Effect of osmolarity on the zero-stress state and mechanical properties of aorta

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1Department of Biomedical Engineering, Surgery, and Cellular and Integrative Physiology, Indiana University-Purdue University Indianapolis, Indianapolis, Indiana; and 2Faculty of Biomedical Engineering, Technion-Israel Institute of Technology, Haifa, Israel

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Guo X, Lanir Y, Kassab GS. Effect of osmolarity on the zero-stress state and mechanical properties of aorta. Am J Physiol Heart Circ Physiol 293: H2328–H2334, 2007. First published June 15, 2007; doi:10.1152/ajpheart.00402.2007.— Some pathological conditions may affect osmolarity, which can impact cell, tissue, and organ volume. The hypothesis of this study is that changes in osmolarity affect the zero-stress state and mechanical properties of the aorta. To test this hypothesis, a segment of mouse abdominal aorta was cannulated in vivo and mechanically distended by perfusion of physiological salt (NaCl) solutions with graded osmolarities from 145 to 562 mosM. The mechanical (circumferential stress, strain, and elastic modulus) and morphological (wall thickness and wall area) parameters in the loaded state were determined. To determine the osmolality-induced changes of zero-stress state, the opening angle was observed by immersion of the sectors of mouse, rat, and pig thoracic aorta in NaCl solution with different osmolarities. Wall volume and tissue water content of the rings were also recorded at different osmolarities. Our results show that acute aortic swelling due to low osmolarity leads to an increase in wall thickness and area, a change in the stress-strain relationship, and an increase in the elastic modulus (stiffness) in mouse aorta. The opening angle, wall volume, and water content decreased significantly with increase in osmolarity. These findings suggest that acute aortic swelling and shrinking result in immediate mechanical changes in the aorta. Osmotic pressure-induced changes in the zero-stress state may serve to regulate mechanical homeostasis.

swelling; opening angle; elastic modulus; strain

Although this hypothesis accounts for chronic remodeling, it remains unclear how the opening angle can change acutely or immediately.

Osmotic pressure plays an important role in controlling the distribution of water across cell membranes, and, thus, the cell volume is closely regulated. Lanir et al. (16) reported the acute effect of swelling on the opening angle of the ventricle as the heart was perfused with different concentrations of mannitol. Their experiments showed that the opening angle of a rat left ventricle segment decreases with an increase in osmolarity. In a parallel theoretical analysis based on detailed morphology of the myocardium, they reasoned that the effects of osmolarity on swelling and opening angle could be due to control of the interstitial fluid volume and pressure. It is unknown whether changes in osmolarity have a similar effect on blood vessels.

The purpose of the present study was to characterize the effect of the osmotic pressure of physiological salt (NaCl) solution on the mechanical properties (opening angle, stress, strain, and elastic modulus) in mouse, rat, and pig. The mouse aorta was used for the stress-strain studies, because the relation is rather simple (linear) in the 30- to 120-mmHg pressure range. Unfortunately, because mouse aortic rings are so small, they cannot be used for measurement of weight changes at different osmolarities. Hence, we opted for a larger species, i.e., the rat. The swine was used because of similarities to the cardiovascular system of the human and, hence, the translation of data. We hypothesized that, similar to the heart, the opening angle decreases as osmolarity increases. We further hypothesized that aortic swelling due to lower osmolarity increases aortic stiffness. We have considered the implications of these findings on regulation of mechanical homeostasis of the vessel wall and, particularly, the intima.

METHODS

Experimental Protocol for Mice

Animal preparation. Ten homozygous inbred male mice (C57BL/6 strain; 23–26 g body wt) were anesthetized with intraperitoneal ketamine (80 mg/kg) and xylazine (8 mg/kg). Heparin (200 U/ml) was used to prevent blood clots in the aorta via a jugular vein catheter. All animal experiments were performed in accordance with national and local ethical guidelines, including the Institute of Laboratory Animal Research guidelines, Public Health Service policy, the Animal Welfare Act, and an approved University of Indiana-

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Purdue University Indianapolis protocol regarding the use of animals in research.

**Mechanical testing.** The mouse was euthanized with an overdose of ketamine and xylazine via the jugular vein. The segment of abdominal aorta from the renal artery to the common iliac bifurcation was carefully exposed, and all the branches of this segment were tightly ligated. The abdominal aorta was then cannulated by a 23-gauge needle at a location close to the renal artery. Water-resistant carbon particles were used to mark the segment of the abdominal aorta for measurement of axial changes. The perfusion medium consisted of four different NaCl solutions supplemented with 0.3 mM EGTA. Osmolarities of 0.45%, 0.9%, 1.35%, and 1.8% NaCl perfusate measured by an automatic osmometer (model 5002, Precision System, Inc) were 145, 282, 425, and 562 mosM, respectively.

The cannulated abdominal aorta was first perfused with 0.9% NaCl solution and then ligated at a distal position near the iliac. The aorta was filled with perfusate for 10 min within the physiological pressure range (100 – 120 mmHg). The aorta was then preconditioned with five cyclic changes in pressure from 0 to 150 mmHg in a triangular form. Perfusion pressure was increased stepwise in 30-mmHg increments from 30 to 150 mmHg. The external geometry of the abdominal aorta, at each pressurized state, was photographed 30 s after the perfusion pressure was increased to obtain the loaded outer diameter and the in vivo axial length. We confirmed that the aortic segment was leak free; hence, the pressure applied was the pressure transmitted to the aortic wall. The ligature was released, and the 0.9% NaCl solution was flushed out. The segment was then perfused with 1.35% NaCl, and the distal part was religated. The pressure-diameter-length relation was recorded after another 10 min of perfusion. The remaining protocol of mechanical testing was performed by perfusion of 1.8% and 0.45% NaCl solutions, respectively. The 0.9% NaCl solution is a physiological perfusate used as the control in this study.

**No-load and zero-stress state.** After the mechanical testing, the marked segments of abdominal aorta were carefully dissected and placed in 0.45% NaCl solution. The segment was cut transversely into five or six rings. Each ring was immersed in the NaCl solutions in which the osmolarities were increased from 145 to 562 mosM for 10 min and then photographed in no-load states. The rings were then transferred to 0.9% NaCl solution and cut radially with scissors to reveal the zero-stress state. The ring opened into a sector and gradually approached a constant opening angle. Each sector was immersed in NaCl solutions of four different osmolarities for 10 min each. The in vitro axial length, inner and outer circumferences, wall thickness (WT), and wall area (WA) in the no-load and zero-stress state at each osmolarity after 10 min of immersion were measured from the images by using a morphometric analysis system (Sigma Scan).

The thoracic aorta was dissected and placed in the 0.9% NaCl solution immediately after the mouse was euthanized. The thoracic aorta was cut into 12–18 rings. Each ring was then cut radially for measurement of the zero-stress state. Each sector was transferred to four NaCl solutions with different osmolarities for 10 min each. The morphological measurements of in vitro axial length, wall cross-sectional area, and opening angle of each ring in the zero-stress state were recorded.

**Wall volume measurements.** Since the mouse aortic rings were too small to be weighed accurately for determination of the wet-to-dry ratio, the volume of the ring was measured morphometrically. Each ring at zero-stress state was photographed in two planes: transversely for determination of the wall cross-sectional area and axially for determination of the length of the ring. The product of wall cross-sectional area and length provides the desired measurement of wall volume. The normalized wall volume was calculated as the quotient of wall volume at each osmolarity to that at standard osmolarity (0.9% NaCl). Similar measurements were made for rat and pig specimens (see below).

**Experimental Protocol for Rats**

**Animal preparation.** Five male Wistar rats (320 – 360 g body wt) were anesthetized with pentobarbital sodium (25 mg/kg ip). Heparin (200 U/ml) was used to prevent blood clots in the aorta via a jugular vein catheter. The rat was euthanized with an overdose of pentobarbital sodium via the jugular vein.

**Zero-stress state.** The thoracic aorta was directly dissected and cut into 12–18 rings. Each ring was then cut radially for documentation of the zero-stress state. The rest of the experimental protocol was identical to that described for the mice (see above).

**Tissue water content.** Similar to the mouse, the aortic rings of the rat are too small for accurate weight measurement. The ascending aorta and arch used for measurement of water content were rapidly cut and placed in 0.9% NaCl solution. The aorta was gently dried with an absorbent tissue and weighed on an analytic balance to obtain the wet weight. The aorta was then transferred to the other three NaCl solutions with different osmolarities for 10 min each, and the wet weight was measured. The aorta was dried to a constant weight in an oven maintained at 37°C for 12 h, and dry weight was measured. The tissue water content was expressed as wet-to-dry weight ratio, which was calculated as the quotient of wet weight at each osmolarity to dry weight.

**Experimental Protocol for Pigs**

**Animal preparation.** Three normal domestic pigs (32–35 kg body wt) were anesthetized with ketamine (33 mg/kg) and atropine (0.05 mg/kg), and anesthesia was maintained with isoflurane (1–2%). Ventilation with 100% O₂ was provided with a respiratory pump, and a midline sternotomy was performed. After induction of anticoagulation with heparin (100 U/kg), pentobarbital sodium (80 mg/kg iv) was injected to ensure deep anesthesia. The heart was then arrested with a saturated KCl solution administered through the jugular vein. The heart was excised and used for another experiment, and the aorta was used for the present study.

**Zero-stress state.** The thoracic aorta was dissected and cut into 12–18 rings. Each ring was then cut radially to reveal the zero-stress state. The rest of the experimental protocol was identical to that described for mice and rats (see above).

**Tissue water content.** Each ring of the pig thoracic aorta was directly measured to obtain the wet-to-dry weight ratio. The experimental protocol was identical to that described for the rat (see above).

**Statistical Analysis**

Each aortic ring was considered an independent statistical sample. Values are means ± SD. Significance of the differences between the groups was evaluated by two-way ANOVA or t-test. The results were considered statistically significant when P < 0.05 (2-tailed).

**RESULTS**

Data for mice, rats, and pigs were analyzed separately. Conclusions were the same for all three species.

The outer diameter of mouse aorta was obtained by direct measurement in the loaded states and increased with pressure (P < 0.01; data not shown). The intima-media thickness of the aortic wall decreased with an increase in pressure as computed from Eq. 1b (see APPENDIX; P < 0.01). Figure 1 shows the effect of perfusate osmolarity on WT and WA of mouse aorta at different pressures. WT decreased with an increase in osmolarity. A significant increase in WT was observed in hypotonic perfusate (0.45% NaCl at 145 mosM) compared with the physiological perfusate (0.9% NaCl at 282 mosM, P < 0.05), which was not significantly different from the other two hypotonic perfusates (1.35% NaCl at 425 mosM and 1.8%
NaCl at 562 mosM). WA decreased linearly with an increase in osmolarity at different perfusion pressures ($P < 0.01$).

Cauchy stress and Green strain increased linearly ($P < 0.01$) with an increase in distension pressure of 30–120 mmHg. The linear least squares method was used to fit data to a curve, and the empirical constants are summarized in Table 1. The effect of osmolarity on circumferential Cauchy stress and Green strain of the mouse aorta at physiological pressure (120 mmHg) is shown in Fig. 2, A and B. The stress and strain in hyposmotic perfusate showed a significant decrease compared with physiological perfusate and hyperosmotic perfusates ($P < 0.05$), which were not significantly different from each other.

The relationship between Green strain and Kirchhoff stress was linear in the pressure range of 30–120 mmHg, and the least-squares fit constants are also summarized in Table 1. The circumferential elastic modulus was computed using Eq. 4 (see APPENDIX). The effect of osmolarity on the circumferential elastic modulus of the mouse aorta is shown in Fig. 2C. The circumferential modulus was significantly increased in the hyposmotic solution (145 mosM) compared with the physiological solution (282 mosM, $P < 0.05$). There was no significant difference, however, in the circumferential modulus between standard osmolarity and higher osmolarities.

The opening angle was defined as the angle subtended by two radii connecting the midpoint of the inner vessel wall. The effect of osmolarity on the opening angle and normalized wall

Table 1. Linear regression for Cauchy stress, Green strain, and circumferential elastic modulus and pressure at different osmolarities in mouse aorta

| Osmolarity, mOsm | Cauchy Stress | | Green Strain | | Elastic Modulus |
|------------------|--------------|------------------|-------------|------------------|
|                  | $\alpha$, kPa/mmHg | $\beta$ | $R^2$ | $\alpha$, 1/mmHg | $\beta$ | $R^2$ | $\alpha$, kPa/mmHg | $\beta$ | $R^2$ |
| 145              | 2.5          | $-57.8$ | 0.99   | $9.1 \times 10^{-3}$ | $8.2 \times 10^{-3}$ | 0.96   | 75.9          | $-0.4$ | 0.96   |
| 282              | 2.9          | $-64.5$ | 0.99   | $1.0 \times 10^{-2}$ | $3.5 \times 10^{-2}$ | 0.97   | 69.3          | $-3.4$ | 0.96   |
| 425              | 3.0          | $-68.7$ | 0.99   | $1.0 \times 10^{-2}$ | $1.1 \times 10^{-2}$ | 0.96   | 71.6          | $-2.2$ | 0.96   |
| 562              | 3.1          | $-70.7$ | 0.99   | $1.0 \times 10^{-2}$ | $2.3 \times 10^{-2}$ | 0.96   | 72.2          | $-2.4$ | 0.96   |

$R^2$ represents the coefficient of the following equation: $y = \alpha x + \beta$, where $y$ represents Cauchy stress, Green strain, or elastic modulus and $x$ represents perfusion pressure.
volume for mouse, rat, and pig aorta and on the water content (wet-to-dry weight ratio) for rat and pig aorta is shown in Fig. 3. The gradual increase in osmolarity from 145 to 562 mosM caused a linearly significant decrease in the opening angle, wall volume, and water content ($P < 0.01$) in all animal aortas. The linear least squares method was used to fit the relationship to a curve, and the empirical constants are summarized in Table 2.

**DISCUSSION**

The major finding of this study is that the opening angle of mouse, rat, and pig aorta increases with a decrease in osmotic pressure. Low osmolarity (swelling) is associated with greater wall thickness and wall volume and increased wall stiffness in the mouse aorta. Acute aortic swelling or shrinking causes immediate mechanical changes in the aorta. Regulation of the zero-stress state through osmotic changes may serve to regulate mechanical homeostasis and can be an early and immediate response to changes in mechanical loading (e.g., hypertension and flow overload) that initiate growth and remodeling of tissue.

### Osmolarity Changes Under Pathological Conditions

The normal effective osmolarity is physiologically maintained at ~285–295 mosM. Hyperosmolarity (300–325 mosM) may occur with conditions such as dehydration, hyperglycemia, or hypernatremia. Hyposmolarity (245–280 mosM) may indicate overhydration or hyponatremia induced by a number of medical conditions, such as pulmonary disease, congestive heart failure, and head injury, and surgery (24). The serum osmolarity in vivo is regulated through integration of neuronal, humoral, and vascular mechanisms. Under normal conditions, a rise in extracellular fluid osmolarity triggers the sensation of thirst and causes release of antidiuretic hormone, which leads to water retention and a subsequent fall in extracellular fluid osmolarity (7). The broad range of osmolarity (145–562 mosM) in this study is not intended to be physiological but, rather, to induce swelling or shrinking of the vessel wall. The latter certainly occurs pathophysiological, and the present findings may explain the mechanism by which residual stress (and associated vessel function) is implicated.

### Morphological and Water Content Changes

It is well accepted that most cells exhibit volume regulatory responses when their water content is perturbed (26). In the presence of nonisosmotic conditions, a consequence of rapid alteration of the intra- or extracellular osmolarity, the passive flow of water causes cell swelling or shrinking (29). The influence of volumetric swelling or shrinking on structural and functional behaviors of tissues or organs in response to osmotic pressure has been extensively studied for many years. Starr et al. (31) found that the edema due to low-osmolarity coronary perfusates leads to increased myocardial water content and altered histological characteristics in the rat left ventricle. Increases in cartilage volumetric swelling, measured as water weight gain, have been reported as an early sign of osteoarthritis (20). Here, we found that the aortic water contents

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**Table 2. Linear regression for relation between opening angle, normalized wall volume, water content, and osmolality in mouse, rat, and pig aorta**

<table>
<thead>
<tr>
<th>Species</th>
<th>Opening Angle</th>
<th>Normalized Wall Volume</th>
<th>Water Content (wt-to-dry wt ratio)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\alpha$, degrees/mosM</td>
<td>$\beta$</td>
<td>$R^2$</td>
</tr>
<tr>
<td>Mouse</td>
<td>$-9.0 \times 10^{-2}$</td>
<td>154.9</td>
<td>0.94</td>
</tr>
<tr>
<td>Rat</td>
<td>$-0.2$</td>
<td>121.0</td>
<td>0.97</td>
</tr>
<tr>
<td>Pig</td>
<td>$-0.1$</td>
<td>107.1</td>
<td>0.93</td>
</tr>
</tbody>
</table>

$R^2$ represents the coefficient of the following equation: $y = \alpha x + \beta$, where $y$ represents opening angle, normalized wall volume, or water content and $x$ represents osmolarity.

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**Fig. 3. Effect of osmolarity on opening angle (A) and normalized wall volume (B) of mouse, rat, and pig aorta and on water content (wt-to-dry ratio, C) of rat and pig aorta. Opening angle, wall volume, and water contents significantly decreased with an increase in osmolarity ($P < 0.01$) in mouse, rat, and pig.**
(wet-to-dry weight ratio) in the rat and pig (Fig. 3C) and the aortic wall volume in the mouse, rat, and pig (Fig. 3B) decreased as osmolarity increased. We also found that lower osmolarity leads to a significant increase in wall thickness and wall area at physiological loading in the mouse aorta (Fig. 1). This observation demonstrates that the blood vessel wall, similar to the myocardium and other connective tissues, can absorb or release water; hence, the volume of the vessel wall immediately changes in response to alterations in osmotic pressure.

Changes in Stress and Strain

To our knowledge, no studies on the influence of osmolarity on mechanical properties of blood vessels have been published. In the present study, the relationship between osmolarity and mechanical properties was quantified by in vivo mechanical testing of the mouse aorta. Our results show that changes in osmolarity affect circumferential stress and strain and the stress-strain relationship and, in turn, alter the circumferential elastic modulus (Fig. 2). The hypotonic (145 mosM) perfusate was found to significantly increase the elastic modulus of the mouse aorta, indicating an increase in aortic stiffness upon swelling. The aortic wall shrinkage due to high osmolarity (425 and 562 mosM), however, did not have a significant effect on aortic elasticity. Similar results were reported in a study of pig and rat myocardium in which crystalloid-induced myocardial edema was associated with an increase in stiffness and a decrease in the filling volume of the left ventricle (2). Pratt et al. (27) also found that interstitial myocardial edema induced by acute transient coronary sinus hypertension depresses contractility and increases diastolic stiffness of the left ventricle in dogs. Contrary to these findings, a recent study of rat intestinal edema showed decreased stiffness and residual stress, which result in intestinal transit delay (28). It is interesting that different tissues or organs respond differently to changes in osmotic pressure consistent with their respective function.

Changes in Zero-Stress State

Knowledge of the zero-stress state is fundamental in mechanics, because all calculations of stress and strain are made in reference to this state. The existence and level of residual stress have been shown to significantly affect arterial (6) and left ventricular (16, 23) wall stress distribution and their function. Biologically, remodeling of the zero-stress state is thought to be an index of the nonuniformity of growth and remodeling (9). A number of studies have addressed chronic changes in the opening angle of blood vessels under various pathological conditions. Matsumoto et al. (21) proposed a significant increase in the opening angle of the rabbit atherosclerotic aorta. The effect of diabetes on the zero-stress state of pulmonary and systemic arteries was an increase in the opening angle that reached a plateau after ~30 days of diabetes (18).

The acute effect of osmolarity on the opening angle of the rat ventricle has been examined by Lanir et al. (16). They found that the opening angle decreased with an increase in osmolarity and reasoned that myocardial swelling affects the opening angle by an increase in the intercellular distance and the associated increase in the collagen struts that interconnect them. Fung and Liu (10) investigated the changes of the opening angle of the rat pulmonary artery in response to acute hypoxic hypertension. Their results showed that the pulmonary opening angle increased immediately and rapidly (in the first 2–12 h and become stable after 2 days) when the rat was exposed to hypoxia-induced pulmonary hypertension. In the present study, we immersed aortic rings in physiological salt solutions of different osmolarities for only 10 min to test the acute osmotic effect. Our data show that the opening angle of the aorta decreases with an increase in osmolarity in mouse, rat, and pig (Fig. 3A). The mean opening angle decreased from 145.1° at 145 mosM to 108.1° at 562 mosM in the mouse, from 98.0° to 22.6° in the rat, and from 96.1° to 53.7° in the pig. Interestingly, the largest change in opening angle for the same change in osmolarity was shown in the rat aorta. The responses of the mouse and pig aorta are very similar, as are the baseline opening angles.

The presently reported effect of tissue swelling on the zero-stress state (the opening angle) may have significant mechanical and biological ramifications. As discussed by Lanir et al. (16), this effect of swelling implies that, under physiological isometric conditions, the zero-stress configuration calculated from the corresponding opening angle of the wall tissue is not truly stress free. The tissue is, rather, under balance between the stress induced by the tissue fibers, on the one hand, and the hydrostatic pressure of the tissue fluid, on the other. Both are regulated by swelling. The mechanical significance of this distinction is that the loaded strain and stress calculated on the basis of this apparent “zero-stress” reference configuration are not their true values. Biologically, this implies that the cells in the tissue space are subjected to a mechanical environment that may be significantly different from these calculated values.

Cell Swelling as a Stimulus for Remodeling

In general, cell membranes are highly permeable to water. Water flow across the membrane is directed by the osmotic pressure gradient. At hypotonic condition, swelling is due to a simple osmotic shift of water across the cell membrane from a hypotonic pericellular environment (35). In humans, low osmolarity induced by hyponatremia leads to transcellular water movement and consequent changes in brain cell volume, which ultimately give rise to cerebral edema (1, 25). Carter et al. (5) found that low-osmolarity coronary perfusates increase the myocardial histological edema score and interstitial space compared with normal osmolarity. A morphological examination of the isolated rat hindquarter preparation shows that muscle tissue exhibited extracellular expansion and intracellular edema followed after hyposmolar fixation (14). In the present study, it is likely that cell swelling and extracellular expansion occur in the aortic wall at low-perfusion osmolarity. The latter is likely dominant.

Morphological and histological examinations have found that subendothelial edema occurs in arteries during the induction of atherosclerosis (3) and the development of preatherosclerotic lesions (11). It is also known that the hyperglycemia in diabetes mellitus enhances the formation of sorbitol from glucose, and the subsequent excessive cellular accumulation of sorbitol has been postulated to cause cell swelling (4). In the case of acute hypoxic hypertension, Fung and Liu (10) reported that the increase in the opening angle seems to coincide with the nonuniform pathological and histological changes in the
vessel wall, such as swelling, blebbing, and thickening of the intima and media, soon after exposure to hypoxia. If the acute alterations in cell volume accompanied by changes in the opening angle and stiffness are not sufficient to restore mechanical homeostasis, a remodeling response may ensue. It has been postulated that conformational changes in plasma membrane-associated molecules, as well as in extracellular matrix and cytoskeleton, may function as sensors and transducers for external mechanical forces applied transiently or chronically to cells (13, 32). Vascular cells are equipped with numerous receptors that allow them to sense and respond to the mechanical stress and translate this mechanical stimulus into an intracellular biochemical signal and, ultimately, a biological response (34). In the present study, it can be hypothesized that the acute changes in osmotic pressure, similar to acute hypertension, can serve as an external mechanical stimulus to cause cell deformation through changes in protein conformation, which lead to altered binding affinities of proteins. This can initiate an intracellular signaling cascade to produce changes in the proteins localized in regions of high stress. Therefore, release of the biochemical signal induced by changes in osmotic pressure ultimately results in the alteration of vascular mechanics and function through mechanochemical transduction.

**Cell Swelling as a Protective Mechanism for the Intima**

The existence of a mechanical homeostatic state in the cardiovascular system is well recognized (15). Acute changes in mechanical loading (e.g., hypertension) alter mechanical homeostasis and incite an immediate response. Cell swelling following acute hypertension can increase the opening angle and the stiffness of the vessel wall. What are the ramifications of these immediate mechanical changes? We recently showed that an increase in opening angle can offload the stress from the intima to the adventitia to protect the endothelial cells (unpublished observations). This novel finding was verified by simulation of wall stress on the basis of transmural measurements of strain and material properties of coronary arteries in reference to the zero-stress state in one- and two-layer (intima-media and adventitia) models, along with a sensitivity analysis of the effect of the opening angle on circumferential stress distribution. It was shown that the larger opening angle shifts the circumferential stress from the intima-media to the adventitia, and the same pattern holds within each layer in the two-layer model (unpublished observations). In addition, the increase in stiffness will further protect the intima, the tensile material properties of which are insignificant compared with those of the media and adventitia in the normal vessel (30). In summary, the effect of cell swelling may be a protective mechanism for the intima in various disease states. Experimental validation of this hypothesis remains a task for the future.

**APPENDIX**

**Biomechanical Analysis**

**Incompressibility condition.** The loaded inner radius and wall thickness of a vessel were determined from the incompressibility assumption. The incompressibility condition for a cylindrical vessel can be expressed as

\[ r_i = \sqrt{r_o^2 - \frac{A_o}{\pi \lambda_c}} \]  

(1a)

where \( r_o \) and \( r_i \) are the outer and inner radii, respectively, in the loaded state, \( \lambda_c = l/l_0 \) is the stretch ratio in the axial direction, where \( l \) and \( l_0 \) represent vessel length in loaded and zero-stress states, respectively, and \( A_0 \) is wall area in the no-load state. WT in the loaded state was computed as the difference between the outer and inner radius of the vessel at various pressures as

\[ WT = r_o - r_i = r_o - \sqrt{r_o^2 - \frac{A_0}{\pi \lambda_c}} \]  

(1b)

where \( r_o \), \( A_o \), and \( \lambda_c \) were measured quantities.

**Strain and stress.** The circumferential deformation of the artery may be described by Green strain (\( \epsilon_0 \)), which is defined as follows

\[ \epsilon_0 = \frac{1}{2} (\lambda_c^2 - 1) \]  

(2)

where \( \lambda_c \) is the midwall circumferential stretch ratio (\( \lambda_c = c/c_{m} \) where \( c \) is the midwall circumference of the vessel in the loaded or no-load state and \( c_{m} \) refers to the midwall circumference in the zero-stress state).

At equilibrium, the average circumferential Cauchy and second Piola-Kirchhoff stress in a cylinder can be computed as

\[ \tau_b = \frac{P r_o}{WT} \]  

(3a)

and

\[ S_b = \frac{\tau_b}{\lambda_c} \]  

(3b)

where \( P \) is the luminal pressure and \( r_o \) and \( \lambda_c \) are the inner radius and the circumferential stretch ratio of the vessel, respectively. **Equations 2 and 3 allow the determination of the circumferential stresses and strains, respectively, for different pressure distensions.**

**Elastic moduli.** Computation of the elastic modulus has been previously described in detail (12). In analogy to an isotropic tube, the circumferential elastic modulus (\( E_{\theta} \)) can be defined as

\[ E_{\theta} = \frac{\Delta \tau_b}{\Delta \epsilon_0} \frac{1}{1 - \frac{\Delta \tau_b}{\Delta \epsilon_c} E_c} \]  

(4a)

where

\[ E_c = \frac{\Delta \tau_b}{\Delta \epsilon_c} + \left( \frac{\Delta \epsilon_0}{\Delta \epsilon_c} \right) \frac{\Delta \tau_b}{\epsilon_0} \]  

(4b)

Since the stress-strain relation was found to be linear, \( E_{\theta} \) and \( E_c \) are constant and independent of pressure.

**GRANTS**

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