59.8 (10.1)</td>
<td>146.4 (41.0)</td>
<td>139.0 (47.7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.2</td>
<td>63.5 (4.3)</td>
<td>172.9 (15.4)</td>
<td>168.1 (23.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Axial</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.0</td>
<td>55.2 (13.8)</td>
<td>76.3 (14.2)</td>
<td>76.6 (9.9)</td>
<td>134.7 (9.1)</td>
<td>130.2 (8.8)</td>
</tr>
<tr>
<td>1.05</td>
<td>56.1 (11.3)</td>
<td>83.3 (17.7)</td>
<td>84.0 (15.7)</td>
<td>138.3 (9.3)</td>
<td>133.7 (9.0)</td>
</tr>
<tr>
<td>1.1</td>
<td>55.6 (12.1)</td>
<td>85.1 (18.4)</td>
<td>85.9 (17.0)</td>
<td>141.6 (9.5)</td>
<td>136.9 (9.2)</td>
</tr>
<tr>
<td>1.15</td>
<td>53.3 (6.3)</td>
<td>97.2 (24.2)</td>
<td>98.7 (19.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.2</td>
<td>54.4 (7.4)</td>
<td>111.5 (11.2)</td>
<td>108.5 (18.5)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are means (SD). W, intact wall; Aw and A, adventitia tube with and without consideration of residual stretches, respectively; MIw and MI, media-intima composite tube with and without consideration of residual stretches, respectively.
graphic measurements of 22 adults (34–77 yr) (3, 12). The value, however, differs significantly for the ICA, where a DTR of 13 was reported (3, 12). For young human CCA, a DTR of 3.8 \( (n = 7, \text{age: 21–35 yr}) \) was reported (17), which is clearly smaller than those found for the DTR of the CCA in the present study. Furthermore, animal CCAs showed smaller DTRs: 5.4 for sheep \( (n = 5) \) and 5.0 for porcine \( (n = 26) (37) \), which may lead to the conclusion that the DTRs of CCAs of young humans and (young) animals are smaller than those from elderly humans. The DTR of the adventitia tubes were determined to be 17.0 and 15.2 for the CCA and the ICA, respectively, which justifies the adopted thin-wall approach, while such high DTRs were not found for the intact wall and the MI tube of the CCA and ICA (see Table 2). No comparable data for carotids are available in the literature. Only a DTR value of 22.7 for human femoral adventitia tubes, which is a similar finding, was documented in the literature (43).

The unloaded average thicknesses for the intact wall were determined to be 1.17 mm (SD 0.16) for the CCA and 0.86 mm (SD 0.06) for the ICA. Animal CCA thicknesses found in the literature were similar for sheep, 0.98 mm (SD 0.18) (5), and smaller for porcine, 0.64 mm (SD 0.09) (37). Unfortunately, no comparable data for carotid adventitia were found in the literature. However, the thickness of human femoral adventitia was stated as 0.41 mm (43), which is similar to the CCA adventitia thickness of 0.47 mm (SD 0.07) in our study. We determined the MI composite thickness to be 0.70 mm (SD 0.13) for the CCA and 0.53 mm (SD 0.10) for the ICA. The MI thickness for aged human CCA found in the literature was 0.72 mm (SD 0.15) (29), which correlates very well with our findings. A value of 0.64 mm (SD 0.14) for younger human CCA [mean age 42.4 mm (SD 16.5)] is reported in Ref. 40. No data were found for the MI thickness of the ICA. A fundamental difference between human and animal arteries is that, not only the adventitia and the media, but also the intima exhibited considerable thickness and mechanical strength (20, 42).

**Residual Stretches and Stress-Free Configuration**

For the first time, data on the significant axial residual stretches of the adventitia and the MI composite in an arterial wall composite are reported (see Table 2). Significant residual stress release in the circumferential direction was observed only for the intact wall of the CCA and ICA. We identified an average circumferential curvature of 0.232 mm\(^{-1}\) (SD 0.072) for the intact wall of the CCA, and 0.179 mm\(^{-1}\) (SD 0.128) for the intact wall of the ICA. This corresponds very well to the opening angle of 130° (SD 15) or to the curvature of 0.224 mm\(^{-1}\) (SD 0.026) for human CCAs \( (n = 8) \) published by Delfino (11). However, a larger average curvature 0.282 mm\(^{-1}\) (SD 0.049) \( (n = 8) \) or 116° (SD 20) was found for human ICAs (11). Opening angles for CCAs of rats [84° (SD 12)] and sheep [85° (SD 27)] were clearly smaller than opening angles of human CCAs (5, 49), which may indicate age dependency of residual stresses (38). Unfortunately, no data about residual stresses of the ICA were found in the literature.

Human aortic adventitia remained flat in the circumferential \([-0.003 \text{ mm}^{-1} (SD 0.029)]\) and axial \([0.0 \text{ mm}^{-1} (SD 0.040)]\) directions (19), which was not the case for human CCAs and ICAs investigated here (see Table 3). Furthermore, the study by Holzapfel et al. (19) has shown that residual deformations are three-dimensional and cannot be described by a single parameter, such as “the” opening angle. Therefore, their quantification and modeling require consideration of both stretching and bending, which are highly layer-specific and axially dependent. Our study confirmed this finding. Apparently, these residual stresses and stretches have the purpose to uniform stress gradients by growth and remodeling in the physiologically loaded, intact arterial tube. The curvature and residual stretch data reveal the stress-free state of the tissues (intact wall, adventitia, MI) and are important for reliable constitutive modeling and finite-element modeling of the intact arterial and layer-specific mechanics.

**Load Deformation Behavior, Stress Analysis, and Implications for Vascular Physiology**

Furthermore, all tissues (except the MI of the CCA in the circumferential direction, Fig. 7A) showed a decrease in circumferential stretches with increasing axial stretches, which indicates the cross talk between both directions. We observed the “inversion feature” of the axial stretch-pressure curves at higher axial stretches only for the intact walls. At this inversion stretch, the axial stretch and force are relatively independent of the transmural pressure. This feature is supposed to be characteristic of all arteries (22). For animal arteries, the axial in situ stretch is approximately equal to the inversion stretch (27, 47), whereas aged human arteries showed significantly smaller axial in situ stretches than inversion stretches (42). To answer the crucial question whether or not the measured axial in situ stretch equals the axial in vivo stretch at mean arterial pressure, we performed in situ inflation tests on human iliac arteries. Based on these findings, we concluded that the “physical operating range” was between the axial in situ stretch and the inversion stretch (42). From an energy-minimizing point of view, it would be best if the axial in vivo stretch in arteries equals approximately the inversion stretch (i.e., arteries do no “axial work” during the cardiac cycle). However, this energetically optimized vessel function was neither confirmed nor disproved in the above-mentioned studies. The inversion feature was not observed for the adventitia and the MI composite up to an axial stretch of 1.30 and 1.25, respectively. If this feature occurs at higher stretches of the adventitia and MI, the difference of the inversion behavior between the intact wall and the adventitia and MI must be caused by the residual stretches and the different mechanical properties of the “layers” and the fact that the adventitia and the MI are bound in the intact wall.

Statistical analysis showed significant negative correlations between age and axial inversion stretches for the CCAs \( (r = -0.67, P = 0.03) \) and for the ICAs \( (r = -0.29, P = 0.04) \). A possible explanation for this correlation is that aged arteries show reduced distensibility, since aging causes histostructural changes, such as loss and degeneration of elastin fibers and laminae, and increase of collagenous material and ground substance (31). This age-dependent phenomenon could be relevant and important for all types of artery surgeries, where the length of the artery could be changed in that extent so that the energetically optimized vessel function is not given anymore. Interestingly, no significant correlations were found between age and external axial inversion forces for the CCAs \( (r = 0.26, P = 0.46) \) and for the ICAs \( (r = 0.45, P = 0.26) \).
We found a significant increase of collagen content with age ($r = 0.99$, $P = 0.01$) in the arteries. Besides that, also a decrease of elastin amount with age is documented in the literature (30), which leads to increasing stiffness of blood vessel.

The mechanical data generated in this study emphasize the adventitia and the MI composite as layers with different mechanical properties, and hence different mechanical functions. In particular, the adventitia is very compliant at low pressures, but carries significant loads under physiological conditions. At higher blood pressures, it becomes the mechanically dominant wall component due to its pronounced stiffening behavior, and, consequently, the adventitia is most appropriate for sensing hypertensive states (43). These insights support the hypothesis of an adventitia-dependent regulation of the arterial smooth muscle tone. The adventitia is suggested to consist of many potential mechanical sensory and regulatory constituents, such as the adipose tissue, fibroblasts, macrophages, vasa vasorum, and terminal nerve fibers. Perivascular adventitial adipose tissue is suggested to release a transferable adventitium-derived relaxing factor in vascular smooth muscle cells (26). Another study suggested that visceral periadventitial adipose tissue controls arterial tone by inducing vasorelaxation via voltage-dependent K$^+$ channel activation in vascular smooth muscle cells (46). Adventitial fibroblasts may be transfected via adenoviral vectors to produce nitric oxide (NO) upon receptor or nonreceptor activation. This single molecule NO is responsible for regulatory processes, such as anti-inflammatory, mitogenic, and contractile activities of the vessel wall, as well as the homeostatic process within the vessel lumen (15). In this regard, it has been shown that NO produced by fibroblasts in the adventitia causes effective vessel relaxation (44). Furthermore, the endothelial cells in the intima may react on elevated mechanical stresses with specific paracrine activity, which could affect the entire vessel wall (43). Additionally, from the sympathetic and vagal efferent nerve terminals, released neurotransmitters may act on vascular smooth muscle directly or through release of endothelium-derived NO (18). It has also been demonstrated that the adventitia removal influenced the arterial wall viscosity and elasticity in in vivo studies, possibly by a smooth muscle-dependent mechanism, since it was not present in in vitro studies (7). On the other hand, the adventitia is suggested to play a key role in the development of atherosclerosis from early intimal hyperplasia to calcification of chronic vascular lesions (18). Thus therapies that focus on the adventitial contribution to the development of atherosclerosis and restenosis may have a strong clinical promise (18). The adventitias investigated in the present study changed into extremely stiff tubes at pressures exceeding the physiological range (see Figs. 6 and 9). This suggests that an overstretch of the media and intima is strongly restricted by the adventitia. Because of this pronounced stiffening, the difference between a “safe” and a “dangerous” balloon diameter during balloon angioplasty is very small. If the nominal diameter of the balloon is too large, the adventitia faces extremely high pressures, which finally might cause even rupture (43).

The biaxial mechanical behavior of human carotid MI tubes have, to our knowledge, never been published before. Interestingly, the diameter change during pressurization of the MI tube seems to be independent of the axial stretch. The typically shown cross talk between the circumferential and axial directions of the intact wall and the adventitia tubes was not observed for the MI tubes. This may be explained by the histological structure of the media, where smooth muscle cells and thin elastic laminae show concentric ring-like structures, which are tied together by radially oriented collagen (see, e.g., Ref. 48). Due to a weak connection between these concentric smooth muscle rings in the media, the circumferential stretch during inflation may show independency of axial stretching. The relatively low burst pressure of $\sim 60$ kPa of the MI tube may lead to damage or rupture of the MI tube during high-pressure loading of the intact wall, e.g., during balloon angioplasty. Therefore, our results emphasize a proper choice of the balloon diameter for a safe and effective angioplasty procedure. Our circumferential pressure-stretch results correspond to diameter-pressure curves of MI tubes from bovine carotid arteries (28). However, the study (28) documents a dependency of the diameter-pressure behavior on the axial prestretch and significant bigger hysteresis. Remarkably, high circumferential and axial stresses occurred in the MI tube at mean physiological pressure (13.3 kPa) (see Table 5).

**Study Limitation**

Only the separation of the intact wall tube in an intact adventitia tube and an intact MI composite tube was feasible. The mechanical dissection of the adventitial layer from the MI composite may cause structural damage. However, this seems not to be the case in view of the mechanical strength of the adventitia and the relative ease of dissection. The MI tubes did show rupture of medial lamellar layers on the outer surface of the media, which, to some aspect, might have influenced the mechanical behavior. However, the number of ruptured medial layers could be limited to three or four. The influence of the connection between adventitia tissue and the MI tissue was not examined in the present study.

The wall thickness $H$ of the intact wall in the load-free tube was determined photogrammetrically from two rings that were taken from the ends of each segment. Since the shape of the vessel is not a perfect cylindrical tube, this may have caused errors. Future developments should allow us to determine the wall thickness or the inner diameter directly at the center of the specimen. An accurate thickness measurement is crucial for stress computations. Reliable thickness measurements of highly deformable thin structures such as the adventitias are especially difficult, and hence more likely to be afflicted with errors. Finally, a relatively small number of arterial specimens were investigated so that an anamnesis-dependent conclusion could not be drawn.

The separation of the vessel wall into the major layers is a step toward modeling the next level of hierarchy in the structure. Each layer can be described by a unique set of material parameters and an appropriate nonlinear, anisotropic constitutive model. A more detailed investigation and determination of the microstructure and the mechanical behavior of the constituents (collagen fibers, elastin, smooth muscle cells, macrophages, etc.), and the combination with the macroscopic behavior of arteries will lead to better, constituent-based, multiscaled, and structure-based constitutive models. With this knowledge, a more satisfactory description of arterial walls, their functionality, adaptation, and remodeling will be feasible.
MECHANICAL PROPERTIES OF HUMAN CAROTID ARTERIES

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DISCLOSURES

I am not aware of financial conflict(s) with the subject matter or materials discussed in this manuscript with any of the authors, or any of the authors’ academic institutions or employers.

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