Duration of no-load state affects opening angle of porcine coronary arteries

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Rehal, Devinder, Xiaomei Guo, Xiao Lu, and Ghassan S. Kassab. Duration of no-load state affects opening angle of porcine coronary arteries. Am J Physiol Heart Circ Physiol 290: H1871–H1878, 2006. First published December 9, 2005; doi: 10.1152/ajpheart.00910.2005.—The zero-stress state of a blood vessel has been extensively studied because it is the reference state for which all calculations of intramural strain and stress must be based. It has also been found to reflect nonuniformity in growth and remodeling in response to chemical or physical changes. The zero-stress state can be characterized by an opening angle, defined as the angle subtended by two radii connecting the midpoint of the inner wall. All prior studies documented the zero-stress state or opening angle with no regard to duration of the no-load state. Our hypotheses were that, given the viscoelastic properties of blood vessels, the zero-stress state may have “memory” of prior circumferential and axial loading, i.e., duration of the no-load state influences opening angle. To test these hypotheses, we considered ring pairs of porcine coronary arteries to examine the effect of duration in the no-load state after circumferential distension. Our results show a significant reduction in opening angle as duration of the no-load state increases, i.e., vessels that are reduced to the zero-stress state directly from the loaded state attain much larger opening angles at 30 min after the radial cut than rings that are in the no-load state for various durations. To examine the effect of axial loading, we found similar reductions in opening angle with duration in the no-load from the in situ state, albeit the effect was significantly smaller than that of circumferential loading. Hence, we found that the zero-stress state has memory of both circumferential and axial loading. These results are important for understanding viscoelastic properties of coronary arteries, interpretation of the enormous data on the opening angle and strain in the literature, and standardization of future measurements on the zero-stress state.

The zero-stress state of a blood vessel has been extensively studied because it is the reference state for which all calculations of intramural strain and stress must be based. The zero-stress state is also the best state in which to study tissue remodeling because any change in structure can be documented without the effect of stress or strain (4). Changes in the zero-stress state and opening angle provide a simple index of the nonuniformity of growth and remodeling (4).

There are tremendous data on the opening angle of blood vessels from different vessels and species. Previous studies have included opening angle measurements along the aortic tree in rats (15) and pigs and dogs (8), the coronary arterial tree of pigs (3, 7) and dogs (11), systemic arteries of cows (27), systemic veins of rats (28), left ventricles of rats (20) and dogs (19), duodenum of guinea pigs (6), trachea of pigs and dogs (9), and pulmonary arteries and veins of humans (10). Significant data are also available on the remodeling of opening angles in various conditions such as hypertension and hypertrophy (16), age and atherosclerosis (25), diabetes (17), and cigarette smoking (18). It is concluded that the opening angle varies significantly along the arterial tree with large scatter; typical values for opening angles along the porcine aortic tree vary between 20° and 160° (7, 15) and between 89° and 128° degrees along the human arterial tree (10).

As described by Fung (4) and others (27), the zero-stress state of a vessel can be revealed by a radial cut of a vessel ring in the no-load state (zero transmural pressure). The vessel is first reduced from the loaded (pressurized) state to a ring in the no-load state by making two transverse cuts to the long axis of the vessel. A subsequent radial cut causes the vessel ring to spring open into a vessel sector that can be characterized by an opening angle, defined as the angle subtended by two radii connecting the midpoint of the inner wall (15). All prior studies have determined the zero-stress state or opening angle with no regard to the duration of the no-load state, i.e., the period between the first two transverse cuts and the radial cut. At some unspecified time after the two transverse cuts, the vessel sector is cut radially and the resulting sector is considered as the zero-stress state, typically 30 min after the radial cut.

Our hypotheses were that, given the viscoelastic properties of blood vessels, the duration of the no-load state may affect the opening angle attained 30 min after the radial cut for both circumferential and axial loading. To test the former hypothesis, silicone elastomer was perfused through the porcine left anterior descending coronary artery (LAD) to distend the vessel to physiological pressure and two adjacent rings were studied. To test the latter hypothesis, similar ring pairs were compared after immediate excision from the in situ state, which has axial prestretch compared with vessel rings cut radially. Our results confirm the proposed hypotheses for circumferential and axial loading. A viscoelastic Kelvin model was used to describe the time course of deformation in the zero-stress state. The limitations and implications of the results are discussed in light of previous and future studies on vascular zero-stress state.

METHODS

Effect of circumferential loading. Six hearts weighing 253 ± 18.9 g from Yorkshire farm pigs of either gender were obtained from a local abattoir on the day of the experiment. Immediately after the pig was killed, the heart was excised and placed in a cold saline bath and transported to our laboratory. The left coronary artery was cannulated and perfused with 6% dextran solution to flush out the blood. The LAD was then perfused at physiological pressure of 100 mmHg with catalyzed silicone elastomer containing Cab-O-Sil (to block the flow of...
through small arteries and capillaries in order to ensure uniform pressure throughout the coronary arterial tree). After the elastomer was allowed to harden for about an hour, the LAD was carefully dissected down to ~0.5-mm diameter branches (epicardial coronary arteries, EPCA). The anterior side of the LAD and its branches were labeled with carbon black before dissection. After dissection, the LAD and its branches were placed in a Ca²⁺-free Krebs solution, maintaining the elastomer in the lumen. The vessels were cut perpendicular to the long axis into rings (length of ~1 mm) with a sharp blade and placed in a Ca²⁺-free Krebs solution aerated with 95% O₂-5% CO₂. Care was taken to maintain the elastomer in the lumen, and ring pairs in situ and no-load states. Length; adjacent rings cut and reduced to zero-stress state from no-load states. Rings cut and reduced to zero-stress state from loaded and no-load states.

A Fig. 1. Schematic of experimental protocol for circumferential loading: vessel distended with hardened elastomer; adjacent rings cut and reduced to zero-stress state from loaded and no-load states. B: axial loading: vessel stretched to in situ length; adjacent rings cut and reduced to zero-stress state from in situ and no-load states.

Effect of axial loading. Six hearts weighing 271 ± 62.8 g from Yorkshire farm pigs of either gender were obtained as described above and transported to our laboratory. After dissection of the LAD EPCA, the two ends of the arterial segments were ligated and stretched to their in situ length for ~1 h in a Ca²⁺-free Krebs solution bath aerated with 95% O₂-5% CO₂. The in situ length was determined by placing markers (carbon black) on the surface of the artery in situ. Similar ring pairs were examined. The first ring of each pair was labeled with carbon black before dissection. After dissection, the LAD and its branches were placed in a Ca²⁺-free Krebs solution aerated with 95% O₂-5% CO₂ as shown schematically in Fig. 1A. In one ring, the elastomer was removed to reduce the ring to the no-load state, the no-load was photographed, and an immediate (within 15–30 s) radial cut was made to reveal the zero-stress state. After 30 min, the zero-stress state was photographed. In the second ring, the elastomer was also removed, the no-load state photographed, and the radial cut made after various durations in the no-load state (from 0.5 to 6 h) for different ring pairs. The zero-stress state was also photographed 30 min after the radial cut (Fig. 1A). Similarly, an additional six hearts weighing 220 ± 43.1 g were used to dissect intramyocardial coronary arteries (IMCA). In summary, in each adjacent ring pair, one ring was reduced to the zero-stress state almost directly from the in situ state, whereas the adjacent ring was reduced to the zero-stress state after some duration (0.5 to 6 h) in the no-load state (Fig. 1B). The use of the two adjacent rings for the former and latter procedures was again randomized.

Zero-stress state. To investigate whether the opening angle attains the same value (from different initial conditions), two sets of rings were digitally photographed over time. With the approach outlined above, a radial cut was made from the loaded state of one ring (from each pair), and the zero-stress state was photographed immediately (within 15–30 s) and followed for a period of 6 h. The elastomer was reduced from the second ring of the pair, and the radial cut was made after either 1 or 3 h in the no-load state. Photographs of the zero-stress state were taken at the same time points. A total of 12 ring pairs were examined from 2 hearts (288 ± 17.7 g) for the 1-h no-load state and another 12 ring pairs from 2 additional hearts (239 ± 12.7 g) for the 3-h no-load state.

Biomechanical analysis. The circumferential deformation of an artery can be described by the midwall circumferential stretch ratio ($\lambda$), defined as:

$$\lambda = \frac{C_{m}^{p}}{C_{m}^{ZSS}}$$

(1)

where $C_{m}^{p}$ is the midwall circumference of the vessel in the loaded state and $C_{m}^{ZSS}$ is the midwall circumference in the zero-stress state. The average circumferential Cauchy stress ($\tau$) can be computed for a cylindrical vessel wall at an equilibrium condition as follows:

$$\tau = \sigma_m \frac{P}{A}$$

where $\sigma_m$ is the midwall stress and $P$ is the pressure.
where $\lambda$ is defined in Eq. 1. Because the loading and dimensional parameters were measured, the stress and deformation were computed.

Viscoelastic model. To model the viscoelastic behavior of the vessel when the stress is suddenly decreased as a step function (radial cut in condition 1, Fig. 1A) from loaded state to zero-stress state, the Kelvin model (or standard linear solid) was used. The Kelvin model is composed of a combination of linear springs (with spring constants $\mu_0$ and $\mu_1$) and a dashpot (with coefficient of viscosity $\eta_1$) as shown in Fig. 2. The stress-strain ($\sigma$-$\varepsilon$) equation for the Kelvin model can be stated as:

$$\sigma + \tau_\varepsilon \frac{d\varepsilon}{dt} = E_0 \left[ \varepsilon + \tau_\varepsilon \varepsilon \right]$$

where $\mu_0$ = ER, $\mu_1$ = $(\tau_\varepsilon - \tau_\sigma)/\tau_\varepsilon$, $\eta_1 = \tau_\varepsilon \mu_1$, and $t$ is time (2). The corresponding creep solution of Eq. 4a was solved for the creep recovery response (i.e., $\sigma = 0$):

$$\varepsilon(t) = \frac{1}{E_0} \left[ 1 - \left( 1 - \frac{\tau_\varepsilon}{\tau_\sigma} e^{-\frac{\tau_\varepsilon}{\tau_\sigma} t} \right) \right] H(t)$$

where $H(t)$ is the heavy-side step function (4). Equation 4a was solved for the creep recovery process (i.e., $\sigma = 0$):

$$\varepsilon(t) = e_0 \left( e^{-\frac{\tau_\varepsilon}{\tau_\sigma} t} \right)$$

where $e_0$ is the initial strain. Experimental data were obtained from the zero-stress state (photographs within 15–30 s after radial cut from loaded state and followed for a period of 6 h). A total of 26 rings were examined from 6 hearts (256 ± 27.7 g).

The material constants for the Kelvin model (ER, $\tau_\sigma$, and $\tau_\varepsilon$) were determined from a curve fit of the strain data with an equation of the form:

$$\varepsilon(t) = A - B e^{-\chi t}$$

This is, of course, analogous to Eq. 4b, with $A = 1/E_0$, $B = (\tau_\varepsilon - \tau_\sigma)/(E_0 \tau_\varepsilon)$, and $\chi = 1/\tau_\varepsilon$. A nonlinear least squares yielded the values of the constants $A$, $B$, and $\chi$, which were used to calculate $E_0$, $\tau_\sigma$, and $\tau_\varepsilon$ as $E_0 = 1/A$, $\tau_\sigma = 1/C$, and $\tau_\varepsilon = (A - B)/CA$. These values were then used to determine $\mu_0$, $\mu_1$, and $\eta_1$ as outlined above. These values were in turn used in Eq. 4c to predict strain, which was compared with the experimental data for the 6-h period. The correlation coefficient of the fit was determined.

**RESULTS**

As described above, the measurements of opening angle from the loaded state were made within 15–30 s of the radial cut. We measured the initial opening angles with a video camera from $t = 5$ s to 1 min. We found that within the first 60 s, the change in opening angle is linear, as shown in Fig. 3 ($n = 6$ rings). Furthermore, we confirmed that the variation in opening angle in the 15- to 30-s interval was small (7.7%; 234°).

**Fig. 3. Temporal variation of opening angle within the first 1 min after the radial cut from the loaded state. Solid line represents a linear fit of average opening angle $\theta$ over 6 rings and is given by $\theta = 1.09r + 201$, where $r$ is time, $R^2 = 0.98$. A 2-way ANOVA statistics analysis reveals significant linear variation ($P < 0.01$).**

**Smooth muscle and osmolarity effects.** To test the effect of smooth muscle cells on the zero-stress state, sodium nitroprusside was used. Twelve rings were cut radially after excision of the LAD from the in situ state. The zero-stress state was photographed after 30 min. The rings were then placed in a Ca$^{2+}$-free Krebs solution with sodium nitroprusside (10$^{-5}$ M), and the zero-stress state was photographed after 10 min. To test for the effect of osmotic pressure, a similar experiment was repeated with varying concentrations of mannitol (0–423 mosM). Twelve rings from the LAD artery were cut radially from the loaded state. The zero-stress state was photographed after 30 min. The rings were placed in solutions with increasing concentrations of mannitol (increasing osmolarity) for 10 min each.

**Data analysis.** The difference in opening angle or strain for circumferential (conditions 1 and 2 in Fig. 1, A and B, respectively) and axial loading (conditions 3 and 4 in Fig. 1, C and D, respectively) were fitted with a nonlinear least squares fit of the form:

$$\Delta X = \alpha (1 - \beta e^{-\chi t})$$

where $X$ represents opening angle or strain and $\alpha$, $\beta$, and $\chi$ are empirical constants obtained from a nonlinear least squares fit. Equation 5a can be expressed as:

$$\Delta X = \Delta X^0 + (\Delta X^0 - \Delta X^\infty) e^{-\ln 2/(\tau_\sigma)}$$

where $\Delta X^0 = \alpha$, $\Delta X^\infty = \alpha (1 - \beta)$, and $t_{1/2} = \ln 2/\chi$. $\Delta X^0$ and $\Delta X^\infty$ represent the difference in either opening angle or strain at $t = 0$ and $t = \infty$, respectively, and $t_{1/2}$ represents the time required for $\Delta X$ to reach 50% of its final value.

**Ordering of coronary arterial branches.** Our group previously developed (12) a diameter-defined Strahler system that provides a unique relationship between vessel diameter and order number for the entire coronary arterial tree. Hence, we classified the following diameters into their respective orders: order 1 ($>2.19$ mm), order 10 (2.18–0.99 mm), and order 9 (0.98–0.55 mm).

**Statistical analysis.** Each arterial ring was considered as an independent sample. Plotted values are mean values with SE bars. The differences in the opening angle or strain were examined with one-way ANOVA. The results were considered significant when $P < 0.05$. 

**RESULTS**

As described above, the measurements of opening angle from the loaded state were made within 15–30 s of the radial cut. We measured the initial opening angles with a video camera from $t = 5$ s to 1 min. We found that within the first 60 s, the change in opening angle is linear, as shown in Fig. 3 ($n = 6$ rings). Furthermore, we confirmed that the variation in opening angle in the 15- to 30-s interval was small (7.7%; 234°).
Data are presented for strain with time. The solid line represents a nonlinear least squares fit of interval (between 15 and 30 s) for all subsequent data presented vs. 217°. Therefore, a manual photograph was used in all states.

**Fig. 4. Circumferential loading (difference between loaded and no-load states).** A: difference (Δ) in opening angle (opening angle measured 30 min after radial cut) with time in the no-load state. B: difference in midwall Green’s strain with time. The solid line represents a nonlinear least squares fit of Eq. 5b presented for orders 11, 10, and 9 epicardial coronary arteries (EPCA) and order 9 intramyocardial coronary arteries (IMCA).

Effect of circumferential loading. The time course of the difference in opening angle ΔOA (between loaded and no-load states) is shown in Fig. 4A (for 3 orders of EPCA and 1 order of IMCA) and fitted with Eq. 5b. The values of ΔOA0, ΔOA−, and t1/2 are presented in Table 1 for EPCA of orders 11, 10, and 9 and IMCA of order 9. The correlation coefficients (R2) of the curve fit are presented in Table 1 as well. The results are statistically significant after 1 h for order 11 (P = 0.013) and after 2 h for both orders 10 and 9 (P = 0.031 and P = 0.041, respectively). The differences in Green’s strain E between loaded and no-load states were also computed and are shown in Fig. 4B. The data were similarly curve fitted by an equation of the form of Eq. 5b with the empirical constants (ΔE0, ΔE−, and t1/2) presented in Table 2. R2 of the curve fit are also presented in Table 2.

To determine whether stress or strain is responsible for the difference in opening angle, correlations of Δstress and Δstrain with ΔOA were examined. The degree of correlation of the data was determined. The relation between ΔOA and Δstress yielded poor correlation coefficients for order 11 (R2 = 0.0252), order 10 (R2 = 0.0612), and order 9 (R2 = 0.157) EPCA. In addition, P values (calculated with Pearson correlation) indicated no relation between ΔOA and Δstrain for order 11 (P = 0.734), order 10 (P = 0.593), and order 9 (P = 0.380) EPCA. The correlations between ΔOA and Δstrain are largely significant, however, as shown in Fig. 5, A–D, for orders 11, 10, and 9 EPCA and order 9 IMCA, respectively (n = 6 for each heart). Table 3 summarizes the results of the linear curve fit of ΔOA and Δstrain for each heart. Although some P values for EPCA are slightly larger than 0.05, P values for the average of all data indicate a positive correlation between ΔOA and Δstrain for order 11 (P = 0.0021), order 10 (P = 0.00978), and order 9 EPCA (P = 0.0088). However, the P value for average order 9 IMCA data indicates no significant correlation between ΔOA and Δstrain (P = 0.447).

Effect of axial loading. Similarly, data for the temporal variation of ΔOA (between in situ and no-load states) were fitted with Eq. 5b and are shown in Fig. 6. The empirical values of the curve fit (ΔOA0, ΔOA−, and t1/2) are presented in Table 4 for EPCA of orders 11, 10, and 9 and IMCA of order 9. The results are statistically significant after 2 h for both orders 11 and 9 (P = 0.031 and P = 0.048, respectively) and after 3 h for order 10 (P = 0.027).

**Table 2. Circumferential loading: difference in strain between loaded and no-load states**

<table>
<thead>
<tr>
<th>Order</th>
<th>ΔE0</th>
<th>ΔE−</th>
<th>t1/2</th>
<th>R2</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td>8.4×10−3</td>
<td>0.22</td>
<td>0.97</td>
<td>0.952</td>
</tr>
<tr>
<td>10</td>
<td>6.5×10−3</td>
<td>0.17</td>
<td>0.67</td>
<td>0.959</td>
</tr>
<tr>
<td>9 (EPCA)</td>
<td>4.2×10−3</td>
<td>0.12</td>
<td>0.56</td>
<td>0.966</td>
</tr>
<tr>
<td>9 (IMCA)</td>
<td>1.4×10−3</td>
<td>0.10</td>
<td>0.23</td>
<td>0.961</td>
</tr>
</tbody>
</table>

Data are empirical coefficients of nonlinear least squares fit of Eq. 5b presented for orders 11, 10, and 9 epicardial coronary arteries (EPCA) and order 9 intramyocardial coronary arteries (IMCA). ΔE, difference in strain between loaded and no-load states; R2, correlation coefficient of the curve fit.

The zero-stress state. Figure 7 shows the temporal variation of opening angle for different initial conditions. The top curve in Fig. 7 corresponds to a loaded ring immediately reduced to zero-stress state. The opening angle is initially large and decreases over time. The two lower curves in Fig. 7 correspond to rings reduced to the zero-stress state from the no-load state for two different durations (1 and 3 h, respectively). The opening angle increases in those cases toward an asymptotic value. The opening angle approaches the same value after 3 h for all three cases.

**Table 1. Circumferential loading: difference in opening angle between loaded and no-load states**

<table>
<thead>
<tr>
<th>Order</th>
<th>ΔOA0</th>
<th>ΔOA−</th>
<th>t1/2</th>
<th>R2</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td>−2.2</td>
<td>130</td>
<td>1.34</td>
<td>0.971</td>
</tr>
<tr>
<td>10</td>
<td>−0.74</td>
<td>55.3</td>
<td>0.67</td>
<td>0.931</td>
</tr>
<tr>
<td>9 (EPCA)</td>
<td>0.49</td>
<td>34.2</td>
<td>1.05</td>
<td>0.943</td>
</tr>
<tr>
<td>9 (IMCA)</td>
<td>0.47</td>
<td>13.4</td>
<td>0.82</td>
<td>0.934</td>
</tr>
</tbody>
</table>

Data are empirical coefficients of nonlinear least squares fit of Eq. 5b presented for orders 11, 10, and 9 epicardial coronary arteries (EPCA) and order 9 intramyocardial coronary arteries (IMCA). ΔOA, difference in opening angle between loaded and no-load states; t1/2, time to reach 50% of final value; R2, correlation coefficient of the curve fit.
Viscoelastic model. Average values for the temporal variation of the difference in strain from Fig. 4B were fitted with Eq. 4b ($R^2 = 0.958$). The resulting Kelvin model parameters for the creep response of the vessel rings cut from the loaded state are $\mu_0 = 4.86$ mmHg, $\mu_1 = 49.7$ mmHg, and $\eta_1 = 5.83$ mmHg·h. The creep recovery response is presented in Fig. 8. The reference state for these strain measurements is taken at $t = 6$ h. The solid vertical line in Fig. 8 represents the step drop in strain after the radial cut. The zero-stress state creep recovery data were compared with the model of Eq. 4c with the model parameters determined from the creep response ($R^2 = 0.874$).

Smooth muscle and osmolarity effects. Vessel rings used to test for the effect of smooth muscle cells had an average opening angle of $107 \pm 17.3^\circ$ after excision from the in situ state (opening angle measured 30 min after radial cut). After treatment with sodium nitroprusside, the rings had an average opening angle of $120 \pm 15.6^\circ$. The difference in opening angle due to nitroprusside was not statistically significant ($P = 0.060$). Similarly, the difference in opening angle due to an osmotic effect was not statistically significant ($233.5 \pm 0.38^\circ$ at 0 mosM and $246.3 \pm 6.7^\circ$ at 423 mosM; $P = 0.333$).

**DISCUSSION**

Effect of duration in no-load state. For EPCA, the circumferential data show a reduction in the opening angles from the

<table>
<thead>
<tr>
<th>Order</th>
<th>Heart 1</th>
<th>Heart 2</th>
<th>Heart 3</th>
<th>Heart 4</th>
<th>Heart 5</th>
<th>Heart 6</th>
<th>Average</th>
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<tbody>
<tr>
<td>11</td>
<td>339</td>
<td>461</td>
<td>653</td>
<td>1040</td>
<td>553</td>
<td>939</td>
<td>742.3</td>
</tr>
<tr>
<td>$R^2$</td>
<td>0.679</td>
<td>0.801</td>
<td>0.812</td>
<td>0.545</td>
<td>0.705</td>
<td>0.548</td>
<td>0.871</td>
</tr>
<tr>
<td>$P$</td>
<td>0.0227</td>
<td>0.0065</td>
<td>0.0056</td>
<td>0.0580</td>
<td>0.0181</td>
<td>0.0572</td>
<td>0.00210</td>
</tr>
<tr>
<td>10</td>
<td>316</td>
<td>474</td>
<td>303</td>
<td>362</td>
<td>283</td>
<td>384</td>
<td>405</td>
</tr>
<tr>
<td>$R^2$</td>
<td>0.750</td>
<td>0.620</td>
<td>0.323</td>
<td>0.604</td>
<td>0.662</td>
<td>0.337</td>
<td>0.767</td>
</tr>
<tr>
<td>$P$</td>
<td>0.0118</td>
<td>0.0281</td>
<td>0.1030</td>
<td>0.0398</td>
<td>0.0259</td>
<td>0.1110</td>
<td>0.00978</td>
</tr>
<tr>
<td>9 (EPCA)</td>
<td>392</td>
<td>150</td>
<td>435</td>
<td>266</td>
<td>375</td>
<td>413</td>
<td>434.4</td>
</tr>
<tr>
<td>$R^2$</td>
<td>0.776</td>
<td>0.352</td>
<td>0.514</td>
<td>0.578</td>
<td>0.509</td>
<td>0.645</td>
<td>0.771</td>
</tr>
<tr>
<td>$P$</td>
<td>0.0088</td>
<td>0.1210</td>
<td>0.0697</td>
<td>0.0472</td>
<td>0.0718</td>
<td>0.0296</td>
<td>0.00880</td>
</tr>
<tr>
<td>9 (IMCA)</td>
<td>181</td>
<td>75.1</td>
<td>−57.8</td>
<td>165</td>
<td>−73.0</td>
<td>−484</td>
<td>−112</td>
</tr>
<tr>
<td>$R^2$</td>
<td>0.150</td>
<td>0.046</td>
<td>0.109</td>
<td>0.395</td>
<td>0.016</td>
<td>0.372</td>
<td>0.120</td>
</tr>
<tr>
<td>$P$</td>
<td>0.389</td>
<td>0.645</td>
<td>0.469</td>
<td>0.130</td>
<td>0.784</td>
<td>0.145</td>
<td>0.447</td>
</tr>
</tbody>
</table>

Data are slope $\alpha$ of correlation obtained from linear curve fit of $\Delta$strain and $\Delta$OA for each heart ($n = 6$) presented for orders 11, 10, and 9 EPCA and order 9 IMCA. $R^2$, correlation coefficient of the linear least squares fit.
loaded to no-load states (larger difference in opening angle with time). This implies that distended vessels attain larger opening angles, with the effect fading after several hours. In addition, our axial data show a reduction in the opening angles from the in situ to no-load states for EPCA. Therefore, we found that the zero-stress state depends on the time period from initial circumferential and axial loading. The circumferential effect is larger than the axial (Figs. 4A and 5, respectively) because the open sector primarily reveals circumferential residual strain. In summary, our results show that the opening angle depends on the duration of the no-load state, i.e., the opening angle is larger after 30 min if pressurized vessels are cut immediately and the opening angle decreases as the duration in the no-load state increases. This is the first report to point out the significance of duration in the no-load state, which has important implications for future zero-stress state (opening angle) studies. It may also explain the significant variability in the previous opening angle measurements reported in the literature (24), because the duration of the no-load state was not controlled.

Role of strain in viscoelastic response. The present data show that there is no correlation between the difference in opening angle and initial Cauchy stress. We found a significant positive correlation, however, between the difference in opening angle (condition 1 minus condition 2) and the initial Green’s strain E for EPCA (Fig. 5, A–C). In general, the slope of the linear curve fit decreases toward the lower orders as seen in Table 3. The slope of the linear curve fit for order 11 is significantly larger than that of order 10 (P = 0.026) and order 9 (P = 0.022). The slopes of orders 10 and 9 were not significantly different (P = 0.784). This implies that a smaller change in strain will cause a larger change in opening angle for order 11 compared with order 10 or 9. Order 9 IMCA showed no correlation between the difference in opening angle and strain (Fig. 5D).

Epicardial vs. intramyocardial arteries. In both circumferential and axial loading, order 9 IMCA showed no temporal variation in the difference in opening angle compared with order 9 EPCA (Figs. 4A and 6, respectively). One obvious difference is that the range of change in strain is significantly smaller for the IMCA vessels compared with the EPCA. This is, of course, due to myocardial tethering, i.e., IMCA vessels are surrounded by myocardium and their deformation and stress are small. Unlike EPCA vessels, whose creep behavior reflects the change in stress, the IMCA vessels have a much smaller change in stress and thus a much smaller creep response.

Zero-stress state. From Fig. 7, it is clear that the opening angle decreases with time for a vessel ring cut from the loaded state, whereas the opening angle increases with time for a vessel ring cut from the no-load state. The former is a novel observation, whereas the latter is well documented (e.g., Ref. 3). Our data suggest that the opening angle will reach the same value after 3 h despite the duration in the no-load state (1 vs. 3 h). Hence, we have shown that the opening angle value depends on two factors: the time in the no-load state and the change in strain.
time after the radial cut. Although the latter is well documented, we would like to call attention to the former.

Here we show that, despite the duration in the no-load state, a unique opening angle can be achieved after 3 h. Because it is common practice to consider the zero-stress state 30 min after the radial cut, the no-load state duration will impact the resulting opening angle. Hence, if the opening angle is measured 30 min after the radial cut, it is essential to specify the duration in the no-load state. After 3 h in the zero-stress state, the duration of the no-load state becomes insignificant. Hence, a possible recommendation would entail the measurement of the opening angle after 3 h rather than 30 min, which would minimize the effect of the duration of the no-load state and serve to standardize future data on the zero-stress state.

**Viscoelasticity.** Several studies have attempted to explain the viscoelastic behavior of blood vessels in relation to the role of smooth muscle cells (8), osmotic effects (13) and microstructural elements such as collagen and elastin (29). Because the opening angle observed after the vessel sectors were placed in sodium nitroprusside did not change significantly, we conclude that the changes in the opening angle are not due to the contraction of smooth muscle cells. This result has also been confirmed by Han and Fung (8), using papaverine.

The effect of osmolarity on the opening angle of the ventricle has been examined by Lanir et al. (13). Because residual strain is caused by the interaction of internal forces related to the microstructure, they reasoned that collagen and elastin fibers in the intercellular matrix may exert forces that may increase the residual strain caused by increased swelling. Their experiments showed that the opening angle from rat left ventricle decreased with increasing osmolarity as the heart was perfused with different concentrations of mannitol, i.e., the opening angle increased with swelling. This signifies that myocardial swelling affects the opening angle by increasing the intercellular distances. In the present study, we immersed vessel rings in different concentrations of mannitol to test for an osmotic effect. The change in opening angle data was not statistically significant. Hence, the osmotic effect is not significant in coronary vessels. It is interesting that the heart and vessels may behave differently in regard to osmotic effects.

The creep response of blood vessels is likely to have a basis in the microstructural components of the ventricle wall (15). Zeller and Skalak (29) reported an increase in opening angle after degradation of matrix components (collagen and elastin). Their results indicate that extracellular matrix components may be responsible for changes in opening angle. They hypothesized that collagen and elastin may play a crucial role in maintaining the opening angle, which is reasonable because collagen and elastin exhibit some viscoelastic properties (4). Purslow et al. (22) tested the hypothesis that time-dependent reorientation of collagen fibers is responsible for viscoelasticity of soft connective tissues. Because no large-scale reorientation of collagen fibers was seen during the time course of creep and stress relaxation, their results indicate that reorientation of collagen fibers is not the principal mechanism underlying the viscoelastic behavior of connective tissue. They (22) and others (29) have suggested that the viscoelastic mechanism resides within the collagen fibers or at the interface between the fibers and the surrounding matrix.

Microstructurally, the ring cut from the loaded state has collagen fibers that are stretched (30). After the loading is relieved (radial cut), the fibers will recoil. In a perfectly elastic body, the recoil would occur instantaneously (no time dependence) and with no loss of energy in the form of friction. Because collagen is a viscoelastic material (29), the retraction will be a time-dependent process. In addition, there will be some internal friction due to the movement of the collagen fibers in the fluid of the surrounding matrix. Obviously, this effect is different if the collagen fibers are not initially stretched as the cut is made from the no-load state. This may also explain the difference between EPCA (large stretch or strain) and IMCA (small strain). This mechanism is speculative and requires validation in future studies.

In cartilage, collagen is thought to contribute significantly to the viscoelasticity in tensile testing (14). In tendons, the primary mechanism for viscoelasticity is thought to be related to glycosaminoglycan composition due to friction of flow through glycosaminoglycan-associated water and/or solid interactions between the extracellular matrix and the collagen fibers (2). In the myocardium, fibrillar collagen is thought to be the major source of viscoelasticity within the extracellular matrix (1, 26). The degree of viscoelasticity has been reported to be proportional to the content of collagen present and the ratio of type I to type III collagens (21).

Smooth muscle cells exhibit even larger hysteresis than collagen (4). To determine the role of collagen, elastin, or passive smooth muscle cells on the viscoelastic behavior of the vessel wall, future investigations are needed. Selective degradation or elimination of each component in turn may provide insight into the contribution of each cell type. Until then, a phenomenological model such as the one presented in Figs. 2 and 8 is needed to represent the viscoelastic behavior of the vessel wall.

**Critique of methods.** Silicone elastomer was used to distend the vessels at physiological pressure to compute the stress and strain at loaded state. Elastomer is an inert material mixed with a catalyst (7% ethyl silicate and 3.5% tin octate) to solidify the material. Our previous studies (7) showed that opening angles of arteries perfused with gelatin (protein matrix) are not significantly different from those of arteries perfused with elastomer. Hence, the inert elastomer does not affect the parameters of interest.

The issue of cell viability during the 6-h duration warrants discussion. Although the tissue was maintained and aerated under physiological conditions, it is unlikely that the cells are completely viable after 6 h. Regardless of cell viability, the pertinent question is: Are the mechanical properties of interest (opening angle) affected during this period? Saini et al. (25) previously examined the aortas of rats and humans to study the effect of time after death on opening angle. They found a slight decrease (10%) in opening angle during the first 24 h but no significant change within the next 48 h. Because the observed changes in this study are greater than 10%, tissue viability during this period will not significantly affect the present conclusions.

**Significance of study.** Extensive data exist in the literature on the opening angle of various vessels in health and disease of different species. Despite the abundance of data, it is difficult to compare the opening angles between vessels of different organs, species, or disease states because of the variability of the initial state of the vessels—the duration of the no-load state is not specified. Because we have shown that the opening angle...
of a blood vessel depends on the duration of the no-load state, it is important to specify this parameter to reduce the variability and simplify the interpretation of experimental measurements.

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