Published in final edited form as:

Cell Mol Bioeng. 2012 December 1; 5(4): 474–487. doi:10.1007/s12195-012-0251-x.

Alterations in Pulse Pressure Affect Artery Function

Danika M. Hayman^{1,3}, Yangming Xiao¹, Qingping Yao^{1,*}, Zonglai Jiang⁴, Merry L. Lindsey^{2,3}, and Hai-Chao Han^{1,3}

¹Department of Mechanical Engineering, University of Texas at San Antonio, China

²Department of Medicine, Division of Geriatrics, Gerontology, and Palliative Medicine, University of Texas Health Science Center at San Antonio, China

³Biomedical Engineering Program, UTSA-UTHSCSA, China

⁴Institute of Mechanobiology & Medical Engineering, Shanghai Jiaotong University, China

Abstract

Pulse pressure changes in response to cardiovascular diseases and interventions, but its effect on vascular wall structure and function is poorly understood. We examined the effect of increased or decreased pulse pressure on artery function, cellular function, and extracellular matrix remodeling. Porcine carotid arteries were cultured under non-pulsatile (100 mmHg), pulsatile (70-130 mmHg), or hyper-pulsatile pressure (50-150 mmHg) for 1 to 3 days. Vasomotor response, wall permeability, cell proliferation, apoptosis, extracellular matrix remodeling, and proteins involved in atherogenesis were examined. Our results showed that hyper-pulsatile pressure decreased the artery response to sodium nitroprusside, basal tone, and wall permeability after three days. Non-pulsatile pressure increased cell proliferation. Neither hyper-pulsatile nor non-pulsatile pressure caused a change in the extracellular matrix or in the expression of matrix metalloproteinase-2 (MMP-2), MMP-9, caveolin-1, or α -actin. Hyper-pulsatile pressure increased monocyte chemotactic protein-1 gene expression. Taken together, these changes indicate that pulse pressure has a limited effect on the artery immediately after its application. Specifically an increase in pulse pressure alters the artery tone and wall permeability while a decrease in pulse pressure alters cell proliferation. Overall these results provide insight into how the artery initially responds to changes in pulse pressure.

Keywords

pulsatile pressure; permeability; cell proliferation; matrix metalloproteinase; monocyte chemotactic protein-1; and ex vivo organ culture; porcine artery

INTRODUCTION

Pulse pressure, the difference between the systolic and diastolic pressure, changes as the result of alterations in cardiac function or arterial compliance due to aging and cardiovascular disease.¹ Increases in pulse pressure are correlated with aging, the development of coronary heart disease, and congestive heart failure; in fact, increased pulse

Address Correspondence to: Dr. Hai-Chao Han, Department of Mechanical Engineering, The University of Texas at San Antonio, San Antonio, TX 78249, Tel: (210) 458-4952, Fax: (210) 458-6504, haichao.han@utsa.edu.

*Visiting scholar from the Institute of Mechanobiology & Medical Engineering, Shanghai Jiaotong University, China

Statement of conflict of interest

The authors have no commercial associations or sources of support that might pose a conflict of interest.

pressure has been proposed as an indicator of cardiovascular disease progression in patients.^{2, 3} However little is known about the early adaptation response of the artery to altered pulse pressure. Conversely, the use of axial flow left ventricular assist devices and cardiopulmonary bypass machines causes a drastically reduced pulsatility in the vasculature, which causes changes in vascular behavior.⁴ Therefore, it is important to understand the effects of an increased or decreased pulse pressure on vascular wall function. Though it has been reported that artery function and remodeling due to the long term changes in pulse pressure may contribute to the development of various diseases *in vivo*,⁵ the early changes and development process are unclear. In addition, the relationship between decreased pulse pressure and atherosclerosis development is unclear. Therefore, it is of clinical interest to investigate the changes caused by variations in pulse pressure that may contribute to the development of atherosclerosis.

One important function of the artery wall is the regulation of permeability. Changes in wall permeability are known to contribute to the development of cardiovascular diseases. Specifically, changes in the permeability of the wall to key molecules such as LDL which has been linked to the development of atherosclerosis are affected by changes in the mechanical environment.^{6, 7} Alterations in transmural pressure, wall shear stress,⁸ and oscillating frequency of the flow⁹ have all been shown to change permeability. Additionally, pulsatile pressure alters the fluid filtration characteristics of the artery wall compared to the steady pressure profiles.¹⁰ The endothelium specifically plays an important role in controlling fluid flux across the arterial wall. A study by Wiley et al. found that the endothelium alters the filtration characteristics of the artery wall between 50 – 100 s after the onset of the change in pressure.¹¹ This temporally regulated change suggests that the artery response to changes in pressure is dynamic as it adapts to its new environment.

Transport of molecules across the endothelial wall can occur by one of two pathways: the transcellular pathway or the paracellular pathway.¹² Transcellular permeability is mediated through clathrin-coated vesicles and caveolae while paracellular permeability is regulated by adherens and tight junctions between the cells. Albumin, lipids, steroid hormones, fat-soluble vitamins and molecules which attach to albumin all cross the endothelium through the transcellular pathway via clathrin-coated vesicles and caveolae.¹² Additionally studies have found that junctional complexes play an important role in altering wall permeability in response to changes in the mechanical environment.¹³ Specifically a study by Conklin et al. found that low shear stress caused both an increase in permeability and a decrease in occludin gene expression.¹⁴ A cell study also found that increased static pressure resulted in a decreased level of vascular endothelial cadherin (VE-cadherin).¹⁵ Finally, the presence of leaky junctions caused by endothelial cell (EC) turnover is also known to affect artery permeability.¹⁶ Specifically, hypertension and shear stress have both been shown to increase permeability through an increase in leaky junctions.^{16, 17} These pathways contribute to changes in permeability in the artery wall.

Proteins involved in regulating permeability and wall remodeling have also been shown to play an important role in the development of atherosclerosis. Specifically caveolae and cavolin-1 have been shown to play an important role in the development of atherosclerosis.¹⁸ Lower caveolin-1 (CAV-1) levels are associated with plaque instability in humans and increased neo-intimal formation in mice, while increased CAV-1 levels were associated with lower levels of matrix metalloproteinase (MMP) activity.¹⁹ Enzyme production is also affected by cyclic stretch and shear flow.²⁰ MMP-2 and MMP-9, gelatinases which degrade collagen, increase in response to increased pressure.²⁰ These studies suggest that enzyme activity is both an important contributor in the atherosclerosis process and is affected by changes in cyclic stress. However, though the remodeling

response to atherosclerosis and different shear stresses has been studied in detail, the remodeling response to changes in pulse pressure alone is not well understood.

One important step in atherosclerosis development is the recruitment of monocytes to the surface of the endothelium.⁶ Various proteins are involved in signaling and attracting monocytes to the endothelium. Monocyte chemotactic protein-1 (MCP-1) has been shown by multiple studies to increase in response to cyclic stretch in isolated ECs.²¹ Intercellular adhesion molecule 1 (ICAM-1) has been shown to increase in response to cyclic stretch and increased pulsatility in cell studies, while vascular cell adhesion molecule 1 (VCAM-1) decreases in response to cyclic stretch.²² These studies suggest that an increase in cyclic stretch, compared to a static control, contribute to the development of atherosclerosis. Therefore, it is important to study these molecular changes in order to better understand the effect of pulse pressure on the arterial wall.

Accordingly, the purpose of this study was to investigate the early stage changes in the artery wall structure and function induced by elevated and diminished pulse pressures. We used an *ex vivo* organ culture model to examine the effect of a change the pulse pressure independently of the flow rate and mean pressure after up to three days. Understanding these early stage adaptations will not only elucidate the effect of acute exposure to altered pulse pressure but also describe early signals in the body which would indicate the development of more long-term problems.

MATERIALS AND METHODS

Artery Harvest and Organ Culture

Common carotid arteries were obtained post-mortem from porcine donors at a local abattoir with the approval of the Texas Department of State Health Service and the University of Texas at San Antonio Institutional Biosafety Committee (IBC). Arteries were transported to the lab in ice cold phosphate buffered saline (PBS) supplemented with antibiotic/antimycotic (1.4%). Within a biosafety cabinet in our lab, the arteries were cleaned of loose connective tissue and checked for leaks before being mounted into an *ex vivo* artery organ culture system.²³

The *ex vivo* organ culture system consisted of a vessel chamber and a flow loop which together created an *ex vivo* environment which mimicked the *in vivo* environment of the artery.^{23, 24} The flow rate through the artery, mean internal pressure, and pulse pressure were controlled independently by the peristaltic pump, downstream clamp, and T-end, respectively (Figure 1). The artery was tied to the cannulae inside the vessel chamber and submerged in media (approximately 100 mL). Perfusion media was added to the reservoir (approximately 250 mL) before the loop and vessel chamber were closed and sealed. Arteries were stretched manually to achieve a physiological axial stretch ratio of 1.5.²⁵ The flow loops were placed in incubators (37°C, 5% CO₂) and cultured for 1 day or 3 days. The media was DMEM supplemented with sodium bicarbonate (3.7 g/L), antibiotic/antimycotic (1.4%), L-glutamine (1%), calf serum (10%), and HEPES buffer (2.5%). The lumen of the artery was exposed to circulating perfusion media. The perfusion media was media supplemented with Dextran (50-55 g/L, average molecular weight of 150 kDa) in order to increase the viscosity of the media to that of normal blood (4 cP).

After the sealed organ culture loops were placed in the incubator the pressure and flow in all organ culture loops were increased gradually to experimental levels. Three pulse pressures were examined: pulsatile (100±30 mmHg), non-pulsatile (100±2 mmHg), and hyper-pulsatile (100±50 mmHg). The mean pressure (100 mmHg), flow rate (160 ml/min), and axial stretch ratio (1.5) were held constant among the groups. Pulsatile pressure and hyper-

pulsatile pressure were achieved by adjusting the size of a T-end tube (Figure 1) while a pulse dampener (Masterflex, Cole-Parmer) was used to achieve non-pulsatile pressure.²³

The fluid flow through the artery was designed to closely mimic the *in vivo* environment.²⁵ The mean flow rate was determined using the following equation:^{25, 26}

$$\tau_w = \frac{4\mu}{\pi} \left(\frac{Q}{r^3} \right). \quad (1)$$

Where τ_w is the mean wall shear stress, μ is the viscosity, Q is the flow rate and r is the lumen radius. Thus, a flow rate of 160 ml/min and a viscosity of 4cP were used for the perfusion to generate a physiological shear stress of 1.5 Pa in a carotid artery with a diameter of 2.1 mm.²⁵ The Reynolds number was calculated from the following equation:

$$Re = \frac{2\rho vr}{\mu} \quad (2)$$

where ρ is the fluid density (1g/cm³) and v is the velocity ($v = Q/\pi r^2$). The Reynolds number is 202 at a flow rate of 160 ml/min which indicates that the artery was experiencing laminar flow. The Womersley number was calculated from the following equation:

$$\alpha = r \left(\frac{\rho \omega}{\mu} \right)^{1/2}, \quad (3)$$

where ω is the angular frequency. At a pulse frequency of 3 Hz, the Womersley number was determined to be 4.56, which is close to what was observed in the carotid *in vivo* (4.40).²⁷ In addition, we observed no oscillation in flow direction despite pressure pulsatility, indicating a zero oscillatory shear index,²⁸ which is consistent with the observation for the common carotid arteries *in vivo*.^{29, 30}

To label new proliferating cells in arteries, 5'-bromo-2'-deoxyuridine (BrdU, Roche) was added to the perfusion media (5μg/ml) 24 hours prior to the conclusion of the culture period in a subset of arteries.²⁴

Vasomotor Response

Vasomotor response was quantified by measuring the artery response to increasing concentrations (10⁻⁷M, 10⁻⁶M, and 10⁻⁵M) of norepinephrine (NE), carbachol (CCH), and sodium nitroprusside (SNP).²⁵ NE, a vasoconstrictor, was added first to fully contract the artery. CCH, an endothelial mediated vasodilator was added second to relax the artery. SNP, an endothelium independent vasodilator was added last to fully relax the artery. Each agonist was allowed to circulate for 10 to 15 minutes under the experimental pressure conditions during which time the change in outer diameter was recorded using a Leica camera (at least 1 image per minute). The outer diameter was measured in at least three locations in each image using ImagePro Plus®.

To quantitatively compare the change in vasomotor function between groups, the maximum diameter change in response to each agonist was calculated. The maximum diameter change was taken as the maximum increase or decrease in diameter during the testing period of each concentration of agonist.

$$\%Diameter \text{ Change} = (D_{max} - D_{baseline}) / D_{baseline} * 100\% \quad (1)$$

Where D_{baseline} is the diameter measured immediately prior to the administration of the agonist and D_{max} is the point of maximum diameter change within each testing period.

Wall Permeability

To investigate the effect that artery remodeling²³ had on permeability we evaluated the change in EBD permeation distance at the end of the three day culture period. The wall permeability was quantified by measuring the permeation distance of Evans blue dye (EBD) through the artery wall and the flux of fluorescein isothiocyanate labeled (FITC) dextran (70kDa) across the artery wall. The 70 kDa molecular weight was chosen since it is the same size as the albumin that would be passing through the wall.³¹ Both tests were performed at the end of the culture period before the arteries were removed from the organ culture system. Prior to both permeability tests the pulse pressure in the experimental groups was adjusted to 100 ± 30 mmHg to eliminate differences in transmural pressure. Fresh arteries were also examined under the pulsatile pressure immediately after being mounted into the organ culture system.

Evans Blue Dye Test—EBD was added to the perfusion media at a final concentration of 3.3 g/L and circulated for one hour (determined from preliminary experiments to achieve partial exudation of the EBD through the wall thickness).³² Segments of the arteries were collected at the end of the test and snap frozen in OCT for sectioning using a cryostat. The radial cross-sections were examined using an Olympus microscope and photographed with an attached digital camera (Q-Color 3, Olympus). The exudation distance of the dye through the artery wall was measured using ImagePro Plus®.

FITC Dextran Test—FITC Dextran was added to the perfusion media on the last day of organ culture at a final concentration of 0.32 g/L and circulated for 12 hours.³³ Samples of both the perfusion media and the chamber media were collected at three time points: $t=0$ hrs, 6 hrs, and 12 hrs. The fluorescence in each sample was measured using a Synergy 2 plate reader with Gen5 software (BioTek Instruments Inc.) at an excitation wavelength of 485 nm and an emission wavelength of 528 nm. The flux across the wall was calculated using the following equation:³⁴

$$Flux = \frac{I_{\text{chamber}}}{I_{\text{reservoir}} * t * A} \quad (2)$$

Where I is the florescence intensity measured by the plate reader, t is the time the FITC Dextran circulated, and A is the surface area of the artery lumen.

EBD Transport under Different Pulse Pressure

The effects of pulsatile pressure, non-pulsatile pressure, and hyper-pulsatile pressure on EBD transport in the absence of possible structural changes were also quantified in fresh arteries. Arteries were cultured overnight at a pulse pressure of 100 ± 30 mmHg to allow the artery to acclimate to the organ culture system. Then the pressure was adjusted to non-pulsatile or hyper-pulsatile pressure, or maintained at pulsatile pressure as a control, after which EBD solution was administrated to the perfusion media and circulated for one hour. Arteries were then harvested and the EBD permeation distance was measured as described above.

Histology

The arteries were fixed under pressure (100 mmHg) in 10% formalin for 24 hours, and subsequently stored in 70% alcohol at 4°C. Later, artery specimens were processed for paraffin embedding and micro-sections (5 μm) were cut from the paraffin blocks. The

sections were deparaffinized with a series of xylene and alcohol (100-70%) washes followed by washes in double distilled water and PBS. Slides were then stained for various proteins and markers including hematoxylin-eosin staining, trichrome staining, and Verhoeff staining and immunohistochemistry.

A Masson's trichrome stain, which stains the collagen blue, the muscle red, and the nuclei purplish-black was used to visualize the collagen in the artery wall. The slides were first treated with Bouins solution (50°C, 5 min), a fixative and then immersed in Weigert's iron hematoxylin solution (50°C, 2 min) to stain the nuclei followed by Biebrich scarlet acid-fuchsin solution (50°C, 5 min) to stain the muscle and collagen. Then the slides were treated with phosphomolybdic-phosphotungstic acid solution (room temperature, 10 min) to remove the red stain from only the collagen. Finally, an aniline blue counterstain (room temperature, 2 min) was used to stain the collagen blue. A Verhoeff elastic stain, which stains elastin dark purple, was used to visualize the elastin components in the artery wall. First the tissue sections were overstained in Verhoeff's working elastic tissue stain (10min) followed by differentiation in 5% sodium thiosulfate (1 min). Finally a van Gieson counterstain was applied (1 min). The stained slides were imaged with an Olympus microscope (CX41) and attached digital camera (Q-Color 3 Olympus). The percent area of both the collagen and elastin was measured using ImagePro Plus.

Immunohistochemistry

ZO-1, Occludin and VE-cadherin Staining—Deparaffinized slides were stained for ZO-1, occludin, and VE-cadherin following vendor instructions. Briefly, slides were treated in boiling citrate buffer solution (10mM, 5min, pH 6.0) for antigen retrieval, treated with DMSO in PBS (10%, 3hr) and then permeabilized with 0.3% Triton X-100 (20 min, room temperature). After non-specific staining was blocked with 10% fetal calf serum (40 min, room temperature), The sections were incubated with primary antibodies specific to the protein of interest: ZO-1 (1:50, Invitrogen), occludin (1:40, Invitrogen), or VE-cadherin (1:50, Santa Cruz Biotechnology) for 1hr at 37°C, followed by incubation with a Cy3 labeled secondary antibody (45min, 37°C, Jackson ImmunoResearch). Then, the slides were counterstained with 4',6-diamidino-2-phenylindole (DAPI, 10min at room temperature) and mounted. The staining intensity was examined both qualitatively and quantitatively. The qualitative assessment was performed by assigning a rank to each picture based on the intensity of the staining. The quantitative assessment was performed by measuring the intensity of the fluorescent staining using Image-J.

Quantification of Cell Proliferation Using BrdU Stain—The BrdU positive cells were detected using a BrdU labeling and detection kit 1 (Roche).³⁵ Briefly, deparaffinized slides were treated in boiling citrate buffer solution (10mM, 1hr) for antigen retrieval. The DNA in the samples was denatured with 2M HCL (30min, room temperature). The sections were then permeabilized with 0.5% Triton X-100 (15min, room temperature). Non-specific staining was blocked with 20 % fetal calf serum (30 min, room temperature). Then, the sections were incubated with an anti-BrdU antibody (1hr, 37°C, Roche) followed by incubation with a FITC labeled secondary antibody (45min, 37°C, Roche). The slides were counterstained with DAPI and mounted.

The sections were then imaged using an Olympus fluorescence microscope (CX-41) and photographed with an attached digital camera (Q-Color 3 Olympus). The filter module was switched between the green excitation filter and the blue excitation filter to visualize the BrdU positive cells and DAPI stained cells respectively. The number of BrdU positive cells was expressed as a percentage of DAPI stained cells. In a few cases the DAPI staining was too weak to allow accurate cell counting due to the treatment of the slides with HCL for 30

minutes during the staining process. In these cases H&E stained serial sections were used to obtain counts of the total cells in the artery wall.

Quantification of Cell Apoptosis Using TUNEL Stain—Cell apoptosis was detected using terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining.³² Briefly, the slides were treated in boiling citrate buffer (10mM, 1hr) and cooled for one hour, followed by treatment in 0.1% Triton-X 100 in PBS to permeabilize the tissue. The slides were then incubated with the TdT and fluorescein labeled nucleotides (Roche cat # 11684795910). The enzyme solution and labeling solution were stored separately and mixed immediately prior to use (5 μ l enzyme solution was mixed with 45 μ l labeling solution for each slide). The slides were then incubated with the TUNEL reaction mixture for one hour at 37°C at full humidity. During the process, slides were washed three times in PBS after each step. The slides were then counterstained with DAPI and mounted. The stained sections were imaged with a fluorescent microscope and photographed as described above. The number of TUNEL positive cells was counted and expressed as a percentage of DAPI stained cells.

Immunoblotting

Artery segments were harvested from each experimental group (n=4) after 3 days, washed in PBS, snap frozen in 70% alcohol and dry ice, and stored at -80°C. Protein extraction was performed by homogenizing the samples in reagent 4 buffer (Sigma). A Bradford assay was run to determine the protein concentration in each sample. Equal amounts of the protein extract (10 μ g) were separated using SDS-PAGE in a 4-12% polyacrylamide gel. The protein was transferred to nitrocellulose paper or Immun-Blot PVDF membranes (Bio-Rad) and incubated with the primary antibody: CAV-1 (1:500 dilution, Santa Cruz Biotechnology), MMP-2 (1:1000 dilution, Chemicon), MMP-9 (1:1000 dilution, Chemicon), α -actin (1:5000 dilution, Sigma) or β -actin (1:5000 dilution, Sigma or Santa Cruz Biotechnology). The immunoblots were then incubated with the secondary antibody: peroxidase labeled goat anti-rabbit IgG (1:5000 dilution, Vector Laboratories) or goat anti-mouse IgG-HRP (1:2500 dilution, Santa Cruz Biotechnology). The protein bands were visualized with the enhanced chemiluminescence detection system (ECL) according to the manufacturer's directions (Amersham GE Healthcare). The immunoblots were imaged using x-ray film and the intensity of the protein bands was measured with ImageJ software. The intensity measurements were normalized to β -actin to account for loading differences between lanes. The relative intensity was normalized to the same fresh sample run on each gel, to allow comparisons between gels.

RNA Isolation and Gene Expression

Artery segments were harvested from fresh arteries and all three experimental groups at 24 hours and 3 days, snap frozen in 70% alcohol and dry ice, and stored at -70°C. RNA was isolated from the tissue samples by homogenization and sonication in TRIZOL reagent (Sigma). The amount of RNA in each sample was measured using the NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific) and the amount of RNA used for each RT reaction was normalized to 1 μ g. The high capacity RNA-to-cDNA kit (Applied Biosystems) was used to convert RNA to cDNA following the protocol provided by the manufacturer. Briefly, RNA was incubated with the RT buffer mix and the RT enzyme mix at 37°C for 60 minutes immediately followed by an incubation at 95°C for 5 min in a Px2 thermal cycler (Thermo Electron Corporation). The amount of cDNA in each sample was quantified using the NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific) and the amount of cDNA used for each PCR reaction was normalized to 50 ng. Quantitative RT-PCR was performed using TaqMan universal master mix II with UNG (Applied Biosystems) and the following preconfigured TaqMan gene expression assays (Applied Biosystems):

MCP-1 (Ss03394377_m1), VCAM-1 (Ss03390914_m1), ICAM-1 (Ss03392385_m1), occludin (Ss03377507_u1), VE-cadherin (Ss03378336_u1), and glyceraldehyde 3-phosphate dehydrogenase (GAPDH, Ss03375435_u1). The PCR reaction was run on a 7900HT real-time PCR system (Applied Biosystems) using standard conditions. The thermal cycling applies to the samples is as follows: UNG incubation (50°C, 2 min), polymerase activation (95°C, 10 min), 40 PCR cycle (95°C for 15 s followed by 60°C for 1 min). The threshold cycles were read at a value of 0.15. Both the CT values and the relative gene expression (calculated using the comparative CT method) were examined.

Statistical Analysis

Data are presented as mean $\pm$ standard error of the mean. Statistical significance ($p < 0.05$) was determined with a one-way or two-way ANOVA followed by post-hoc analysis with a Tukey HSD and Student's t-test using JMP software.

RESULTS

Hyper-pulsatile Pressure Decreases Artery Response to SNP and Basal Tone

After three days in culture the arteries in all pulse groups ($n=8$ each) responded strongly to both NE and SNP though the response to CCH was less robust (Figure 2 top). There was no difference in the artery response to NE or CCH due to either an increase or decrease in pulse pressure. However, the response of the hyper-pulsatile arteries ($7.8 \pm 5.3\%$ relaxation) to SNP was significantly decreased when compared to the responses of the pulsatile ($23.7 \pm 12.6\%$ relaxation) and non-pulsatile arteries ($35.8 \pm 13.7\%$ relaxation). No difference was observed between the pulsatile and non-pulsatile artery response to SNP.

The basal tone in arteries cultured under hyper-pulsatile pressure was significantly decreased compared to the pulsatile and the non-pulsatile groups (Figure 2 bottom). No difference in tone was observed between the pulsatile and non-pulsatile arteries.

Hyper-pulsatile Pressure Decreases EBD Permeability

In arteries cultured for three days under different pulse pressures and then tested under pulsatile pressure, EBD permeation distance decreased significantly in the hyper-pulsatile group compared to both the pulsatile and non-pulsatile groups ($n=5$ for each group, Figure 3 top). Additionally, the EBD permeation distance in the fresh group ($n=3$) was significantly lower than the non-pulsatile and pulsatile groups. However, for arteries pre-conditioned under pulsatile pressure overnight and then tested under pulsatile, non-pulsatile or hyper-pulsatile pressures ($n=3$ each group), there was no difference in EBD permeation distance among groups. Combined, these data indicated that the difference in permeation distance was due to structural changes in the artery and not the difference in the driving pressure pulsatility.

In addition, there was no difference in the flux of FITC labeled dextran across the artery wall among arteries cultured under pulsatile, non-pulsatile or hyper-pulsatile pressure ($n=3$ each group) for 3 days (Figure 3 bottom).

ZO-1, Occludin, and VE-cadherin were not Affected by Pulse Pressure

Qualitative comparison of occludin and ZO-1 staining indicated that there was no significant difference in staining intensity, while qualitative comparison of VE-cadherin staining suggested that the non-pulsatile group may exhibit a lower level of staining compared to the fresh arteries. However, a quantitative comparison of staining intensity of arteries ($n=3$ in each group) indicated that there was no significant difference in VE-cadherin staining between the non-pulsatile (258853 ± 47393 , pixel intensity), pulsatile (227417 ± 104856),

hyper-pulsatile (286196 ± 87946) or fresh groups (303203 ± 90665). These results indicate that there was no change in the concentration of these adherens and tight junction proteins under different pulse pressure.

Non-pulsatile Pressure Increases Cell Proliferation

BrdU-positive cells were seen in most sections examined, suggesting that some cell proliferation occurs in all groups after 3 days (Figure 4a). Though cell proliferation was observed in both the intima and media of the artery wall, we were able to differentiate between SMC and EC proliferation by examining the location of the cell and the shape of the nuclei. SMC nuclei were elongated and aligned with the elastin fibers in the media, while EC nuclei were rounded and present only in the intima. The BrdU index in the intima of the non-pulsatile group (n=5) was significantly higher compared to that in the pulsatile group (n=3) and hyper-pulsatile group (n=4), indicating that pulse pressure has an effect on endothelial cell proliferation (Figure 4b). However, no difference in BrdU index in the media was observed among these groups. No differences in BrdU index in the intima or media were observed for the hyper-pulsatile group compared to the normal pulsatile group. Additionally, in a separate set of arteries (n=4) cultured under pulsatile pressure for 3 days without dextran, there was no difference detected in the BrdU index in either the intima or media compared to the pulsatile group with dextran, indicating dextran had no effect on cell proliferation.

TUNEL Staining (Apoptosis) was not Affected by Pulse Pressure

TUNEL staining indicates cell apoptosis occurs in arteries cultured under all pulse pressure groups. However, there was no statistical difference among the groups (n=4) though the non-pulsatile group exhibited a slightly higher level of apoptosis in the intima compared to the pulsatile and hyper-pulsatile groups (Figure 5). In addition, in a separate set of arteries (n=4) cultured under pulsatile for 3 days without Dextran, there was no difference detected in the TUNEL index in either the intima or media compared to the pulsatile group with dextran, indicating dextran had no effect on cell apoptosis.

Effect of Pulse Pressure on MMP-2, MMP-9, Collagen and Elastin

No significant change in ECM remodeling was observed in arteries after three days in organ culture under either non-pulsatile or hyper-pulsatile pressure. The levels of MMP-2 and MMP-9 in the pulsatile, non-pulsatile and hyper-pulsatile groups (n=4 each), showed no statistical difference (Figure 6). However, the MMP-2 antibody detected two bands in the experimental groups but only one band was detected in the fresh artery group (Figure 6b). The fresh groups exhibited a significantly higher level of pro MMP-2 compared to the non-pulsatile group, and a significantly lower level of active MMP-2 compared to all three groups (Figure 6c).

The trichrome staining of slides from arteries in the non-pulsatile (n=5), pulsatile (n=3), and hyper-pulsatile (n=6) groups demonstrated no qualitative difference in collagen content in terms of the percentage of area, among these groups. Similarly, Verhoeff staining of slides from arteries in the non-pulsatile (n=5), pulsatile (n=4), and hyper-pulsatile (n=6) groups demonstrated no qualitative difference in elastin content among these groups.

CAV-1 and α -actin Expression

Western blots of samples from arteries cultured under all pulse groups (n=4) demonstrated that there was no significant increase in CAV-1 or α -actin expression due to non-pulsatile or hyper-pulsatile pressure. Additionally, the distribution of CAV-1 did not change due to either an increase or decrease in pulse pressure (Figure 7).

MCP-1, VCAM-1, ICAM-1, VE-cadherin, and Occludin Gene Expression

Gene expression was measured for fresh arteries (n=4) and all experimental groups at 24 hours (n=5) and at 3 days (n=6 in the hyper-pulsatile group and 4 in all other groups). There were no significant differences in VE-cadherin or occludin gene expression levels among the different pulse pressure groups at either 24 hours or three days. Additionally, no significant differences were detected in VCAM-1 or ICAM-1 among pulse pressure groups at either 1 day or three days.

However, pulse pressure had a significant effect on the MCP-1 CT values (Figure 8 top). Hyper-pulsatile pressure decreased MCP-1 CT values which indicate an increase in MCP-1 levels. There was no difference between the 1 day and 3 day samples, and there was no significant interaction between pulse pressure and culture time. Significant differences were observed in the gene expression levels of GAPDH, the housekeeping gene, between some of the groups (Figure 8 bottom). There was a significant increase in GAPDH from 1 day to 3 days. Additionally, the fresh group had significantly higher levels of GAPDH compared to all other experimental groups.

DISCUSSION

In summary, we investigated the effect of a change in pulse pressure on artery structure and function by examining arteries cultured under hyper-pulsatile and non-pulsatile pressure after one day and three days. Our results showed that while hyper-pulsatile pressure affects artery function and non-pulsatile pressure affects cellular function, neither an increase nor decrease in pulsatility caused ECM remodeling. Specifically we found that hyper-pulsatile pressure decreased the artery response to SNP, basal tone, and wall permeability, while non-pulsatile pressure caused an increase in cell proliferation in the intima.

Our results indicate that the artery function changes in response to altered pulse pressure after only three days. One specific change is that hyper-pulsatile pressure decreased the artery response to SNP. Since SNP acts directly on the SMCs to induce vessel relaxation this change in SNP response in the absence of a change in NE response indicates that the initial SMC tone has decreased in response to hyper-pulsatile pressure. While this decrease in function is a possible indicator that the SMCs may be moving toward a more synthetic phenotype a corresponding increase in cell proliferation or levels of collagen and elastin in the artery wall were not observed. Additionally, the level of α -actin did not change between the groups or time points, indicating that there was not a significant change in phenotype. Further study is needed to elucidate the underlying mechanism by examining additional markers of the SMC contractile phenotype. For example, calponin and smoothelin are two markers which are commonly used to examine the SMC contractile phenotype.³⁶⁻³⁸

Our results also indicate that artery permeability is affected by an increase in pulse pressure. Exposure to hyper-pulsatile pressure results in an increase in EBD permeability. These results agree with the literature which states that an increase in cyclic pressure decreases permeability across endothelial cell layers.³⁹ EBD travels across the artery wall in two possible states: bound to albumin in the media or as free molecules dissolved in the media.⁴⁰ EBD bound to albumin is a large molecule which passes intracellularly through the artery wall via vesicular transport,¹² while unbound EBD is significantly smaller and passes through the intercellular junctions between the cells.¹² The observed increase in EBD permeation distance could be due to the permeation of EBD bound to albumin (from calf serum) in the perfusion media; however the 70 kDa FITC dextran flux across the wall was not changed by different PP after 3 days. The 70 kDa FITC dextran was used as an inert size analog for albumin.³¹ If the observed change in permeability was due to a change in albumin flux across the wall then a change in 70 kDa FITC dextran should have been observed as

well. No change in dextran flux across the wall was observed indicating that there was no change in the permeability of 70 kDa molecules across the wall. One possible reason is that the outer layer (adventitia) was not affected by the differences in pulse pressure and thus the dextran flux showed no difference among groups. Additionally, we examined the levels of CAV-1 which is critical for transport of albumin across the wall,¹² and no change was observed. Since CAV-1 is directly involved in the transport of albumin across the wall, this further indicates that albumin flux across the wall was not changed.

Since albumin flux was not changed, a possible explanation for the observed change in EBD permeation distance is that it is the free dye moving across the wall and not the dye bound to albumin. In this case the molecules are small enough to pass through the intercellular pathway, through the tight and adherens junctions.¹² After it was determined that the FITC dextran flux did not change we tested the hypothesis that unbound EBD was moving across the wall via the intercellular pathway. However, our gene expression data indicated that there was no upregulation in VE-cadherin or occludin gene expression at either 24 hours or three days. Additionally, we saw no difference in ZO-1, VE-cadherin, or occludin staining at 3 days. This indicated that it was not an upregulation in ZO-1, VE-cadherin, or occludin that was decreasing permeation distance.

At the same time this does not fully eliminate changes in the intercellular pathways as a possible mechanism behind the change in permeation distance. For example the intercellular pathway is partially regulated by Ca^{2+} ,¹² a primary determinant of SMC contractility.⁴¹ Ca^{2+} increases intercellular permeability by initiating a signaling pathway which results in the initiation of myosin light chain kinase and therefore the formation of actomyosin contractile units and stress fibers.¹² This activation of contractile units exerts a force on the junctions between the cells and therefore increases intercellular permeability.¹² Our vasomotor results indicate that the hyper-pulsatile group exhibits a weaker response to SNP and a weaker basal tone which corresponds with the observed decrease in permeability.

Additionally, we observed that the fresh arteries exhibited a decreased EBD permeation distance as well. We suspect that this was because the arteries were placed in the organ culture system and tested on the same day under pulsatile pressure and did not have time to regain their tone. This suggests that decreases artery contractility may be related to the observed decrease in EBD permeation distance.

Finally, it is also possible that the observed difference in permeation distance is the result of EBD binding to the ECM components of the wall.⁴² The study by Yao et al. found that the IEL fenestrae size decreased in the hyper-pulsatile group after three days.²³ This alters a barrier in the wall which not only slows down flow through the wall but also binds to the free EBD molecules. Therefore it is possible that the observed decrease in permeability in the hyper-pulsatile arteries is the result of increased binding at the IEL compared to the pulsatile and non-pulsatile groups. We did observe binding of the EBD to the elastin in the artery wall on sections that had been fixed in formalin, paraffin embedded, and subsequently labeled with BrdU or TUNEL assays. However, the changes in the IEL cannot explain the decreased permeation distance observed in the fresh arteries compared to the experimental groups. Unbound EBD should move more easily through the fresh arteries since the IEL has not yet remodeled to exhibit smaller IEL fenestrae.²³ Future research should further investigate the mechanisms behind these changes in function.

A change in cell proliferation was also detected after three days. An increase in cell proliferation in the intima of arteries cultured under non-pulsatile pressure was observed after three days. It is difficult to compare our results to the existent cell studies which have investigated the effect of cyclic stretch on cell proliferation due to the differences in

experimental design and conditions. A cell culture study by Zheng et al. compared the effect of cyclic stretch (10% elongation, 30 cycles/min) on EC proliferation to the effect of static stretch (10% elongation).⁴³ Both types of stretch increased cell proliferation and tube formation; however, cyclic stretch resulted in a longer tube length and more branching. Another cell study by Shin et al. also found that exposure to a cyclic pressure of 60/20 mmHg increased EC proliferation in a VEGF-C dependent manner compared to the static control (0.15 mmHg).⁴⁴ These results do not agree with our results. Our results indicate that static stretch (non-pulsatile pressure) increases cell proliferation compared to the cyclic stretch control (pulsatile group), which is the opposite of the results observed in the other two studies. These differences may be due to the differences in experimental conditions between studies. For example the studies described above^{43, 44} were cell culture studies while our study was carried out on intact tissue. Additionally, the mean and pulsatile components of the cyclic pressure applied in the study by Shin et al. was lower compared to the groups in our study. Meanwhile the study by Zheng et al. did not apply pressure to the cells, only stretch. Finally, each study defined the control group differently compared to our control group, the cells in arteries were under stretch generated by the pressure while the cells in vitro were initially stretch-free. However, despite these differences in experimental design and results all the studies described, including this study, consistently indicate that a change in pulse pressure affects cell proliferation.

The lack of significant change in SMC proliferation is surprising since cyclic stretch is known to alter SMC proliferation; however, cell studies in the literature describe both increases and decreases in proliferation of SMCs exposed to cyclic stretch.⁴⁵ Additionally, very little cell proliferation is observed in the normal intact artery⁴¹ and so this absence of a change in cell proliferation may be due to the fact that the basic artery structure and function is maintained. Still, more uniformity in the experimental designs between studies is necessary to allow meaningful comparison.

Additionally, arteries cultured under pulsatile pressure without dextran demonstrated unchanged cell proliferation and apoptosis. This is consistent with findings in the literature which suggest that in arteries Dextran does not cause an inflammatory response, like that observed in the colon, but may even serve to prevent the inflammatory response.^{46, 47}

Another observed change in cell function was an increase in MCP-1 after exposure to hyper-pulsatile pressure. However, no changes in ICAM-1 or VCAM-1 were observed, additional molecules involved in monocyte adhesion. A longer term study by Eberth et al. found that an increase in pulsatility caused an increase in MCP-1.⁴⁸ Therefore it is likely that monocyte recruitment is affected by hyper-pulsatile pressure; however, despite the fact that we observed an increase in MCP-1 it was not accompanied by a corresponding increase in adhesion molecules. We also observed changes in GAPDH levels; however, previous research has shown that GAPDH does not change with cyclic stretch and is often used as a housekeeping gene.⁴⁹ This suggests that the changes we observed could be the result of differences between samples rather than experimental conditions. Therefore, although this study suggests that monocyte recruitment is increased in response to an increase in pulse pressure, further study is necessary to verify these results.

Finally, no change in artery remodeling was detected after three days in culture. There were no observed changes in collagen or elastin between the groups after three days. Longer term studies have observed some changes after 7 and 10 days suggesting that a longer organ culture period would be necessary to observe changes in structure.^{23, 48}

Our study examined the porcine carotid artery; however, we expect the results of this study to be applicable to other elastic arteries of a similar size in the cardiovascular network.

Based on our results, both an increase and decrease in PP have a limited effect on the artery. These changes occur early after the initial application of an altered PP. Given that aging and cardiovascular diseases are correlated with increased pulse pressure in the body, understanding the mechanisms of the initial changes that occur will improve our ability to prevent them. Conversely, the results of this project will help describe the early changes in artery structure and function that occur as a result of decreased PP. This will allow engineers to make a more informed decision when designing these steady flow devices.

Conclusions

Our results demonstrate changes in artery and cell function that occur as a result of changes in pulse pressure and offer an initial analysis of the mechanisms behind the observed changes in vessel structure and function that are due to difference in PP. These results improve our understanding of the initial changes that occur due to an increase or decrease in PP. The long term application of these results is to improve the ability of the clinician to treat cardiovascular disease and the ability of the engineer to design cardiovascular devices. Overall these results provide insight into how the artery responds to initial changes in PP. Further work to more fully elucidate these changes would provide valuable information for designing treatments of cardiovascular disease and mechanical interventions.

Acknowledgments

This work was supported by the National Science Foundation under award number CBET-0602834 (HCH) and the National Institutes of Health (HL095852 to HCH, F31HL096448 to DH, and HL075360 and NHLBI HHSN 268201000036C (N01-HV-00244) for the UTHSCSA Cardiovascular Proteomics Center to MLL). It was also partially supported by Grant 10928206 from the NSF of China. We thank Dr. Colleen Witt and the UTSA Research Center in Minority Institution (RCMI) Advanced Imaging Core for providing us the access and technical support for confocal microscopy. We also thank Dr. John Zhang, Dr. Rogelio Zamilpa, Dr. Quixia Dai, Ms. Monica Gireud, Ms. Trevi Ramirez, Ms. Jamie Parra, and Ms. Erin Dunne for their help in this study.

REFERENCES

1. Benetos A, et al. Influence of age, risk factors, and cardiovascular and renal disease on arterial stiffness: clinical applications. *Am J Hypertens.* 2002; 15:1101–8. [PubMed: 12460708]
2. Haider AW, Larson MG, Franklin SS, Levy D. Systolic blood pressure, diastolic blood pressure, and pulse pressure as predictors of risk for congestive heart failure in the Framingham Heart Study. *Ann Intern Med.* 2003; 138:10–6. [PubMed: 12513039]
3. O'Rourke MF. Isolated systolic hypertension, pulse pressure, and arterial stiffness as risk factors for cardiovascular disease. *Curr Hypertens Rep.* 1999; 1:204–11. [PubMed: 10981067]
4. Nishimura T, et al. Diminished vasoconstrictive function caused by long-term nonpulsatile left heart bypass. *Artif Organs.* 1999; 23:722–6. [PubMed: 10463496]
5. Amar J, Chamontin B. Cardiovascular risk factors, atherosclerosis and pulse pressure. *Adv Cardiol.* 2007; 44:212–22. [PubMed: 17075210]
6. Lusis AJ. Atherosclerosis. *Nature.* 2000; 407:233–41. [PubMed: 11001066]
7. Tarbell JM. Mass transport in arteries and the localization of atherosclerosis. *Annu Rev Biomed Eng.* 2003; 5:79–118. [PubMed: 12651738]
8. Jo H, Dull RO, Hollis TM, Tarbell JM. Endothelial albumin permeability is shear dependent, time dependent, and reversible. *Am J Physiol.* 1991; 260:H1992–6. [PubMed: 1905493]
9. Friedman MH, Henderson JM, Aukerman JA, Clingan PA. Effect of periodic alterations in shear on vascular macromolecular uptake. *Biorheology.* 2000; 37:265–77. [PubMed: 11145073]
10. Alberding JP, Baldwin AL, Barton JK, Wiley E. Onset of pulsatile pressure causes transiently increased filtration through artery wall. *Am J Physiol Heart Circ Physiol.* 2004; 286:H1827–35. [PubMed: 14726305]

11. Alberding JP, Baldwin AL, Barton JK, Wiley E. Effects of pulsation frequency and endothelial integrity on enhanced arterial transmural filtration produced by pulsatile pressure. *Am J Physiol Heart Circ Physiol*. 2005; 289:H931–7. [PubMed: 15833802]
12. Minshall RD, Malik AB. Transport across the endothelium: regulation of endothelial permeability. *Handb Exp Pharmacol*. 2006:107–44. [PubMed: 16999218]
13. Berardi DE, Tarbell JM. Stretch and Shear Interactions Affect Intercellular Junction Protein Expression and Turnover in Endothelial Cells. *Cell Mol Bioeng*. 2009; 2:320–331. [PubMed: 20161517]
14. Conklin BS, Vito RP, Chen C. Effect of low shear stress on permeability and occludin expression in porcine artery endothelial cells. *World J Surg*. 2007; 31:733–43. [PubMed: 17372666]
15. Ohashi T, Sugaya Y, Sakamoto N, Sato M. Hydrostatic pressure influences morphology and expression of VE-cadherin of vascular endothelial cells. *J Biomech*. 2007; 40:2399–405. [PubMed: 17261311]
16. Ogunrinade O, Kameya GT, Truskey GA. Effect of fluid shear stress on the permeability of the arterial endothelium. *Ann Biomed Eng*. 2002; 30:430–46. [PubMed: 12085996]
17. Wu CH, et al. Transendothelial macromolecular transport in the aorta of spontaneously hypertensive rats. *Hypertension*. 1990; 16:154–61. [PubMed: 2379948]
18. Frank PG, Hassan GS, Rodriguez-Feo JA, Lisanti MP. Caveolae and caveolin-1: novel potential targets for the treatment of cardiovascular disease. *Curr Pharm Des*. 2007; 13:1761–9. [PubMed: 17584106]
19. Rodriguez-Feo JA, et al. Caveolin-1 influences vascular protease activity and is a potential stabilizing factor in human atherosclerotic disease. *PLoS ONE*. 2008; 3:e2612. [PubMed: 18596970]
20. Chesler NC, Ku DN, Galis ZS. Transmural pressure induces matrix-degrading activity in porcine arteries ex vivo. *Am J Physiol*. 1999; 277:H2002–9. [PubMed: 10564157]
21. Wang DL, et al. Mechanical strain induces monocyte chemotactic protein-1 gene expression in endothelial cells. Effects of mechanical strain on monocyte adhesion to endothelial cells. *Circ Res*. 1995; 77:294–302. [PubMed: 7614716]
22. Barron V, et al. The effect of physiological cyclic stretch on the cell morphology, cell orientation and protein expression of endothelial cells. *J Mater Sci Mater Med*. 2007; 18:1973–81. [PubMed: 17554597]
23. Yao Q, et al. Alterations of pulse pressure stimulate arterial wall matrix remodeling. *J Biomech Eng*. 2009; 131:101011. [PubMed: 19831481]
24. Lee YU, Luo J, Sprague E, Han HC. Comparison of artery organ culture and co-culture models for studying endothelial cell migration and its effect on smooth muscle cell proliferation and migration. *Ann Biomed Eng*. 2010; 38:801–12. [PubMed: 20033777]
25. Han HC, Ku DN. Contractile responses in arteries subjected to hypertensive pressure in seven-day organ culture. *Ann Biomed Eng*. 2001; 29:467–75. [PubMed: 11459340]
26. Ku DN. Blood flow in arteries. *Ann Rev Fluid Mech*. 1997; 29:399–434.
27. Milner JS, Moore JA, Rutt BK, Steinman DA. Hemodynamics of human carotid artery bifurcations: computational studies with models reconstructed from magnetic resonance imaging of normal subjects. *J Vasc Surg*. 1998; 28:143–56. [PubMed: 9685141]
28. Moore JE Jr. et al. Fluid wall shear stress measurements in a model of the human abdominal aorta: oscillatory behavior and relationship to atherosclerosis. *Atherosclerosis*. 1994; 110:225–40. [PubMed: 7848371]
29. Ku DN, Giddens DP, Zarins CK, Glagov S. Pulsatile flow and atherosclerosis in the human carotid bifurcation. Positive correlation between plaque location and low oscillating shear stress. *Arteriosclerosis*. 1985; 5:293–302. [PubMed: 3994585]
30. Wu SP, et al. Wall shear rates differ between the normal carotid, femoral, and brachial arteries: an in vivo MRI study. *J Magn Reson Imaging*. 2004; 19:188–93. [PubMed: 14745752]
31. Nerem RM, Mosberg AT, Schwerin WD. Transendothelial transport of ¹³¹I-albumin. *Biorheology*. 1976; 13:71–7. [PubMed: 938743]
32. Hayman, D. Biomedical Engineering. University of Texas at San Antonio; San Antonio, TX: 2011. Effect of pulse pressure on arterial wall remodeling.

33. Phelps JE, DePaola N. Spatial variations in endothelial barrier function in disturbed flows in vitro. *Am J Physiol Heart Circ Physiol*. 2000; 278:H469–76. [PubMed: 10666077]
34. Karmakar N. Interaction of transmural pressure and shear stress in the transport of albumin across the rabbit aortic wall. *Atherosclerosis*. 2001; 156:321–7. [PubMed: 11395028]
35. Lee YU, Hayman D, Sprague EA, Han HC. Effects of axial stretch on cell proliferation and intimal thickness in arteries in organ culture. *Cell & Mol Bioeng*. 2010; 3:286–295.
36. Gimona M, Herzog M, Vandekerckhove J, Small JV. Smooth muscle specific expression of calponin. *FEBS Lett*. 1990; 274:159–62. [PubMed: 2253769]
37. van der Loop FT, et al. Differentiation of smooth muscle cells in human blood vessels as defined by smoothelin, a novel marker for the contractile phenotype. *Arterioscler Thromb Vasc Biol*. 1997; 17:665–71. [PubMed: 9108778]
38. Hu JJ, et al. Time courses of growth and remodeling of porcine aortic media during hypertension: a quantitative immunohistochemical examination. *J Histochem Cytochem*. 2008; 56:359–70. [PubMed: 18071063]
39. Shin HY, Bizios R, Gerritsen ME. Cyclic pressure modulates endothelial barrier function. *Endothelium*. 2003; 10:179–87. [PubMed: 13129821]
40. Lindner V, Heinle H. Binding properties of circulating Evans blue in rabbits as determined by disc electrophoresis. *Atherosclerosis*. 1982; 43:417–22. [PubMed: 7115470]
41. Humphrey, JD. *Cardiovascular Solid Mechanics: Cells, Tissues and Organs*. Springer; New York: 2002.
42. Heinle H, Lindner V. The binding of Evans blue to collagen and elastin in elastic tissue. *Arch Int Physiol Biochim*. 1984; 92:13–7. [PubMed: 6207787]
43. Zheng W, Christensen LP, Tomanek RJ. Differential effects of cyclic and static stretch on coronary microvascular endothelial cell receptors and vasculogenic/angiogenic responses. *Am J Physiol Heart Circ Physiol*. 2008; 295:H794–800. [PubMed: 18586890]
44. Shin HY, Gerritsen ME, Bizios R. Regulation of endothelial cell proliferation and apoptosis by cyclic pressure. *Ann Biomed Eng*. 2002; 30:297–304. [PubMed: 12051615]
45. Haga JH, Li YS, Chien S. Molecular basis of the effects of mechanical stretch on vascular smooth muscle cells. *J Biomech*. 2007; 40:947–60. [PubMed: 16867303]
46. Gombocz K, et al. Influence of dextran-70 on systemic inflammatory response and myocardial ischaemia-reperfusion following cardiac operations. *Crit Care*. 2007; 11:R87. [PubMed: 17697359]
47. Schuerholz T, Marx G. Dextran-70 to modulate inflammatory response after cardiopulmonary bypass: potential for a novel approach? *Crit Care*. 2007; 11:163. [PubMed: 17850682]
48. Eberth JF, et al. Time course of carotid artery growth and remodeling in response to altered pulsatility. *Am J Physiol Heart Circ Physiol*. 2010; 299:H1875–83. [PubMed: 20852047]
49. Barber RD, Harmer DW, Coleman RA, Clark BJ. GAPDH as a housekeeping gene: analysis of GAPDH mRNA expression in a panel of 72 human tissues. *Physiol Genomics*. 2005; 21:389–95. [PubMed: 15769908]

Figure 1. Schematic of *ex vivo* organ culture system. The T-end was used to achieve pulsatile and hyper-pulsatile pressure while a pulse dampener dome was used in place of the T-end to achieve non-pulsatile pressure.

Figure 2.

The cumulative vasomotor responses (*top*) and vasomotor tone (*bottom*) for arteries cultured under different pulse pressure for three days. Values are mean±SE, n=8 for all groups.

*p<0.05 vs. pulsatile arteries; +p<0.05 vs. non-pulsatile arteries

Figure 3.

Top: Comparison of EBD permeation distances (mean±SE in mm) in arteries in fresh (n=3), pulsatile (n=5), non-pulsatile (n=5), and hyper-pulsatile (n=5) groups. Bottom: Comparison of the flux (mean±SE in arbitrary unit/mm²/min) across the artery wall of pulsatile (n=5), non-pulsatile (n=4), and hyper-pulsatile (n=7) groups. *p<0.05 vs. pulsatile arteries; +p<0.05 vs. non-pulsatile arteries; # p<0.05 vs. fresh arteries

Figure 4.

Representative images of BrdU stained sections for pulsatile, non-pulsatile, and hyper-pulsatile arteries (Top to bottom). Representative SMC nuclei are indicated with a circle while representative EC nuclei are indicated with an arrow. Bargraph: Comparison of the BrdU indices for the endothelium of arteries cultured under various pulse pressures. * $p < 0.05$ vs. pulsatile arteries

Figure 5.

Representative images of TUNEL stained sections of pulsatile, non-pulsatile, and hyper-pulsatile arteries (Top to bottom). Bargraph: Comparison of the TUNEL indices for the media of arteries (mean±SE, n=4 for each group) cultured under various pulse pressures. *p<0.05 vs. pulsatile arteries

Figure 6. Representative western blots illustrating the levels of (a) MMP-9 and (b) MMP-2 in fresh, pulsatile, non-pulsatile, and hyper-pulsatile arteries. (c) Comparison of the relative intensities of pro and active MMP-2 bands of all groups (n=4 each). *p<0.05 vs. pulsatile arteries

Figure 7. Distribution of CAV-1 in arteries cultured under (a) pulsatile, (b) non-pulsatile, and (c) hyper-pulsatile pressure. CAV-1 is stained green while the cell nuclei are stained red with propidium iodide. (d) A representative negative control image with propidium iodide only is shown.

Figure 8.

Top: The average threshold cycle for MCP-1 is shown for all groups. Bottom: The average threshold cycle for GAPDH is shown for all groups. * $p < 0.05$, # $p < 0.05$ vs. fresh arteries