Effects of elastin degradation and surrounding matrix support on artery stability

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Lee AY, Han B, Lamm SD, Fierro CA, Han HC. Effects of elastin degradation and surrounding matrix support on artery stability. Am J Physiol Heart Circ Physiol 302: H873–H884, 2012. First published December 9, 2011; doi:10.1152/ajpheart.00463.2011.—Tortuous arteries are often associated with aging, hypertension, atherosclerosis, and degenerative vascular diseases, but the mechanisms are poorly understood. Our recent theoretical analysis suggested that mechanical instability (buckling) may lead to tortuous blood vessels. The objectives of this study were to determine the critical pressure of artery buckling and the effects of elastin degradation and surrounding matrix support on the mechanical stability of arteries. The mechanical properties and critical buckling pressures, at which arteries become unstable and deform into tortuous shapes, were determined for a group of five normal arteries using pressurized inflation and buckling tests. Another group of nine porcine arteries were treated with elastase (8 U/ml), and the mechanical stiffness and critical pressure were obtained before and after treatment. The effect of surrounding tissue support was simulated using a gelatin gel. The critical pressures of the five normal arteries were 9.52 kPa (SD 1.53) and 17.10 kPa (SD 5.11) at axial stretch ratios of 1.3 and 1.5, respectively, while model predicted critical pressures were 10.11 kPa (SD 3.12) and 17.86 kPa (SD 5.21), respectively. Elastase treatment significantly reduced the critical buckling pressure (P < 0.01). Arteries with surrounding matrix support buckled into multiple waves at a higher critical pressure. We concluded that artery buckling under luminal pressure can be predicted by a buckling equation. Elastin degradation weakens the arterial wall and reduces the critical pressure, which thus leads to tortuous vessels. These results shed light on the mechanisms of the development of tortuous vessels due to elastin deficiency. Arteries are subjected to significant mechanical loads from blood pressure, surrounding tissue tethering, and body movement. The strength and stability of arteries under these loads are essential to the maintenance of normal arterial function. While the mechanical stress and strength of blood vessels have been well documented, the mechanical stability of arteries is less understood.

Arteries and arterioles often become tortuous, leading to hemodynamic changes and various clinical complications (10, 40). For example, tortuous internal carotid arteries can lead to stroke, vertigo, syncope, blackout, persistent tinnitus, and other cerebrovascular deficiencies in patients (1, 27, 32, 57). Clinical studies have shown that artery tortuosity is associated with hypertension, atherosclerosis, aging, and other pathological changes (10, 24, 25, 32, 40, 47, 48, 50), suggesting that mechanical factors may play an important role.

Recent theoretical analysis by our group and others suggested that mechanical instability occurs in arteries and subsequently leads to tortuosity (16, 17, 19, 28, 44). Artery instability due to buckling may be a possible mechanism for the development of tortuous arteries. Therefore, it is of clinical importance to understand the buckling behavior of arteries under luminal pressure.

Mechanical stability of arteries depends on their wall stiffness (14, 16, 17). Elastin is an important extracellular matrix component for arterial elasticity and stiffness, and elastin degradation weakens the arterial wall (11). Elastin deficiency has been associated with tortuous arteries in patients with arterial tortuosity syndrome and Loeye-Dietz syndrome, as well as in mice with an elastin gene knockout (7, 36, 49, 52). Elastin degradation has also been associated with tortuous cerebral arteries due to increased blood flow (5, 25). In addition, elastin degradation and arterial tortuosity occur concomitantly in the aged population (13, 46). These lines of evidence suggested that elastin degradation may play a role in the development of tortuous arteries, but the underlying mechanisms are unclear. Therefore, it is important to study the effect of elastin on the mechanical stability of arteries.

Accordingly, the objectives of this study were to determine the critical buckling pressure of porcine carotid arteries, to test the predictive value of the artery buckling model, and to determine the effects of elastin degradation and surrounding matrix support on the mechanical buckling of arteries. We tested the hypothesis that arteries may become mechanically unstable under luminal pressure, and that elastin degradation reduces the critical pressure, thus leading to artery tortuosity.

MATERIALS AND METHODS

Specimen preparation. Porcine common carotid arteries (the segment distal to the sinus but proximal to the bifurcation of inner and outer carotid arteries) were harvested postmortem from 6- to 7-mo-old farm pigs (100–150 kg) at a local abattoir. After being rinsed with phosphate-buffered saline (PBS) (Dulbecco’s PBS, Sigma Chemical, St. Louis, MO), the specimens were placed into PBS solution and transported to our laboratory in an iced cooler. Once in the laboratory, the arteries were cleaned by removing excess connective tissue and were rinsed again with PBS. The in vitro free lengths were measured while the vessels were afloat in PBS solution. The arteries were then
mounted onto a luer stopper at one end, attached to a 10-ml plastic syringe at the other end, and were inflated briefly to check for leaks. The inflated diameter and length of the arteries were monitored to ensure the inflation pressure applied was lower than 100 mmHg.

Inflation testing and determination of the stress-strain relationship. To evaluate the mechanical stiffness of the porcine arteries, we measured their elongation and diameter inflation under internal pressure. Briefly, one end of the arteries was mounted to a cannula in the tissue chamber (Fig. 1, top) used in our laboratory’s previous studies (16, 21), while the other end was tied onto a luer stopper and allowed to move freely. The cannula was connected to a pressure meter and a syringe pump filled with PBS. The arteries were preconditioned by gradually inflating them with PBS to a pressure of 200 mmHg and deflating to 0 mmHg for several cycles (8, 33). After that, the arteries were slowly inflated with PBS, at a rate of ~2 mmHg/s, using the syringe pump and allowed to expand freely in both radial and axial directions. The inflated outer diameter and axial length of the arteries were measured simultaneously as the luminal pressure increased. The pressure and axial force can thus be expressed as functions of the strains (Eqs. A9 in the APPENDIX). The detailed derivations of the equation are given in [insert reference to the equation].

Based on the experimental observation, the arterial walls were assumed as cylindrical thick-walled tubes with an orthotropic nonlinear elastic wall characterized by the Fung strain energy function (15, 26). The pressure and axial force can thus be expressed as functions of the strains (Eqs. A9 and A10 in the APPENDIX). The material constants were determined by fitting these equations with the experimental pressure-diameter-length data using the nonlinear “Isqnonlin” function in Matlab.

For a group of five normal arteries (normal group), the experimental data were fit with the three-dimensional (3D) Fung strain energy function first. Then, for comparison, the data were also fit with the two-dimensional (2D) Fung strain energy function (b3 = b5 = b6 = 0 in Eq. A4) using the thin-walled cylindrical model, as previously described for veins (30).

Buckling testing and measurement of the critical pressure. To determine the critical pressure, arteries were tied at both ends onto cannulas inside the tissue chamber (Fig. 1, bottom). The arteries were stretched axially to achieve designated axial stretch ratios and then gradually pressurized with PBS using the syringe pump, while being photographed at pressure increments of 5–10 mmHg until a large deflection (lateral displacement) of 5–10 mm was reached at the middle of the vessels. A preliminary reading of the critical pressure was recorded when arteries began to exhibit visually detectable deflection. This process was repeated three times for each artery, and the process was also recorded with a SONY digital camera. The camera was orientated to take photographs from either the top view or the side view, depending on the direction of the buckling. If the artery was buckled in a direction other than the horizontal or vertical planes, we rotated the artery with both cannulas so that the buckling would be perpendicular to the camera lens (there was no contact between the arteries and the chamber walls).

Later, the lateral displacements of the arteries under pressure were measured from the video and photos using ImagePro Plus (Media Cybernetics). First, the positions of the central lines of the arteries at all pressure levels were determined by averaging the coordinates of the two edges of the arteries. Then the deflection was determined as the maximum lateral displacement of the central line (at the middle of the vessel length) from its baseline position at zero pressure. The critical pressure was determined to be the pressure when the deflection became detectable (~0.5 mm) from the initial baseline.

For the five arteries in the normal group, the buckling tests were done at a series of axial stretch ratios in the range of 1.0–1.7 at a step of 0.1, which covers physiological (1.5 in vivo) and subphysiological ranges (21), to evaluate the effect of axial stretch ratio on the critical pressure.

Model prediction of critical pressure. The critical buckling pressure (p<sub>cr</sub>) was determined using the buckling equation

\[ p_{cr} = \frac{E}{\pi r_i^2} \left( \frac{2\pi}{L} \right) H \]

where N is the axial force, and H is the “bending force” given as:

\[ N = \pi r_i^2 p + \pi \int_{r_i}^{r_e} \left[ (2 + 2E_i)\left(b_3E_{b3} plus b_5E_{b5} plus b_6E_{b6}\right) \right. \]

\[ \left. - (1 + 2E_i)\left(b_3E_{b3} plus b_4E_{b4} plus b_5E_{b5}\right) \right] \]

\[ \left. - (1 + 2E_i)\left(b_4E_{b4} plus b_5E_{b5} plus b_6E_{b6}\right) \right] \] \cdot \left( b_0 e^{0.5} \right) \cdot \frac{r^2}{r_i^2} \cdot \frac{r}{r_i} \]

where

\[ Q = b_1 E_{b1}^2 + b_3 E_{b3}^2 + b_5 E_{b5}^2 - 2b_4 E_{b4} E_{b5} + 2b_5 E_{b5} E_{b6} + 2b_6 E_{b6} E_{b6} \]

\[ J_z = 2g_2 \left[ \left( 1 + 2E_i \right) g_z - \left( 1 + 2E_i \right) g_z \right] + g_2 \]

\[ + \left( 1 + 2E_i \right) b_2 - \left( 1 + 2E_i \right) b_5 \]

\[ J_y = 2g_2 \left[ \left( 1 + 2E_i \right) g_y - \left( 1 + 2E_i \right) g_y \right] \]

\[ + \left( 1 + 2E_i \right) b_4 - \left( 1 + 2E_i \right) b_5 \]

\[ g_0 = b_1 E_{b1} + b_3 E_{b3} + b_5 E_{b5} \]

\[ g_z = b_3 E_{b3} + b_4 E_{b4} + b_5 E_{b5} \]

\[ g_r = b_1 E_{b1} + b_3 E_{b3} + b_5 E_{b5} \]

\[ (3c) \]

where \( L \) is the vessel length; \( r_i \) and \( r_e \) are the lumen and outer radii, respectively; \( E_{b1}, E_{b3}, \text{ and } E_{b5} \) are the circumferential, axial, and radial components of Green strain, respectively; and \( b_0, b_1, \ldots, \text{ and } b_6 \) are material constants. This equation was developed for arteries with two fixed ends as used in the experimental setting following our previous model (17, 18). The detailed derivations of the equation are given in the APPENDIX.

For each artery, the critical pressures at different axial stretch ratios were determined based on its own material constants and initial
dimensions determined from the experimental measurements (17, 18). Residual stresses were ignored in the model simulations (an opening angle of 2\(\theta_0\) = 360° was assumed, see Fig. A1), since a pilot study using rabbit data demonstrated little effect of the opening angle on the critical pressure of arteries (6, 18) (see Discussion).

**Elastase treatment.** To explore the possible effect of arterial wall matrix components on arterial critical pressure, a group of nine arteries (elastase group) was treated with elastase, as described previously (11). Briefly, arteries were filled with elastase [8 U/ml, Lyophilized (ESL), Worthington Biochemical, Lakewood, NJ] in the lumen for 60 or 90 min after the baseline inflation and buckling tests were done. Statistical testing showed no significant difference between the elastase treatment groups for 60 or 90 min. After treatment, the arteries were washed with PBS to remove residual elastase, and the pressurized inflation test and buckling test were performed again following the same protocols as described above. The buckling tests for the elastase group were done at only three axial stretch ratios of 1.1, 1.3, and 1.5 to reduce the testing time, since testing of the normal group had depicted the effect of axial stretch ratio on the critical pressure in the full range of 1.0 to 1.7. A subset of arteries in this group was used for opening angle measurement and elastin quantification.

**Opening angle measurement.** To determine the effect of elastase treatment on the residual strain in the carotid arteries, we measured the opening angles of a subset of five arteries from the elastase treatment group. Arterial rings (short segments of 2–3 mm in axial length) were cut from the distal and proximal ends of the specimens before elastase treatment and compared with rings cut from the middle after elastase treatment. For the opening angle measurement, arterial ring segments were arranged in PBS (25°C) in a Petri dish and then cut open by radial cuts (20, 22). After the radial cut, the rings popped opened into C-shaped sectors, and the sectors were allowed to stabilize for 30 min to fully release the residual stress (20, 22). The configurations of the sectors were then photographed with a digital camera. The angle between the two lines from the middle of the inner wall to the two tip of the inner wall (20, 22) was measured for each sector using ImagePro Plus. This angle was then converted to the opening angle 2\(\theta_0\) based on the relation that this angle plus \(\theta_0\) is equal to 180° (6, 18).

**Buckling test of arteries within gelatin matrix.** To illustrate the effect of surrounding tissue support, an additional eight arteries (gel group) were placed into gelatin after baseline buckling tests in PBS at a stretch ratio of 1.3. The gelatin had an elastic modulus of 7.4 ± 7 kPa, to mimic the tissue stiffness reported in the literature (58). To prepare the gelatin matrix, gelatin powder (Knox Original Gelatine, Kraft) was added to heated water (37°C) at a concentration of 9 g/200 ml water. The liquid gelatin mixture was then poured into the testing box to submerge the artery and cooled for 3 h until it formed a solid gel. The stiffness of the gel was controlled by the concentration and was premeasured using compression testing. The buckling tests were performed to measure the critical pressure. Then the gel was gently removed and washed. The critical pressure and the buckled mode shape of the arteries were measured again for comparison.

**Measurement of arterial initial dimensions.** After inflation and buckling tests, all arteries were removed from the chamber, and short ring segments (~2 mm in axial length) were cut for cross-sectional dimension measurements. The lumen diameter, outer diameter, and wall thickness were measured from the cross-sectional surfaces under a dissecting microscope and photographed. For each ring segment, the diameters were measured at two radial locations 90° apart, and the wall thickness was measured from four locations 90° apart. The segments were then fixed in 10% formalin for histology.

**Histology.** The specimens were fixed overnight in formalin and then processed and embedded in paraffin blocks. Sections (5 \(\mu\)m in thickness) were cut and processed for hematoxylin-eosin staining. For arterial specimens treated with elastase, consecutive sections were processed for hematoxylin-eosin staining, Verhoff staining, and trichrome staining.

**Photometric measurement of elastin and collagen contents.** The elastin and collagen contents were measured for arterial cross sections with Verhoff staining and trichrome staining, respectively. Eight to twelve locations from two to four cross sections of each artery, each including the intima, media, and adventitia, were measured. The ratio of the area of the collagen or elastin to the total tissue area was determined using the histogram analysis feature in ImagePro Plus. The percentage of elastin was determined as the ratio of elastin to tissue areas. The same procedure was repeated for collagen.

**Measurement of elastin content using Fastin assay.** The change of elastin content due to elastase treatment was further quantified in a subgroup of four arteries in the elastase group using a Fastin assay (31). Briefly, equal segments ~4 mm in length were cut from arteries before and after elastase treatment. Elastin was extracted by putting each sample into a microcentrifuge tube with 0.25 N oxalic acid solution and immersion inside a 100°C water bath for 1 h. The sample was then centrifuged for 10 min, and the supernatant was removed. This process was repeated 10 times to completely dissolve the tissue sample. A Fastin assay kit (Biocolor Fastin Assay Kit, CLRF2000, Accurate Chemical and Scientific, Westbury, NY) was then used to quantify the elastin content using a microplate reader, according to the manufacturer’s specifications. The elastin content is specified as microgram (\(\mu\)g) elastin per milligram (mg) tissue.

**Statistical analysis.** All values are presented as means (SD) (standard deviation). Statistical significance between means was determined by having achieved a significance level \(P\) value < 0.05. A normalcy test (Instat) was used to find out if the two elastin groups (60 and 90 min) could be combined. A paired Student’s \(t\)-test was used to determine the significance in the elastin study. The \(t\)-test and a power analysis were done using JMP software (SAS Institute).

**RESULTS**

A total of 22 arteries were examined in three groups. These arteries included either left or right common carotid arteries from 30 animals (the rest were either used for other studies or disposed due to branch or leakage). Five arteries (normal group) were tested to determine the effect of axial stretch ratio and to test the predictive value of the 3D and 2D theoretical models. Nine arteries (elastase group) were examined before and after elastase treatment to detect the effect of elastin degradation, and eight arteries (gel group) were tested in PBS and in a gelatin matrix to illustrate the effect of surrounding tissue matrix support.

**Stress-strain relationship of normal arteries (normal group).** The lengths of the five arteries in the normal group ranged from 47 to 57.2 mm, with an average external radius of 3.0 mm. Dimensions of these vessels are summarized in Table 1. When pressurized with one end free, the artery segments enlarged and elongated simultaneously (Fig. 2). The length (and accordingly axial stretch ratio) increased consistently with increasing pressure, while the radius increase slowed down after 10–15 kPa (Fig. 2).

<table>
<thead>
<tr>
<th>Table 1. Summary of the initial dimensions of the arteries tested (normal group)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Artery ID</strong></td>
</tr>
<tr>
<td>5</td>
</tr>
<tr>
<td>6</td>
</tr>
<tr>
<td>7</td>
</tr>
<tr>
<td>8</td>
</tr>
<tr>
<td>9</td>
</tr>
<tr>
<td>Mean (SD)</td>
</tr>
</tbody>
</table>

Values are in mm. ID, artery identification number.

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The material constants obtained by fitting the data with Eqs. A9 and A10 in the APPENDIX are summarized in Table 2. The corresponding material constants obtained by fitting the data with the 2D strain energy function using the thin-walled vessel model are listed in Table 3.

**Critical pressure of normal arteries.** All artery segments buckled when the luminal pressure exceeded critical values. Initially, the arteries inflated, and their diameters increased but did not deflect laterally. When the luminal pressure reached critical pressure levels, arteries deflected from the baseline and became buckled (Fig. 3). The deflection initiated at the critical pressure and increased as the pressure continued to increase, which demonstrated a nonlinear postbuckling behavior (Fig. 3, bottom). One interesting observation was that, once an artery buckled at a specific location and direction under increasing pressure, it would buckle in the same location and direction under repeating pressure loads. The critical pressures were obtained at several levels of axial stretch ratios (1.0–1.7, at a step of 0.1), and the results are summarized in Fig. 4. It is seen that the critical pressure increased as the axial stretch ratio increased.

Critical pressures of these arteries were also calculated using buckling equation (Eq. 1) with vessel dimensions and

### Table 2. Material constants for three-dimensional Fung strain energy function (normal group)

<table>
<thead>
<tr>
<th>ID</th>
<th>$b_0$</th>
<th>$b_1$</th>
<th>$b_2$</th>
<th>$b_3$</th>
<th>$b_4$</th>
<th>$b_5$</th>
<th>$b_6$</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>55.45</td>
<td>0.304</td>
<td>0.001</td>
<td>0.297</td>
<td>0.518</td>
<td>0.318</td>
<td>0.241</td>
</tr>
<tr>
<td>6</td>
<td>43.00</td>
<td>0.220</td>
<td>0.001</td>
<td>0.217</td>
<td>0.342</td>
<td>0.155</td>
<td>0.140</td>
</tr>
<tr>
<td>7</td>
<td>31.02</td>
<td>0.189</td>
<td>0.147</td>
<td>0.636</td>
<td>0.738</td>
<td>0.856</td>
<td>0.001</td>
</tr>
<tr>
<td>8</td>
<td>70.10</td>
<td>0.148</td>
<td>0.021</td>
<td>0.001</td>
<td>0.428</td>
<td>0.338</td>
<td>0.108</td>
</tr>
<tr>
<td>9</td>
<td>330.0</td>
<td>0.076</td>
<td>0.001</td>
<td>0.096</td>
<td>0.210</td>
<td>0.175</td>
<td>0.068</td>
</tr>
</tbody>
</table>

$b_0$–$b_6$, Material constants.
material constants given in Tables 1, 2, and 3 for each individual artery. The results are shown as solid curves in Fig. 4. It is seen that the model-predicted critical pressures matched well with the experimental results, with an average correlation coefficient of $r = 0.84$. At an axial stretch ratio of 1.3, for example, the critical pressures measured were 9.52 kPa (SD 1.53), while the buckling equation using the 3D model predicted the critical pressures of 10.11 kPa (SD 3.12). Both 2D and 3D buckling models predicted a trend of nonlinear increase of critical pressure as the stretch ratio increased. The 2D model tended to overestimate the critical pressure at the higher stretch ratios, whereas the 3D model was more consistent in its predictions.

Effect of elastase treatment on arterial wall mechanical properties (elastase group). Verhoff staining, which stains elastin in dark purple, clearly showed elastin degradation in elastase treated artery segments compared with untreated segments (Fig. 5). Before elastase treatment, the internal elastic lamina and other elastic fibers were well organized throughout the wall. After elastase treatment the internal elastic lamina was nearly fully removed, and the other elastin fibers were fragmented throughout the wall. Trichrome staining showed no difference in collagen content before and after elastase treatment visually and by photometric measurement ($n = 7$, $P$ = nonsignificant). Both photometric measurement ($n = 7$, $P < 0.01$ at a power of 0.91) and Fastin assay ($n = 4$, $P < 0.05$, at a power of 0.98) demonstrated a significant 50–60% reduction in elastin content after elastase treatment (Fig. 5, bottom).

Slight changes in the overall vessel dimensions were also observed after elastase treatment. The vessel lengths of the nine arteries were $44.6 \pm 8.0$ mm before treatment and $43.4 \pm 8.0$ mm after treatment; outer diameters were $6.5 \pm 0.7$ mm before treatment and $6.9 \pm 0.7$ mm after ($P < 0.05$); and wall thicknesses were $1.19 \pm 0.41$ mm before treatment and $0.90 \pm 0.29$ mm after. The differences were statistical insignificant for length and wall thickness.

Pressure inflation tests showed that arteries deformed more under pressure after elastase treatment compared with before treatment, indicating that arterial walls became less stiff post-elastase treatment (Fig. 6). The corresponding material constants of the 3D Fung strain energy changed after the elastase treatment (Table 4). The opening angles $\theta_0$ of arteries were significantly smaller in the elastase-treated arteries ($P < 0.05$).

Effects of elastase on the critical buckling pressure (elastase group). The degradation of elastin reduced the critical buckling pressure. For arteries treated by elastase, the critical buckling pressure significantly decreased posttreatment compared with pretreatment (Fig. 7). At an axial stretch ratio of 1.5, for example, the average critical pressure of arteries was 18.29 kPa (SD 4.58) pretreatment and 9.58 kPa (SD 3.68) posttreatment, respectively ($n = 9$, $P < 0.01$). The theoretical model accurately predicted the critical pressure before elastase treatment.
but underestimated the critical pressure after elastase treatment.

The effects of matrix support on the buckling behavior of arteries (gel group). While arteries in PBS (without surrounding matrix support) buckled into one sinusoidal wave, arteries in an elastic matrix buckled into multiple waves (Fig. 8). The buckling pressure was higher when arteries buckled inside an elastic matrix compared with arteries that buckled in PBS. At an axial stretch ratio of 1.3, the buckling pressures of the eight arteries were 11.10 kPa (SD 3.02), 22.50 kPa (SD 7.41), and 8.9 kPa (SD 2.48) at the baseline (in PBS), in gel, and after the removal of the gel (in PBS), respectively. The critical pressure in gel was significantly higher than in PBS (P < 0.01). These findings are consistent with our laboratory’s previous theoretical predictions of arteries buckling inside a matrix (17).

DISCUSSION

The stability of long arteries under internal pressure has rarely been studied, since arteries are commonly considered stable under luminal pressure and axial tension. Our results clearly demonstrated that arteries buckle and become tortuous under luminal pressure. The critical buckling pressure depended on the axial stretch ratio and wall stiffness. Elastase treatment reduced the stiffness of the arterial wall and its critical pressure, which thus makes arteries vulnerable to instability. In contrast, surrounding tissue matrix support enhanced the artery’s stability by increasing the critical pressure. These results are consistent with the conclusions from our previous model analysis, and the buckling equation accurately predicted the critical pressure of the arteries.

One interesting conclusion from these results is that arteries could buckle and become tortuous not only at hypertensive blood pressure but also at physiological pressures due to a weakened wall or reduced axial strain. Arteries are commonly considered stable under their high internal pressure and axial tension. Here we demonstrated that high luminal pressure and/or reduced axial tension can cause instability of arteries. Arteries in vivo are subjected to longitudinal tensions that change significantly due to aging and cardiovascular disease (37). The reduction in axial strain below physiological levels has been shown to induce tortuosity in rabbit carotid arteries in vivo (28). Arterial elongations due to excessive growth in collateral arteries also lead to tortuosity (5, 12). In contrast, significant axial stretch in normal arteries helps maintain the stability of arteries against buckling. Clinical studies have shown that tortuous arteries are often associated with hypertension and reduced axial stretch. Buckling may occur after vascular surgeries due to reduced stretch ratios or reduced surrounding tissue support after surgery (23, 56). Surgical treatment to shorten the redundant arteries in patients usually shows very positive outcomes in symptomatic patients with tortuous arteries (2, 27), indicating that an increasing axial stretch ratio increases arterial stability and prevents buckling, as predicted by our model. Therefore, axial tension is essential in maintaining the stability of arteries and vascular grafts in preventing tortuosity.

Fig. 5. Top: histology sections (×10) with Verhoff staining demonstrating the elastin (in black) in an artery before (left) and after (right) elastase treatment. Breakdown of elastin is evident after the treatment. Middle: histology sections with Trichrome staining demonstrating collagen (in blue) before and after elastase treatment. Bottom: quantification of protein concentrations. The left bar graph represents the color metric quantification of elastin and collagen area ratios [mean (SD), n = 7], and the right bar graph shows the elastin concentration [mean (SD), n = 4] measured using Fastin assay. *P < 0.05, **P < 0.01.
Compared with experimental measurement, the 3D thick-wall cylindrical model with the Fung strain energy function accurately predicted the critical pressure for the arteries tested whereas the 2D thin-walled model showed a tendency to overestimate the critical pressure at higher stretch ratios.

It has been shown that elastase treatment reduces wall stiffness (11). Our results confirmed that the arteries were softer post-elastase treatment, although the changes were less significant in the axial direction than in the circumferential direction. A 2D analysis confirmed that the incremental modulus decreased after elastase treatment (data not shown). Our new finding is that weakened arterial walls also compromise the mechanical stability of arteries by reducing the critical pressure and lead to bent buckling that normally will not happen under physiological pressures. Furthermore, our results from the gel group demonstrated that arteries buckle into sinusoidal shapes of multiple waves when matrix support is

**Fig. 6. Effect of elastase treatment on the mechanical properties of arteries (elastin group). Comparison of the outer diameter (circles) and vessel length (triangles) of an artery plotted as functions of luminal pressure before (open) and after (solid) elastase treatment. Solid curves are the fitting results using the Fung 3D strain energy function. Bottom bar graph: comparison of opening angles of arteries [mean (SD), n = 5] before and after elastase treatment. **P < 0.05.

Compared with experimental measurement, the 3D thick-wall cylindrical model with the Fung strain energy function accurately predicted the critical pressure for the arteries tested whereas the 2D thin-walled model showed a tendency to overestimate the critical pressure at higher stretch ratios.

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**Fig. 7. Top: comparison of the critical pressure of arteries [mean (SD), n = 9] measured before and after elastase treatment (elastin group). Bottom: comparisons of experimental measurements with theoretical predictions (Fung 3D) after elastase treatment. **P < 0.01 vs. before treatment. λ + 1.1, λ + 1.3, and λ + 1.5: axial stretch ratios of 1.1, 1.3, and 1.5, respectively.
ELASTIN DEGRADATION LEADS TO ARTERIAL INSTABILITY

Fig. 8. The effect of surrounding tissue support on artery buckling. Photographs are of an artery buckled under internal pressure in PBS solution (A) and then inside gelatin gel (B). The critical pressures were 13.3 and 30.6 kPa in PBS and in gel, respectively. The axial stretch ratio was 1.3, and the gel modulus was 4.4 kPa. Bar graph: a comparison of critical pressures of arteries [mean (SD), n = 8] at baseline (in PBS), in gel, and after gel removal (in PBS). **P < 0.01.

Around the artery. This phenomenon can be explained using our previous model equation of critical pressure (17):

\[
p_{cr} = \frac{N}{\pi r_i^2} + \frac{EI}{\pi r_i^4} \left( \frac{n \pi}{l} \right)^2 + \frac{k_s}{\pi r_i^3} \left( \frac{l}{n \pi} \right)^2
\]

where \(k_s\) is the modulus of the surrounding matrix, \(EI\) is the bending rigidity of the artery, and \(n\) is the wave number of the buckling mode shape (17, 19). According to this equation, without surrounding matrix support \((k_s = 0)\), \(p_{cr}\) is minimal at \(n = 1\); with matrix support \((k_s > 0)\), the second term in the equation increases with increasing wave number \(n\), while the third term decreases. So a minimum buckling pressure is reached at a wave number \(n > 1\). Therefore, arteries buckle into multiple wave shapes within the surrounding tissue matrix, and matrix support increases the critical pressure. In fact, strong tissue support can prevent artery buckling (17). In contrast, a straight artery may develop into wavy tortuous shapes when the surrounding tissues are weakened by degenerative diseases. For example, it was reported that tortuous arterioles occurred with the development of small lacuna cavities (status lacunaris) (41).

The model predictions were vessel specific, using the vessel dimensions and material constants as input. Therefore, variations in vessel diameter and length did not affect, but demonstrated, the predictive value of the model. Note that the pressure diameter curve was obtained while the vessels were free to expand longitudinally. Arterial diameter-pressure relations were often obtained at fixed length in the literature (11, 15). Here we used a free-end inflation test to obtain both axial and cross-sectional deformations, since our previous model demonstrated that arterial buckling depends on both axial and cross-sectional stiffness (17, 18).

One limitation of the study was that the pressure was static while arteries in vivo are under pulsatile pressure and flow. Both theoretical modeling and experimental measurements were done under static pressure for simplicity to capture the main feature of artery buckling and for consistency between the model and experiment. Further work needs to be done to study artery buckling behavior under pulsatile pressure, although a theoretical analysis of artery dynamic stability was recently given by Rachev and Gleason (44, 45). Meanwhile, the use of static model provides an effective approach to estimate the buckling pressure. It is similar to the use of mean pressure to estimate the main stress and strain in arteries, although arteries are subject to pulsatile pressure. Furthermore, in small arteries and arterioles, as well as veins, the pressure can be approximated as a steady pressure as the pulse pressure is dampened down.

The buckling model was developed for the general situation that included the opening angle. However, our previous simulations using rabbit data from Chuong and Fung (6) showed that the opening angle had very little effect on the critical pressure. The difference reported in our laboratory’s 2008 paper (18) was due to an error, and corrections were made in an erratum. Thus, in the current simulations, we did not consider the effect of the opening angle.

Another limitation is that we only tested artery buckling under one gel stiffness to illustrate the effect of gel support vs. without gel support (in PBS). According to our theoretical analysis (Eq. 4) (17), gel stiffness affects the critical pressure. Further studies are needed to experimentally determine the relationship between gel stiffness and critical pressure.

The elastase used for the elastase group was not highly purified elastase. Although there may be some collateral damages in addition to elastin breakdown, no change in collagen content was observed in the elastase-treated arteries, while both photometric measurement and Fastin assay confirmed a 50–60% reduction in the elastin content. The results achieved our goal of illustrating that ECM breakdown will reduce the buckling pressure of arteries.

The 3D model simulations were able to accurately predict the buckling of arteries before elastase treatment, but underestimated the critical pressure after elastase treatment. The small changes in wall dimensions due to elastase treatment were already accounted for in the model simulation by using the posttreatment wall dimensions. We speculate that there may be two possible reasons: first, the lumen layer of the arteries became loose and wavy after elastase treatment, which may have led to errors in the lumen diameter measurement posttreatment. Second, we also observed that the lumen layer of the arterial wall was slightly longer than the outer layer when cut into short ring segments after elastase treatment, indicating a difference in axial length across the layers of the vascular wall (31). This nonuniformity of axial length across the lumen may affect the buckling behavior of the arteries, which was not
considered in the buckling equations. Further studies are needed to examine this effect.

While our models predicted a lower than physiological critical pressure for porcine arteries, arteries in vivo normally have a higher critical pressure due to higher axial stretch ratios and surrounding tissue support (17, 18). This was illustrated in our results with surrounding matrix support. Arteries with matrix support buckled with a higher pressure of 22.5 kPa, which is above the normal pressure 16 kPa. Thus arteries in vivo do not buckle under normal conditions. Buckling is accelerated if the arteries are under higher pressures, such as in hypertensive patients, lower stretch ratios shown in aged individuals, and decreased elastin, which is shown in diseased arteries, as well as aged arteries (10, 28, 53). The present study suggests that mechanical instability could be an underlying mechanism to trigger the development of tortuous vessels.

The current results broaden our understanding of vascular biomechanics and shed light on the stability of arteries and veins. A high prevalence of tortuosity has been observed in vessels with hypertensive pressure, diminishing axial stretch, and other vascular diseases (10, 24, 37, 40). We demonstrated that arteries buckle under hypertensive pressure or reduced axial stretch ratios. Buckling would change luminal blood flow and wall stress and result in remodeling of the arterial wall (38, 43, 48, 55). Understanding the biomechanics of blood vessel buckling may have wide implications in vascular physiology and pathology, as well as vascular surgery.

In conclusion, arteries buckle due to a decrease in stretch ratio, wall stiffness, and/or an increase in internal pressure, and buckled arteries exhibit tortuous shapes. Mechanical buckling could be a possible mechanism that leads to artery tortuosity, which is observed in many clinical patients.

APPENDIX

Deformation of cylindrical vessel under pressure and axial tension. Arteries are modeled as thick-walled, circular cylinders under internal pressure \( p \) and axial (longitudinal) tension \( N \) with an axial elongation of stretch ratio \( \lambda_z^0 \) (17). We denote the cylindrical coordinates of a material point in the arterial wall to be \( (R, \Theta, Z) \) in the stress-free state (open sector, with an opening angle of \( 2\Theta_0 \)), and \( (r, \phi, z) \) in the loaded state (Fig. A1). Thus,

\[
r = r(R, p), \quad \phi = \frac{\pi \Theta}{\Theta_0}, \quad z = \lambda_z^0 Z \quad (A1)
\]

The stretch ratios in the radial, circumferential, and axial directions are

\[
\lambda_r = \frac{\partial r}{\partial R}, \quad \lambda_\phi = \frac{r}{\Theta_0}, \quad \lambda_z = \lambda_z^0 \quad (A2)
\]

The corresponding Green strains \( (E_r, E_\phi, E_z) \) are

\[
E_r = \frac{1}{2}(\lambda_r^2 - 1), \quad E_\phi = \frac{1}{2}(\lambda_\phi^2 - 1), \quad E_z = \frac{1}{2}(\lambda_z^2 - 1) \quad (A3)
\]

It is seen that the strains are axisymmetric. The circumferential, axial, and radial strains depend on \( r \) only, and the axial strain is a constant. The arterial wall was assumed to be incompressible, homogenous, orthotropic, nonlinear elastic and described by the Fung strain energy function (15, 17):

\[
w_0 = \frac{1}{2}b_0e^{d_0} + K\left[ (1 + 2E_r)(1 + 2E_\phi)(1 + 2E_z) - 1 \right]
\]

\[
Q = b_1E_r^2 + b_2E_\phi^2 + b_3E_z^2 + 2b_4E_rE_\phi + 2b_5E_rE_z + 2b_6E_\phi E_z \quad (A4)
\]

wherein \( w_0 \) is the strain energy density, \( b_0 \) and \( b_1\ldots b_6 \) are material constants, and \( K \) is a Lagrangian multiplier. Accordingly, the stress in the radial, axial, and circumferential directions can be expressed as:

\[
\sigma_r = \left( 1 + 2E_r \right) \frac{\partial w_0}{\partial E_r} + K \quad (A5)
\]

\[
\sigma_\phi = \left( 1 + 2E_\phi \right) \frac{\partial w_0}{\partial E_\phi} + K \quad (A6)
\]

\[
\sigma_z = \left( 1 + 2E_z \right) \frac{\partial w_0}{\partial E_z} + K
\]

The equation of equilibrium in the straight axisymmetric vessel become

\[
\frac{\partial \sigma_r}{\partial r} + \frac{\sigma_r - \sigma_\phi}{r} = 0 \quad (A6)
\]

With boundary conditions

\[
\sigma_r(r_i) = -p; \quad \sigma_r(r_e) = 0 \quad (A7)
\]

where \( r_i \) and \( r_e \) are lumens and outer radii. Integrating Eq. A6 from \( r_e \) to \( r \) and combining with Eqs. A5 and A7 yields

\[
sigma_r = \int_{r_e}^{r} \left[ \left( 1 + 2E_r \right) \frac{\partial w_0}{\partial E_r} - \left( 1 + 2E_r \right) \frac{\partial w_0}{\partial E_\phi} \right] \frac{d\xi}{\xi}
\]

\[
\sigma_\phi = \left[ \left( 1 + 2E_\phi \right) \frac{\partial w_0}{\partial E_\phi} - \left( 1 + 2E_r \right) \frac{\partial w_0}{\partial E_r} \right] + \int_{r_e}^{r} \left( 1 + 2E_\phi \right) \frac{\partial w_0}{\partial E_\phi} - \left( 1 + 2E_r \right) \frac{\partial w_0}{\partial E_r} \frac{d\xi}{\xi}
\]

\[
\sigma_z = \left[ \left( 1 + 2E_z \right) \frac{\partial w_0}{\partial E_z} - \left( 1 + 2E_r \right) \frac{\partial w_0}{\partial E_r} \right] + \int_{r_e}^{r} \left( 1 + 2E_\phi \right) \frac{\partial w_0}{\partial E_\phi} - \left( 1 + 2E_r \right) \frac{\partial w_0}{\partial E_r} \frac{d\xi}{\xi}
\]

where \( \xi \) is the radial coordinate between \( r_e \) and \( r \). Letting \( r = r_i \) in the first equation of Eq. A8, we have

\[
r = r(R, p), \quad \phi = \frac{\pi \Theta}{\Theta_0}, \quad z = \lambda_z^0 Z
\]

\[
AJP-Heart Circ Physiol • doi:10.1152/ajpheart.00463.2011 • www.ajpheart.org
\]
p = \int_{r_1}^{r_2} \left[ (1 + 2E_r)(b_1E_o + b_2E_z + b_3E_r) \\
- (1 + 2E_r)(b_6E_o + b_7E_z + b_8E_r) \right] b_0 e^p \frac{d\xi}{\xi} \tag{A9}

The resultant of the stresses on a cross section is only an axial tension N. Integrating \( \sigma_c \) over the cross-sectional area that yields axial tension N:

\[
N = \pi r_1^2 p + \pi \int_{r_1}^{r_2} \left[ 2(1 + 2E_r)(b_1E_o + b_2E_z + b_3E_r) \\
- (1 + 2E_r)(b_6E_o + b_7E_z + b_8E_r) \right] b_0 e^p r dr \tag{A10}
\]

**Buckling equations for artery segments with fixed ends.** For arteries with fixed ends, our experimental observations and theoretical analysis demonstrated that arteries buckle into a cosine shape (19). The buckling mode shape of the central axis of the vessel is given by:

\[
u_c = C \left[ \frac{1}{2} - \cos \left( \frac{2\pi n \zeta}{l} \right) \right] \tag{A11}
\]

where \( C \) is a small constant. By assuming that the cross sections of the arteries remain circular when arteries deflect, the radial, circumferential, and axial coordinates of point \((r, \phi, z)\) after deflection \( u_c \) are:

\[
\begin{align*}
\rho &= r(R, p) + u_c \cdot \cos \phi; \\
\phi &= \frac{\pi \Theta}{\Theta_0} \\
\zeta &= z - \frac{u_c}{r} \cdot r \cos \phi
\end{align*} \tag{A12}
\]

The deformation gradient and strain components can be determined accordingly (17). By neglecting the high-order terms of small deflection \( u_c \), the only nonzero incremental strain component due to deflection \( u_c \) is the axial incremental strain:

\[
\Delta \varepsilon_\text{ax} = - \left( \frac{\partial \zeta}{\partial Z} \right)^2 \frac{\partial^2 u_c}{\partial z^2} r \cos \phi \tag{A13}
\]

This equation indicates that the small bend at buckling leads to non-axisymmetric axial strain, but the circumferential and radial strains remain axisymmetric, and the shear strains remain zero. Accordingly, the change in axial stress can be determined by substituting \( \varepsilon_\text{ax} + \Delta \varepsilon_\text{ax} \) in the third equation in Eq. A8, and thus the bending moment \( M(z) \) can be obtained by integrating the axial stress over the cross-sectional area \( A \):

\[
M(z) = \int_A \sigma \cdot dA = \int_A (r \cos \phi) (\Delta \varepsilon_\text{ax}) \cdot dA \tag{A14}
\]

that yields

\[
M(z) = \left( \frac{2\pi n}{L} \right)^2 H \cdot \frac{C}{2} \cdot \cos \left( \frac{2\pi n \zeta}{l} \right) \tag{A15a}
\]

with

\[
H = \pi \int_{r_1}^{r_2} \left[ J_1 \cdot \frac{1}{3} J_0 b_0 e^p r^3 dr + \frac{\pi r_1^3}{3} \int_{r_1}^{r_2} [J_0 b_0 e^p r^3 dr \right. \\
J_2 = 2g_r \left[ (1 + 2E_2)g_r - (1 + 2E_2)g_r \right] + 2g_z + (1 + 2E_2) b_3 - (1 + 2E_2) b_3 \tag{A15b} \\
J_3 = 2g_r \left[ (1 + 2E_2)g_r - (1 + 2E_2)g_r \right] + (1 + 2E_2) b_3 - (1 + 2E_2) b_3 \tag{A15a}
\]

wherein

\[
Q_0 = \frac{1}{2} \int_0^l q(z) dz = 0 \tag{A17}
\]

Therefore, the bending moment \( M(z) \) at axial location \( z \) can be determined by

\[
M(z) = M_0 - N = \frac{C}{2} \left[ 1 - \cos \left( \frac{2\pi n \zeta}{l} \right) \right] - \frac{1}{2} \int_0^l q(\xi) d\xi \tag{A18}
\]

Taking Eq. A16 into Eq. A18 and integrating yields

\[
M(z) = (-N + \frac{p \pi r_1^2}{2}) \cdot \frac{2\pi n}{L} \cdot \cos \left( \frac{2\pi n \zeta}{l} \right) \tag{A19}
\]

Combining Eqs. A19 and A15a yields:

\[
\frac{N + \left( \frac{2\pi n}{L} \right)^2 H}{\pi r_1^2} \tag{A20}
\]

Thus, when the pressure reaches the level given in this equation, the artery will achieve equilibrium at the bent shape, and thus buckling occurs. Therefore, this equation (with \( n = 1 \)) determines the critical buckling pressure of arteries with both ends fixed.

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