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Perivascular tethering modulates the geometry and biomechanics of cerebral arterioles

Samantha M. Steelman¹, Qiaofeng Wu², Hallie P. Wagner², Alvin T. Yeh², and Jay D. Humphrey²

¹Department of Systems Biology and Translational Medicine, Texas A&M Health Science Center

²Department of Biomedical Engineering, Texas A&M University

Abstract

Recent studies have renewed interest in the effects of perivascular tethering on vascular mechanics, particularly growth and remodeling. We quantified effects of axial and circumferential tethering on rabbit pial arterioles from the ventral occipital lobe of the brain. The homeostatic axial pre-stretch, which is influenced by perivascular tethering, was measured in situ to be 1.24 ± 0.04 . Using a cannulated microvessel preparation, wall mechanics were then quantified in vitro for isolated arterioles at low (1.10) or normal (1.24) values of axial stretch and for tethered arterioles having perivascular support. Axial stretch did not significantly affect changes in circumferential stretch or stress upon pressurization, but circumferential tethering caused arteriolar geometry to change from a circular cross section at normal pressure to an elliptical one at pressures above and below normal. Calculations suggested that the observed levels of ellipticity could cause a modest decrease in volumetric blood flow but also a pronounced variation in shear stress around the circumference of the arteriole. An elliptical cross-section could thus increase vascular resistance or promote luminal remodeling at pressures different from normal. This characterization of effects of perivascular tethering on pial arterioles should prove useful in future studies of roles of perturbed cerebral blood flow on the propensity of the cerebral microcirculation to remodel.

Keywords

vascular remodeling; cerebral microvasculature; shear stress; ellipticity

1. Introduction

The structural stiffness of a blood vessel depends on the composition and thickness of its wall and the surrounding perivascular tissue (Fung et al., 1966; Humphrey and Na, 2002; Liu et al., 2008; Singh and Devi, 1990). Although “perivascular tethering” is seldom considered, it contributes indirectly to circumferential stiffness and directly to axial stiffness,

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Address Correspondence to: Jay D. Humphrey, Ph.D., Department of Biomedical Engineering, 337 Zachry Engineering Center, 3120 TAMU, Texas A&M University, College Station, Texas 77843-3120, jhumphrey@tamu.edu Phone: (979) 845-5558, Fax: (979) 845-4450.

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Conflict of Interest

None declared.

the latter via the in vivo axial pre-stretch that manifests as retraction upon excision. This axial pre-stretch is a critical modulator of the biomechanics (Humphrey et al., 2009; Patel and Fry, 1966; Van Loon, 1977), and arteries exhibit marked and rapid growth and remodeling (G&R) responses when perturbed from a normal stretch (Gleason et al., 2007; Jackson et al., 2002, 2005). Because G&R play an integral role in many disease processes, it is important to understand how tethering influences such responses in all vessels. Despite many studies of the biomechanics of cerebral arterioles (e.g., Baumbach et al., 2002, 2003, 2004; Chillon and Baumbach, 2004), none have considered the effects of the perivascular tissue.

Using an in vitro tissue preparation, we found that axial tethering tended to limit circumferential stretch of pial arterioles at higher pressures but otherwise had little effect. Asymmetrical circumferential tethering caused these arterioles to become elliptical in cross section at pressures different from normal. Predicted hemodynamics suggested that volumetric flow may not be affected much by lumen ellipticity, but shear stress could vary widely around the circumference. From these data and computations, we submit that perivascular tissue offers modest mechanical protection of pial arterioles against transient fluctuations in perfusion pressure, but may contribute to overall G&R in response to sustained alterations in hemodynamics. To our knowledge, this is the first study to characterize effects of perivascular tethering in cerebral or non-cerebral arterioles.

2. Methods

All animal procedures were approved by the Institutional Animal Care and Use Committee of Texas A&M University. A surgical plane of anesthesia was induced (acepromazine maleate 2 mg/kg IM and sodium pentobarbital 30 mg/kg IV) in male New Zealand White rabbits (n = 29), which were then euthanized by exsanguination. The brain was removed and placed in a physiologic buffered saline (PBS) solution at 4°C.

2.1 Isolated and Tethered Pial Arterioles

To characterize and quantify biomechanical effects of perivascular tethering on pial arterioles, we categorized tethering by that which acts primarily in axial versus circumferential directions. Axial tethering (or, prestretch) appears to arise largely from biological growth during development, but is maintained in part by perivascular tissue; circumferential tethering, on the other hand, results from radial tractions imposed on the vessel by the surrounding perivascular tissue. Different levels of axial tethering were simulated in vitro by changing the axial stretch of isolated arterioles during pressurization tests. Preliminary studies revealed little change in the size of blocks of brain parenchyma upon excision, hence arterioles embedded in native perivascular tissue were assumed to remain at their in vivo axial stretch and no additional stretch was applied during in vitro pressurization tests. Effects of circumferential tethering (i.e., extravascular support provided by brain parenchyma and pia mater) were determined by the direct comparison of naturally embedded versus isolated arterioles.

Isolated arterioles (100–150 μ m diameter, 1–2 mm length) were cannulated and secured (using 11-0 suture) on glass micropipettes in cold PBS within a vessel chamber (Living Systems, Inc), checked for leaks, stretched axially, pressurized to 40 mmHg, and equilibrated at 37°C for 30 minutes. An inverted microscope and video camera measured inner diameter and wall thickness, and standard transducers measured luminal pressure, all of which were sampled and stored using LabView. Passive pressure-diameter relationships were obtained using a standard calcium-free PBS containing 2 mM EGTA (ethylene glycol tetraacetic acid), a calcium chelator.

Tethered arterioles were studied while embedded within an $\sim 1 \text{ cm}^3$ piece of tissue that was dissected en-block from the ventral occipital lobe of the brain and placed in cold PBS. One arteriole was chosen from each block based on the following criteria: no more than three branches, a distance of at least 0.4 cm from the cut edges of the tissue, and a lack of visible damage to the pial membrane. Branches were ligated using 11-0 suture and proximal and distal ends were transected approximately 2 mm apart. The tissue block was then transferred to a vessel chamber containing cold calcium-free PBS with EGTA and the transected ends were cannulated on glass micropipettes containing one to two microliters of a 1% crystal violet solution in 25% methanol to distinguish it from surrounding tissue. The bath was warmed to 37°C and the vessel was observed using a dissection microscope and video camera; diameter was measured at a central location, distant from cut tissue edges, ligated branches, and cannulated ends. Data were collected as described above.

Another subset of arterioles was used to investigate the mechanics before and after removal of perivascular tissue. Inner diameters were measured at 0, 20, and 40 mmHg both while the arterioles were in the intact brain and after isolation and cannulation.

2.2 Nonlinear Optical Microscopy (NLOM)

The cross-sectional geometry of tethered arterioles was determined at 0, 3, 30, and 60 mmHg using a NLOM system described previously (Larson and Yeh, 2006); NLOM uses second harmonic generation to detect the fine structure of fibrillar collagen. Three-dimensional imaging stacks were created in MetaVue (Universal Imaging Corp.) and rotated to allow en-face viewing of each arteriole.

2.3 Biomechanical Metrics

In vitro axial stretch of isolated vessels was calculated as $\lambda_z^c = \ell^c / L$, where ℓ^c is the loaded length and L is the unloaded length at $\sim 2 \text{ mmHg}$, which prevented the vessel from collapsing. In situ axial stretch was determined by measuring the length of an arteriolar segment between two branches before and after transection and removal of perivascular tissue; the ratio of in situ (ℓ^h) to excised (L) length yielded the homeostatic value (λ_z^h). Circumferential stretch was calculated as $\lambda_\theta = a/A$, where a is the inner radius under loaded conditions and A is the inner radius at the nearly unloaded ($\sim 2 \text{ mmHg}$ and length L) state. Because cannulated vessel diameters were originally measured only after vessels had been stretched to $\lambda_z^c = 1.10$ or 1.24 , diameters were measured in a subset of vessels at $\lambda_z^c = 1.00$, 1.10 , and 1.24 ; ratios describing mean changes in diameter upon stretch were used to approximate unloaded diameters from the partially loaded states. In vitro circumferential stress in isolated vessels was calculated as $\sigma_\theta = Pa/h$, where P is the luminal pressure, a the current inner radius, and h the average of the two measured current wall thicknesses ($h = (h_1 + h_2)/2$) at any given P .

In situ circumferential stress was inferred to calculate the effective outer radial traction due to tethering, that is, the outer pressure P_o . Note, therefore, that the stress must be the same in isolated and tethered vessels when subjected to the same deformation because $\sigma_\theta = \hat{\sigma}_\theta(\lambda_\theta, \lambda_z)$. When circular and at the same deformation,

$$\sigma_\theta^{\text{tethered}} \equiv \frac{(P_i - P_o)r_i}{r_o - r_i} - P_o = \frac{Pa}{h} \equiv \sigma_\theta^{\text{isolated}} \quad (1)$$

where r_i and r_o are inner and outer radii, respectively, in the tethered vessel. Hence,

$$P_o = \frac{P_i r_i}{r_o} - \frac{Pa(r_o - r_i)}{hr_o}. \quad (2)$$

The circumferential stress - stretch behavior of isolated arteries extended at $\lambda_z^c = 1.24$ was then fit (not modeled) with an exponential function using Matlab's built in 'fit.m' and model 'exp1'. Outer radius was used instead of inner radius to calculate circumferential stretch due to better R^2 values ($R^2=0.638$ using $\lambda_\theta^{r_i}$ vs. $R^2=0.875$ using $\lambda_\theta^{r_o}$). This fit allowed the outer pressure to be estimated easily based on data matched from the two experiments.

Finally, major (α) and minor (β) axes of the luminal cross-section in tethered arterioles, parallel and perpendicular to the pia mater, respectively, were measured at multiple pressures using NLOM and Image J software. Ellipticity was defined as the ratio $\varepsilon = \alpha/\beta$. A corresponding circular radius under non-tethered conditions was calculated under the assumption that the cross-sectional area (CSA) of tethered and untethered arterioles would equal: $CSA = \pi\alpha\beta = \pi a^2$, which was solved for a , thus giving a predicted circular radius and diameter ($d = 2a$) for each tethered arteriole. Volumetric flow Q through elliptical (e) and circular (c) tubes is given by (Humphrey and Delange, 2004),

$$Q_e = \frac{-\pi}{4\mu} \left(\frac{dp}{dz} \right) \frac{\alpha^3 \beta^3}{\alpha^2 + \beta^2} \quad \text{and} \quad Q_c = \frac{-\pi a^4}{8\mu} \left(\frac{dp}{dz} \right), \quad (3)$$

where μ is the fluid viscosity and dp/dz is the luminal pressure gradient along the length of the vessel. Shear stress (τ) within elliptical and circular lumens is similarly given by

$$\tau_e(x_1, y_1) = \frac{dp}{dz} \frac{\alpha^2 \beta^2}{\alpha^2 + \beta^2} \sqrt{\frac{x_1^2}{\alpha^4} + \frac{y_1^2}{\beta^4}} \quad \text{and} \quad \tau_c(x_1, y_1) = \frac{dp}{dz} \frac{a}{2}, \quad (4)$$

where (x_1, y_1) are rectangular coordinates describing a unique location at the vessel wall. The elliptical relations recover those for circular lumens when $\alpha = \beta = a$.

2.4 Statistics

Quantities are presented as mean $\pm$ standard error. Statistics were performed using R software, a commonly used statistical freeware package (<http://www.R-project.org>). ANOVA for repeated measures or t-tests were used as appropriate, and paired t-tests were used to analyze data from the paired arteriole experiments. In all cases, $p < 0.05$ was considered significant.

3. Results

Measured in situ axial stretch λ_z^h was 1.24 ± 0.04 ($n = 15$), which did not correlate with either segment diameter ($R^2 < 0.001$, $p = 0.96$) or length ($R^2 < 0.001$, $p = 0.79$). To delineate effects of axial tethering, we compared the passive biomechanics of isolated arterioles stretched axially until just straight when pressurized, which yielded a value of $\lambda_z^c \sim 1.10$ (1.09 ± 0.01 , $n = 5$), to that measured at the in situ stretch ($\lambda_z^c = 1.24$). Inner diameter did not differ significantly between these stretches (Fig. 1), despite a tendency for increased axial stretch to limit circumferential stretch at 60 mmHg ($n = 5$, $p = 0.068$; Fig. 2A). Likewise, circumferential stress did not differ significantly between these groups (Fig. 2B). Vessels at $\lambda_z^c = 1.10$ had a higher average wall thickness (12.78 ± 0.29 vs. 11.39 ± 0.41 μm ; $p < 0.01$)

and cross-sectional area (6875.21 ± 377 vs. $5995.39 \pm 253 \mu\text{m}^2$; $p < 0.05$). Wall thickness decreased with pressure in both groups ($p < 0.05$), although cross-sectional area did not change in either group (consistent with incompressibility).

Passive biomechanics of isolated vessels at $\lambda_z^c = 1.24$ were compared with those of vessels tethered by surrounding tissue. Diameters of tethered vessels were $\sim 20 \mu\text{m}$ smaller (average $20.50 \mu\text{m}$, range $13.43 - 31.32 \mu\text{m}$) than those of isolated vessels at all distending pressures ($n = 5$, $p < 0.01$; Fig. 1). The shape of the normalized diameter-pressure relationship of tethered arterioles differed from that of isolated arterioles at both $\lambda_z^c = 1.10$ and 1.24 ; the former described an almost sigmoidal curve, suggesting a nonuniform effect of tethering over the pressures tested.

The inner diameter of each unpressurized arteriole was measured in the intact brain as well as after the arteriole was isolated, cannulated, slightly pressurized ($\sim 2 \text{ mmHg}$) to prevent collapse, and stretched axially to its individually measured in situ length. Arteriolar diameters were significantly larger with intact perivascular tethering than after removal of surrounding tissue (68.50 ± 2.33 vs. $49.45 \pm 2.43 \mu\text{m}$; $p < 0.05$; $n = 7$). Similar results were seen in arterioles pressurized to 20 and 40 mmHg ($n = 3$, data not shown). One possible explanation for these findings was a change of cross-sectional shape to an ellipse at pressures above and below the normal value of 30 mmHg, which could manifest as an increase in diameter if viewed from the surface of the brain.

To test the hypothesis that arterioles became elliptical at pressures different from normal, we used NLOM to generate 3-dimensional reconstructions of pressurized, tethered arterioles. Using collagen structure to demarcate inner and outer boundaries of the vessel wall, arteriolar geometry was quantified from the cross-sectional view of each 3-D reconstruction (Fig. 3). Calculated ellipticity ($\epsilon = \alpha/\beta$) was 2.44 ± 0.29 , 1.23 ± 0.27 , 1.02 ± 0.06 , and 1.08 ± 0.03 at 0, 3, 30, and 60 mmHg, respectively ($n = 3$ at each pressure). This ellipticity was then used to calculate the minor axis length (β) for each major axis measurement (α) made in unpressurized, tethered arterioles; α and β were also used predict what the diameter would have been if untethered and perfectly circular. The predicted diameter was $43.85 \pm 1.49 \mu\text{m}$ whereas the measured diameter was $49.45 \pm 2.43 \mu\text{m}$ ($p = 0.06$, $n = 7$; data not shown). The small but consistent under-prediction of diameter (5/7 arterioles) was likely due in part to the low pressure ($\sim 2 \text{ mmHg}$) used to prevent the cannulated, untethered arterioles from collapsing. We also predicted the circular diameter of each tethered arteriole at 3, 30, and 60 mmHg using data from the unpaired experiments. As expected, circumferential stretches calculated using the predicted values of d at 30 and 60 mmHg were virtually identical to those from isolated arterioles (Table 1).

The pressure-like traction exerted on arterioles by perivascular tethering (P_o) was calculated using a Laplace type derivation, hence results are applicable only to vessels having a circular cross section. We thus calculated P_o at a luminal pressure of 30 mmHg, the normal value. Outer pressure ranged from -11.04 to 2.36 mmHg , with a mean of -4.63 and a 95% confidence interval of -14.11 to 4.84 mmHg ($n = 4$), which was not significantly different from 0 ($p = 0.22$).

Calculated volumetric flow rate and shear stress reflect effects of ellipticity on the cerebral circulation as a whole (flow) and on the vessel itself (shear stress). Results are expressed as a ratio of elliptical to circular cases. These ratios are thus independent of confounding variables such as transmural pressure and blood viscosity; a value of 1.000 indicates no effect of ellipticity. Blood flow in an elliptical arteriole was calculated to remain essentially the same as that in a circular arteriole at 3, 30, and 60 mmHg (0.979, 1.000, and 0.997,

respectively). At 0 mmHg ($\epsilon = 2.44$), this ratio dropped to 0.702, indicating a possible ~30% increase in vascular resistance caused by ellipticity.

Shear stress (τ), an important modulator of vascular tone and structure, was calculated along 180° of the circumference of the vessel. Shear stress varied the most when $\epsilon = 2.44$ (Fig. 4), ranging from 0.45 along the major axis to 1.10 on the minor axis. Variations in less elliptical arterioles were not as marked, with an almost perfectly normal distribution at $\epsilon = 1.01$ (30 mmHg).

4. Discussion

We examined, for the first time, how perivascular tissue may influence the biomechanics of pial arterioles and focused on passive behaviors because the maximum dilatory capacity is of most importance to cerebrovascular mechanics. Our data suggest that circumferential, not axial, tethering has the greatest effect on cerebral arteriolar mechanics. A decrease in axial stretch from the in vivo value did not affect circumferential stretch or stress significantly, which stands in contrast to data published by our group on rat cremaster arterioles (Guo et al., 2007). It is possible, however, that the “in vivo length” in the cremaster constitutes a range, not a single value, for it normally stretches well past its resting length (cf. Spurgeon et al., 1978). In contrast, it is unlikely that pial arterioles change length acutely in vivo. In this study, arterioles at $\lambda_z^c = 1.10$ represented the lowest level of stretch possible in an isolated vessel that did not bend or buckle when pressurized to 60 mmHg. This method of stretching is commonly used to set the reference length in cannulated arteriole studies, but it may not be physiologically meaningful. Nevertheless, our findings suggest that under stretching pial arterioles probably does not significantly affect data obtained during acute experiments.

Investigators who use the cranial window technique to study cerebral arteriolar mechanics in situ (e.g., Baumbach et al., 2002, 2003, 2004) implicitly assume that perivascular tethering does not distort the vessels into a non-circular shape. Our data suggest that this may not always be the case and, perhaps more importantly, that distortion from circular may have important implications for both short-term and long-term regulation of cerebral blood flow. An elliptical lumen may promote arteriolar collapse when perfusion pressure drops, such as during sudden postural changes or an orthostatic challenge. Albeit not significantly different from 0, the mean in situ outer pressure $P_o \sim -4.5$ mmHg suggests that perivascular tethering at normal pressures may help maintain the lumen patent, which could be protective. It is important to note, nonetheless, that effects on blood flow and resistance depend upon the magnitude of ellipticity achieved by the vessel; values we measured in the rabbit may be greater or less than those in humans. While we expect that these phenomena could be observed in humans, pathophysiological implications necessarily depend on the propensity of human pial arterioles to deform asymmetrically.

Finally, the long-term maintenance of arteriolar structure may be influenced by perivascular tethering. At non-physiological pressures, wall shear and circumferential stresses could vary around the circumference of an elliptical arteriole, as seen in Figure 4. Given that blood vessels adapt to alterations in wall shear and circumferential stress, this non-uniform distribution of stresses could stimulate vascular G&R at non-physiological pressures. Several studies show that even a modest change in shear stress can alter local production of nitric oxide and endothelin-1 (Noris et al., 1995; Uematsu et al., 1995) and potentially affect arteriolar structure and function (Bakker et al., 2002; Sorop et al., 2003). In addition, a recent study by Kassab and colleagues found that hypertrophic remodeling only occurred in the untethered region of coronary veins (Choy et al., 2006). These findings suggest that vessels on the surface of organs such as the heart and brain may be more prone to mechanically-

induced G&R than other vessels that are surrounded uniformly by tissue. In fact, results from the present study suggest that the protection offered to pial arterioles by perivascular tethering is modest.

To our knowledge, this is the first study of perivascular tethering of arterioles. We have shown that the unique mechanical coupling of the cerebral vasculature to the brain may render the pial circulation vulnerable to acute changes in perfusion pressure. An elliptical lumen caused by the constraint of overlying meninges could promote arteriolar collapse or vascular G&R at non-physiological pressures (e.g., Yamakawa et al., 2003). Future studies investigating the applicability of our findings to cerebral arteries, as well as comparisons to arterioles from other essential vascular beds such as the heart and kidney, would prove enlightening.

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References

- Bakker EN, van der Meulen ET, van den Berg BM, et al. Inward remodeling follows chronic vasoconstriction in isolated resistance arteries. *J Vasc Res.* 2002; 39:12–20. [PubMed: 11844933]
- Baumbach GL, Sigmund CD, Bottiglieri T, et al. Structure of cerebral arterioles in cystathionine beta-synthase-deficient mice. *Circ Res.* 2002; 91:931–937. [PubMed: 12433838]
- Baumbach GL, Sigmund CD, Faraci FM. Cerebral arteriolar structure in mice overexpressing human renin and angiotensinogen. *Hypertension.* 2003; 41:50–55. [PubMed: 12511529]
- Baumbach GL, Sigmund CD, Faraci FM. Structure of cerebral arterioles in mice deficient in expression of the gene for endothelial nitric oxide synthase. *Circ Res.* 2004; 95:822–829. [PubMed: 15388643]
- Chillon JM, Baumbach GL. Effects of indapamide, a thiazide-like diuretic, on structure of cerebral arterioles in hypertensive rats. *Hypertension.* 2004; 43:1092–1097. [PubMed: 15007039]
- Choy JS, Dang Q, Molloy S, et al. Nonuniformity of axial and circumferential remodeling of large coronary veins in response to ligation. *Am J Physiol Heart Circ Physiol.* 2006; 290:H1558–H1565. [PubMed: 16299258]
- Fung YC, Zweifach BW, Intaglietta M. Elastic environment of the capillary bed. *Circ Res.* 1966; 19:441–461. [PubMed: 5914856]
- Gleason RL, Wilson E, Humphrey JD. Biaxial biomechanical adaptations of mouse carotid arteries cultured at altered axial extension. *J Biomech.* 2007; 40:766–776. [PubMed: 16750537]
- Guo H, Humphrey JD, Davis MJ. Effects of Biaxial Stretch on Arteriolar Function In Vitro. *Am J Physiol Heart Circ Physiol.* 2007
- Humphrey JD, Na S. Elastodynamics and arterial wall stress. *Ann Biomed Eng.* 2002; 30:509–523. [PubMed: 12086002]
- Humphrey, JD.; Delange, SL. An introduction to biomechanics: solids and fluids, analysis and design. New York: Springer-Verlag; 2004.
- Humphrey JD, Eberth JF, Dye WW, Gleason RL. Fundamental role of axial stress in compensatory adaptations by arteries. *J Biomech.* 2009; 42:1–8. [PubMed: 19070860]
- Jackson ZS, Gotlieb AI, Langille BL. Wall tissue remodeling regulates longitudinal tension in arteries. *Circ Res.* 2002; 90:918–925. [PubMed: 11988494]
- Jackson ZS, Dajnowiec D, Gotlieb AI, et al. Partial off-loading of longitudinal tension induces arterial tortuosity. *Arterioscler Thromb Vasc Biol.* 2005; 25:957–962. [PubMed: 15746437]
- Larson AM, Yeh AT. Ex vivo characterization of sub-10-fs pulses. *Opt Lett.* 2006; 31:1681–1683. [PubMed: 16688260]

- Liu Y, Zhang W, Kassab GS. Effects of myocardial constraint on the passive mechanical behaviors of the coronary vessel wall. *Am J Physiol Heart Circ Physiol*. 2008; 294:H514–H523. [PubMed: 17993601]
- Noris M, Morigi M, Donadelli R, et al. Nitric oxide synthesis by cultured endothelial cells is modulated by flow conditions. *Circ Res*. 1995; 76:536–543. [PubMed: 7534657]
- Patel DJ, Fry DL. Longitudinal tethering of arteries in dogs. *Circ Res*. 1966; 19:1011–1021. [PubMed: 5928541]
- Singh SI, Devi LS. A study on large radial motion of arteries in vivo. *J Biomech*. 1990; 23:1087–1091. [PubMed: 2277043]
- Sorop O, Spaan JA, Sweeney TE, et al. Effect of steady versus oscillating flow on porcine coronary arterioles: involvement of NO and superoxide anion. *Circ Res*. 2003; 92:1344–1351. [PubMed: 12764025]
- Spurgeon TL, Kitchell RL, Lohse CL. Physiologic properties of contraction of the canine cremaster and cranial preputial muscles. *Am J Vet Res*. 1978; 39:1884–1887. [PubMed: 749569]
- Uematsu M, Ohara Y, Navas JP, et al. Regulation of endothelial cell nitric oxide synthase mRNA expression by shear stress. *Am J Physiol*. 1995; 269:C1371–C1378. [PubMed: 8572165]
- Van Loon P. Length-force and volume-pressure relationships of arteries. *Biorheology*. 1977; 14:181–201. [PubMed: 912047]
- Yamakawa H, Jezova M, Ando H, et al. Normalization of endothelial and inducible nitric oxide synthase expression in brain microvessels of spontaneously hypertensive rats by angiotensin II AT1 receptor inhibition. *J Cereb Blood Flow Metab*. 2003; 23:371–380. [PubMed: 12621312]

Figure 1.

Percentage of maximal passive outer diameters for isolated pial arterioles at two different levels of axial stretch (1.10 and 1.24) as well as for arterioles with perivascular tissue intact (tethered). Values are mean $\pm$ SEM; $n=5$ for each group.

Figure 2. Circumferential stretch (panel A) and circumferential stress-stretch relationships (panel B) for isolated, cannulated arterioles at two different levels of axial stretch (1.10 and 1.24). Circumferential stretch was calculated using the nearly unloaded diameter of each vessel (measured at $P = 2-3$ mmHg and $\lambda_z^c = 1.00$). Values are mean $\pm$ SEM; $n = 5$ for each group.

Figure 3. Cross-sectional views of a tethered arteriole generated from 3D reconstructions of nonlinear optical microscopy images. The arteriole was pressurized to 0, 3, 30, and 60 mmHg. Numbers represent calculated values of ellipticity ($\epsilon = \alpha/\beta$) at each pressure.

Figure 4.

Shear stress for an elliptical cross-section normalized to that for a circular cross-section having equal area (τ_e/τ_c) shown over 180° of the circumference. Values of ellipticity correspond to cerebral arteriolar shape at 0 ($\epsilon = 2.44$), 3 ($\epsilon = 1.23$), 30 ($\epsilon = 1.01$), and 60 ($\epsilon = 1.17$) mmHg, with $\epsilon = 1.00$ signifying a circle.

Table 1

Measurements of the major axis (α) were used to estimate values of the minor axis (β) and then to predict the diameter (d) for a given cross sectional area. Circular diameter was then used to predict circumferential stretch (λ_0) at each pressure, which was compared with values measured in isolated arterioles. Data are presented as mean $\pm$ SEM (n = 6).

Pressure (mmHg)	Dimension (μm)			Stretch	Stretch
	α (Measured)	β (Calculated)	d (Predicted)	λ_0 (Predicted)	λ_0 (Measured)
3	133.2 $\pm$ 10.2	108.3 $\pm$ 8.3	120.1 $\pm$ 9.2	1.00 $\pm$ 0.00	1.00 $\pm$ 0.00
30	170.3 $\pm$ 15.2	168.6 $\pm$ 15.0	169.4 $\pm$ 15.1	1.41 $\pm$ 0.06	1.42 $\pm$ 0.06
60	188.2 $\pm$ 13.4	174.2 $\pm$ 12.4	181.1 $\pm$ 12.9	1.52 $\pm$ 0.06	1.50 $\pm$ 0.07