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THE EFFECT OF ENDOTHELIAL DYSFUNCTION ON AORTIC MECHANICS AND EXTRACELLULAR MATRIX MICROSTRUCTURE DURING AGE-RELATED VASCULAR REMODELING

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INTRODUCTION

The endothelium, comprised of a single layer of endothelial cells, is the innermost lining of blood vessels providing the crucial interface between blood and the arterial wall. "Endothelial dysfunction," characterized as a reduction in the bioavailability of the major vasodilator nitric oxide (NO), is associated with abnormal vascular remodeling and correlated with cardiovascular pathologies such as hypertension and atherosclerosis. [1]

Based on previous studies, endothelial dysfunction can lead to arterial wall thickening and arterial stiffening, which involves changes in extracellular matrix (ECM) structure or composition and, consequently, alterations in the passive mechanical properties.[2] [3] Additionally, acute NO deficiency leads to increased contractile tone; but long-term deficiency may reduce the overall contribution of the smooth muscle cells (SMCs) to the mechanical environment. The microstructure of the ECM, especially collagen fiber organization, plays an essential role in aortic mechanics. However, very few studies have been performed on the impact that endothelial dysfunction has on the microstructure of the ECM, especially collagen fiber organization, during age-related vascular remodeling that could be used to decipher changes in macroscopic mechanical properties. Thus, a mouse model of direct genetic deletion of endothelial nitric oxide synthase (NOS3) was used in this project to manifest endothelial dysfunction to study the alterations of collagen fiber organization as well as changes in active and passive biomechanics over the time course of vascular remodeling.

METHODS

We used groups of endothelial NO synthase (NOS3) knockout (KO), NOS3 heterozygous (Het), and wild type (WT) B6 mice (controls) to study the time course of vascular remodeling between 6 wks to 12 mo. Multiphoton second harmonic generation (SHG) microscopy was used to image collagen fibers through the wall of the descending thoracic aorta (DTAo). The axial (0°) direction was defined by the average orientation of endothelial cell nuclei; the corresponding perpendicular direction was defined as circumferential (90°). An image-processing protocol was then developed to reconstruct collagen fibers in 3D space. Reconstructed fibers were classified into three fiber families, with one axially-oriented, one circumferentially-oriented, and helically-oriented families (axial= 0-22.5° and 157.5-180°, circumferential= 67.5-112.5°; helical= 22.5-67.5° and 112.5-157.5°) using a custom MATLAB code. The mean normalized frequency for each fiber family and average undulation associated with the family were calculated.

Active and passive mechanical tests of DTAo taken from mice of each genotype were conducted on a customized murine artery inflation-extension testing apparatus. For active tests, maximal contraction of SMCs was achieved by adding phenylephrine into the bathing medium (Krebs solution) to achieve a final concentration of $2x10^{-5}$ M. The medium was temperature-controlled within 37-38°C and continuously aerated

with 95% O_2/5% CO_2. Arteries were then preconditioned with 5 inflation/deflation cycles to minimize viscoelastic effects. For data acquisition, each DTAo sample was extended and held at three different axial stretch ratios which are 1.5, 1.6 (corresponding to the in-vivo stretch ratio), and 1.7, respectively. At each stretch ratio, the arterial sample was pressurized from 10 to 160mmHg in increments of 10 mmHg using in 3 consecutive quasi-static inflation cycles with the axial force, luminal pressure, and axial stretch recorded. The passive mechanical response was assessed by exchanging the perfusion and test chamber medium with phosphate buffered saline (PBS) containing 10^{-5} M sodium nitroprusside (SNP) to fully relax the SMCs. Passive mechanical preconditioning and inflation-extension testing were performed in an identical manner to that described above.

RESULTS

As shown in Figure 1 (a.), there is a slight, non-significant increase in the relative number of circumferentially oriented fibers (p value = 0.116) and a similarly small decrease in axially oriented fibers with age in WT mice (p=0.128). There are no obvious trends in heterozygotes (b.), while in KO (c.) there is a significant increase in the number of circumferentially oriented fibers with age (p=0.035). The trend in KO mice indicates that more dramatic collagen fiber reorganization occurs with endothelial dysfunction during age-related vascular remodeling.

The active and passive mechanical responses of different genotypes at 12 mo are compared in Figure 2. In both (a.) and (b.), the mechanical responses of DTAo from all genotypes exhibit J shaped stress-stretch behavior in the circumferential direction. In (a.), the active mechanical response shows slight difference between genotypes, while in (b.) there is very little variation among genotypes under SMC relaxed state. Based on (c.), the SMC active stress generation in circumferential direction at common circumferential stretch 1.6 in KO is lower than both WT and Het which suggests diminished SMC contraction in KO at 12mo. In addition, corresponding lumen area compliance at a common loading condition (100 mmHg) of KO vessels is lower than WT and Het under SMC relaxed states in (d.), indicating increased aortic stiffness in KO for passive mechanical property. However, the sample size is only two, so more data is required to determine whether these differences are statistically significant.

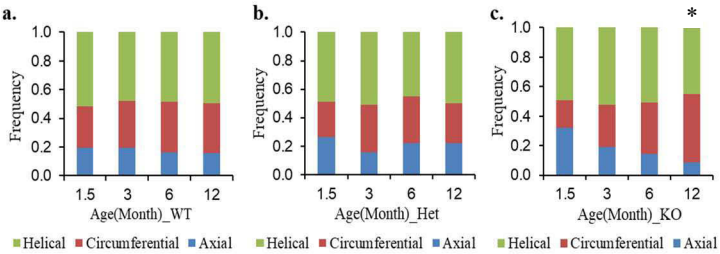

Fig 1. Changes in distribution of collagen fiber families with age in each genotype. WT mice shown in (a.), Het in (b.) and KO in (c.). The mean frequency of three fiber families is represented using different colored bars in the graph. N=3-6 for each group. * indicates significant difference (p<0.05) between age groups in KO mice.

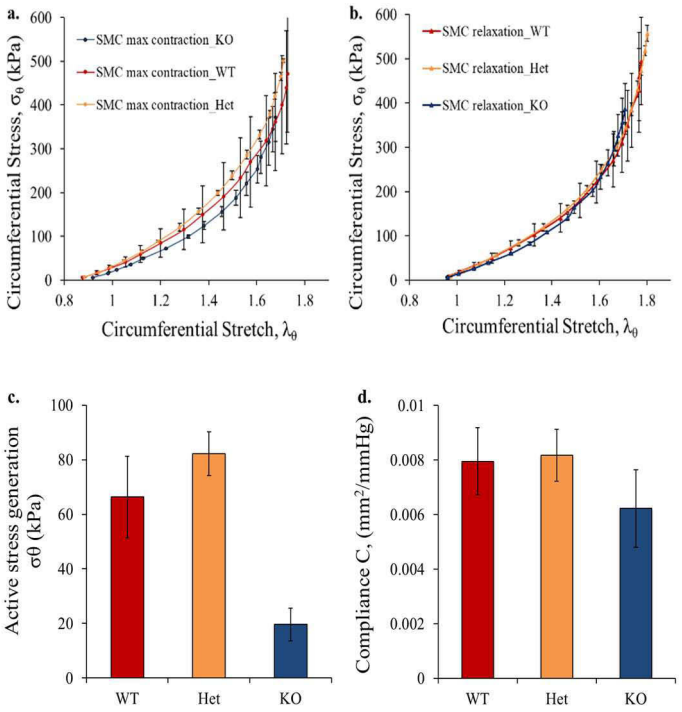

Fig 2. Comparison of circumferential stress-stretch relationships at in-vivo axial stretch, 1.6, for different genotype groups (n=2) at 12 mo under SMC maximally contracted state in (a.) and under SMC relaxed state in (b.). Corresponding SMC active stress generation at common circumferential stretch λθ=1.6 in (c.). Lumen area compliance (C, mm²/mmHg) at common loading condition (100 mmHg) under SMC relaxed state in (d.)

DISCUSSION

Preliminary results demonstrate that there is a trend in collagen fibers shifting towards circumferential from axial orientation with age in KO mice compared to the other two genotypes. However, the sample size for active and passive mechanical study is currently too small, so the effect of endothelial dysfunction on mechanical properties is still uncertain. Thus, active and passive biaxial mechanical tests of all genotypes will be completed in the future to compare mechanical properties between genotypes and time points.

ACKNOWLEDGEMENTS

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