Mechanical response of human subclavian and iliac arteries to extension, inflation and torsion

Gerhard Sommer\textsuperscript{a}, Christoph Benedikt\textsuperscript{a}, Justyna A. Niestrawska\textsuperscript{a}, Gloria Hohenberger\textsuperscript{b}, Christian Viertler\textsuperscript{c}, Peter Regitnig\textsuperscript{c}, Tina U. Cohnert\textsuperscript{d}, Gerhard A. Holzapfel\textsuperscript{a,b,c,⇑}

\textsuperscript{a}Institute of Biomechanics, Graz University of Technology, Austria
\textsuperscript{b}Department of Orthopedics and Trauma Surgery, Medical University Graz, Austria
\textsuperscript{c}Institute of Pathology, Medical University Graz, Austria
\textsuperscript{d}Clinical Department of Vascular Surgery, Medical University Graz, Austria
\textsuperscript{⇑}Faculty of Engineering Science and Technology, Norwegian University of Science and Technology, Trondheim, Norway

**Abstract**

Peripheral vascular trauma due to injuries of the upper and lower limbs are life-threatening, and their treatment require rapid diagnosis and highly-qualified surgical procedures. Experienced surgeons have recognized that subclavian arteries, affected by injuries of the upper limbs, require a more careful handling due to fragility than common iliac arteries, which are may be affected by injuries of the lower limbs. We investigated these two artery types with comparable diameter to evaluate the differences in the biomechanical properties between subclavian and iliac arteries. Human subclavian and common iliac arteries of 14 donors either from the right or the left side (age: 63 yrs, SD: 19, 9 female and 5 male) were investigated. Extension-inflation-torsion experiments at different axial strains (0–20\%), transmural pressures (0–200 mmHg) and torsion (±25\°) on preconditioned arterial tubes were performed. Residual stresses in both circumferential and axial direction were determined. Additionally, the microstructure of the tissues was determined via second-harmonic generation imaging and by histological investigations. At physiological conditions (\(p_t = 13.3\) kPa, \(\lambda_0 = 1.1\)) common iliac arteries revealed higher Cauchy stresses in circumferential and axial directions but a more compliant response in the circumferential direction than subclavian arteries. Both arteries showed distinct stiffer behavior in circumferential than in axial direction. Circumferential stiffness of common iliac arteries at physiological conditions increased significantly with aging (\(r = -0.67\), \(p = 0.02\)). The median inversion stretches, where the axial force is basically independent of the transmural pressure, were determined to be 1.05 for subclavian arteries and 1.11 for common iliac arteries. Both arteries exhibited increased torsional stiffness, when either axial prestretch or inflation pressure was increased. Residual stresses in the circumferential direction were significantly lower for subclavian arteries than for common iliac arteries at measurements after 30 min (\(p = 0.05\)) and 16hrs (\(p = 0.01\)). Investigations of the collagen microstructure revealed different collagen fiber orientations and dispersions in subclavian and iliac arteries. The difference in the collagen microstructure revealed further that the adventitia seems to contribute significantly to the passive mechanical response of the tested arteries at physiological loadings. Histological investigations indicated pronounced thickened intimal layers in subclavian and common iliac arteries, with a thickness comparable to the adventitial layer. In conclusion, we obtained biomechanical differences between subclavian and common iliac arteries, which possibly resulted from their different mechanical loadings/environments and respective in vivo movements caused by their anatomical locations. The biomechanical differences explored in this study are well reflected by the microstructure of the collagen and the histology of the investigated arteries, and the results can improve trauma patient care and endovascular implant design.

**Statement of Significance**

During surgical interventions surgeons experienced that subclavian arteries (SAs) supplying the upper extremities, appear more fragile and prone to damage during surgical repair than common iliac arteries (CIAs), supplying the lower extremities. To investigate this difference in a systematic way the aim of this...
1. Introduction

The prevalence of vascular injuries in which peripheral traumas are life-threatening, and require rapid diagnosis and highly-qualified surgical procedures, has increased in the recent decades [1–3]. Up to now the literature lacks detailed information on possible post-traumatic differences between upper and lower limbs after arterial extremity trauma. Therapeutic algorithms have advanced through wartime experiences and led to a decrease in the amputation rates from 53 to 1.5% over the past seven decades [4]. Experienced surgeons have emphasized that the subclavian artery (SA) might be more sensitive to vascular interventions in comparison to the common iliac artery (CIA). This could be traced back to different mechanical loads acting on the arteries. SAs are subjected to compression due to their anatomical location between the first rib and the clavicle, and to bending caused by abduction of the arms [5]. Additionally, they may also face torsion due to cyclic respiratory forces [6]. CIAs undergo twisting, shortening, and bending movements during hip flexion [7]. CIAs are of particular interest as they are prone to atherosclerosis resulting in frequent endovascular treatments [8]. Surgeons, patients and health insurance companies are interested in interventions free of complications, which reduce the number of secondary operations avoiding further burdens to the patients and additional costs for the public health system.

To predict changes resulting from interventions, exact knowledge of the biomechanical properties of these arteries is essential to determine the extent of differences in the biomechanical properties. We thus investigated these two artery types with the aid of state-of-the-art biomechanical experiments. Furthermore, we investigated whether the higher sensitivity of the SA compared to CIA can be derived from their biomechanical properties as these data can enhance surgical techniques and patient safety as well as endovascular implant design. We performed extension-inflation-torsion experiments, since they mimic the in vivo multiaxial loading conditions of arteries very well [9]. Moreover, extension-inflation tests are appropriate to determine constitutive equations of arteries and data of these tests are rare for people over 50 years [8]. Only two studies could be found which investigated the mechanical properties of human SA and CIA. The study [10] determined the planar biaxial mechanical response of human SAs and CIAs, whereas [8] documented the extension-inflation response of human iliac arteries. To our knowledge, the present study is the first utilizing extension-inflation-torsion tests for the determination of the mechanical behavior of human SAs and CIAs. In addition to extension-inflation-torsion behavior, residual stresses, as these are important for determining the zero-stress state of arteries [11], the collagen microstructure, and the histology of the tested arteries were also determined and compared.

The extension-inflation properties of the investigated arteries are provided specifically as pressure vs circumferential stretch, pressure vs axial stretch, and axial Cauchy stress vs axial stretch behavior. The axial inversion stretches, at which the axial force is independent of the transmural pressure, and circumferential distensibilities/compliances were determined to allow a direct comparison of the mechanical properties of SA and CIA. Circumferential and axial Cauchy stresses were calculated and plotted vs the applied transmural pressures. Torsional properties of the arteries were compared in terms of their shear moduli and axial Cauchy stress vs the amount of shear behavior. Residual stresses in circumferential and axial directions of the vessels are quantified by measuring opening angles. To evaluate the microstructure of the investigated arteries collagen fiber orientations and dispersions were determined from second-harmonic generation (SHG) imaging and compared with each other. Geometrical measures and contents in structural proteins, i.e. collagen, elastin and smooth muscle cells were determined from histological analyses and compared with each other.

2. Materials and methods

A total of 15 pairs of human SAs and CIAs (62.7 ± 18.6 yrs, mean ± SD, range 43–96. 9 female and 5 male) were harvested and investigated. From patient VIII both left- (VIII L) and right-side arteries (VIII R) were taken. Amenities of the donors are listed in Table 1. Only straight segments without palpable circumferential wall hardening were used. For the present study, the use of autopsy material from human subjects was approved by the Ethics Committee of Medical University of Graz, Austria, with the approval number 27–460 ex 14/15. We kept the arteries refrigerated at 4 °C and humidified them with phosphate-buffered saline solution at all times to minimize tissue autolysis and degradation. For each donor one SA and one CIA from the same site were harvested and subsequently tested. All arteries were tested within 36 hrs after excision from the body.

A typical SA and CIA from the same patient, divided in the segments A, B, C, D and E used for different investigations, are depicted in Fig. 1. The longest piece C (≈ 60 mm) was used for the extension-inflation-torsion experiments, segments A (≈ 1.5 cm) and B (≈ 3 mm) were used to determine residual stresses in the axial and circumferential directions (see also Section 2.2), respectively. Segment B (≈ 3 mm) was also used for thickness measurements. For a detailed description of the thickness measurement method, the reader is referred to [12]. On segments E and F (≈ 10 mm), microstructural investigations via second-harmonic generation (SHG) imaging and histological investigations were carried out.

2.1. Extension-inflation-torsion tests

For the determination of the biomechanical properties for SAs and CIAs, extension-inflation-torsion tests were utilized. In contrast to planar biaxial tests, extension-inflation-torsion tests mimic the physiological loading conditions of arteries very well as they preserve the in vivo ultrastructure [9]. During such experiments arteries were loaded simultaneously through axial forces Fz, transmural pressures p, and torques M. An axial elongation or extension in particular led to an increase in axial force and stress, the
inflation pressure induced a change in circumferential and axial stretches/forces, and the torsion resulted in a torque and shear deformations/stresses in the arterial wall.

2.1.1. Preparations

Segments that were as straight as possible were prepared from the arteries for the extension-inflation-torsion tests (Fig. 1, segment C). Loose connective tissue on the adventitia was removed to enable precise deformation measurements with the videoextensometer. Existing branches were sealed using surgical threads to ensure no leaking during inflation. The straight arterial tube was cannulated with two diameter-matching end adapters, which were glued with cyanoacrylate adhesive gel, and additionally, fixed via cords to ensure tightness between adapters and artery. Thereafter, the artery was inserted in the extension-inflation-torsion testing apparatus (Fig. 2).

Proper zero adjustment of axial load, pressure and torque is crucial for extension-inflation-torsion tests of soft biological tissues. Proper zero adjustment of the axial load was achieved by fixing the artery first to the upper shaft, submerging to artery to a marked level on the upper shaft, and setting the axial force then to zero. Thereafter, the artery was fixed to the lower shaft and the artery was submerged to the same marked level. If the axial force was still zero the artery was inserted without axial load. For proper zero adjustment of the pressure, the valve on the upper shaft was opened and pressurized until the water level in the hollow upper shaft was equal to the water level in the tissue bath. The pressure was then set to zero, and the valve was closed to measure the 'transmural' pressure. Torsion-free inserting of the artery was achieved by ensuring a zero twisting angle of the upper shaft (measured by the laserextensometer) when fixing the artery to the lower shaft during axial load adjustment. After zero loadings adjustments, axial gage markers were painted onto the arteries with a special tissue ink (Fig. 2(b)), and the tempered tissue bath was raised to the marked level covering the whole artery in 37°C phosphate-buffered saline (PBS).

2.1.2. Experimental setup

Extension-inflation-torsion tests were performed on a novel and custom-built device, see Fig. 2. Extension was achieved via spindle-driven crossheads moving in opposing directions, allowing a fixed position of the gage region of the specimen. A motor driven spindle pump (Model 205571, Dustec; Wismar, Germany) was installed at the back of the testing machine and generated the inflation pressure. Torsion was achieved by an additional stepper motor connected to the twistable lower shaft of the device.

Axial force was measured by a 20 N class 1 strain-gauge load cell (model Xforce HP 063926, Zwick/Roell; Ulm, Germany) with an accuracy of 0.2% of the nominal force specified by the manufacturer. Inflation pressure was measured by 5 bar class 1 strain-gauge pressure transducer (model TP16R, AEP transducer; Cognento, Italy) with an accuracy of 0.15% of the nominal pressure. The PC-based videoextensometer utilizes a CCD camera (model μEye UI-3243-CP-M-GL, IDS; Obersulm, Germany) with a resolution 1280 × 1024 px and a frame rate of 60 fps for simultaneous measurements of circumferential and axial deformations within gage markers (Fig. 2(b)). This class 0.5 instrument supports interactive monitor-based selection of the measuring zone and automatically traces axial gage markers and vessel edges using the contrast of the white artery with respect to the black edges. The applied torque was measured by means of a cantilevered pivot bearing (model 5008–800, Riverhawk; New Hartford, USA) with a spring rate of 0.82 g/cm° and the laserextensometer, which measured the twist of the pivot bearing. The laserextensometer utilizes a laser and a CCD camera (model μEye UI-3483-CP-M-GL, IDS; Obersulm, Germany) with a resolution of 2560 × 1920 px and a frame rate of 60 fps. The temperature of the tissue bath was maintained at 37 ± 0.1°C by a heater-circulator unit (model EcoSilver, Lauda; Lauda-Königshofen, Germany).

2.1.3. Preconditioning

Preconditioning was performed first in axial, then in circumferential direction, and finally a torsion was applied. In particular, each specimen was elongated cyclically four times up to \( z_k = 1.1 \), then the specimen was elongated once more and held at \( z_k = 1.1 \). At this axial stretch, the specimen was cyclically inflated five times to 13.3 kPa (100 mmHg) and held at 13.3 kPa, where the specimen was subjected to two twisting cycles ±25° for torsional preconditioning. Preliminary tests on SAs and CIAs showed that inversion stretches, at which the axial force is basically independent of the transmural pressure, were determined to be between 1.08 and 1.12. Moreover, four elongation and inflation cycles were adequate

### Table 1

<table>
<thead>
<tr>
<th>Cause of death</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>
<th>XIV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>82</td>
<td>52</td>
<td>96</td>
<td>58</td>
<td>50</td>
<td>88</td>
<td>79</td>
<td>61</td>
<td>50</td>
<td>49</td>
<td>46</td>
<td>48</td>
<td>92</td>
<td>43</td>
</tr>
<tr>
<td>Sex</td>
<td>m</td>
<td>f</td>
<td>f</td>
<td>m</td>
<td>f</td>
<td>f</td>
<td>f</td>
<td>f</td>
<td>m</td>
<td>f</td>
<td>f</td>
<td>m</td>
<td>f</td>
<td>m</td>
</tr>
<tr>
<td>Cause of death</td>
<td>Ca</td>
<td>LE</td>
<td>LE</td>
<td>CS</td>
<td>TP</td>
<td>AML</td>
<td>LE</td>
<td>LE</td>
<td>Ca</td>
<td>AID</td>
<td>AR</td>
<td>VT</td>
<td>Ca</td>
<td>LE</td>
</tr>
<tr>
<td>Segment</td>
<td>A</td>
<td>B</td>
<td>C</td>
<td>D</td>
<td>E</td>
<td>A</td>
<td>B</td>
<td>C</td>
<td>D</td>
<td>E</td>
<td>A</td>
<td>B</td>
<td>C</td>
<td>D</td>
</tr>
</tbody>
</table>

*Note: I–XIV: donor number; AID: autoimmune disease; AML: acute myeloid leukemia; AR: aneurysm rupture; Ca: cardiac arrest; CRI: cardiorespiratory insufficiency; CS: coronary sclerosis; CT: cardiac tamponade; GHD: global heart dilatation; LE: lung emphysema; MI: myocardial infarction; MOF: multi-organ failure; RVD: right ventricular dilatation; SAH: subarachnoid hemorrhage; TP: tumor progression; VT: vein thrombosis; V-tach: ventricular tachycardia.*
For the achievement of proper preconditioning. In contrast, only two twisting cycles were necessary to precondition the arteries in regard to torsion.

2.1.4. Testing protocol

Extension-inflation-torsion tests with continuous recording of axial force, transmural pressures, outer diameter, axial gage length, twisting angle and torque were performed. Arterial segments were tested at axial stretches ranging from $z_2 = 1.0$ to $1.2$ in 0.05 increments. At each axial stretch the pressure was increased stepwise from 0 to 26.7 kPa (200 mmHg) in 3.3 kPa increments, and at each pressure step a twist in the range of $\pm 25^\circ$ was applied to the artery. For the tests, the elongation rate was 10 mm/min, the inflation rate was 1 kPa/s, and the torsion rate was 2/ $s$.

In this study we wanted to cover the physiological loading regime with a mean arterial pressure of 100 mmHg (13.3 kPa) but also supra-physiological conditions in hypertensive patients or during hard physical activities, e.g., weight lifting, with arterial pressures up to 200 mmHg (26.7 kPa). Within this pressure domain we do not expect any damage to the tissue. Similarly, the maximal axial stretch of 1.2 and the twisting angle of $\pm 25^\circ$ used in this study are also considered to be not harmful to arteries.

We tried to conduct all tests on all specimens, but for different reasons it was not always possible. In some cases, for no and small axial prestretches ($z_2 = 1.0$ and 1.05), pressurization under isometric conditions (arterial segments were forced to keep their lengths) led to buckling of the arterial walls. If buckling occurred then the biomechanical data were only considered for larger axial prestretches where the arterial segment showed no buckling. Tightening the artery in a satisfactory manner turned out to be a difficult task; sometimes the artery began to leak at higher pressures, which also resulted in an incomplete data set. Hence, biomechanical data for small axial prestretches and higher pressures are thus lacking for such cases in the related tables (Tables 3 and 4), see the section on the results.

To determine residual stresses in the arteries, opening angles, labeled as $\alpha$ and $\beta$, were measured for circumferential and axial strips, respectively. The circumferential opening angle $\alpha$ was defined as the angle between the two lines proceeding from the center of the specimen to the end tips of the arc (Fig. 3(a)) [13]. The axial opening angle $\beta$ was measured by cutting a $15 \times 2$ mm axial strip from a 15 mm arterial segment, and measuring the angle of sector that resulted from outward bending of the intima of the axial strip (Fig. 3(b)) [14]. Larger values of $\alpha$ correspond to larger circumferential residual stresses, whereas larger values of $\beta$ correspond to smaller axial residual stresses. For proper opening angle measurements, circumferential rings and axial strips were glued with pinpoint size cyanoacrylate adhesive onto small hollow plastic tubes (diameter of 7 mm), which served as supports in the tissue bath. The axial strip was glued at the intima, because it bends outwards. All preparations and measurements were performed in PBS at room temperature.

Immediately after placing the ring and the strip in the bath images were taken with a digital camera. Afterwards the circumferential ring was cut and another image was taken. Additional images were taken from both circumferential and axial strips every 30 min for a duration of three hours. Further images were taken after six and twelve hours to illustrate the long-term residual stress release. The opening angles were measured photogrammetrically using the Adobe Illustrator CS4 software package (San José; California, USA).

2.3. Second-harmonic generation (SHG) imaging of the microstructure

The microstructure of the arterial walls, i.e. collagen fiber orientation and dispersion, was determined using SHG imaging. The thickness of the arterial wall ($\sim 1.4$ mm) limits the application of optical microscopy techniques. In order to increase the penetration...
depth of the laser rays of a multiphoton microscope (MPM), the specimens were optically cleared.

For optical clearing the first preparation step was to remove water from the specimen by means of an ascending ethanol series according to the following protocol: 50. 70. 95%, 2 x 100% ethanol. Each step of the protocol lasted 45 min. In the second step the tissue was optically cleared using a benzyl alcohol to benzyl benzoate (BABB) mixture [15]. The dried arteries were then placed in a 1:1 ethanol:BABB solution for 4 hrs before putting them in pure BABB-solution, in which they remained for at least 12 hrs prior to imaging. All preparations were performed at room temperature.

SHG imaging of collagen fibers was performed using an imaging-setup consisting of a picosecond laser source integrated into a Leica SP5 confocal microscope (Leica Microsystems, Inc., Mannheim, Germany). The laser was tuned to 880 nm to enable SHG inducing of collagen. For the detection of the second-harmonic signal, a BP 465/170 emission filter in combination with a non-descanned detector in epi-mode (backward detection) was used. The images were acquired using a Leica HCX IRAPO L 25 x 0.95 water objective with a working distance of 2.5 mm for deep tissue imaging. The resulting image (z-stacks) are comprised of two-dimensional (2D) images recorded from the previously cleared specimen, and each stack contained sectional images with a field of view of 620 x 620 µm in the (x, y)-plane. In depth (z-direction), a step size, i.e. a distance between images in the z-stack of 5 µm was used. Representative 2D images obtained via SHG can be seen in Fig. 4.

2.3.1. Analysis of SHG images

SHG images were analyzed by extracting data from z-stacks of three-dimensional images. Here, a Fourier power spectrum analysis and wedge filtering were applied, as described in [15], yielding discrete angular distributions of relative amplitudes, resembling the fiber orientations. These were defined utilizing the same coordinate system as introduced in [16, 17], see, e.g., Fig. 2 in [17]. This coordinate system was characterized by the basis vectors \( e_1 \) (circumferential direction), \( e_2 \) (axial direction) and \( e_3 \) (radial direction). The general fiber direction in the unloaded reference configuration was described by a unit vector \( \mathbf{N} \), which was defined by the angles \( \phi \) and \( \theta \), referred to respectively as the in-plane and out-of-plane angles. In-plane and out-of-plane fiber orientations were fitted with a bivariate von Mises distribution 

\[
\rho(\phi, \theta) = \rho_{ip}(\phi)\rho_{op}(\theta),
\]

assuming that in-plane and out-of-plane dispersions are independent [18]. Following [16] the specific choice for the von Mises distribution was

\[
\rho_{ip}(\phi) = \frac{\exp[a \cos 2(\phi - \mu)]}{I_0(a)}
\]

\[
\rho_{op}(\theta) = 2\sqrt{\frac{2b}{\pi}} \exp[b(\cos 2\theta - 1)] \text{erf}(\sqrt{2b}),
\]

where \( \mu \) is the angle between the circumferential direction \( e_1 \) and the mean fiber direction and \( I_0(a) \) is the modified Bessel function of the first kind of order 0. Here \( a \) and \( b \) are concentration parameters defining the shape of the distributions and are obtained by fitting to the discrete angular distributions of relative amplitudes. Following [16] we define two scalar quantities to measure the in-plane \( \langle \kappa_{ip} \rangle \) and out-of-plane \( \langle \kappa_{op} \rangle \) distribution as

\[
\kappa_{ip} = \frac{1}{2} \frac{I_1(a)}{2I_0(a)}
\]

\[
\kappa_{op} = \frac{1}{2} \frac{1}{8b^2} \frac{1}{4} \sqrt{2} \exp(-2b) \frac{\text{erf}(\sqrt{2b})}{\pi b \text{erf}(\sqrt{2b})},
\]
where \(0 \leq K_{ip} \leq 1\) and \(0 \leq K_{op} \leq 1/2\). A value of \(K_{op} = 0.5\) resembles isotropy in-plane, if \(K_{ip} = 0\) the fibers are perfectly aligned. If \(K_{op} = 0.5\) all fibers lie in-plane, if \(K_{op}\) approaches a value of \(1/3\) all fibers are dispersed out-of-plane.

2.4. Histology

Histological investigations were performed to obtain the collagen, elastin, and smooth muscle cell contents as also geometric dimensions such as diameter and thickness values of the tested arteries. The small arterial segments used (segments E in Fig. 1) were thus fixed in 4% formaldehyde solution (pH 7.4), embedded in paraffin, sectioned at 4 \(\mu\)m and stained. For collagen hematoxylin and eosin (H&E) staining was used. Hematoxylin binds to the DNA and stains the nuclei dark blue or violet. Eosin binds to positively charged amino acids and stains the collagen pale pink. Elastica van Gieson (EVG) staining was applied to stain elastin in the arteries. Stained sections were examined under a light microscope and assessed for differences in contents and dimensions between the SAs and CAs. They were imaged and analyzed by the use of an image analysis routine within the Aperio ImageScope software package (version 12.0). Semiquantitative analysis of elastin content was performed by two pathologists and the following categories were used: +++ = 90–100% of expected normal elastin content (as referred to A. subclavia elastin content), ++ = 50–90% of normal elastin, + = 1–50% of normal elastin, and 0 = no elastin. For diameter and thickness measurements, a ring (height \(\approx 3\) mm) was cut with a sharp trimming blade, which was aligned as perpendicular as possible to the long axis of the arterial segment. Careful embedding of the rings and serial sections including different stains were performed to receive appropriate cross sections of the vessels and to ensure that a true thickness was obtained in histological evaluation by the participating pathologists.

2.5. Mechanical data analysis

2.5.1. Extension-inflation behavior

Kinematics of both initial (load-free) and current (loaded) configurations during extension-inflation tests are described in Fig. 5. The initial configuration is described by the sample length \(L\), gauge length \(Z\), inner radius \(R_i\) and outer radius \(R_o\). Corresponding measures in the current configuration are \(L, Z, r_i, r_o\). In the initial configuration the axial force \(F_z\), inflation pressure \(p_i\) and torque \(M_t\) are zero.

\[
\begin{align*}
\text{Initial configuration} & \quad \text{Current configuration} \\
F_z = 0 & \quad F_z \\
p_i = 0 & \quad p_i \\
M_t = 0 & \quad M_t \\
\end{align*}
\]

Fig. 5. Kinematics of the artery during tests: (a) initial (load-free) configuration with inherent residual stresses, (b) current (loaded) configuration with applied axial force \(F_z\), transmural pressure \(p_i\), and torque \(M_t\).

Note that due to residual stresses the load-free initial configuration is not stress free.

To estimate circumferential and axial stresses in the arterial wall for comparison purposes, the tubular specimens were assumed to be circular cylindrical, thin-walled, homogeneous, and incompressible [19]. ‘Averaged’ circumferential \((\sigma_{\theta\theta})\) and axial Cauchy stresses \((\sigma_{zz})\) of a loaded tube can then be calculated by means of global equilibrium as

\[
\sigma_{\theta\theta} = p_i \left( \frac{r_o^2}{R_i^2} - 1 \right), \quad \sigma_{zz} = \frac{p_i (r_o - h)^2 + F_z}{4 \pi (2 R_i - h)}.
\]

where \(p_i\) is the transmural pressure, \(F_z\) the axial load, \(r_o\) is the outer radius, and \(h\) the wall thickness of the loaded tube, respectively [20]. The wall thickness in the current configuration was calculated using \(h = r_o - r_i\), where \(r_i\) is determined by using the incompressibility assumption via

\[
r_i = \sqrt{r_o^2 - R_i^2} - \frac{F_z}{4 \pi (2 R_i - h)}.
\]

As a further measure to compare the extension-inflation properties of arteries the so-called circumferential distensibility \(\Delta \lambda_\theta\) was calculated from the pressure vs circumferential stretch plots. Hence, \(\Delta \lambda_\theta\) is defined as the difference between the circumferential stretches at \(p_i = 0\) kPa \((\lambda_{\theta0})\) and \(p_i = x\) kPa \((\lambda_{\theta x})\) through

\[
\Delta \lambda_\theta = \lambda_{\theta x} - \lambda_{\theta0},
\]

with \(x\) as either 13.3 kPa (100 mmHg) or 26.7 kPa (200 mmHg). The stretch difference \(\Delta \lambda_\theta\) is determined at an axial prestretch \(\lambda_{zz} = 1\), as this value is closest to the so-called inversion stretch \(\lambda^*\). At \(\lambda^*\) the axial force \(F_z\) and the axial stretch \(\lambda_{zz}\) are basically independent of the transmural pressure \(p_i\). This feature of arteries was first described on animals by Weizsäcker et al. [21] using the term crossover length, and then also shown on humans by Schulze-Bauer et al. [8]. The existence of such a stretch is a clear evidence for the anisotropic characteristics of arteries.

2.5.2. Torsional behavior

The twisting angle \(\theta\) of the arterial segment during torsion tests is calculated by \(\theta = \theta_{\text{motor}} - \theta_{\text{pivot}}\), where \(\theta_{\text{motor}}\) is the torsion angle applied by the stepper motor (SMT in Fig. 2) at the lower end of the artery and \(\theta_{\text{pivot}}\) is the torsion angle measured by the cantilevered pivot bearing (TT in Fig. 2) at the upper end of the artery. The torque in a cylindrical tube can then be calculated by

\[
M_t = 2 \pi \int_{r_i}^{r_o} \sigma_{\theta\theta} r^2 dr,
\]

wherein the differential force \(\sigma_{\theta\theta} \pi r^2 dr\) was multiplied by the moment arm \(r\) and integrated over the entire cross section [22]. The torque \(M_t\) was linearly proportional to \(\theta / L\) in the range of the applied twist (\(\pm 25^\circ\)). Hence, we can assume that the average shear stress \(\sigma_{\theta\theta}\) is linearly proportional to \(\theta / L\) in this range. With the definition of the shear strain \(\epsilon_{\theta\theta} = \theta / 2L\) at the radius \(r\) the linearity implies so that

\[
\sigma_{\theta\theta} = 2 G \epsilon_{\theta\theta} = G \frac{\theta}{L}.
\]

where \(G\) is the shear modulus of the arterial wall, \(\theta\) is the angle of twist and \(L\) denotes the vessel segment length. Inserting Eq. (7) into (6) we obtain

\[
M_t = 2 \pi G \frac{\theta}{L} \int_{r_i}^{r_o} r^2 dr = \pi G \frac{\theta}{2L} (r_o^4 - r_i^4) = J G \frac{\theta}{L}.
\]

\[
J = \pi (r_o^4 - r_i^4)/2,
\]
where \( J \) denotes the polar moment of inertia. For the evaluation of the torsion data we assume constant wall thickness along the whole length of the arterial segments.

### 2.6. Statistical analysis

Medians and interquartile ranges (IQR) were calculated for the load-free geometries, inversion stretches, Cauchy stresses, shear moduli, opening angles, and microstructural parameters as we cannot assume normal distributions due to the small sample cohort. Moreover, outliers can severely affect the mean and standard deviation. Significant correlations between the median values were tested by using the Mann-Whitney U-test. To detect differences between measurements from SA and CIA we performed paired tests using the Wilcoxon signed-rank test. For both tests, differences were considered statistically significant if \( p \)-values were less than 0.05, corresponding to a 95% confidence. The mean and SD values were also calculated in order to be able to compare the results in this study with other studies in the literature. All statistical analyses were performed using MATLAB [23].

### 3. Results

#### 3.1. Extension-inflation-torsion behavior

##### 3.1.1. Geometry

Outer diameters \( D_o \) and thicknesses \( H \) of the load free SAs and CIAs are summarized in Table 2. Outer diameters and thicknesses were \( 7.2 \pm 2.2 \) mm and \( 1.3 \pm 0.5 \) mm for SA, and therefore smaller as for CIA, with values of \( 11.2 \pm 2.4 \) mm and \( 1.9 \pm 0.4 \) mm. The median diameter-to-thickness ratios resulted in \( 5.9 \pm 3.6 \) for SA and \( 5.6 \pm 2.1 \) for CIA.

##### 3.1.2. Preconditioning

In general during ‘axial’ preconditioning axial stresses were larger for CIA than for SA and the nonlinear (stiffening) behavior was also more pronounced in CIA than in SA. At large, four ‘axial’ preconditioning cycles were sufficient for both arteries. During ‘circumferential’ preconditioning the SA showed a stiffer behavior in the circumferential direction than the corresponding CIA. A point of interest here is that the axial stretches decreased slightly during circumferential preconditioning for both arteries. In general, during axial and circumferential preconditioning the hysteresis area decreased with an increasing cycle number for both SA and CIA. Four ‘circumferential’ preconditioning cycles were sufficient for both arteries. In contrast, only two twisting cycles were sufficient for preconditioning the arteries regarding torsion.

#### 3.1.3. Extension-inflation behavior

Representative pressure vs circumferential stretch and pressure vs axial stretch behavior due to inflation up to \( 26.6 \) kPa for different axial prestretches \( (\bar{z}_k = 1.0–1.2) \) for SAs and CIAs are shown in Fig. 6. For the sake of clarity, only the loading paths are shown. In the pressure-circumferential stretch plots (Fig. 6(a) and (b)) the SA was less compliant in the circumferential direction than the corresponding CIA. A point of interest here is that increasing axial prestretch led to a softer response for the SA and to a stiffer response for the CIA in the circumferential direction, indicating a different anisotropic nature and microstructure of the two artery types. In the pressure-axial stretch plot (Fig. 6(c)), the SA elongated at axial prestretches \( \bar{z}_k = 1.0, 1.05 \) and 1.1 and shortened at \( \bar{z}_k = 1.15 \) and 1.2 with inflation, which indicates that the inversion stretch \( \bar{z}_I \) of the SA from patient II is between \( \bar{z}_k = 1.10 \) and 1.15. In Fig. 6(d) the iliac artery elongated at \( \bar{z}_k = 1.0 \) and at 1.05, the length remained constant during inflation, showing that the stretch 1.05 is close to the inversion stretch \( \bar{z}_I \). At larger axial stretches \( (\bar{z}_k = 1.1 \) and 1.15) the artery shortened during inflation due to exceeded inversion stretch.

##### 3.1.4. Inversion stretch

The point of intersection of the axial force vs the axial stretch curves for all constant values of pressures, in Fig. 7, corresponds to the inversion stretch of the artery [8]. This common point is highly characteristic and was found in all arteries investigated by us so far. In the representative axial force-axial stretch plots (Fig. 7) the inversion stretch \( \bar{z}_I \) was larger for SA than for CIA. In Table 3 the determined inversion stretches for both SA and CIA are listed. Inversion stretches were larger for SA \( (\bar{z}_I = 1.1) \) than for CIA \( (\bar{z}_I = 1.05) \), but not significant different \( (p = 0.22) \). No significant linear correlations with age were found for inversion stretches of neither CIA \( (r = -0.40, p = 0.18) \) nor SA \( (r = -0.14, p = 0.7) \).

### Table 2

Load-free geometries, i.e., outer diameter \( D_o \), wall thickness \( H \), and their ratio \( D_o/H \), for SAs and CIAs.

<table>
<thead>
<tr>
<th>Specimen</th>
<th>SA</th>
<th>CIA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( D_o )</td>
<td>( H )</td>
</tr>
<tr>
<td>I</td>
<td>9.1</td>
<td>1.0</td>
</tr>
<tr>
<td>II</td>
<td>6.6</td>
<td>0.9</td>
</tr>
<tr>
<td>III</td>
<td>7.5</td>
<td>1.7</td>
</tr>
<tr>
<td>IV</td>
<td>8.5</td>
<td>1.3</td>
</tr>
<tr>
<td>V</td>
<td>4.5</td>
<td>1.1</td>
</tr>
<tr>
<td>VI</td>
<td>9.2</td>
<td>1.1</td>
</tr>
<tr>
<td>VII</td>
<td>12.3</td>
<td>1.4</td>
</tr>
<tr>
<td>VIII L</td>
<td>6.7</td>
<td>1.7</td>
</tr>
<tr>
<td>VIII R</td>
<td>7.2</td>
<td>1.5</td>
</tr>
<tr>
<td>IX</td>
<td>6.7</td>
<td>0.6</td>
</tr>
<tr>
<td>X</td>
<td>5.5</td>
<td>1.3</td>
</tr>
<tr>
<td>XI</td>
<td>8.6</td>
<td>2.0</td>
</tr>
<tr>
<td>XII</td>
<td>11.7</td>
<td>2.0</td>
</tr>
<tr>
<td>XIII</td>
<td>6.8</td>
<td>1.1</td>
</tr>
<tr>
<td>XIV</td>
<td>6.9</td>
<td>1.5</td>
</tr>
<tr>
<td>Median</td>
<td>7.2</td>
<td>1.3</td>
</tr>
<tr>
<td>[Q1: Q3]</td>
<td>[6.7:8.9]</td>
<td>[1.1:1.6]</td>
</tr>
<tr>
<td>Mean</td>
<td>7.9</td>
<td>1.3</td>
</tr>
<tr>
<td>SD</td>
<td>2.1</td>
<td>0.4</td>
</tr>
</tbody>
</table>
3.1.5. Circumferential distensibility

The circumferential distensibility $\Delta \lambda_{\theta}$ was introduced to allow a comparison of the circumferential mechanical properties/stiffness for SA and CIA during inflation (see Section 2.5). Circumferential distensibilities for SA and CIA at an axial stretch $\lambda_z = 1.1$ and at transmural pressures of 13.3 and 26.7 kPa are summarized in Table 4. We found larger circumferential distensibilities for CIA than for SA at both pressure values, but the differences were not significant (13.3 kPa: $p = 0.55$; 26.7 kPa: $p = 0.98$). For some donors, however, substantial differences in the circumferential distensibilities between SA and CIA were observed. On the one hand, the SA of donor XI revealed with $\Delta \lambda_{\theta} = 0.03$ a much stiffer behavior than the corresponding CIA with $\Delta \lambda_{\theta} = 0.2$, while on the other hand, the SA of donor XIV were with $\Delta \lambda_{\theta} = 0.16$ more compliant than the corresponding CIA with $\Delta \lambda_{\theta} = 0.07$.

3.1.6. Stress analysis

In Fig. 8 circumferential $\sigma_{\theta\theta}$ and axial Cauchy stresses $\sigma_{zz}$ are plotted as functions of inflation pressures for different axial prestretches. Both circumferential and axial Cauchy stresses increase with increasing prestretch $\lambda_z$. The circumferential stress-pressure behavior (Fig. 8(a) and (b)) appears to be less dependent on the axial prestretch than the corresponding axial stress-pressure behavior (Fig. 8(c) and (d)). Calculated circumferential Cauchy stress-pressure slope values $k_\theta$ were clearly greater than corresponding axial slope values $k_z$ for both arteries. For the circumferential direction, slope values are similar for all axial prestretches, but corresponding slope values for the axial direction increase distinctly with increasing axial prestretches.

At physiological conditions ($p_t = 13.3$ kPa, $\lambda_z = 1.1$), the Cauchy stresses in the circumferential $\sigma_{\theta\theta}$ and the axial directions $\sigma_{zz}$, the circumferential $k_\theta$ and the axial stress-pressure slopes $k_z$, and the corresponding slope ratios $SR$ are summarized in Table 5. Median values of the circumferential stresses $\sigma_{\theta\theta}$ were similar for SA and CIA ($p = 0.56$), however, median axial stresses were significantly greater for CIA than for SA ($p = 0.02$). Similarly, median values of $k_\theta$ were equal for SA and CIA, whereas the median of $k_z$ was remarkably greater for CIAs than for SAs ($p = 0.20$). The stiffer axial behavior of CIAs compared to SAs was also observed in [10], where the mean axial stretch $\lambda_z$ at 13.3 kPa was lower for CIA than for SA. Slope values $k_{\theta/z}$ between SA and CIA were significant different ($p = 0.04$). Moreover, median circumferential-to-axial stress slope ratios $SR = k_{\theta/z}$ were greater than two for both arteries SA ($SR = 2.8$) and CIA ($SR = 2.2$), which indicates that SA and CIA were more compliant in the axial than in the circumferential direction.

3.1.7. Torsional behavior

Representative torque vs rate of twist behavior for SA and CIA at a constant pressure of 13.3 kPa and different axial prestretches $\lambda_z$ are illustrated in Fig. 9(a) and (b). The curves show the torque behavior during a torsion in the range $\pm25^\circ$. For the sake of clarity, the entire torsion cycles were only plotted for $\lambda_z = 1.0$, for other axial prestretches the average of loading and unloading curves is shown. Hysteresis areas during torsion cycles were pronounced for all axial prestretches. For the applied range of twist the linear relationship between torque and rate of twist was observed for every specimen. The slopes of the curves in the plots of Fig. 9(a) and (b) correspond to the shear modulus $G$, where a higher (absolute) value of $G$ is associated with a higher torsional stiffness of the artery. During torsion tests the shear moduli for SAs and CIAs increased with increasing axial prestretch and increasing inflation pressure (Table 6). No significant correlation between shear modulus and age was observed for SA and CIA.

---

Fig. 6. Representative mechanical responses of preconditioned SA (a), (b) and CIA (c), (d) taken from patient II subjected to transmural pressure up to 26.6 kPa and different axial prestretches $\lambda_z = 1.0$–1.2 in 0.05 increments. Transmural pressure is plotted against circumferential stretch in (a) and (c), and axial stretch in (b) and (d). Color legend for all panels is given in (a).
For determination of the inversion stretches summarized for the purpose of comparison in Table 6. Shear moduli against axial stretches for different pressures of intersection is equivalent with the inversion stretch. Shear moduli were always smaller for CIA than for SA, but the differences were also not statistically significant.

Representative axial Cauchy stress vs amount of shear behavior is plotted for SA and CIA in Fig. 9(c) and (d), respectively. Symmetric behavior with the greatest axial Cauchy stress $\sigma_{zz}$ at maximum absolute values of the amount of shear $\gamma$ was shown for iliac arteries (Fig. 9(d)). However, a slightly non-symmetric behavior was exhibited for SAs (Fig. 9(c)).

### 3.1.8. Inter-specimen variation, paired data trends, circumferential and axial compliances, influence of age

In general, the load-deformation behavior showed high inter-specimen variations. Paired comparisons for morphological and mechanical measurements were performed to detect statistical differences between SA and CIA. Therefore, geometrical and extension/inflation/torsion data such as diameter, thickness, inversion stretch, circumferential distensibility, stress, and shear modulus were compared between SA and CIA from the same donor. These paired comparisons revealed no statistical significant differences between SA and CIA.

In Fig. 10, original data of the loading branches of the specimens from a young donor XIV and an old donor XIII are presented in the axial Cauchy stress vs circumferential stretch graphs. For each specimen, 25 data points are plotted. They are connected with five lines, which indicate pressure levels (in kPa: 0, 6.7, 13.3, 20, 26.7). Additionally, five lines indicate different axial prestretches (1.0, 1.05, 1.1, 1.15, 1.2). Thus, the compliance of the specimens in both circumferential and axial directions can be viewed at once. In particular, a long horizontal and a short vertical relationship correspond to a compliant artery in both circumferential and axial directions. For these special cases, the young SA revealed a distinctly larger circumferential compliance than the old SA, whereas axial compliances/stiffnesses were similar. In contrast, the young CIA showed a similar circumferential compliance than the old CIA, but the young CIA revealed a distinctly larger axial compliance/stiffness than the old CIA.

By comparing all tested specimens, Pearson’s correlation coefficients between age and circumferential distensibility at 13.3 kPa were not significant for SA ($r = -0.50$, $p = 0.17$) but significant for CIA ($r = -0.67$, $p = 0.02$), which revealed decreasing circumferential distensibility of the CIA with advancing age of the donors. The correlation between age and axial Cauchy stress range under physiological conditions was revealed to be insignificant for both SA ($r = 0.21$; $p = 0.45$) and CIA ($r = 0.14$; $p = 0.70$).

### 3.2. Residual stresses

The change of opening angles $\alpha$ and $\beta$ over time of circumferentially and axially oriented strips of SA and CIA are shown in Fig. 11(a) and (b). For both circumferential and axial strips we observed a pronounced nonlinear residual stress release over time. The opening angles of the circumferential/axial strips increased/decreased very rapidly at the beginning of the tests, within the first 60 min, and reached equilibrium after approximately 6 hrs.

Opening angles $\alpha$ and $\beta$ of circumferential and axial strips, respectively, of all samples for SA and CIA after 30 min and after 16 hrs are presented in Table 7. The SA revealed significantly smaller opening angles $\alpha$ and, therefore, smaller circumferential residual stresses than CIA, after both 30 min ($p = 0.02$) and 16 hrs ($p = 0.001$).
3.3. Microstructure

Fig. 12 shows representative intensity plots of the collagen fiber direction and distribution throughout the wall of one SA and one CIA of the same donor (patient II). The SA shows a thin intima and two counter-rotating fiber families in the media with some dispersion around the circumferential direction. The adventitial collagen in this sample reveals a low dispersion, pointing towards the axial direction. By contrast, the CIA shows a comparatively dispersed fiber structure in the intima, followed by fibers oriented very close to the circumferential direction ($\mu = 0^\circ$) throughout the media. The adventitia is relatively wavy and more dispersed in the CIA specimen compared to the SA, showing also a mean fiber direction towards the axial direction.

Table 8 shows a summary of all structural parameters and Fig. 13 shows box-and-whisker plots of the in-plane dispersion parameter $j_{ip}$, the out-of-plane dispersion parameter $j_{op}$ and the mean fiber angle $\mu$ for the media and adventitia for both SA and CIA at $\lambda_z = 1.1$.
The in-plane dispersion $\kappa_{ip}$ of the CIA media (0.17 ± 0.08) was significantly lower compared to the SA media (0.23 ± 0.05) ($p = 0.03$). The dispersion parameter $\kappa_{ip}$ of the adventitia for SA and CIA were not significantly different, while $\kappa_{ip}$ was significantly lower in the media for the CIA (0.17 ± 0.08) compared to the adventitia of the CIA (0.25 ± 0.04) ($p = 0.001$). No significant difference between the layers of the SA could be found. The out-of-plane dispersion parameter $\kappa_{op}$ showed no significant differences in any of the compared cases. Interestingly, the mean fiber angle $\mu$ was significantly lower in the CIA media (3.4 ± 1.5°) compared to the CIA adventitia (53.2 ± 22.5°) ($p < 0.001$), but showed no significant difference between the layers of the SA. Furthermore, the angle $\mu$ was significantly lower for the SA adventitia ($10.8 \pm 20.3^\circ$) compared to the CIA adventitia ($p = 0.03$), but significantly greater for the SA media ($11.1 \pm 20.4^\circ$) compared to the CIA media ($p < 0.001$).

### 3.4. Histology

Representative photomicrographs of cross-sections for SAs and CIAs from donor II stained with H&E are shown in Fig. 14. In Fig. 14(a) the media of the SA is thick and shows up to 70 layers of concentric elastic fibers (colored in black), which indicate that the SAs are of the elastic type. In Fig. 14(b) the media of the CIA is comparably thin with only a few elastic fibers, which suggests that the CIAs are of the muscular type. The higher elastin content in the media of SA is clearly visible as the micrograph (Fig. 14(a)) appears darker compared with the micrograph representing the CIA media (Fig. 14(b)).

Geometrical dimensions, i.e. outer and inner diameters, thicknesses of the wall and its layers as well as contents of collagen, smooth muscle cells and elastin of tested arteries obtained from histological investigations are shown in Table 9. Due to a slight

### Table 5

<table>
<thead>
<tr>
<th></th>
<th>SA (n = 10)</th>
<th>CIA (n = 10)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\sigma_{r}$ (kPa)</td>
<td>Median 45.4 [34.2;53.8]</td>
<td>Median 45.5 [45.2;57.8]</td>
<td>0.56</td>
</tr>
<tr>
<td>$\sigma_{zz}$ (kPa)</td>
<td>Median 34.5 [31.0;58.8]</td>
<td>Median 41.1 [19.1; 19.1]</td>
<td>0.02</td>
</tr>
<tr>
<td>$k_{r}$</td>
<td>Mean ± SD 3.4 ± 1.2</td>
<td>Mean ± SD 3.5 ± 1.1</td>
<td>1.00</td>
</tr>
<tr>
<td>$k_{z}$</td>
<td>Mean ± SD 1.4 ± 1.1</td>
<td>Mean ± SD 1.7 ± 1.6</td>
<td>0.20</td>
</tr>
<tr>
<td>SR</td>
<td>Mean ± SD 2.8 ± 1.4</td>
<td>Mean ± SD 2.3 ± 1.2</td>
<td>0.31</td>
</tr>
</tbody>
</table>

**Fig. 9.** Representative torsional behavior at different axial stretches for SA and CIA (patient II') in terms of torque vs rate of twist in (a) and (b) and axial Cauchy stress vs amount of shear in (c) and (d). At every axial stretch level, the inflation pressure $p_{i}$ was held constant at 13.3 kPa when the artery was twisted by ±25°. Dashed curves in (a) and (b) represent the entire loading-unloading cycle whereas solid curves represent the average of loading and unloading branches. The slope of the (solid) curves in diagrams (a) and (b) corresponds to the shear modulus $G$. Color legend for all panels is given in (a). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)
shrinking during histological preparations, dimensions of the stained tissues were smaller than the respective dimensions measured by the videotensometer (see Table 2). On average, SAs had smaller diameters and thicknesses than CIAs. Intimal hyperplasia thickness was greater in CIAs than in SAs. Interestingly, the intima was relatively thick in SAs and CIAs, with a similar extent to the adventitia. On average, the collagen content was less in SAs (44.3%) than in CIAs (51.4%), whereas the content of smooth muscle cells was higher in SAs (51.4%) than in CIAs (48.6%). Elastin content was determined to be higher in SAs than in CIAs. A high amount of elastin is a prerequisite for the elasticity of arteries and their associated Windkessel effect, which helps to maintain a relatively constant pressure in the arteries despite the pulsating nature of the blood flow.

Table 6
Shear moduli $G$ at different axial prestretches $\lambda_z$ (at constant $p_i = 13.3$ kPa) and at different transmural pressures $p_i$ (at constant $\lambda_z = 1.1$); $p$-values indicate possible statistical difference between medians of $G$ for SAs and CIAs, while $n$ indicates the number of considered specimens.

<table>
<thead>
<tr>
<th>$\lambda_z$</th>
<th>SA Median</th>
<th>SA Mean</th>
<th>CI Med</th>
<th>CI Mean</th>
<th>$n$</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0</td>
<td>435</td>
<td>576</td>
<td>6</td>
<td>188</td>
<td>422</td>
<td>9</td>
</tr>
<tr>
<td>1.05</td>
<td>497</td>
<td>714</td>
<td>11</td>
<td>334</td>
<td>627</td>
<td>11</td>
</tr>
<tr>
<td>1.1</td>
<td>654</td>
<td>963</td>
<td>12</td>
<td>445</td>
<td>781</td>
<td>10</td>
</tr>
<tr>
<td>1.15</td>
<td>848</td>
<td>1365</td>
<td>9</td>
<td>763</td>
<td>970</td>
<td>6</td>
</tr>
<tr>
<td>1.2</td>
<td>1303</td>
<td>1606</td>
<td>7</td>
<td>873</td>
<td>1046</td>
<td>4</td>
</tr>
</tbody>
</table>

Fig. 10. Axial Cauchy stress vs circumferential stretch behavior of selected experimental data of SAs (a), (b) and CIAs (c), (d) of a rather young donor XIV of age 43 (a), (c) and of an old donor XIII of age 92 (b), (d). Data points are at the intersections of parametric lines that specify constant axial stretches $\lambda_z$ and inflation pressures $p_i$. Plots show both circumferential stretches and axial stresses of selected specimens, and indicate remarkable differences between SA and CIA, and a clear correlation between age and distensibility/stiffness. Note that no data were available for CIA at $p_i = 26.7$ kPa of specimen XIII in (d).
4. Discussion

During surgical interventions surgeons experienced that SAs supplying the upper extremities appear more fragile and prone to damage during surgical repair than CIAs, supplying the lower extremities. To investigate this difference in a systematic way the aim of this study was to compare the biomechanical properties of these two arteries from the same donors in terms of geometry, extension-inflation-torsion behavior, residual stresses, microstructure, and histology. To the knowledge of the authors, this is the first study reporting extension-inflation-torsion data of human SAs and CIAs under physiological and supra-physiological loading conditions. In regard to cardiovascular medicine the material behavior of aged human arteries is of crucial interest. Moreover, the investigation of SA is important as it can help to improve surgical procedures at this challenging location. Over the long-term it might well be of value in the construction of artificial arteries for substituting native arteries. In addition, the analysis of mechanical stresses can improve design and material choice for endovascular implants to optimize long-term implant function.

4.1. Geometry

In this study the average outer diameters of SAs and CIAs \((n = 17)\) were 7.9 and 10.6 mm, respectively (Table 2). This is in good agreement with values for human CIA of 11.3 mm \((n = 16)\) and 9.9 mm \((n = 9)\) documented in [8,10]. However, a slightly larger mean outer diameter was reported for human SA \((D_o = 10.7 \text{ mm}, n = 12)\) in [10]. Average wall thickness values were 1.3 mm for SA and 1.8 mm for CIA (Table 2). Similar wall thickness values were found for human SA \((1.6 \text{ mm}, n = 12)\) and CIA \((2.0 \text{ mm}, n = 16)\) in [10]. A slightly thinner arterial wall was observed for human iliac arteries \((1.4 \text{ mm}, n = 9)\) in [8]. The average outer diameter to wall thickness ratio \(D_o/H\) was 6.4 for SA and 5.9 for CIA (Table 2). These ratios are in good agreement with 6.8 for human SA and 5.7 for human CIA published in [10]. However, a slightly larger ratio of 7.6 was found for human iliac arteries in [8].

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Opening angle (\alpha)</th>
<th>Opening angle (\beta)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>30 min</td>
<td>16 hrs</td>
</tr>
<tr>
<td>I</td>
<td>SA</td>
<td>CIA</td>
</tr>
<tr>
<td>II</td>
<td>100</td>
<td>115</td>
</tr>
<tr>
<td>IV</td>
<td>128</td>
<td>152</td>
</tr>
<tr>
<td>VI</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VII</td>
<td>135</td>
<td>134</td>
</tr>
<tr>
<td>VIII</td>
<td>101</td>
<td></td>
</tr>
<tr>
<td>IX</td>
<td>93</td>
<td>186</td>
</tr>
<tr>
<td>X</td>
<td>69</td>
<td></td>
</tr>
<tr>
<td>XI</td>
<td>65</td>
<td>159</td>
</tr>
<tr>
<td>XII</td>
<td>87</td>
<td>142</td>
</tr>
<tr>
<td>XIII</td>
<td>93</td>
<td>110</td>
</tr>
<tr>
<td>XIV</td>
<td>72</td>
<td>133</td>
</tr>
<tr>
<td>Median</td>
<td>93</td>
<td>138</td>
</tr>
<tr>
<td>IQR</td>
<td>30</td>
<td>43</td>
</tr>
<tr>
<td>Mean</td>
<td>90</td>
<td>130</td>
</tr>
<tr>
<td>SD</td>
<td>26</td>
<td>42</td>
</tr>
</tbody>
</table>
During the cardiac cycle, the axial stretch is approximately equal to the inversion stretch, i.e. arteries consume no ‘axial work’ in vivo.

4.2. Extension-inflation-torsion behavior

A particular axial stretch or inversion stretch \( \lambda \), where the axial force is basically independent of the transmural pressure, was observed for all tested arteries. This ‘inversion feature’ was highly characteristic [21,22] and found in all investigated arteries by us so far [8,12]. In average \( \lambda \) of the SA was 1.1 (see Table 3), the corresponding value of the CIA was 1.07, which is in good agreement with 1.08 reported in [8]. No values of \( \lambda \) for SA could be found in the literature. The axial in situ stretch of the artery is of crucial importance for finite element studies of in vivo arterial biomechanics. Interestingly, the axial in situ stretch is approximately equal to the inversion stretch in animal arteries [24,21], whereas aged human arteries showed smaller axial in situ stretches than inversion stretches [8]. However, from an energy-saving point of view, the axial in vivo stretch would be optimal when approximately equal to the inversion stretch, i.e. arteries consume no ‘axial work’ during the cardiac cycle.

At physiological conditions \((\lambda_p = 1.1, p_i = 13.3 \text{ kPa})\) SA was with \(\Delta\lambda_p = 0.10\) in average slightly stiffer in the circumferential direction than for CIA, with \(\Delta\lambda_p = 0.12\). Distinct larger mean values of \(\Delta\lambda_p\) were found for SA \((\sim 0.23)\) than for CIA \((\sim 0.16)\), however, from planar biaxial extension tests [10]. This discrepancy could be explained due to different experimental approaches and their essentially different boundary conditions. For the CIA a significant negative correlation between age and circumferential distensibility \((r = -0.67, p = 0.02)\) was obtained, which indicates a decreasing compliance of iliac arteries with advancing age. A similar correlation was observed for human iliac arteries in the study [8]. A correlation of this kind was not observed for human SA.

Mean Cauchy stresses in circumferential and axial directions at physiological conditions \((\lambda_p = 1.1, p_i = 13.3 \text{ kPa})\) were strikingly larger in CIA than in SA (see Table 5), which is in agreement with data from planar biaxial tests in [10]. The mean ‘physiological’ axial stress of 41.1 kPa obtained for CIA coincided well with the mean value of 42.2 kPa reported in [8] for CIA at physiological conditions \((F_z = 0.7 \text{ N}, p_i = 13.3 \text{ kPa})\). However, the mean ‘physiological’ circumferential stress of 50.9 kPa for CIA was larger than the corresponding stress of 43.5 kPa documented in [8]. Unfortunately, no ‘physiological’ Cauchy stresses could be found for SA in the

Table 8

<table>
<thead>
<tr>
<th>Specimen</th>
<th>In-plane dispersion (k_{ip})</th>
<th>Out-of-plane dispersion (k_{op})</th>
<th>Mean angle (\mu)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SA A</td>
<td>SA M</td>
<td>CIA A</td>
</tr>
<tr>
<td>I</td>
<td>0.23</td>
<td>0.19</td>
<td>0.20</td>
</tr>
<tr>
<td>II</td>
<td>0.27</td>
<td>0.27</td>
<td>0.25</td>
</tr>
<tr>
<td>III</td>
<td>0.20</td>
<td>0.21</td>
<td>0.26</td>
</tr>
<tr>
<td>IV</td>
<td>0.22</td>
<td>0.20</td>
<td>0.24</td>
</tr>
<tr>
<td>VIII I</td>
<td>0.27</td>
<td>0.23</td>
<td>0.31</td>
</tr>
<tr>
<td>IX</td>
<td>0.22</td>
<td>0.24</td>
<td>0.26</td>
</tr>
<tr>
<td>XII</td>
<td>0.25</td>
<td>0.28</td>
<td>0.23</td>
</tr>
<tr>
<td>XIII</td>
<td>0.24</td>
<td>0.23</td>
<td>0.25</td>
</tr>
<tr>
<td>XIV</td>
<td>0.21</td>
<td>0.21</td>
<td>0.21</td>
</tr>
<tr>
<td>Median</td>
<td>0.23</td>
<td>0.23</td>
<td>0.25</td>
</tr>
<tr>
<td>IQR</td>
<td>0.04</td>
<td>0.05</td>
<td>0.04</td>
</tr>
<tr>
<td>Mean</td>
<td>0.24</td>
<td>0.23</td>
<td>0.24</td>
</tr>
<tr>
<td>SD</td>
<td>0.02</td>
<td>0.03</td>
<td>0.03</td>
</tr>
</tbody>
</table>
literature. The circumferential stress-pressure slopes were equal for SAs \((k_h = 3.4)\) and CIAs \((k_h = 3.5)\), whereas axial stress-pressure slopes were remarkably greater for CIAs \((k_z = 1.7)\) than for SAs \((k_z = 1.4)\) \((p = 0.20)\) (see Table 5). The stiffer axial behavior of iliac arteries compared to SAs was also observed in biaxial extension tests [10], where the average axial stretches at mean arterial pressure were less for CIA than for SA. The corresponding slope ratios were larger than two for SA \((SR = 2.8)\) and CIA \((SR = 2.3)\), which indicates that both arteries bear more load in circumferential than in axial direction.

Pronounced hysteresis formation was observed during cyclic twisting of the arteries (Fig. 9(a) and (b)). In comparison, much smaller hystereses were obtained during torsion \((\pm 17^\circ)\) of rat thoracic aortas [25], although a higher rate of twist \(\sim 5.7^\circ\) s was applied. This discrepancy could be explained by the differences in shear properties of young rat aortas and aged human arteries. During torsion tests the shear modulus of SAs and CIAs increased with increasing axial prestretch and increasing inflation pressure (Table 6). A similar behavior was shown for rat thoracic aortas [25] and for porcine coronary arteries [26]. The study [27] reported a shear modulus of 220 kPa for human carotid arteries from very young donors at 120 mmHg, and in situ stretch. In contrast, we obtained a clearly larger average shear moduli at 120 mmHg and \(\lambda_z = 1.1\), with 1272 kPa for SA and 806 kPa for CIA, which indicates an increasing torsional stiffness with age. The axial Cauchy stress turned out to be slightly nonlinear with respect to an increasing torsion or amount of shear (Fig. 9(c) and (d)). A similar behavior was observed for the majority of SAs and CIAs and was also observed in other studies [9,25,28].

### 4.3. Residual stresses

The opening angles of circumferential and axial strips were determined in order to evaluate residual stresses in circumferential and axial directions of the investigated arteries. For circumferential strips of the CIA we found larger opening angles \((130^\circ \pm 39^\circ, \text{after } 30 \text{ min})\) than with respect to [8] \((94^\circ \pm 41^\circ)\) and [10] \((91^\circ \pm 36^\circ)\). The mean opening angle of CIA \((94^\circ \pm 27^\circ)\) for SA after 30 min is in good agreement with the value of 102 ± 26° published for SA in [10]. For both time ranges (30 min and 16 hrs), the mean opening angles \(\beta\) of axial strips from SA \((219^\circ, 30 \text{ min}; 221^\circ, 16 \text{ hrs})\) were smaller than the corresponding opening angles of CIA \((247^\circ, 30 \text{ min}; 258^\circ, 16 \text{ hrs})\). However, the differences were not statistically significant \((p_{30 \text{ min}} = 0.92, p_{16 \text{ hrs}} = 0.73)\). No opening angles of axial strips for SA and CIA were found in the literature.

### 4.4. Microstructural investigation

The in-plane collagen fiber dispersion defined by \(k_{ip}\) was significantly higher in the adventitia of the CIA than in the media of the CIA. A structural difference of this kind was not observed for the layers in the SA. Furthermore, the collagen fibers were more aligned (less dispersed) in the media of the CIA than in the media of the SA. Collagen fibers in the media of the CIA \((\mu = 3^\circ)\) were aligned closer to the circumferential direction than in the media of SA \((\mu = 11^\circ)\). On the other hand, the collagen fibers in the adventitia of the CIA were more aligned in the axial direction \((\mu = 53^\circ)\) compared to the fibers in the adventitia of the SA, which were more...
aligned towards the circumferential direction ($\mu = 11^\circ$). These results and the larger circumferential distensibility of the CIA than of the SA reveal that the adventitia would appear to contribute significantly to the passive mechanical response of arteries under physiological pressure and axial prestretch. The lower inversion stretch obtained for the CIA ($\lambda_z = 1.05$) compared to the SA ($\lambda_z = 1.11$) might be explained with the axially oriented collagen fibers in the CIA adventitia in comparison to circumferentially oriented fibers in the SA adventitia. The larger axial stresses in the CIA with respect to the SA at physiological conditions (13.3 kPa and $\lambda_z = 1.1$) is in accordance with the preferred axial collagen fiber orientation in the adventitia.

The circumferential to axial stress slope ratio larger than 2 for both SA ($SR = 2.8$) and CIA ($SR = 2.4$), indicates a more compliant behavior in the axial than in the circumferential direction, and can be explained by the, in general, preferred collagen fiber orientation in the wall to the circumferential direction. The higher ratio of the SA compared to the CIA results possibly from the smaller fiber angle in the adventitia of the SA. Moreover, the larger shear moduli for SA and CIA can possibly be explained by the higher dispersed collagen fibers and fewer fibers oriented to the circumferential direction in the SA compared to the CIA. The significantly lower opening angles in the SA than CIA might be explained by the higher dispersion and larger fiber angle, i.e. less fibers are aligned in the circumferential direction, in the media of the SA. The more pronounced intimal hyperplasia in the CIA with respect to the SA was also observed in the SHG images. In general, the intima was more pronounced in SHG images in the CIA, but almost non-existent in the SA.

4.5. Histological investigation

The average inner and outer diameters were clearly smaller for the SA ($D_i: 4.5$ mm; $D_o: 6.1$ mm) in comparison to the CIA ($D_i: 6.3$ mm; $D_o: 7.9$ mm) and the elastic fibers quantity was higher in the SA. Since this is an artery from the elastic type, the quantity of elastic fibers was increased in the SA in comparison to the CIA. The percentage of intimal hyperplasia was at a mean of 83% in the CIA specimens and on average 71% in the SA. To our knowledge, the literature does not present any comparable data. It was difficult to obtain tissue samples from a diverse group of donors. The predominated primary diseases and the causes of death of the donors had cardiovascular reason (see Table 1).

In some cases for no and small axial prestretches ($z_b = 1.0$ and 1.05), pressurization under isometric conditions (arterial segments were forced to keep their lengths) led to buckling of the arterial wall.
walls. If buckling occurred biomechanical data were only considered for larger axial prestretches where the arterial segment showed no buckling. Biomechanical data for small axial prestretches are thus lacking for cases such as these. Tightening the artery in a satisfactory manner proved to be a difficult task; sometimes the artery began to leak at higher pressures with the result that an incomplete data set only was obtained. Hence, the stated statistical measures (medians, quartiles, means and standard deviations) are based on different numbers of specimens and are therefore difficult to compare.

The arterial wall thickness is a crucial factor for stress calculations. However, reliable thickness measurements of the arterial wall are extraordinarily difficult to make due to the highly deformable adventitia, and hence data in this area might be afflicted with errors.

Another shortcoming of performing in situ experiments is that the contraction states of the arteries investigated were not determined. The vessel tone in vivo, i.e. the contraction of the smooth muscle cells, may have been higher than postmortem after vessel inflation. However, it has been demonstrated (for canine arteries) that passive and active vessel responses converge at pressures between 150 and 200 mmHg (20.0 and 26.7 kPa) [29]. Moreover, in situ experiments the smooth muscle cells may undergo rigor mortis in the absence of adequate nutrient and oxygen supply, which might effect the mechanical properties of the tested arteries. The perivascular loose connective tissue/tethering might also effect the mechanical properties of the arteries. To minimize this effect, we tried to remove the same amount of loose connective tissue from the adventitia for every artery.

The glue for mounting the strips on the cylinders might have some influence on the opening angle measurements. However, the choice of cyanoacrylate adhesive would appear to be the most reliable solution since this adhesive does not diffuse into the surrounding tissue. The potential effect of the adhesive is, therefore, very localized, and does not influence the global deformation behavior of the tissue samples. In some cases, while cutting the ring or preparing the axial strip, single layers were separated from each other. Moreover, it was difficult to measure the opening angles of non-circular-shaped strips. In both above-mentioned cases it was difficult to define the locations of the measuring points, which might have led to measurement errors.

5. Conclusion

The present study aimed at evaluating the differences in the biomechanical properties of SAs and CIAs, which are often injured during trauma to the upper and lower limbs. SAs were determined to be stiffer than circumferential loadings and torsion/twisting, whereas CIAs showed a higher axial stiffness. The higher circumferential and torsional stiffness of the SAs might be an explanation for the more susceptible nature of SAs during surgical repair after injury. Moreover, the current increase in vascular injuries and diseases is resulting in an urgent demand for appropriate experimental data to permit a better understanding of the main characteristics of arterial functions in physiology and pathophysiology. The experimental data presented here are thus intended to derive new constitutive model parameters for SAs and CIAs incorporating extension, inflation, torsion and residual stress data and also microstructural and histological data. This comprehensive set of data will be able to validate state-of-the-art constitutive models or even derive new material models able to predict more realistic stresses and strains in finite element simulations of, e.g., surgical interventions of SA injury repair.

Acknowledgement

The authors are indebted to Dr. Heimo Wolinksi, Institute of Molecular Biosciences, BioTechMed-Graz, University of Graz, Austria, and the Institute of Science and Technology, Klosternerbarg, Austria, for their valuable support in SH imaging. In addition, we would like to thank A. Donnerer and his team from the Institute of Pathology, Medical University of Graz, for their valuable support during tissue harvesting.

References


