Artery buckling stimulates cell proliferation and NF-κB signaling

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Artery buckling stimulates cell proliferation and NF-κB signaling. Am J Physiol Heart Circ Physiol 307: H542–H551, 2014. First published June 14, 2014; doi:10.1152/ajpheart.00079.2014.—Tortuous carotid arteries are often seen in aged populations and are associated with atherosclerosis, but the underlying mechanisms to explain this prevalence are unclear. Artery buckling has been suggested as one potential mechanism for the development of tortuous arteries. The objective of this study, accordingly, was to determine the effect of buckling on cell proliferation and associated NF-κB activation in arteries. We developed a technique to generate buckling in porcine carotid arteries using long artery segments in organ culture without changing the pressure, flow rate, and axial stretch ratio. Using this technique, we examined the effect of buckling on arterial wall remodeling in 4-day organ culture under normal and hypertensive pressures. Cell proliferation, NF-κB p65, IκB-α, ERK1/2, and caspase-3 were detected using immunohistochemistry staining and immunoblot analysis. Our results showed that cell proliferation was elevated 5.8-fold in the buckling group under hypertensive pressure (n = 7, P < 0.01) with higher levels of NF-κB nuclear translocation and IκB-α degradation (P < 0.05 for both). Greater numbers of proliferating cells were observed on the outer curve side of the buckled arteries compared with the outer curve side (P < 0.01). NF-κB colocalized with proliferative nuclei. Computational simulations using a fluid-structure interaction model showed reduced wall stress on the inner side of buckled arteries and elevated wall stress on the outer side. We conclude that arterial buckling promotes site-specific wall remodeling with increased cell proliferation and NF-κB activation. These findings shed light on the biomechanical and molecular mechanisms of the pathogenesis of atherosclerosis in tortuous arteries.

LARGE- AND MEDIUM-SIZED ARTERIES often become tortuous in the aged population (8, 20, 40). Specifically, tortuous carotid arteries are observed in 4–45% of angiograms, with a greater prevalence in the aged population than in the young population (8, 56). Tortuous carotid arteries exhibit a higher incidence of atherosclerosis compared with nontortuous arteries (8, 20, 46), but the underlying mechanisms are poorly understood. Aging is known to promote hypertension, reduce axial tension, and increase the risk for atherosclerosis and other cardiovascular diseases (15, 31, 40, 44, 58). Our recent studies (17–19) have shown that arteries lose stability and buckle into a tortuous shape ex vivo due to hypertensive pressure, reduced axial tension, or weakened arterial wall structure. Artery buckling has been suggested as a possible mechanism leading to tortuosity (18, 20, 21). Buckling-induced curving alters the distributions of fluid shear stress and wall stress in arteries (20, 28), which could stimulate arterial wall remodeling (11, 14, 28). Therefore, it is important to determine how arteries remodel after buckling to understand the mechanism of tortuous artery development.

In addition, it is of clinical interest to determine whether artery buckling stimulates atherosclerosis, since 80% atherosclerosis in carotid and coronary arteries occurs at curving and branching areas where shear stress is lower and wall stresses are elevated (29, 39). Cell proliferation and intima hyperplasia are key initiating events in atherosclerosis development (27, 29). Previous studies from many groups, including our own, have demonstrated that altered axial stretch, shear stress, and pulse pressure stimulate cell proliferation in porcine carotid arteries (22, 24, 28, 32, 36, 59). Therefore, it is of clinical interest to investigate whether increased cell proliferation occurs in buckled arteries.

NF-κB is a key nuclear transcription factor that regulates a variety of inflammatory and proliferative gene responses in cells that contribute to arterial remodeling and atherosclerosis (2, 37, 43). NF-κB activation in endothelial cells may play an important role in the pathogenesis of atherosclerosis, especially during the early stages of disease progression (16). In addition, disturbed blood flow and acute alteration of shear stress promote arterial inflammation and wall remodeling through the induction of NF-κB expression in endothelial cells (5, 6).

Therefore, the objective of this study was to investigate cell proliferation and the associated NF-κB activation in buckled carotid arteries. We examined buckling-induced site-specific wall remodeling in curved porcine carotid arteries using a well-established organ culture model (22, 36).

MATERIALS AND METHODS

Ex vivo arterial organ culture. Porcine common carotid arteries were obtained from farm pigs (~100 kg) after death at a local abattoir with approval from the Texas Department of State Health Service and the University of Texas at San Antonio Institutional Biosafety Com-
mittee. Arteries were transported to our laboratory in ice-cold PBS and set up in an ex vivo perfusion organ culture system as previously described in detail (36, 59). Briefly, arteries were cleaned and mounted at their in situ length to diameter-matched stainless steel cannulae in vessel chambers filled with bath medium. Medium perfusion to the arteries was driven by a peristaltic roller pump at a controlled flow rate (160 ml/min) and pulse frequency (2.5 Hz). Perfusion and bath media consisted of cell culture medium DMEM (Sigma) supplemented with sodium bicarbonate (3.7 g/l, Sigma), L-glutamine (2 mM, Sigma), calf serum (10%, Sigma), HEPES (25 mM, GIBCO), and antibiotic-antimycotic solution (1%, Sigma). Dextran (50 g/l, Sigma) was added to the perfusion medium to adjust its viscosity close to that of human blood (4 cP). Bromodeoxyuridine (BrdU; 5 μg/ml, Roche) was added to the perfusion medium at the first day of the experiment to label the nuclei of newly proliferated cells. Flow loops were then placed in 5% CO₂ incubators at 37°C.

To facilitate buckling, long arterial segments (40–60 mm) were used in the experimental groups, whereas short arterial segments (15–25 mm) were used to avoid buckling in control groups. This design was based on our previous theoretical analysis, which showed that longer segments have lower critical buckling pressure than shorter segments (17, 18). With the use of a long segment, the critical pressure of the experimental group was reduced to a level below the perfusion pressure while using a short segment increased the critical pressure of the control arteries to a level above the perfusion pressure. Consequently, when the perfusion pressure was gradually increased to set values of 100 and 200 mmHg, the long segments buckled, whereas the short segments remained straight. This approach allowed us to achieve buckling without altering the flow rate and pressure and thus to distinguish the effects of buckling from the effects of pressure or flow rate. All arteries were maintained for up to 4 days under a physiological flow rate of 160 ml/min and a subphysiological axial stretch ratio of 1.3 to facilitate artery buckling (17, 18). The subphysiological axial stretch ratio was used to mimic the loosening of axial tension in tortuous arteries in vivo. Both buckling and control groups (without buckling) were cultured under normotensive pressure (120 ± 20 mmHg).

To further investigate the combined effects of artery buckling and hypertension, another group of arteries was cultured under hypertensive pressure (230 mmHg). A separate control group was used to distinguish the effects of buckling from the effects of pressure or flow rate.

Immunohistochemistry staining. Immunohistochemistry staining was conducted as previously described (36, 57). Arterial specimens were flushed with sterile ice-cold PBS and pressure fixed in 2% paraformaldehyde in PBS. A partial lengthwise incision was made to mark the outer curve. Fixed arteries were then transferred in 30% sucrose in PBS until processed for embedding in OCT compound.

For immunofluorescent staining, 5-μm frozen cross-sections were treated with 0.01 M citrate buffer (pH 6.0), permeabilized with 0.5% Triton X-100 in PBS, blocked with 10% FBS in PBS, and processed for labeling with the following primary antibodies: mouse anti-BrdU (catalog no. 11296736001, Roche), rabbit anti-NF-κB (catalog no. 372, Santa Cruz Biotechnology), rabbit anti-CD31 (catalog no. 28364, Abcam), and rabbit anti-α-smooth muscle actin (catalog no. 5694, Abcam). For costaining with multiple primary antibodies, the primary antibodies species were matched so that the corresponding FITC-conjugated (green) or Cy3-conjugated (red) secondary antibodies (Molecular Probes) could be used to distinguish the different primary antibodies. 4’,6-Diamidino-2-phenylindole dihydrochloride was used to counterstain nuclei. Immunofluorescence staining was performed with a Qcolor3 camera under an Olympus CX41 fluorescence microscope.

Fig. 1. Top: photographs of a straight (control) artery and a buckled artery, both maintained in an ex vivo organ culture system for 4 days. Bottom: schematic of a buckled artery illustrating the buckling deflection (δ), end distance (L), and inner and outer curve sides.

Apoptosis was determined using caspase-3 immunoblotting and terminal deoxynucleotidyl transferase dUTP nick-end labeling (TUNEL). For TUNEL staining, frozen sections were dried and fixed as described above. After being permeabilized with 0.2% Triton X-100, sections were stained with TUNEL kit reagents (Roche Applied Science). Nuclei were counterstained with 4’,6-Diamidino-2-phenylindole dihydrochloride. Quantification of the percentage of TUNEL-positive cells was performed using a cell count under fluorescent microscopy, with images taken at ×10 magnification.

Endothelial protein extraction and immunoblot analysis for NF-κB p65, IκB-α, ERK1/2, and caspase-3 measurements. From each artery, two tissue samples were harvested for fresh and 4-day cultured arteries. One sample was used for whole cell lysates of the arterial wall in Laemmli sample buffer (catalog no. 161-0737, Bio-Rad) to detect ERK1/2 and caspase-3 by immunoblot analysis. The other sample was used to culture endothelial cells, which were gently scraped from the arterial wall. Harvested cells were divided into two aliquots, one aliquot in Laemmli sample buffer for whole cell lysates to detect total IκB-α using Western blot analysis and another aliquot in cold PBS for nuclear extraction of NF-κB p65. The extraction was done by isolating nuclear fractions using NE-PER nuclear and cytoplasmic extraction reagents (catalog no. 78833, Thermo Fisher Scientific) as described below.
Table 1. Arterial segment unloaded dimensions and organ culture conditions for the experimental groups

<table>
<thead>
<tr>
<th></th>
<th>Normotensive</th>
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<th>Hypertensive</th>
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<tr>
<td></td>
<td>Straight arteries</td>
<td>Buckled arteries</td>
<td>Straight arteries</td>
<td>Buckled arteries</td>
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<tr>
<td>Sample size</td>
<td>5</td>
<td>5</td>
<td>7</td>
<td>7</td>
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<tr>
<td>Unloaded</td>
<td></td>
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<tr>
<td>length, mm</td>
<td>19.6 ± 2.4</td>
<td>59.8 ± 4.8</td>
<td>21.4 ± 1.8</td>
<td>39.0 ± 4.0</td>
</tr>
<tr>
<td>Outer diameter, mm</td>
<td>4.2 ± 0.2</td>
<td>4.6 ± 0.3</td>
<td>6.9 ± 0.3</td>
<td>6.4 ± 0.3</td>
</tr>
<tr>
<td>Inner diameter, mm</td>
<td>1.8 ± 0.1</td>
<td>2.0 ± 0.2</td>
<td>3.9 ± 0.2</td>
<td>3.4 ± 0.2</td>
</tr>
<tr>
<td>Thickness, mm</td>
<td>1.2 ± 0.2</td>
<td>1.3 ± 0.1</td>
<td>1.5 ± 0.2</td>
<td>1.5 ± 0.2</td>
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<tr>
<td>Tortuosity index</td>
<td>0.05 ± 0.01</td>
<td>0.12 ± 0.01</td>
<td>0.06 ± 0.01</td>
<td>0.23 ± 0.02</td>
</tr>
<tr>
<td>Lumen pressure, mmHg</td>
<td>120 ± 20</td>
<td>120 ± 20</td>
<td>230 ± 30</td>
<td>230 ± 30</td>
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<tr>
<td>Flow rate (ml/min)</td>
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<td>160</td>
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<tr>
<td>Axial stretch ratio</td>
<td>1.3</td>
<td>1.3</td>
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*P < 0.01 vs. normotensive straight arteries; †P < 0.01 vs. hypertensive straight arteries.

Briefly, endothelial cells collected in cold PBS were resuspended, homogenized in cytoplasmic extraction reagent (CER) I, and incubated on ice for 10 min. CER II was then added, and extracts were further incubated on ice for 1 min. Extracts were then centrifuged for 5 min, and the supernatant (cytoplasmic fraction) was transferred to a new tube. Nuclear extraction reagent (NER) was added to the nuclear pellet, and the nuclear pellet was incubated on ice for 40 min, with vortexing every 10 min. The extract was centrifuged for 10 min, and the supernatant (nuclear fraction) was transferred to a new tube. In addition, protease inhibitor cocktail (Roche) was added to the nuclear extraction fraction and whole cell lysates to prevent protein degradation. Isolated nuclear extraction fractions and whole cell lysates were stored at −80°C until immunoblot analysis.

Immunoblot analysis was conducted as previously described (57). Briefly, genomic DNA in whole cell lysates was sheered via sonication on ice three times (5 s each). Nuclear extractions and whole cell lysates were loaded onto SDS polyacrylamide gels for electrophoresis separation. The separated protein bands were blotted onto Immun-Blot polyvinylidene difluoride membranes (Bio-Rad) for detection with the following primary antibodies: rabbit anti-NF-kB p65 (catalog no. 372, Santa Cruz Biotechnology), rabbit anti-total IkB-α (catalog no. 371, Santa Cruz Biotechnology), rabbit anti-total ERK1/2 (catalog no. 4695, Cell Signaling), rabbit anti-phospho-ERK1/2 (catalog no. 4370, Cell Signaling), rabbit anti-caspase-3 (catalog no. 9665, Cell Signaling), mouse anti-β-actin (catalog no. 47778, Santa Cruz Biotechnology), and mouse anti-GAPDH (catalog no. CB1001, Millipore). Primary antibody bindings were detected with the corresponding secondary antibodies conjugated with horseradish peroxidase (Amersham GE Healthcare) and visualized using standard ECL detection (Amersham GE Healthcare). Immunoblot results were quantified using densitometry performed with ImageJ (National Institutes of Health), with values normalized to the densitometry of β-actin and GAPDH.

Arterial stress analysis using the fluid-solid interaction model. To illustrate shear stress and wall stress in the buckled arteries, we performed computational simulations using a fluid-solid interaction (FSI) model of arteries with the mean dimensions and loads of each group shown in Table 1 using ADINA software (version 8.9, ADINA R&D). To eliminate the effect of dimensional differences between buckled and control groups, an additional simulation was done for a vessel identical to the buckled artery of the hypertensive group but half of its length to avoid buckling (“hyper-half length”).

Arterial walls were modeled as a nonlinear anisotropic thick-walled model using the following Holzapfel strain-energy function (1, 25):

\[
W = \sum_{n=1}^{3} \left[ \frac{\mu_n}{\alpha_n} \left( \lambda_1^{\alpha_1} + \lambda_2^{\alpha_2} + \lambda_3^{\alpha_3} - 3 \right) \right] + \frac{k_1}{2k_2} \left( \exp[k_2(J_6 - 1)^2] + \exp[k_2(J_6 - 1)^2] - 2 \right) \tag{2}
\]

where \( W \) is the Holzapfel strain-energy function; \( \mu_n \) and \( \alpha_n \) are the Ogden material constants; \( \lambda_1, \lambda_2, \) and \( \lambda_3 \) are the principal stretch ratios in the radial, circumferential, and axial directions; \( k_1 \) and \( k_2 \) are the anisotropic constants; and \( J_4 \) and \( J_6 \) are the square of stretch ratios along the two diagonal fiber directions (\( J_4 = J_6 = \lambda_1^2 \sin^2 \beta + \lambda_2^2 \cos^2 \beta \), where \( \beta \) is the angle between the fiber and the axial direction of the artery). These material constants were determined by fitting the experimental data of pressurized inflation test of a porcine carotid artery (artery 4) from a previous study (38). Values yielded from the

Fig. 2. Effect of artery buckling on cell proliferation. A: comparison of the percentage of bromodeoxyuridine (BrdU)-positive cells (mean ± SE, \( n = 5 \)) in straight and buckled arteries after being cultured under normotensive pressure (NT) for 4 days. B and C: comparisons of the percentage of BrdU-positive cells (mean ± SE, \( n = 7 \)) in straight and buckled arteries cultured under hypertensive pressure (HP) and the corresponding BrdU-positive cells in intimal, medial, and adventitial layers, respectively. **P < 0.01.
fitting used in all simulations were $\alpha_1 = 1.5$, $\alpha_2 = 7$, $\alpha_3 = 0.5$, $\mu_1 = 3.200$, $\mu_2 = 7.800$, $\mu_3 = 5.100$, $k_1 = 15.09$ kPa, $k_2 = 1.06$, and $\beta = 58.7^\circ$. The wall thickness was also assumed to be the same among groups. Buckling was induced by adding an imperfection of the tiny initial curvature and gradually increasing the lumen pressure as previously described (7, 60).

A steady fluid flow rate of 160 ml/min at the inlet, a pressure of 120 mmHg (normotensive) or 230 mmHg (hypertensive) at the outlet, and an initial axial stretch ratio of 1.3 were used in the simulations. The fluid was modeled as a Newtonian fluid with a specific gravity of 1.01 and viscosity of 4 cP.

Statistical analyses. All values are presented as means ± SE. Statistical significance between means was determined using Student’s t-test for two group comparisons or two-way ANOVA for multiple group comparison. The significance level was set at $P < 0.05$.

RESULTS

Arterial buckling was achieved under both hypertensive and normotensive pressure conditions. Using long artery segments for the buckling group and a short segment for the control group, we were able to achieve buckling in the buckling group and avoid buckling in the control group while maintaining the same pressure, flow, and axial stretch ratio in both groups (Fig. 1). Vessel dimensions, hemodynamic conditions, and tortuosity indexes are shown in Table 1 for all groups. The tortuosity index of buckled arteries was significantly higher under hypertensive pressure ($0.23 ± 0.02$) compared with the normotensive pressure group ($0.12 ± 0.01$, $P < 0.01$).

Fig. 3. Cross-sectional images (×40 magnification, lumens on the right) illustrating 4',6-diamidino-2-phenylindole dihydrochloride (DAPI) nuclei counterstaining (blue) and BrdU-labeled nuclei (green fluorescence) in conjunction with CD31 staining (red) for endothelial cells (ECs) and anti-α-actin staining (red) for smooth muscle cells in straight and buckled arteries after being cultured under hypertensive pressure for 4 days.
Cell proliferation was increased by buckling under hypertensive pressures. In normotensive groups, anti-BrdU staining showed similar numbers of positive cells as straight controls, indicating that buckling in the absence of elevated pressure did not stimulate proliferation (Fig. 2A). In hypertensive pressure groups, the percentage of BrdU-positive cells was significantly higher in buckled arteries (9.8 ± 1.5%) compared with straight control arteries (1.7 ± 0.3%, P < 0.01; Fig. 2B). BrdU-positive cells were present mainly in endothelial cells in the intima and smooth muscle cells in the media of the arterial wall, as confirmed by anti-CD31 staining and anti-α-actin staining (Fig. 3). Percentages of BrdU-positive cells in both intimal and medial layers were significantly higher in buckled arteries compared with the corresponding layers in straight arteries (Fig. 2C). Meanwhile, TUNEL staining and caspase-3 immunoblot results demonstrated that there were no differences in apoptotic cell death between buckled arteries and control arteries (Fig. 4). These results suggest that arterial buckling under hypertensive pressure significantly promoted cell proliferation. Furthermore, the percentage of BrdU-positive nuclei in hypertensive controls (straight arteries) was similar to that of normotensive controls (see Fig. 2), suggesting that increased pressure alone did not cause an increase in cell proliferation in the arteries.

Cell proliferation was significantly higher on the inner curve side of hypertensive buckled arteries compared with the outer curve side. BrdU-positive cells also demonstrated a nonuniform variation along the circumference in hypertensive buckled arteries (Fig. 5A). Further measurements showed that the percentage of BrdU-positive cells was more than threefold higher on the inner curve side (16.1 ± 2.5%) than on the outer curve side (4.9 ± 0.9%) of buckled arteries under hypertensive pressure (P < 0.01; Fig. 5, B and C). Both inner and outer curve sides demonstrated more cell proliferation than straight controls.

**NF-κB is significantly activated in hypertensive buckled arteries.** Immunohistochemical staining demonstrated that NF-κB p65 nuclear translocation occurred in both intimal and medial regions of the artery (Fig. 6). This translocation was increased significantly in endothelial cells of the intimal layer (7.0 ± 1.4% vs 2.1 ± 0.8%, P < 0.05; Fig. 6A) in buckled arteries compared with straight arteries but not in smooth muscle cells of the medial layer. Immunoblot results confirmed increased NF-κB p65 nuclear translocation in buckled arteries (Fig. 6, B and D).

An additional independent indicator of NF-κB activation, IκB-α degradation, was also significantly higher in buckled arteries (Fig. 6, D). Further measurements showed that the percentage of BrdU-positive cells was significantly higher on the inner curve side (16.1 ± 2.5%) than on the outer curve side (4.9 ± 0.9%) of buckled arteries under hypertensive pressure (P < 0.01; Fig. 5, B and C). Both inner and outer curve sides demonstrated more cell proliferation than straight controls.

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arteries compared with either fresh or straight arteries (Fig. 6, C and E). Of note, NF-κB colocalized with BrdU-positive nuclei in buckled arteries (Fig. 7). Meanwhile, immunoblot analysis demonstrated no differences in ERK1/2 activation as measured by the ratio of phospho-ERK1/2 to total ERK1/2 among fresh, straight, and buckled groups (Fig. 8). Together, these results demonstrated that hypertensive arterial buckling resulted significant NF-κB activation in the intima but not ERK1/2 activation.

Wall stress and lumen shear stress in buckled arteries. FSI simulations demonstrated that lumen shear stress, circumferential stress, and longitudinal stress became nonaxisymmetric in buckled arteries (Fig. 9), whereas straight (control) arterial segments were subjected to symmetric axial and circumferential tensile stresses and lumen shear stress. Buckling deflection created lower circumferential and longitudinal stresses on the inner curve side of the artery than on the outer curve side, especially in hypertensive arteries (Fig. 9).

DISCUSSION

The present study evaluated artery buckling-induced cell proliferation and NF-κB activation using an ex vivo organ culture model. The key findings of this study were that 1) artery buckling stimulated increased cell proliferation, 2) the increase in proliferation was predominantly induced in the inner curve side under hypertensive lumen pressure, and 3) this increase in cell proliferation was associated with NF-κB activation in the arterial wall as demonstrated by increased IκB-α degradation and NF-κB p65 nuclear translocation that colocalized with BrdU-positive nuclei.

Model novelty. Arteries could buckle and become tortuous due to hypertensive pressure and/or reduced axial strain (17, 18). Our previous model demonstrated that long arterial segments have lower critical buckling pressure (17, 18). Hence, we used long artery segments to facilitate buckling in this study. The length of control arteries (straight cylinders) was shorter, but the length does not affect the flow and wall stresses as well as biological activities in the vessels. This is because...
Thus the stress difference between inner and outer curve sides was much less than in hypertensive arteries. In addition, the results demonstrated low cell proliferation in both normotensive and hypertensive controls with no statistical difference between them. The low cell proliferation in normotensive and hypertensive controls is consistent with our previous measurements (23), although previous measurements demonstrated some difference between the two. Our present results are also consistent with a previous report (33) showing that hypertensive pressure per se did not stimulate cell proliferation. Further statistical analysis demonstrated that there was an interaction between buckling and hypertensive pressure that causes a synergistic effect on cell proliferation.

Changes in mechanical stresses are sensed by vascular cells (endothelial cells and smooth muscle cells) through several different signaling pathways, including the NF-κB pathway (37, 43). The signal transduction involves proteins related to inflammation, proliferation, and oxidative stress, including ROS (55). Although ROS were not determined in the present study, a previous study (55) has demonstrated that NF-κB is an oxidant mechanosensitive transcription factor regulated by flow shear stress and that production and accumulation of ROS with disturbed shear stress associates with activation of NF-κB and increased DNA synthesis. Therefore, one possible mechanism for uneven remodeling in the buckled artery may be linked to ROS-mediated activation of NF-κB. Potential specific molecules targeting NF-κB may be a strategy in the prevention and treatment of tortuosity-associated atherosclerosis.

Relevance to atherosclerosis. Previous studies (8, 47, 54) have demonstrated a strong association between arterial tortuosity and increased atherosclerotic lesions. It has been shown that curved regions of coronary arteries have more lesions than straight regions (54) and that inner sides of curved segments of femoral arteries were more atherosclerotic than outer sides, whereas both were more atherosclerotic than straight segments (47). Consistent with the literature, our present results demonstrated that arterial buckling increased cell proliferation that was significantly higher at the inner curve side, suggesting that buckling led to site-specific remodeling with elevated cell proliferation that might be associated with atherosclerosis. Previously, Van Epps and colleagues (53) have reported that arterial segments exposed to cyclic flexure significantly increased cell apoptosis and macromolecular permeability in a similar ex vivo organ culture system. However, they did not observe an increase in cell proliferation. The difference may be due to differences in the arteries (femoral vs. carotid), loading conditions (flexure under cyclic axial stretch vs. buckling under pulsatile pressure at fixed axial stretch), and duration of the experiments (12 h vs. 4 days).

In addition, Stein et al. (48) demonstrated that cyclic flexion increased lumen wall stress of the inner curve, which contributed to the progression of atherosclerotic plaques in human coronary arteries. Using magnetic resonance images and FSI model analysis, Tang et al. (51) recently demonstrated cyclic bending significantly increased maximum stress in atherosclerotic plaques. Our present results showed a similar pattern of stress change with increased cell proliferation. Taken together, the data suggest that buckling-induced stress could be a mechanism for the high prevalence of atherosclerosis in tortuous arteries, but further studies are needed.
While alterations in wall stress and shear stress in buckled arteries could be a possible mechanism leading to atherosclerosis, the development of atherosclerosis could potentiate wall stress and shear stress effects, leading to an increased level of buckling. It is generally believed that long-standing hypertension and atherosclerosis can cause elongation of arteries that reduces axial stretch, resulting in buckling of the vessels (40, 42, 49, 56). Clinical observations showed that atherosclerotic arteries tend to be more tortuous than other arteries and that tortuosity influences the development of atherosclerosis (8, 46). The two-way interaction between atherosclerosis and buckling stresses may be a possible biomechanical mechanism for the progression of atherosclerosis in the tortuous artery.

Relevance to aging. Clinical studies (8, 42) have shown that carotid artery buckling and tortuosity are highly associated with hypertension, atherosclerosis, and aging. Individuals aged 50 yr or older have a higher prevalence of artery carotid artery buckling or kinking, and women have a greater incidence of artery buckling than men (8, 49, 56). It is well known that aging affects arterial structure and function (31, 40). Arterial wall stiffness increases with aging due to increases in collagen content and cross-linking, a high collagen-to-elastin ratio, increased medial wall thickness, and vascular calcification (9, 10, 12, 30, 31). In addition, it has been reported that sex affects the mechanical properties, biochemical contents, and wall structure of arteries (41). Our recent theoretical analysis showed that structure and mechanical stiffness of the arterial wall directly elevate the critical buckling pressure, and thus the aged-related changes and sex differences in vascular structure and mechanical properties could be a possible explanation for age-related artery buckling and its sex differences.

In addition, axial tension in arteries reduces with the loss of elastin in aged animals and humans (34, 40). Both reduced axial tension and elastin degradation with aging decrease arterial wall stiffness and can trigger artery buckling (4, 17, 35). Here, we showed that artery buckling could trigger arterial wall remodeling. This remodeling could be a mechanism that lead to tortuosity and the associated atherosclerosis observed in carotid arteries in the aged population (8).

There have been many reports of buckling of carotid arteries, which describes the tortuous shape of arteries under in vivo blood flow and pressure, since the 1930s (20, 49, 52). Here, we studied the buckling of arteries under lumen pressure and flow, in which the curved shape resulted from the buckling deformation. There are naturally curved vessels, such as the arch of aorta, where the curved shape is natural (the curved shape remains after the pressure load is removed). It will be interesting to study site-specific remodeling in the arch of aorta with aging in the future to compare remodeling in natural and buckling-induced curved vessels. In addition, it would be interesting to determine whether cell proliferation and NF-κB activation are increased in human arteries.

In conclusion, our results demonstrated that artery buckling stimulates cell proliferation possibly through a NF-κB-medi-
artered mechanism. Buckling-induced cell proliferation indicates that buckling may contribute to the atherosclerosis observed in tortuous arteries. Further studies are needed to determine the longer-term effects of artery buckling and the adaptation process.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


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