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Time Course of Geometrical and Mechanical Changes in a Mouse Model of Marfan Syndrome

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Abstract

Ascending thoracic aortic aneurysm (ATAA) can be asymptomatic for many years, but it can also result in a patient's death if not acted upon early. The cost to surgically act upon an ATAA has a very high risk. Thus, an accurate assessment of ATAA failure risk is a major clinical need. To find evidence of patients with ATAA risks, biomarkers may be used to predict the abnormality. These biomarkers include geometrical, genetic, microstructural, and biofluids information. To obtain this data, experimental and computational models are used. This paper reports experimental data for 3-month-old male and female mutant mice that are a model of ATAA associated with Marfan syndrome and corresponding control wild type mice. The results include the unloaded diameter and lengths of different segments of the aorta, the thickness of the aortic wall, the diameter change of the aorta with pressure, and circumferential stress versus stretch plots. From statistical analysis, we found that ascending and superior abdominal aorta are significantly different according to genotype and by sex, for the diameter increase due to pressure. However, further experiments are required to statistically analyze the descending and inferior abdominal aorta. Lastly, the stretch ratio vs. stress graphs indicates trends of a higher Young's Modulus in mutant mice with Marfan syndrome than wild type mice. This suggests that the stresses experienced by the aorta in the mutant mice are higher than wild type mice.

Introduction

Ascending thoracic aortic aneurysm (ATAA) is a major cardiovascular health problem characterized by a dilated aorta that may eventually fail through dissection or rupture. ATAA has an incidence of about 1 per 10,000 people [1], rupture incidence of roughly 3.5 per 100,000 person-years [2], and mortality rates near 70% if rupture occurs [3]. ATAA is associated with Marfan Syndrome (MFS), caused by mutations in fibrillin-1 [4,5], an extracellular matrix protein necessary for elastic fiber assembly in the large arteries [6]. ATAA repair surgery is difficult and dangerous and up to 60% of ATAA dissections occur before the surgical criteria are reached [7], indicating that the current guidelines are inadequate.

There are two main extracellular matrix (ECM) proteins in the aorta, elastin and collagen. Elastin allows the large arteries to reversibly expand and relax with every cardiac cycle [8]. Elastin is the major protein that imparts the property of elasticity to tissues. Elastin is a cross-linked polymer that is part of the elastic fibers. This structure is assembled outside of the cells and is linked to many other ECM proteins [9]. Elastic fibers mainly contribute in the low-stress region of aortic loading.

Elastin is expressed at high levels during development, but low levels later in life. With a lifetime of loading and increased loading with high blood pressure, the elastin starts to plastically deform. This explains why repair of elastic fibers is incomplete in the adult period and why the elastin protein must have a long half-life [10, 11].

Collagen fibers are initially in a crimped state until the elastin fibers are stretched to the max. Once the collagen fibers start to uncrimp they are the more dominant fiber in the aorta and contribute to the high stress region of aortic loading. Collagen contains 1–4 cross-links per collagen unit, and elastin contains 15–20. This high degree of cross-linking is important for elastin's recoil properties because it is more elastic than collagen and contributes to its longevity [11]. From measuring circumferential stress and strain we can analyze the Young's Modulus. This is a measure of the elasticity of a vessel from a ratio of stress to its strain.

Mouse models of ATAA can be used to understand how the wall structure and mechanics, including the organization and contributions of elastin and collagen, change with disease progression. They can also be used to identify geometrical, genetic, microstructural, and biofluids biomarkers of disease outcomes. As a first step toward identifying biomarkers, we used mutant mice with low levels of fibrillin-1 expression that get ATAAs associated with Marfan Syndrome. We removed the entire aorta and measured the circumferential mechanical behavior of different aortic segments. We also measured the unloaded dimensions to calculate stress and stretch ratios. Male and female mice were used and preliminary analyses examined differences between the genotypes and sexes.

Methods

3-month-old male and female mice were used. They were genotyped by PCR to determine the presence or absence of the mutation that reduces fibrillin-1 levels. The mice were euthanized by carbon dioxide inhalation in compliance with the Institutional Animal Care and Use Committee.

The entire aorta was dissected to perform mechanical testing and was separated into 4 different sections, the ascending, descending, superior abdominal, and inferior abdominal. These vessels were then mounted onto the myograph and mechanical tests were performed.

The mechanical testing consisted of six protocols, which started with three inflation cycles from 0 to 150 mmHg in increments of 15 mmHg for 10 s/step at three different constant axial stretch ratios and then three axial stretch cycles at approximately 20 μm/s at three different constant pressures of 50, 100, and 150 mmHg. From these mechanical tests the lumen pressure, loaded outer diameter, and axial force was recorded. The axial stretch was calculated from the starting and end stretch ratios, assuming a constant stretch rate. After collecting the data from the mechanical tests, the vessels were removed from the myograph and 200–300 μm thick rings were cut and imaged. From ImageJ software, we measured the inner and outer diameter of the rings by tracing the boundaries and forming ellipses to find the average diameters. The unloaded dimensions were also recorded because are necessary to determine the stretch and stress on the wall in the circumferential, radial, and axial directions. Only the first protocol was used for the pressure-diameter comparisons.

To analyze circumferential stretch ratios vs stress, mathematical calculations are performed, and the results are for the first protocol only [12]. From the data collected, the third loading cycle was used for analysis. Compliance was calculated from the average change in diameter per 15 mmHg pressure step. The stretch ratios (λ) in each direction (λ =circumferential, z = axial, and r = radial),

$$\lambda_\theta = \frac{1}{2}\left(\frac{d_i}{D_i} + \frac{d_o}{D_o}\right), \lambda_z = \frac{1}{L}, \lambda_r = \frac{1}{\lambda_\theta \lambda_z}$$

where d_i was calculated from the deformed and undeformed dimensions by conservation of volume, and the mean stresses (σ) in the circumferential and axial directions were determined assuming an incompressible cylinder with no shear,

$$\sigma_\theta = \frac{Pd_i}{d_o - d_i}, \sigma_z = \frac{4f + Pd_i^2}{\pi(d_o - d_i)}.$$

Results and Discussion

Unloaded Diameter and Thickness

Here the comparison between arteries, genotypes, and sexes is shown. Figure 1 shows the unloaded diameter of the aortic segments for 3-month-old male and female, mutant and wild type mice.
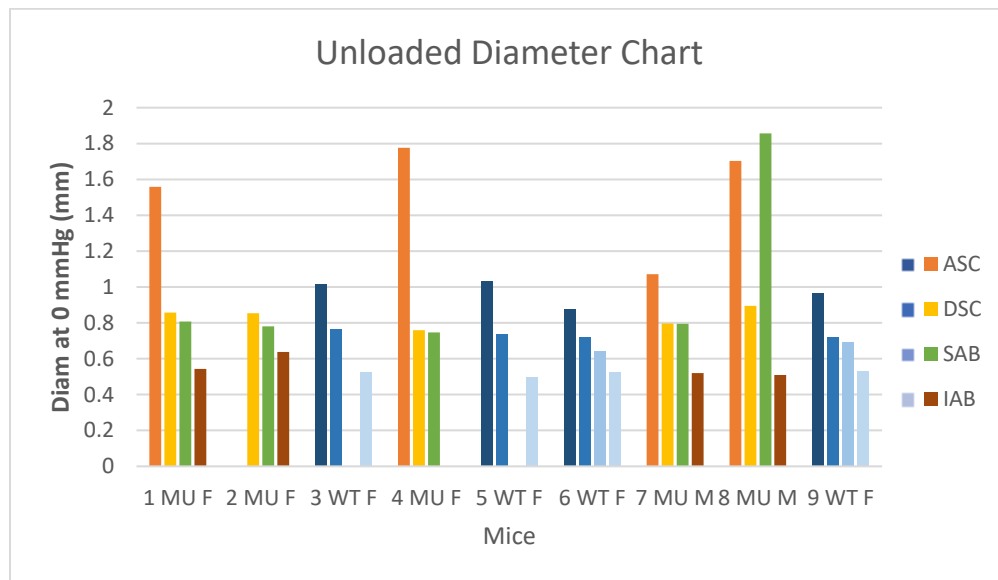

Figure 1 – Comparison of unloaded diameters for 3-month-old mice. Where MU is for mutants, WT is for wild type, M/F (Male/Female) represents sex, ASC (Ascending), DSC (Descending), SAB (Superior Abdominal), and IAB (Inferior Abdominal). Mice are separated by number.

The ascending segment of the aorta has the largest unloaded diameter for all genotypes and sexes. However, in mouse 8, the male mutant mouse has an elevated superior diameter due to an aneurysm, which made it larger than all the other vessels.

Comparing the wild-type mice to the mutants, the ascending, descending, superior, and inferior sections of the mutant aorta are larger than the wild-type mice. This suggests that aneurysms are already

apparent at 3 months of age and that diameter dilation is occurring in all aortic segments, although most notably in the ascending aorta.

Figure 2 shows the thickness of each aortic segment. On average, the thicknesses of the vessels are similar and show minimal differences.
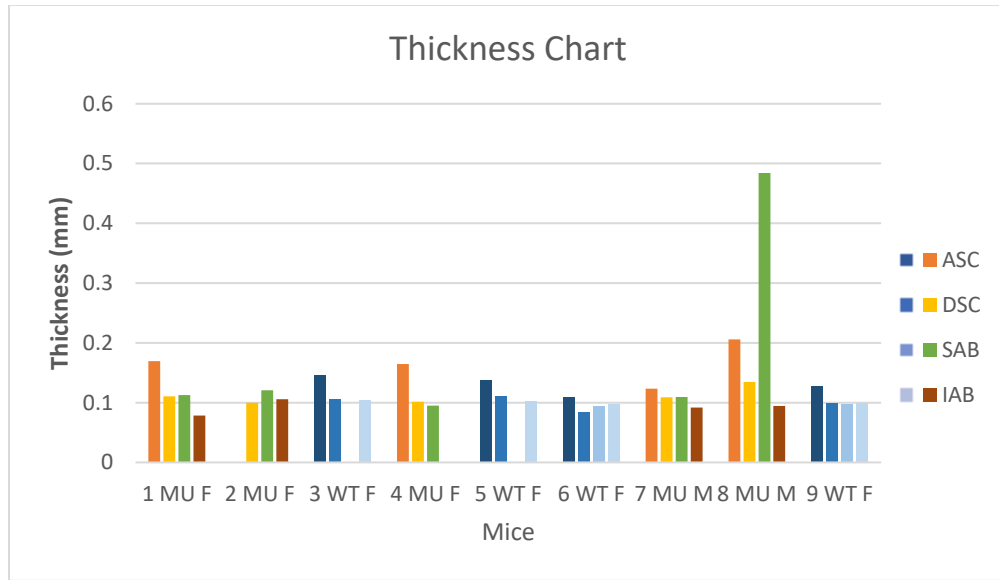

Figure 2 – Comparison of vessel thickness for 3-month-old mice. Where MU is for mutants, WT is for wild type, M/F (Male/Female) represents sex, ASC (Ascending), DSC (Descending), SAB (Superior Abdominal), and IAB (Inferior Abdominal). Mice are separated by number.

However, in mouse 8 the thickness drastically increases in the ascending and superior abdominal vessel. This is due to an aneurysm in the aorta of that section. This aneurysm can be seen in Figure 3, which compares the superior abdominal section of mouse 8 to a normal vessel at the same zoom.
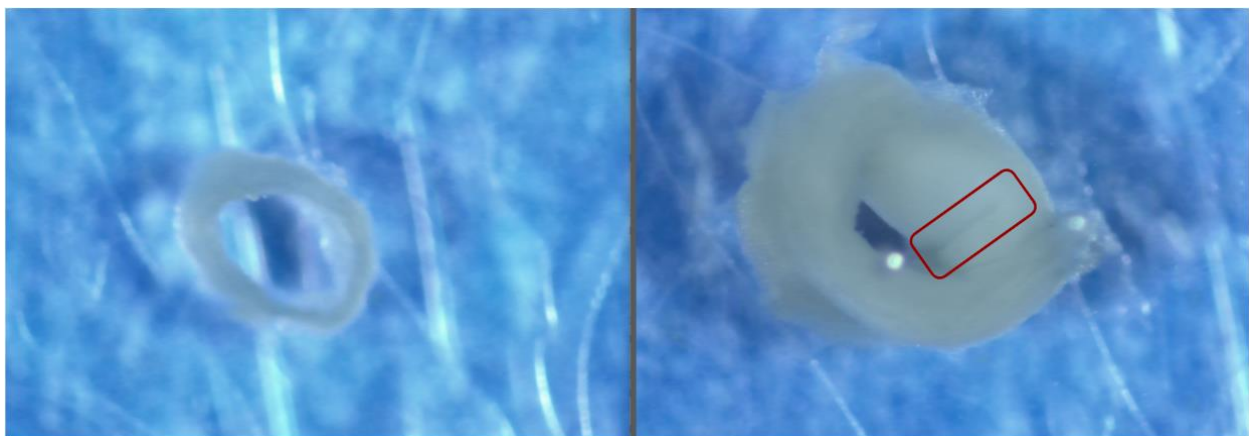

Figure 3 – Normal SAB vessel thickness (Right) vs. SAB from mouse 8 experiencing aneurysm (Left)

There is clearly an increase in thickness in the SAB vessel from mouse 8 in Figure 3. The circled red portion in the figure shows how the vessel has expanded and started to fold over itself showing interesting mechanical remodeling in response to the aneurysm.

Pressure vs. Diameter

During the mechanical tests, the vessels were inflated to 150 mmHg, and their average diameter increase can be seen in Figures 4, 5, and 6. These plots are nonlinear because elastin is dominant at low pressures, then collagen starts to uncrimp and expand, and then becomes dominant at higher pressures. This can be observed as an inflection point in the curves. Using t-tests we can determine whether genotype or sex have significant effects on the diameter at each pressure.
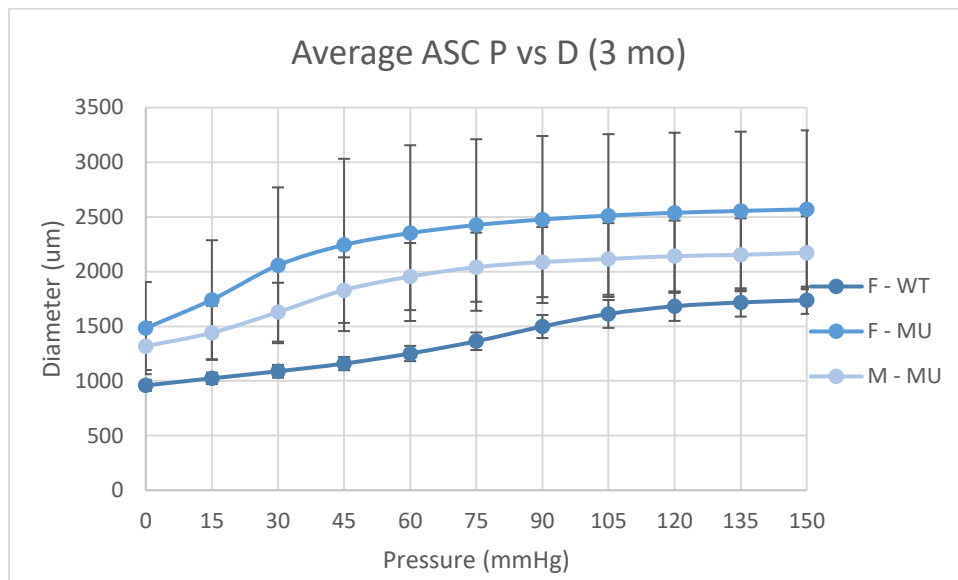

Figure 4 – Diameter change of ascending section due to inflation. Where MU is for mutants, WT is for wild type, and M/F (Male/Female) represents sex.

The ascending wild type mice vessels show a small linear increase in diameter as pressure rises (Figure 4). This indicates that the elastin fibers dominate up to higher pressure values than the mutant mice. The wild type data is linear until about 90 mmHg, while the mutant data is linear until about 30 mmHg. These inflection points show whether elastin or collagen is more dominant.

Rough comparison of the curves suggests that since the standard deviation bars on the mutant male and female mice cross, then sex does not have a significant effect. However, a calculated p-value of 0.017 is less than 0.05 provides significant evidence to suggest that the sex has effect on the pressure vs. diameter of the ascending aorta for 3 month mutated mice. For statistical significance of whether sex has effect, further experimental analysis is required. Also, from comparing the female wild type mice to mutant mice a p-value of 3.19E-06, which is much less than 0.05, suggests that there is significant evidence to prove that genotype does have effect on the ascending aorta for 3 month mice.
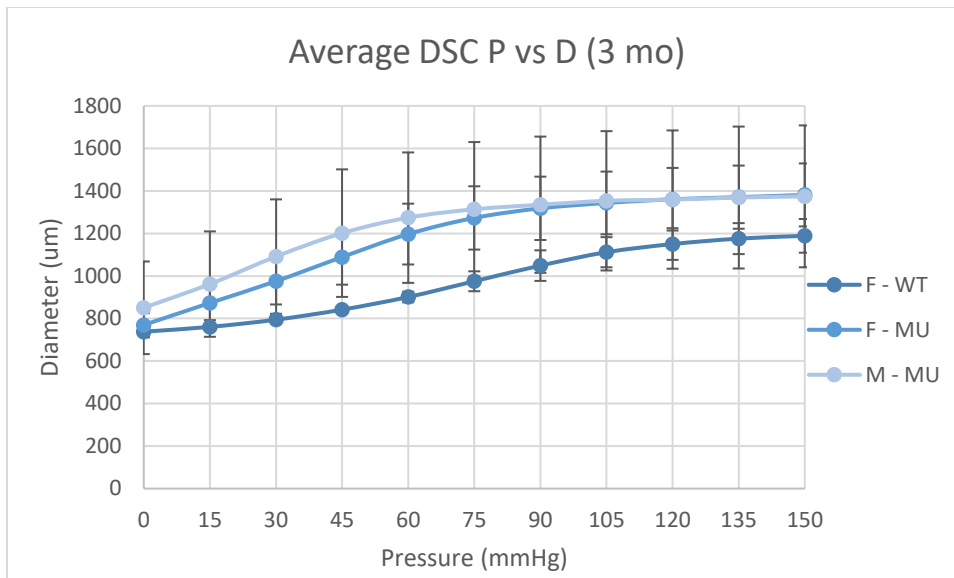
Figure 5 – Diameter change of descending section due to inflation. Where MU is for mutants, WT is for wild type, and M/F (Male/Female) represents sex.

This trend is similar for the descending aorta (Figure 5). However, unlike the ascending section, the male and female mutated mice are consistent with each other. The elastin in the wild-type mice is acting until an inflection point at about 75 mmHg of pressure. The mutated mice show dominant elastin fibers until an inflection point of about 45 mmHg. Statistical analysis shows that since the standard deviation bars on the mutant male and female mice cross, they are independent of each other, and their sex does not have much effect. Also, a calculated p-value of 0.57 is more than 0.05 providing insignificant evidence to suggest that the sex does not influence the descending aorta for 3-month mutated mice.

When comparing the female wild type mice to mutant mice a p-value of 0.023, which is less than 0.05, suggests that there is significant evidence to prove that genotype does affect the descending aorta for 3-month mice.
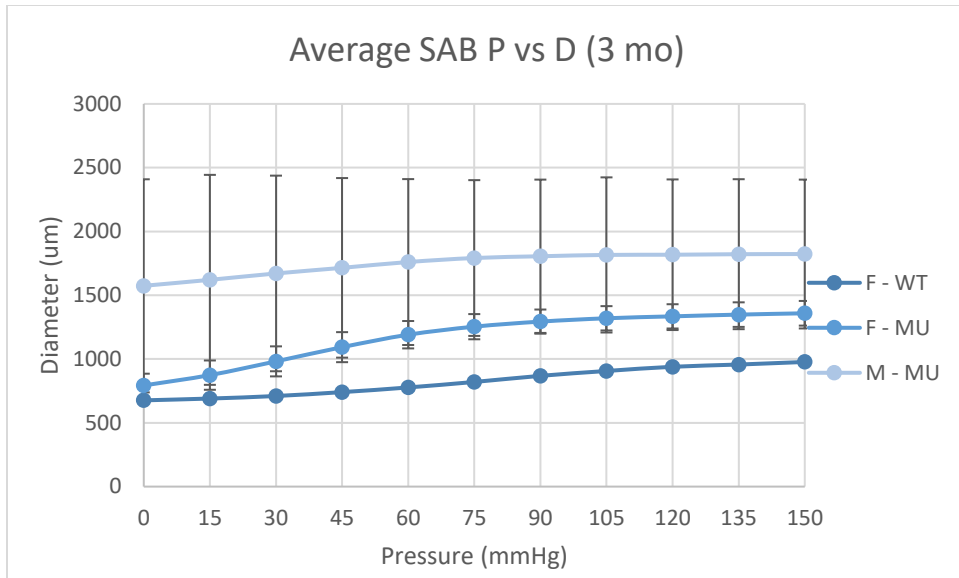
Figure 6 – Diameter change of superior abdominal section due to inflation. Where MU is for mutants, WT is for wild type, and M/F (Male/Female) represents sex.

In Figure 6, of the superior abdominal vessel, the wild-type mice have the smallest diameter. This is consistent with the other vessel sections. However, it is difficult to analyze at what pressure the collagen starts to uncrimp and becomes the dominating fiber. Statistical analysis shows that since the standard deviation bars on the mutant male and female mice cross, they are independent of each other, and their sex does not have much effect. However, a calculated p-value of 3.2E-08 is less than 0.05 providing significant evidence to suggest that the sex does have an effect on the superior abdominal aorta for 3-month mutated mice. From comparing the female wild type mice to mutant mice a p-value of 8.22E-08, which is much less than 0.05, suggests that there is significant evidence to prove that genotype does have an effect on the ascending aorta for 3-month mice.
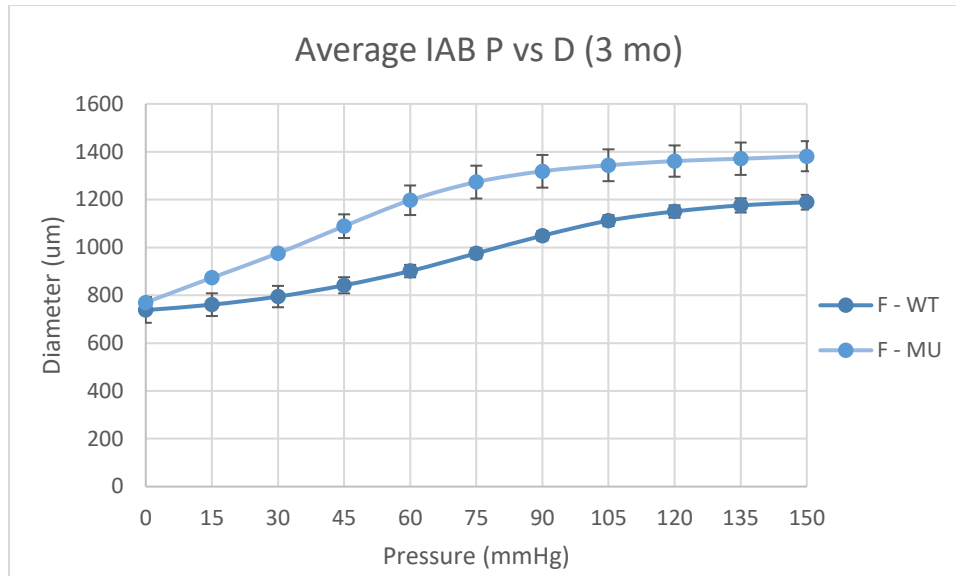
Figure 7 – Diameter change of inferior abdominal section due to inflation. Where MU is for mutants, WT is for wild type, and M/F (Male/Female) represents sex.

Next, in Figure 7, the inferior abdominal section of the aorta has consistent data with the other pressure versus diameter findings, that the overall diameter of the wild-type mice is smaller than the mutated mice when its pressure increases. Also, the elastin fibers in the wild-type mice show a higher dominance in pressure than the mutated mice. Overall, the mutated mice consistently show a larger diameter when pressure increases and a lower dominance of elastin fibers as pressure increases. There is not enough data to determine if sex has an effect on the diameter of the inferior abdominal vessel. However, from comparing the female wild type mice to mutant mice a p-value of 0.008, which is less than 0.05, suggests that there is significant evidence to show that genotype does have effect on the ascending aorta for 3-month mice.

Stretch VS Stress

The stretch versus stress charts shown below in Figures 8, 9, 10, and 11 show the average stress experienced by the mice compared to their stretch ratio. Material stiffness is an indicator of how stress increases in response to increases in strain [13]. The Youngs Modulus of the vessels increases proportionally to the slope. Also, when the sloping trend increases noticeably, the collagen uncrimp having an increase in stress acting on the cell walls. This is due to the quality of the elastin fibers being more elastic with a larger amount of cross links. Statistical analysis cannot be performed on this data due to parameter differences in the x and y coordinates. However, from analyzing the curve trends broad analysis of stretch vs stress ratios can be observed.
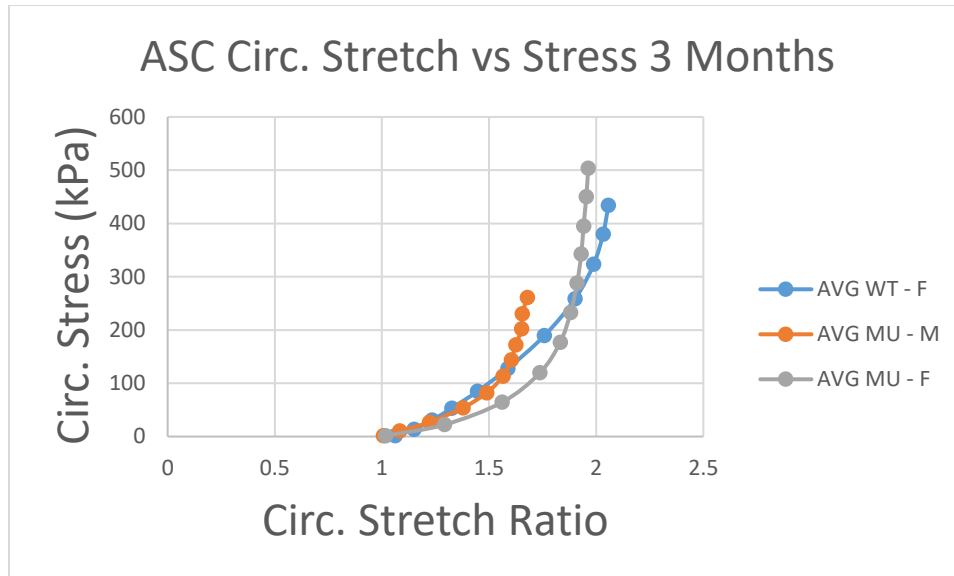
ASC Circ. Stretch vs Stress 3 Months

Figure 8 – Stretch versus stress of ascending aorta. Where MU is for mutants, WT is for wild type, and M/F (Male/Female) represents sex.

The data of the ascending aorta in Figure 8 plots the average circumferential stretch vs stress ratio of the mutant and wild type female mice to be larger than the male mutant mice. However, a large slope increase can be seen in the mutant mice for males starting at a stretch ratio of about 1.6 for male and 1.8 for female mice.

One of the main functions of the proximal aorta is to store energy elastically during systole and to use this energy to work on the blood during diastole [14,15]. Fibrillin- 1 deficient aortas experience a loss in their ability to store this energy. This is most commonly due to hypertension where, in mice with Marfan syndrome, the elastic fibers are impaired and break down faster [16]. Thus, increase in slope represents the elastin fiber's dominance to shift over to collagen fibers in the mutated mice more quickly than wild type mice (Figures 8, 9, 10, 11).
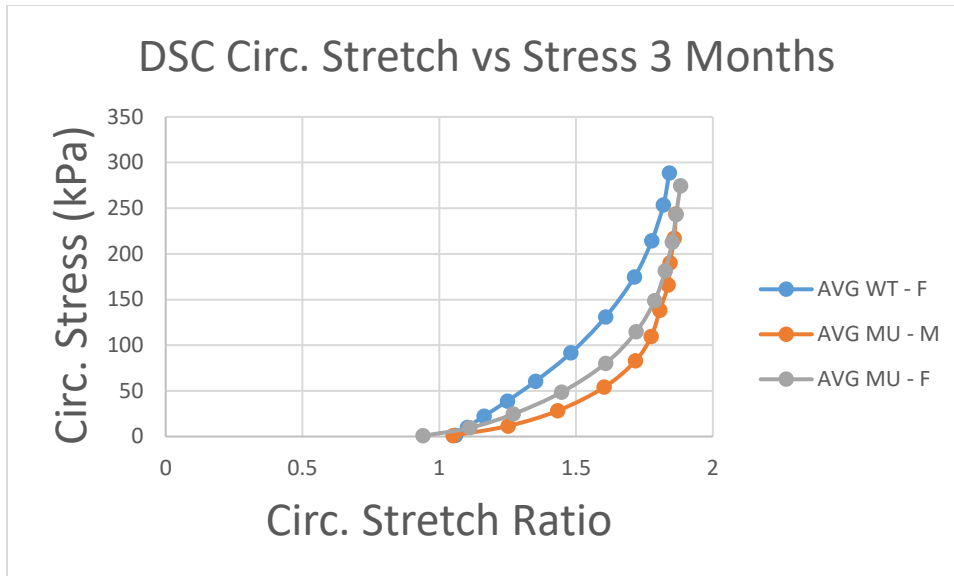
Figure 9 – Stretch versus stress of descending aorta. Where MU is for mutants, WT is for wild type, and M/F (Male/Female) represents sex.

The graph of descending aorta stretch vs. stress ratio indicates that the mutant mice of both sex types have a large linear increase after a circumferential stretch ratio of about 1.75. The slope is larger after this point, also indicating a larger Young's Modulus and higher stress experience.
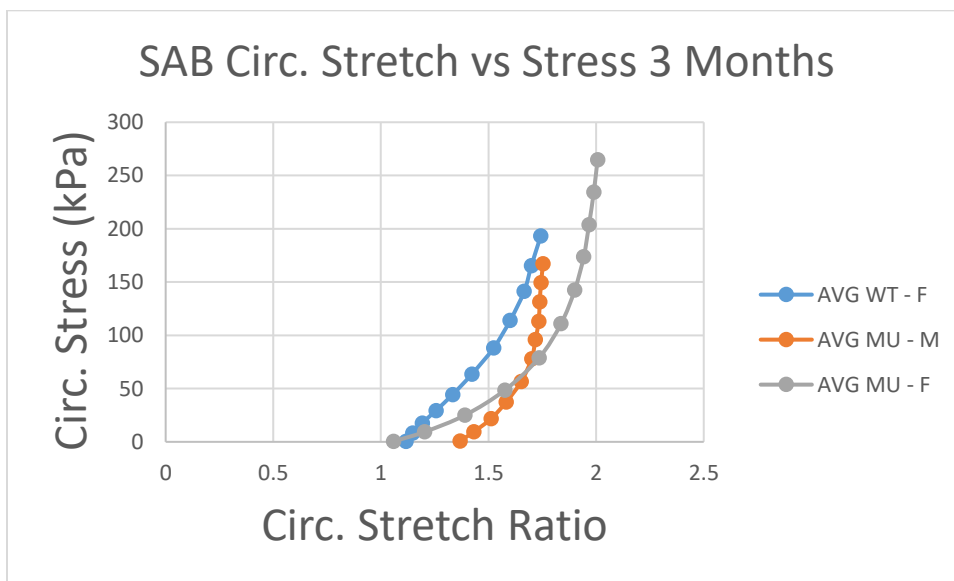

Figure 10 – Stretch versus stress of superior abdominal aorta. Where MU is for mutants, WT is for wild type, and M/F (Male/Female) represents sex.

In Figure 10, the graph of SAB stretch vs. stress ratio indicates that the mutant male mice have a linear increase after a stretch ratio of about 1.75. In comparison, the mutant female mice increase their slope slightly after. This graph indicates that the mutant male mice have the largest Young's Modulus once their elastin fiber is stretched out.
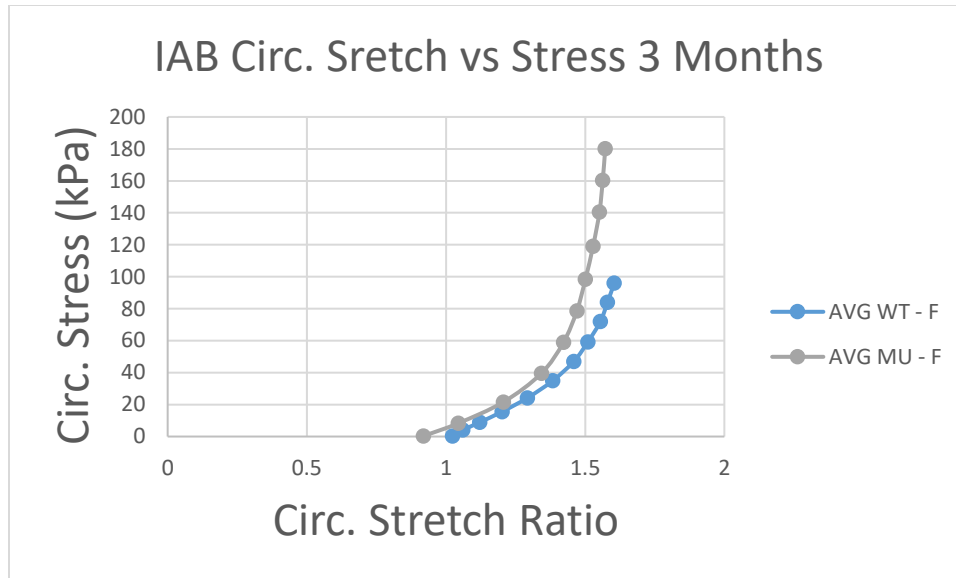
Figure 11 – Stretch versus stress of inferior abdominal aorta. Where MU is for mutants, WT is for wild type, and M/F (Male/Female) represents sex.

Finally, the graph of the inferior abdominal aorta stretch vs stress ratio indicates a change in the trends seen in the other sections of the aorta. Here the mutant female mice experience more stress at a lower stress ratio. However, it is still consistent that the slope of the mutant mice increases at a higher rate than the wild-type mice. To perform statistical analysis, further computational modeling is needed. From this modeling, Young's Modulus will be analyzed and compared between the mice.

The results we found were as expected because, from previous studies, computational growth and remodeling models show that aneurysmal expansion can proceed from highly local losses of elastic fiber integrity and that the aneurysmal wall tends to become circumferentially stiffer due to a turnover of collagen having a preferred orientation toward circumferential [17]. Also, previous studies show that the turnover of collagen will exacerbate the increase in stiffness, not initiate it, even if this is a failed attempt by the cells to limit the expansion [13]. This is consistent with our findings of mice with Marfan syndrome experiencing an increased amount of stress.

Conclusion

In summary, ATAA analysis can be experimentally found through measured circumferential mechanical behavior of different aortic segments and unloaded dimensions to calculate stress and stretch ratios. We found that for the entire aorta, there is significant evidence that genotype does have an effect on the vessel's mechanics and its material properties. Also, we found that for the ascending, superior abdominal, and inferior abdominal segments, the sex of the mice does have an effect on the aorta. However, for the descending segment, there is not enough statistically significant evidence to show that sex has an effect on the aorta. To gain statistical significance on the effect of sex, more experimental analysis is required. Further analysis shows that smaller vessels like the superior and inferior abdominal have less elastin, and it is difficult to analyze when collagen starts to uncrimp. To further analyze the statistics on the stretch vs. stress curves, computational modeling is necessary to compare nonlinear,

anisotropic material properties to get an accurate assessment. This explains that mutations in genotypes and the sex of mice has an effect on the failure risk of the aorta because we found that mutant mice experience more stress and have a larger diameter than wild type mice without Marfan syndrome. The next steps in these studies involve finding constitutive models to create multiphysics models and link disease progression in mice to humans. From this, we will be able to extrapolate how human aorta mechanics will change over time to understand ATAA's and stop them from happening before it causes problems to the patient.

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