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Abrupt increase in rat carotid blood flow induces rapid alteration of artery mechanical properties

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Abstract

Vascular remodeling is essential to proper vessel function. Dramatic changes in mechanical environment, however, may initiate pathophysiological vascular remodeling processes that lead to vascular disease. Previous work by some of our group has demonstrated a dramatic rise in matrix metalloproteinase (MMP) expression shortly following an abrupt increase in carotid blood flow. We hypothesized that there would be a corresponding change in carotid mechanical properties. Unilateral carotid ligation surgery was performed to produce an abrupt, sustained increase in blood flow through the contralateral carotid artery of rats. The flow-augmented artery was harvested after sham surgery or 1, 2, or 6 days after flow augmentation. Vessel mechanical response in the circumferential direction was then evaluated through a series of pressure-diameter tests. Results show that the extent of circumferential stretch (normalized change in diameter) at *in vivo* pressure levels was significantly different ($p < 0.05$) from normo-flow controls at 1 and 2 days following flow augmentation. Measurements at 1, 2, and 6 days were not significantly different from one another, but a trend in the data suggested that circumferential stretch was largest 1 day following surgery and subsequently decreased toward baseline values. Because previous work with this model indicated a similar temporal pattern for MMP-9 expression, an exploratory set of experiments was conducted where vessels were tested 1 day following surgery in animals treated with broad spectrum MMP inhibitors (either doxycycline or GM6001). Results showed a trend for the inhibitors to minimize changes in mechanical properties. Observations demonstrate that vessel mechanical properties change rapidly following flow augmentation and that alterations may be linked to expression of MMPs.

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Keywords

vascular disease; vascular remodeling; mechanotransduction; vessel mechanical properties; matrix metalloproteinases; rat carotid artery

1. Introduction

Vascular remodeling in response to changes in mechanical environment is a fundamental homeostatic process for proper blood vessel function. This remodeling process involves a balance of destructive and constructive processes to break down and rebuild the vessel wall to accommodate new loading conditions. For example, flow-induced outward vascular remodeling is an adaptive process through which blood vessels increase their lumen to normalize hemodynamic shear stresses that have increased with blood flow (Gibbons and Dzau 1994; Guyton and Hartley 1985; Kamiya and Togawa 1980; Kamiya *et al* 1988; Langille and O'Donnell 1986). The basic mechanisms of this process may play a role in pathophysiological remodeling of arteries observed in atherosclerosis, aortic aneurysms, intracranial aneurysms, brain arteriovenous malformations, and vascular sequelae of head trauma (Gibbons and Dzau 1994; Glagov *et al* 1987; Hashimoto *et al* 2001; Hashimoto *et al* 2006; Hoi *et al* 2008).

Tissue remodeling appears to be controlled by the orchestrated effects of a number of cytokines and proteinases, including matrix metalloproteinases (MMPs) (Abbruzzese *et al* 1998; Flamant *et al* 2007; Galis and Khatri 2002; Tronc *et al* 2000). In a previous study using rats, we showed that inflammatory cells, such as macrophages, play critical roles in adaptive outward remodeling of the common carotid artery in response to an increase in blood flow (Nuki *et al* 2009). In this model, the time course of up-regulation of MMPs coincided with that of macrophage infiltration. More importantly, inhibition of MMPs by SB-3CT reduced flow-induced outward remodeling; flow-induced outward remodeling was also reduced in MMP-9 knockout mice (Ota *et al* 2009). These findings indicate that MMPs, produced by macrophages, play an important role in flow-induced outward vascular remodeling.

Because blood vessels remodel to return cells to homeostatic levels of stress following changes in mechanical environment (Humphrey 2008), it seems logical that dynamic changes in mechanical properties would accompany remodeling. Such changes have not, however, received much attention in the literature, especially in response to increased flow. It is thus not known if such changes occur, how subtle they may be, or what time scale they may occur over in the previously referenced model of outward remodeling. Definition of the magnitude and timing of any alteration in mechanical properties will lend further insight into remodeling phenomena, including issues related to the development of vascular pathophysiology.

Given the role of MMPs in extracellular matrix protein degradation and their observed up-regulation following an abrupt increase in blood flow (Nuki *et al* 2009), we hypothesized that such a change in flow would also produce a change in carotid artery mechanical properties. To test this hypothesis, we used the described model of carotid ligation in the rat. In this model, ligation of the left common carotid artery augments blood flow in the right common carotid artery (Nuki *et al* 2009; Ota *et al* 2009). Results from pressure-diameter tests on the flow-augmented right common carotid show that the mechanical characteristics of the vessel change rapidly following an increase in blood flow and suggest that MMP expression may be at least partly responsible for this alteration.

2. Materials and Methods

2.1 Animals, Surgeries, and Treatments

As has been described previously (Nuki *et al* 2009), the left common carotid artery of adult male Sprague-Dawley rats (300–350g, 10–12 weeks old) was ligated and the incision closed, according to institutional guidelines. The right common carotid artery was harvested at 1, 2, or 6 days after flow-augmentation. Vessels were also obtained from animals not undergoing flow-augmentation surgery for control measurements. Because changes in the properties of carotid arteries from these animals appeared to be temporally correlated with MMP expression, as measured in prior experiments using the same ligation model (Nuki *et al* 2009), a follow-up set of experiments was designed to investigate this possible link. In this second set of experiments, animals received one of two broad spectrum MMP inhibitors, GM6001 or doxycycline. GM6001 (10mg/kg) was given intraperitoneally one day before the flow-augmentation and at every 24 hours following. Doxycycline (40 mg/kg/day) was given through drinking water over the same interval as previously described (Lee *et al* 2006; Pyo *et al* 2000). Vessels from animals receiving no inhibitor were also tested for comparison. All vessels in the second set of experiments were resected one day after flow-augmentation, the time point at which we observed maximum changes in mechanical properties. In both sets of experiments, four animals were generally used for each group, leading to four successful tests each at 0, 2, and 6 days (3 successful tests at 1 day) in the first set of experiments. For the second set of experiments, 7, 4, and 4 successful tests were run on untreated, doxycycline-treated, and GM6001-treated cases, respectively. Prior to resection, the *in vivo* length of the carotid was measured. Vessels typically retracted from 11–12 mm to an unloaded length of 8–9 mm upon resection.

2.2 Mechanical Apparatus and Testing

To precisely characterize the mechanical response of blood vessels, we have developed an isolated vessel testing system that provides measurement of forces and deformations under a variety of axial and circumferential loading conditions. The apparatus and associated procedures have been described previously (Monson *et al* 2008). In brief, cross-sections (approximately 0.5 mm thick) were cut from the ends of each sample, and their images were captured to a computer for subsequent diameter and wall-thickness measurement; no repeatable differences were apparent between the two sections, so measurements were averaged for each specimen. The remaining arterial segment was then tied at each end to blunt, 25-gauge needles that had been grooved to minimize slippage during axial stretch. The needles were attached to acrylic blocks whose positions were controlled using a computer-controlled servo-hydraulic load frame [MTS, Model 858 Mini-Bionix] (Fig. 1). Passages in the blocks allowed fluid flow and pressure measurement. To further seal each specimen, a small amount of cyanoacrylate was applied between the vessel ends and the needles. During preparation and testing, specimens were perfused and bathed in physiological saline (Dulbecco's Modified Eagle's Medium; DMEM) bath. One of the specimen mounting blocks was connected to a saline delivery system, a linear actuator [Ultramotion, Digit] that drove the position of a syringe. A 200 kPa pressure transducer [Honeywell, MicroSwitch 26PCDFM6G] was mounted on the other block. Applied loads were measured using an 11 N load cell (Transducer Techniques, MDB 2.5), and displacement was monitored through the linear variable displacement transducer (LVDT) built into the load frame. Vessel outer diameter was determined through measurements of video recorded (3 frames/second) using a digital camera (Pixelink, PL-A641), with an attached high-powered zoom lens (FOV: 1.4–17.6 mm). All non-video data were recorded using a standard data acquisition (DAQ) card.

After attachment to the apparatus, each specimen was preconditioned through a series of 3 to 4 tests in which the vessel was stretched axially to target values just over *in vivo* levels to avoid conditions of buckling, and its length was held constant for approximately two minutes while pressure in the vessel was cycled 5 times between 6.7 and 20.0 kPa (50 and 150 mmHg). Following these initial procedures, another test was conducted in which specimen length was reduced sufficiently to induce buckling and then increased to the maximum value utilized during preconditioning, all while a valve connected to the upper block was open so as to maintain zero pressure; this test provided for identification of the unloaded reference configuration. Finally, the vessel was subjected to a series of three pressurization tests nearly identical to the cyclic preconditioning sequences, each having a different value of axial stretch. Data from the loading phase of the final cycle in each of these three tests were used for determination of mechanical properties.

2.3 Data Analysis

Considering the vessels to be homogenous circular cylinders, specimen stretch λ_i was defined as

$$\lambda_\theta = \frac{d_i + d_o}{D_i + D_o}, \lambda_z = \frac{l}{L}, \quad (1)$$

where $i = \theta, z$ correspond to the circumferential and axial directions, respectively, and d_i, d_o , and l represent specimen inner diameter, outer diameter, and length in the current configuration; D_i, D_o , and L are corresponding values in the unloaded state and were determined from digital images of specimen cross-sections using measurement software (National Instruments, Inc.; Vision Assistant). The tissue was assumed to be incompressible to allow calculation of inner diameter in the current configuration. Mean Cauchy stress T_i was defined as

$$T_\theta = P_i \frac{d_i}{d_o - d_i}, T_z = \frac{\lambda_z}{A} \left(F + \frac{\pi}{4} d_i^2 P_i \right), \quad (2)$$

where P_i is internal pressure, F is measured axial load, and A is vessel cross-sectional area, to satisfy the global equilibrium equations.

Outcome parameters (Table 1) were derived from the resulting circumferential stress-stretch curves and analyzed statistically. The chosen parameters were designed to capture vessel mechanical response around *in vivo* conditions. Mixed-effects linear regression analysis was applied separately for each parameter to test for statistically significant changes at various days after carotid ligation or for different drug treatments, depending on the set of data, while accounting for multiple tests on each specimen and controlling for the contribution of axial stretch (Statacorp; Stata IC 10). Data from the first set of experiments were evaluated using the model specified by Equation 3,

$$param = ctrl + \sum_{i=3} (\beta_{1i} * I_days_i) + \beta_2 * \lambda_{zc} + \beta_3 * \lambda_{zc}^2 \quad (3)$$

where $param$ refers to the parameter value being predicted, $ctrl$ refers to the mean value of $param$ in control specimens, I_days_i corresponds to 1, 2, or 6 days after surgery and, for example, has values of 1, 0, and 0 for a specimen tested 1 day after surgery, λ_{zc} is axial stretch, centered about its mean, and β_{1i}, β_2 , and β_3 are coefficients whose values are

adjusted to optimize the fit of the model to the data. Data from the MMP inhibitor experiments were analyzed using the same equation except with I_drug_i replacing I_days_i to allow comparison of no drug treatment to the use of one of the inhibitors.

3. Results

Figure 2 shows the outer diameter-pressure response of a typical specimen for three different levels of axial stretch. As expected, larger axial stretch values resulted in smaller diameters such that the diameter-pressure response curve shifted downward. This anticipated effect of axial stretch was observed for all specimens, confirming the need to account for the influence of axial stretch on measured vessel properties, as shown in the statistical model (Eq. 3).

In comparison to normo-flow controls, carotid arteries exposed to flow augmentation for 1 or 2 days demonstrated a marked increase in circumferential stretch (normalized change in diameter; Fig. 3); this increased stretch was present at pressures of both 80 and 120 mmHg. Figure 3 presents three points for each specimen, each collected at a different level of axial stretch. Accounting for multiple tests with various levels of axial stretch on each specimen, the increase in circumferential stretch was statistically significant ($p < 0.05$) after both 1 and 2 days of increased flow, illustrated by observing that the 95% confidence intervals shown in Figure 4 for I_days_1 and I_days_2 do not cross the mean value (0.0 on the x-axis) of circumferential stretch for control specimens. Changes after 6 days of augmented flow were nearly significant. Figure 4 additionally shows that both axial stretch and its square also had significant, or near significant, influence on circumferential stretch values in the tests, though their effect was to reduce the level of stretch, consistent with Figure 2. Statistically predicted changes in stretch shown in Figure 4 correspond to an increase of 0.1 in axial stretch and its square. Of the outcome parameters identified for study in Table 1, only circumferential stretch was found to significantly change as a result of flow augmentation.

Although circumferential stretch values were significantly different from non-ligated controls at both 1 and 2 days after surgery, results from 1, 2, and 6 days were not statistically different from one another. Figures 3 and 4 both, however, illustrate a trend of circumferential stretch values being highest at day 1 and then returning toward baseline values with time. Notably, the timing of this trend is similar to the expression of MMP-9 in carotid arteries subjected to flow augmentation in the same model, reported elsewhere (Nuki *et al* 2009). In that report, MMP expression was greatest 1 day following ligation surgery, was somewhat reduced at 2 days, and returned to near baseline values by 7 days. This correspondence motivated additional experiments to explore a possible link between the observed change in mechanical properties and expression of MMP-9.

In order to test whether increased levels of MMPs were responsible for the observed mechanical property changes, we assessed effects of two broad spectrum MMP inhibitors on circumferential stretch. As shown in Figure 5, both doxycycline and GM6001 resulted in vessels with lower mean circumferential stretch values, but differences from untreated controls were not statistically significant. Nevertheless, doxycycline appeared to exert a stronger influence than GM6001. These results suggest a possible role for MMPs in the observed mechanical property changes, but more experiments are clearly needed to establish this connection. While it may be tempting to compare the data of Figure 5 to that of Figure 4, it is important to note that the average axial stretch value in the second dataset was higher than that in the first. This likely explains the lower circumferential stretch values seen in the untreated data of Figure 5 relative to that at 1 day in Figure 4.

Cross-section analysis revealed no changes in unloaded vessel dimensions, including outer diameter, cross-sectional area, and stress-free opening angle, as a function of either time after ligation or drug treatment. Accordingly, there was no evidence of structural remodeling in dimensional changes of cross-sections in the zero-load configuration.

4. Discussion

The objective of this work was to explore whether measureable changes in carotid artery mechanical properties occur following an abrupt, sustained increase of blood flow. Results indicate that statistically significant alterations occur rapidly but then subside with time. Initial exploratory studies suggest that MMPs may contribute to the observed changes. These findings lend new insight into how blood vessels respond to changes in their mechanical environment.

To our knowledge, the reported rapid change in vessel properties, followed by a return to baseline behavior, has not been previously reported. While this temporal pattern mirrors previously reported expression of MMP-9 in the same model, it is not clear which components of the vessel wall are responsible for the changes in properties. In discussing this question, it is important to note that smooth muscle activity was not chemically eliminated in experiments. Tests were performed in a standard physiological salt solution (DMEM), considered appropriate by many for measurement of passive properties (Humphrey 2002), and in the absence of flow and, thus, any endothelium-dependent smooth muscle signaling. Data for determining properties were also obtained following preconditioning, eliminating some smooth muscle contraction (Fridez *et al* 2001), but these conditions don't remove the possibility of a myogenic response at the basal tone level.

Langille (1996) previously suggested that remodeling in response to a change in flow occurs in two phases – an immediate vascular smooth muscle response followed by later structural alterations in the media. The observed increase in circumferential stretch indicates that carotid arteries previously exposed to flow augmentation expanded more under physiological pressure in pressure-diameter tests than did arteries in which flow was normal before resection. The observed increase in circumferential stretch at 1 day appears to be consistent with smooth muscle-induced dilation in response to increased flow, but it is not clear why this dilation would decrease over the next few days while the increased level of flow persisted.

Consistent with Langille's timeline, no structural remodeling was observed during the period studied, but this would not rule out all structural alteration. Given the increase in circumferential stretch, it is notable that E_{iv} , the stiffness of the circumferential stress-stretch curve at 100 mmHg, did not change significantly with flow augmentation. These two results together are consistent with a shift of the circumferential stress-stretch curve to the right, a lengthening of the toe region of the curve without significant alteration of the rest of its shape. Because elastin has been shown to define the low-stiffness response of blood vessels (Roach and Burton 1957), it may be that the mechanism producing the observed alterations affects elastin fibers more than other structural elements of the vascular wall, such as collagen. Given the long time required for elastin synthesis (Humphrey 2009), however, the rapid recovery of control behavior could not be accomplished through replacement of elastin, but contributions from other components may compensate.

Increase in circumferential stretch at 1 day could also be the result of damage to the vessel wall by overload. Cope and Roach (1977) observed changes in toe-region elastic properties of isolated human cerebral vessels subjected to over-pressures *in vitro*, though the pressures applied in their study were much higher than those present here. Other investigators have

reported changes in the internal elastic lamina following increased flow in the carotid or basilar arteries (Hoi *et al* 2008; Tronc *et al* 2000; Wong and Langille 1996), though large flow changes were required to produce fragmentation of the lamina. Hoi *et al* (2008) reported remodeling activity in the basilar artery of rabbits following both unilateral and bilateral ligation of the carotid arteries, but only bilateral ligation resulted in internal elastic lamina fragmentation. In contrast, Nuki *et al* (2009) showed that luminal diameter increased only slightly during the first 5 minutes after ligation in the current model, so it is unlikely that passive overload occurred. Additionally, it is unlikely that a significantly damaged vessel could return to baseline behavior over a small number of days. Nevertheless, histological study of these vessels would help rule out such damage in future work.

Given these considerations, it is not currently possible to determine the cause for the observed change in properties. It appears, however, that inflammation resulting from the rapid change in mechanical environment produces an alteration in either expected smooth muscle response or the way structural components of the wall interact with one another, or both; related work has suggested that structures coupling extracellular matrix to smooth muscle cells may be disrupted in vascular injury (Jamal *et al* 1992). Future investigation should include appropriate chemicals to manipulate smooth muscle contributions and allow clear distinction between active and passive response.

While the reported data suggest a trend for maximum increase in circumferential stretch at day 1, followed by a decrease back toward baseline levels, the existence and timing of this trend should be further explored with experiments at earlier and later times. Because results at 1 and 2 days were not statistically different from each other and no measurements were made earlier than 1 day, it may be that maximum change occurs at a time other than one day following flow augmentation. In order to more fully understand the vascular response, it is also important to define whether the trend of recovery suggested by the data actually occurs.

Although neither inhibitor treatment demonstrated a statistically significant effect on mechanical properties, both tended to produce lower circumferential stretch ratios than comparable controls, with doxycycline being more influential than GM6001. In contrast to GM6001, doxycycline is less selective and produces effects other than MMP inhibition, including modulation of various aspects of inflammation (Beekman *et al* 1997; Kim *et al* 2005; Lee *et al* 2003), possibly contributing to its relatively greater influence.

The potential significance of such a rapid change in vessel properties is not known. The observed increase in circumferential stretch for a given level of pressure, however, could indicate a loss of vascular integrity with potential for subsequent dysfunction or initiation of disease. Although none of the carotid arteries tested in this study displayed any tendency to not recover from the increase in compliance, mechanical thresholds for the initiation of such instability should be defined. Additionally, while the initiating mechanical event in this study was an abrupt change in shear stress, the approach of this research should be extended to examine response to other types of changes in mechanical environment, such as those that occur with trauma.

In summary, mechanical properties of carotid arteries change rapidly following exposure to augmented flow. Trends suggest that the affected properties may return to baseline values over time and that the timing of changes may be mediated by MMPs. The reported findings further define how blood vessels respond to changes in their mechanical environment and are believed to lend insight into processes associated with the development of vascular disease and dysfunction.

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References

- Abbruzzese TA, Guzman RJ, Martin RL, Yee C, Zarins CK, Dalman RL. Matrix metalloproteinase inhibition limits arterial enlargements in a rodent arteriovenous fistula model. *Surgery*. 1998; 124:328–334. [PubMed: 9706156]
- Beekman B, Verzijl N, de Roos JA, Koopman JL, TeKoppele JM. Doxycycline inhibits collagen synthesis by bovine chondrocytes cultured in alginate. *Biochem. Biophys. Res. Commun.* 1997; 237:107–110. [PubMed: 9266839]
- Cope DA, Roach MR. Effects of high static pressures on human cerebral arteries in vitro. *Stroke*. 1977; 8:254–257. [PubMed: 847792]
- Flamant M, Placier S, Dubroca C, Esposito B, Lopes I, Chatziantoniou C, Tedgui A, Dussaule JC, Lehoux S. Role of matrix metalloproteinases in early hypertensive vascular remodeling. *Hypertension*. 2007; 50:212–218. [PubMed: 17515450]
- Fridez P, Makino A, Miyazaki H, Meister JJ, Hayashi K, Stergiopulos N. Short- Term biomechanical adaptation of the rat carotid to acute hypertension: contribution of smooth muscle. *Ann. Biomed. Eng.* 2001; 29:26–34. [PubMed: 11219505]
- Galis ZS, Khatri JJ. Matrix metalloproteinases in vascular remodeling and atherogenesis: the good, the bad, and the ugly. *Circ. Res.* 2002; 90:251–262. [PubMed: 11861412]
- Gibbons GH, Dzau VJ. The emerging concept of vascular remodeling. *N. Engl. J. Med.* 1994; 330:1431–1438. [PubMed: 8159199]
- Glagov S, Weisenberg E, Zarins CK, Stankunavicius R, Kolettis GJ. Compensatory enlargement of human atherosclerotic coronary arteries. *N. Engl. J. Med.* 1987; 316:1371–1375. [PubMed: 3574413]
- Guyton JR, Hartley CJ. Flow restriction of one carotid artery in juvenile rats inhibits growth of arterial diameter. *Am. J. Physiol.* 1985; 248:H540–H546. [PubMed: 3885757]
- Hashimoto T, Mesa-Tejada R, Quick CM, Bollen AW, Joshi S, Pile-Spellman J, Lawton MT, Young WL. Evidence of increased endothelial cell turnover in brain arteriovenous malformations. *Neurosurgery*. 2001; 49:124–131. [PubMed: 11440433]
- Hashimoto T, Meng H, Young WL. Intracranial aneurysms: links among inflammation, hemodynamics and vascular remodeling. *Neurol. Res.* 2006; 28:372–380. [PubMed: 16759441]
- Hoi Y, Gao L, Tremmel M, Paluch RA, Siddiqui AH, Meng H, Mocco J. In vivo assessment of rapid cerebrovascular morphological adaptation following acute blood flow increase. *J. Neurosurg.* 2008; 109:1141–1147. [PubMed: 19035734]
- Humphrey, JD. *Cardiovascular Solid Mechanics : Cells, Tissues, and Organs*. New York: Springer; 2002.
- Humphrey JD. Mechanisms of arterial remodeling in hypertension: coupled roles of wall shear and intramural stress. *Hypertension*. 2008; 52:195–200. [PubMed: 18541735]
- Humphrey JD. Coupling hemodynamics with vascular wall mechanics and mechanobiology to understand intracranial aneurysms. *Int J Comput Fluid Dyn.* 2009; 23:569–581. [PubMed: 20526461]
- Jamal A, Bendeck M, Langille BL. Structural changes and recovery of function after arterial injury. *Arterioscler Thromb.* 1992; 12:307–317. [PubMed: 1547190]
- Kamiya A, Togawa T. Adaptive regulation of wall shear stress to flow change in the canine carotid artery. *Am. J. Physiol.* 1980; 239:H14–H21. [PubMed: 7396013]
- Kamiya A, Ando J, Shibata M, Masuda H. Roles of fluid shear stress in physiological regulation of vascular structure and function. *Biorheology*. 1988; 25:271–278. [PubMed: 3196824]

- Kim HS, Luo L, Pflugfelder SC, Li DQ. Doxycycline inhibits TGF-beta1-induced MMP-9 via Smad and MAPK pathways in human corneal epithelial cells. *Invest. Ophthalmol. Vis. Sci.* 2005; 46:840–848. [PubMed: 15728539]
- Langille BL, O'Donnell F. Reductions in arterial diameter produced by chronic decreases in blood flow are endothelium-dependent. *Science.* 1986; 231:405–407. [PubMed: 3941904]
- Langille BL. Arterial remodeling: relation to hemodynamics. *Can. J. Physiol. Pharmacol.* 1996; 74:834–841. [PubMed: 8946070]
- Lee CZ, Yao JS, Huang Y, Zhai W, Liu W, Guglielmo BJ, Lin E, Yang GY, Young WL. Dose-response effect of tetracyclines on cerebral matrix metalloproteinase-9 after vascular endothelial growth factor hyperstimulation. *J. Cereb. Blood Flow Metab.* 2006; 26:1157–1164. [PubMed: 16395286]
- Lee YC, Lane KB, Zoia O, Thompson PJ, Light RW, Blackwell TS. Transforming growth factor-beta induces collagen synthesis without inducing IL-8 production in mesothelial cells. *Eur. Respir.* 2003; 22:197–202.
- Monson KL, Barbaro NM, Manley GT. Biaxial response of passive human cerebral arteries. *Ann. Biomed. Eng.* 2008; 36:2028–2041. [PubMed: 18855141]
- Nuki Y, Matsumoto MM, Tsang E, Young WL, van Rooijen N, Kurihara C, Hashimoto T. Roles of macrophages in flow-induced outward vascular remodeling. *J. Cereb. Blood Flow Metab.* 2009; 29:495–503. [PubMed: 19002198]
- Ota R, Kurihara C, Tsou TL, Young WL, Yeghiazarians Y, Chang M, Mobashery S, Sakamoto A, Hashimoto T. Roles of matrix metalloproteinases in flow-induced outward vascular remodeling. *J. Cereb. Blood Flow Metab.* 2009; 29:1547–1558. [PubMed: 19513084]
- Pyo R, Lee JK, Shipley JM, Curci JA, Mao D, Ziporin SJ, Ennis TL, Shapiro SD, Senior RM, Thompson RW. Targeted gene disruption of matrix metalloproteinase-9 (gelatinase B) suppresses development of experimental abdominal aortic aneurysms. *J. Clin. Invest.* 2000; 105:1641–1649. [PubMed: 10841523]
- Roach MR, Burton AC. The reason for the shape of the distensibility curves of arteries. *Can. J. Biochem. Physiol.* 1957; 35:681–690. [PubMed: 13460788]
- Tronc F, Mallat Z, Lehoux S, Wassef M, Esposito B, Tedgui A. Role of matrix metalloproteinases in blood flow-induced arterial enlargement: interaction with NO. *Arterioscler Thromb. Vasc. Biol.* 2000; 20:E120–E126. [PubMed: 11116076]
- Wong LC, Langille BL. Developmental remodeling of the internal elastic lamina of rabbit arteries: effect of blood flow. *Circ. Res.* 1996; 78:799–805. [PubMed: 8620599]

Figure 1.
Mechanical testing apparatus

Figure 2.
Outer diameter-pressure response for a single control specimen at three different levels of fixed axial stretch

Figure 3. Circumferential stretch ratios at pressures of 80 (λ_{C80}) and 120 (λ_{C120}) mmHg for normoflow control arteries and those resected after 1, 2, or 6 days of augmented flow (three points per specimen)

Figure 4.

Confidence intervals (95%) of predicted change in λ_C from control values for each of the independent variables used in the statistical model. Intervals not crossing the control mean value ($x=0.0$) are statistically significant ($p<0.05$). Intervals shown for the continuous variables λ_{ZC} and λ_{ZC}^2 are the predicted change in circumferential stretch for a change of 0.1 in the independent variable.

Figure 5. Circumferential stretch ratios at pressures of 80 and 120 mmHg for vessels resected at 1 day in animals receiving no treatment and in those receiving either doxycycline and GM6001 (three points per specimen)

Table 1

Parameters considered for mechanical response analysis

Parameter	Definition
ΔD_o	change in outer diameter; $80 < P < 120$ mmHg
$\Delta \lambda_c$	change in circumferential stretch; $80 < P < 120$ mmHg
$\Delta P / \Delta D_o$	change in pressure divided by change in outer diameter; $95 < P < 105$ mmHg
E_{iv}	simple line fit of circumferential stress-stretch curve for $95 < P < 105$ mmHg
λ_{C80}	circumferential stretch value at $P = 80$ mmHg (see Eq. 1a)
λ_{C120}	circumferential stretch value at $P = 120$ mmHg (see Eq. 1a)