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AGE-RELATED COLLAGEN REMODELING OCCURS IN THE ABSENCE OF HYPERTENSION IN AORTAS OF NOS3 HETEROZYGOUS MICE

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INTRODUCTION

The endothelium, composed of a single layer of endothelial cells, is the innermost lining of vessels, acting as the interface between blood and the arterial wall. "Endothelial dysfunction" is defined as reduction or loss of bioavailability of endothelial-derived nitric oxide (NO), a condition that precedes or accompanies several cardiovascular pathologies associated with aging, such as atherosclerosis. [1] [2]

NO plays an important role in regulating vascular tone and maintaining vascular homeostasis as a vasodilator. Thus, we hypothesize that decreased NO production may induce collagen fiber reorientation and increased collagen production, to shift load from smooth muscle cells to the extracellular matrix, eventually leading to vascular remodeling. The aim of this project is to study the impact of NO deficiency on hemodynamic parameters, collagen content, and collagen fiber orientation during age-related vascular remodeling using a mouse model.

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. In-vivo hemodynamic factors including blood pressure (BP) and volumetric flow rate were tracked at each time point. Diameter and blood velocity in mouse descending thoracic aorta (DTAo) and abdominal aorta (AAo) were measured by ultrasound to obtain volumetric flow rates. Mouse BP was monitored by tail-cuff plethysmography. We used multiphoton second harmonic generation (SHG) microscopy to image collagen fibers through the wall thickness of DTAo and AAo. The axial (90°) direction was defined by the average orientation of endothelial cell nuclei; the corresponding perpendicular direction was defined as circumferential (0°). An image-processing protocol was then developed to reconstruct collagen fibers in 3D space. Reconstructed fibers were used to obtain fiber undulation and fiber angle distribution. In addition, picrosirius red staining was performed on sections of DTAo and AAo. Histological images were taken using brightfield microscopy (20X) to measure total vessel wall area and cross-polarized transmitted light (20X) to exploit the collagen birefringence to quantify the fractional collagen content in these two regions.

RESULTS

As shown in Figure 1, both systolic and diastolic blood pressure in NOS3 KO mice are significantly higher than in either Het or WT mice beginning at 3 months of age, whereas there is no significant difference between Het and WT mice. BP significantly increases with time in NOS3 KO mice, while there are no significant differences with age in Het or WT mice.

Representative SHG images of DTAo are illustrated in Figure 2a. and 2b.; in 2b., the reference directions based on averaged orientation of endothelial cell nuclei were used in the following collagen fiber angle measurements. In Figure 2c, preliminary data of relative collagen fiber angle in the adventitia of DTAo in Het mice shows a shift from more axially oriented

fibers towards more circumferentially oriented fibers in the θ-Z plane with age (from 6wks to 6mo). Representative histology of DTAo at 6wks, 3mo and 6mo in Het mice is compared in Figures 3a. and 3b. As shown in Figure 3c., the fractional collagen content of corresponding samples shows a significant increase with age. These results suggest that NO deficiency could lead to vascular remodeling associated with collagen fiber reorientation and increased collagen production, even in the absence of hypertension.

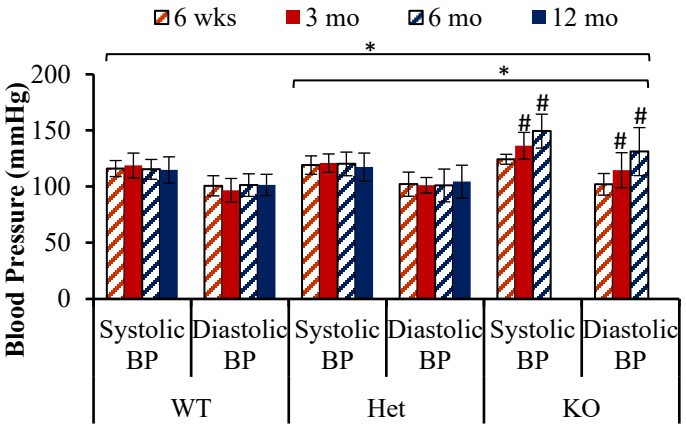

Fig. 1. Blood pressure (systolic and diastolic BP), N=10 (5 male, 5 female) for each genotype. # indicates significant difference vs 6 wks BP in KO group, * indicates significant difference between genotypes.

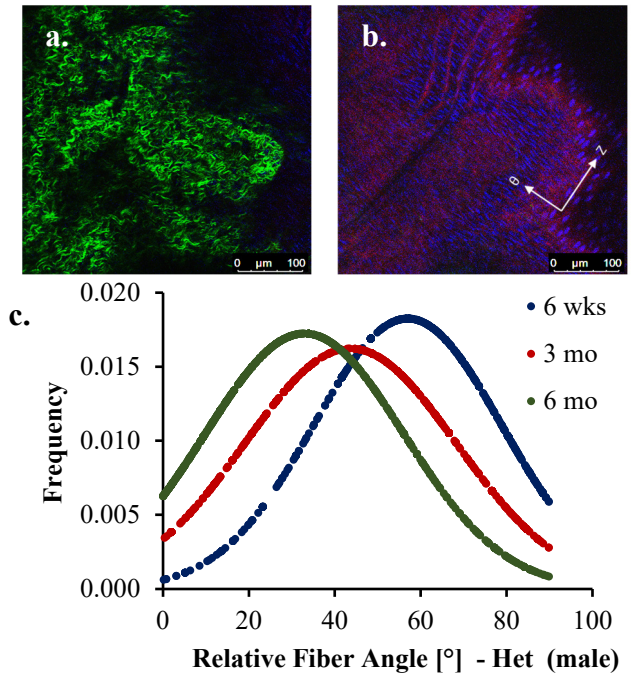

Fig. 2. Representative MP-SHG images of DTAo (a.) Green represents the collagen fibers in adventitia under SHG excitation (b.) Blue shows nuclei of endothelial cells and smooth muscle cells and red shows elastin in media. (c.) Collagen fiber angle distribution in adventitia of DTAo for different age groups in Het male mice, N=1 for each group.

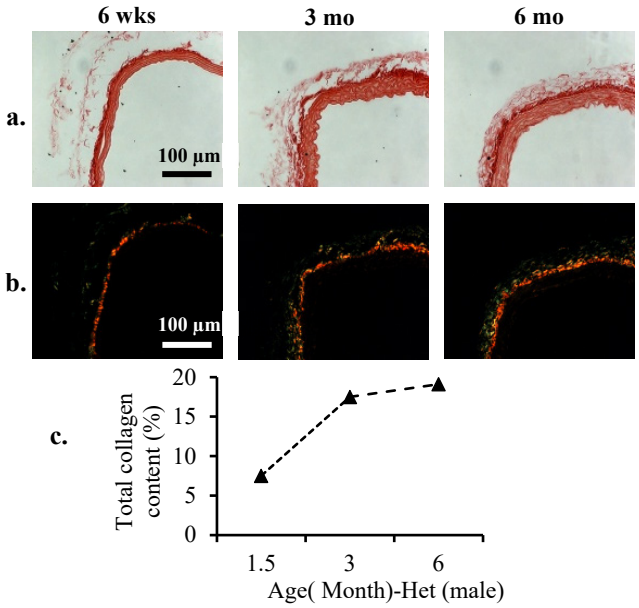

Fig.3.Representative histological images of 6 wks, 3mo and 6mo old Het male mice DTAo sections in (a.) and (b.). Brightfield images (20X) in (a.) were used to obtain total area of vascular sections, while in (b.) cross-polarized light images (20X) were employed to visualize the collagen fibers. (c.) Quantitation of fractional collagen content in DTAo for different age groups in Het male mice, N=2 for each group.

DISCUSSION

Preliminary results demonstrate that blood pressure in NOS3 KO mice is significantly higher than in either Het or WT mice. BP significantly increases with time in NOS3 KO mice, while there are no significant differences with age in Het or WT mice. In Het mice, relative collagen fiber angle in adventitia of DTAo shows a shift from a more axially oriented direction towards a more circumferentially oriented direction in the θ-Z plane with age. Concurrently, the fractional collagen content in DTAo of Het mice shows a significant increase with age. We conclude that the reduction of NO can lead to vascular remodeling associated with collagen fiber reorientation and increased collagen production, even in the absence of hypertension.

For future work, the analysis of collagen fiber orientation and histological study of DTAo and AAo of all genotypes will be completed for larger group sizes. In addition, active and passive biaxial mechanical tests will be performed on DTAo and AAo of all genotypes.

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