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2005 AHA Greater Southeast Affiliate Predoctoral Fellowship
Progress Report 2
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Abdominal aortic aneurysms (AAA) are characterized by localized vessel inflammation. This localized vessel disease is likely caused by complex interplay between altered normal vessel mechanics and gene/protein regulation. Our ongoing objective is to define the early molecular and mechanical events that shift a healthy aorta into a dilating, aneurysmal aorta.

Specific Aim 1: Define the fundamental biomechanical changes in the pre-aneurysmal aorta.

The objective of this specific aim is to determine the biomechanical changes of the aorta prior to aneurysm formation. The hypothesis is that alterations of the aorta before aneurysm formation are detectable and quantifiable. The rationale is to incorporate the events leading to full aneurysm formation into a timeline of aortic vessel changes in shape and microstructure. The expectation is that pre-aneurysmal events include 1) a global reduction in aortic vessel compliance and 2) a reorganization of elastin and collagen structure in the matrix.

Whole aorta inflation tests were utilized to measure pressure-diameter relationships, with resulting compliance as the mechanical metric. Our first model of AAA formation included 16 week C57Bl/6 male mice treated with angiotensin II (angII), beta-aminoproprionitrile (bAPN), an inhibitor of lysyl oxidases, and fed high fat chow. This experimental model has 100% occurrence of AAA formation. The second model lacked bAPN, serving as a more physiologic model of AAA formation. For the third model $ApoE^{-/-}$ mice treated with angII and high fat diet were also investigated as a AAA model due to the hyperlipidemia-induced baseline inflammatory state. Aortas were harvested at 3, 6, 9, & 12 days post treatment for inflation. A transmural pressure ramp was applied from 5 to 135 mmHg and the concomitant diameter change was measured at the level of the celiac artery and 7^{th} intercostal arteries. Baseline maximum compliance values of control aortas at the celiac artery and the 7^{th} intercostal artery pair were $5.5 \times 10^{-3} \pm 0.0017$ and $6.2 \times 10^{-3} \pm 0.0021$ mm/mmHg, respectively, corresponding with published compliance measurements in male C57Bl/6 mice. Pressure-diameter relationships revealed significant difference relative to control at 12 days in the C57Bl/6-angII/bAPN model only (Figure 1). However, histological results did reveal microstructural changes in elastin structure and collagen accumulation in all models compared to control (Figure 2, all 12 day timepoints). Because mechanical differences were only detected in the most aggressive model of AAA, the results suggest that early molecular and microstructural events most likely direct mechanical behavior in physiological aneurysm formation.

Next, aortic stretch ratio, as a representation of in vivo axial tension, was measured in the C57Bl/6 models to investigate mechanical behavior in the longitudinal direction. Stretch ratio is the ratio of unloaded in vivo length to unloaded ex vivo length. At 12 days, the stretch ratio of both the angII and angII/bAPN models were reduced (Figure 3), indicating that pre-aneurysmal aortas have reduced unloaded axial tension, and suggesting that mechanical changes manifest first in the axial direction, and subsequently in the circumferential direction. More sophisticated axial testing with a myograph will be performed in Dr. Rudy Gleason's lab at Georgia Tech beginning on

June 27. These tests will determine the loaded (or pressurized) axial behavior, in both the circumferential and longitudinal directions, of the aorta, providing data representative of the in vivo state.

Specific Aim 2: Determine the functional importance of oxidative stress in modulating the biomechanical changes in the aorta that occur prior to aneurysm formation.

Based on previous evidence of increased reactive oxygen species (ROS) production in established aneurysms, we hypothesize that ROS produced by smooth muscle cells in the vessel wall provide a critical signal that potentiates the mechanical changes in the pre-aneurysmal aortic wall. Our expectation is that increased generation of ROS predisposes the vessel to matrix remodeling, which decreases aortic compliance and subsequently promotes AAA formation. Our mouse model for this aim has smooth muscle cell specific over-expression of catalase in an apoE knock-out background. These mice are currently being bred and aged in our laboratory. Breeding has been successful and mechanical studies with these animals will begin in July when the animals are 16 weeks old.

Specific Aim 3 (revised): Determine the mechanical significance of adventitial collagen in AAA formation.

The original objective of this aim was to develop a first-order model to predict aneurysm outcome. The hypothesis was that we can predict the mechanical behavior of pre-aneurysmal aortas with computer-based modeling. However, while performing our compliance and axial testing we have become greatly intrigued with the contribution of the adventitia to the development of AAA (and vascular disease in general). Indeed our preliminary studies utilizing collagenase digestion of adventitial collagen suggest that the adventitia may minimize axial force generation in the axial direction under hydrodynamic pressure. In the experiment protocol, aortas were harvested as usual and subject to compliance and axial testing. At the end of the experiment, the media bath was replaced with collagenase solution (175 U/ml) for 15 minutes. After adventitial collagenase digestion, fresh media was exchanged and the same compliance and axial tests were performed. Figure 4 shows histological staining of a control aorta before and after collagenase digestion. Preliminary pressure versus force behavior of a control aorta (Figure 5, no AAA treatment) before and after collagenase treatment shows that force generation is elevated after collagenase digestion. Controlled studies will be performed along with the circumferential and longitudinal studies outlined in Aim 1.

To update my professional activities, for the third semester I was a teaching assistant for BMED 2210 Conservation Principles in Biomedical Engineering. This past spring semester was my best experience with teaching yet. I had the opportunity to teach 3 course sessions and taught/managed my own weekly recitation session with a smaller group of students. I also presented my current work in a poster session at the 2007 ATVB meeting in Chicago and in an oral presentation at the Annual Emory Cardiology Research Symposium on June 8 this month.

Significant Changes since previous Progress Report:

As discussed in the Progress Report above, we expect that the modeling aim originally outlined in Aim 3 will be replaced with a study to investigate the mechanical contribution of the adventitia to AAA formation. We became very interested in the adventitia this past year and even were invited to write a review on the adventitia in vascular inflammation. This manuscript is currently under final review. We believe that defining adventitial function in AAA development will have an even greater impact in the scope of AAA treatment and vascular disease than the originally proposed model because it may reveal new drug targeting avenues for AAA prevention. It has implications to possibly stabilize AAA dilation from the outside of the vessel. Indeed I am extremely happy to have been awarded the AHA Greater Southeast Affiliate Predoctoral Fellowship for the third year. By June 2008, the end of the three year award period, I expect to have completed all proposed aims.

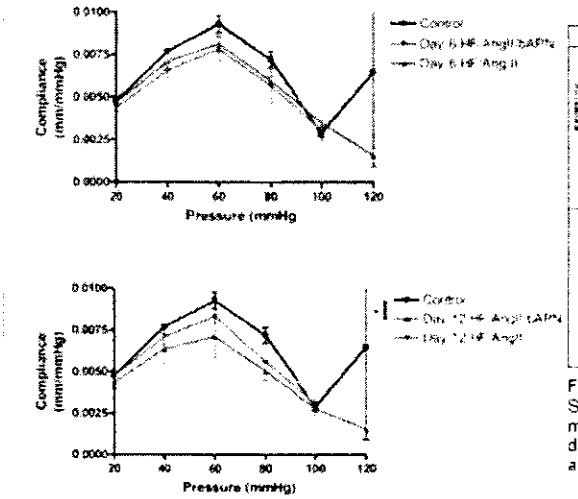


Figure 1 Pressure versus compliance behavior is significantly altered by 12 days of angII-bAPN AAA treatment

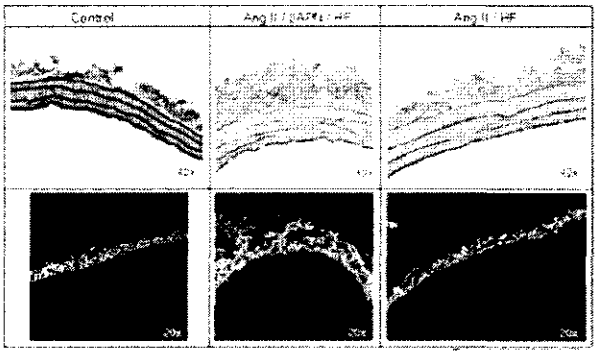


Figure 2 Van Gieson Elastin Stain (top panel) and Picro Sirius Red Staining (bottom panel) of both C57 AAA models. Both models show significant medial elastin degradation while the angII-bAPN model show increased adventitial collagen deposition

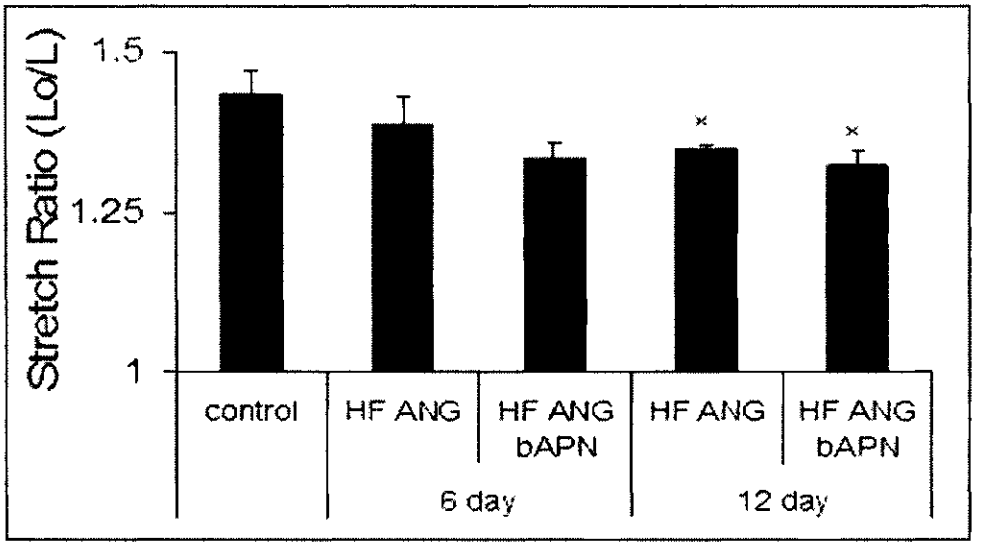
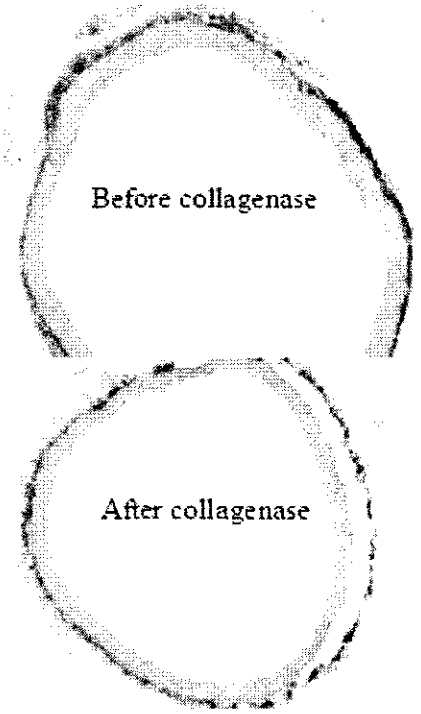


Figure 3 Aortic stretch decreases with treatment and time, indicating that pre-aneurysmal aortas have reduced in vivo axial tension in the unloaded state



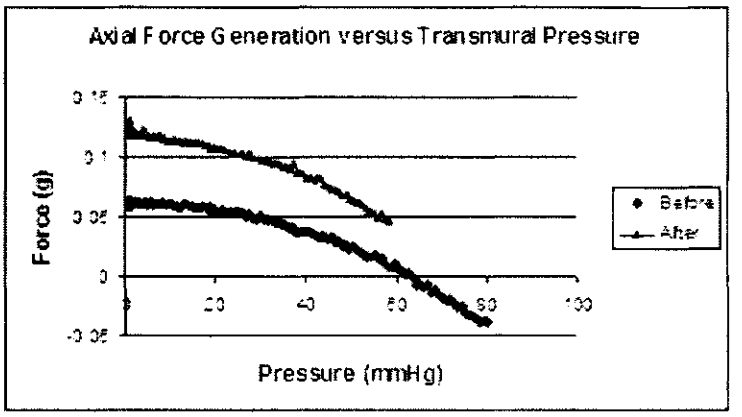


Figure 5. Digestion of the aortic adventitia increases the magnitude of force generated in the longitudinal direction in the loaded state.

Figure 4. Collagen staining of aortic cross sections before and after adventitial collagen digestion with collagenase solution.