MATERIALS AND METHODS:

Materials and Methods are available in the online-only Data Supplement.

Animals 14 young (9.3±1.0 year old) and 15 old (26±0.4 year old) male Macaca fascicularis and Rhesus macaque monkeys were studied. The monkeys were fed a primate diet containing 5% to 6% fat, 18% to 25% protein, and 0.2% to 0.3% sodium chloride. The animals used in the current study were maintained in accordance with the Guide for the Care and Use of Laboratory Animals (National Research Council, 8th Edition 2011). Rutgers University and University of Wisconsin Primate Research Center approved all IACUC protocols.

Hemodynamics For surgery, monkeys were tranquilized with ketamine hydrochloride (2 to 3 mg/kg IM), anesthetized with thiamylal sodium (5 to 10 mg/kg IV), and maintained with isoflurane (0.5 to 1.5 vol · 100 mL^{-1} in oxygen). Tygon catheters were implanted in the descending aorta and in the left atria. Two pairs of piezoelectric crystals were implanted; one pair on the descending thoracic aorta and the other on the abdominal aorta.

Measurements were made in the conscious state, using a tether system carried by the monkeys in their cage (Fig. 1). Data were recorded on Chart 7 software (AD Instruments, CO) with a Powerlab (AD Instruments, CO) data acquisition system. Aortic pressure was measured with the implanted aortic catheter and strain gauge manometer; diameters were measured with piezoelectric ultrasonic dimension crystals. Aortic stiffness (β) was computed in this manner: ln(systolic pressure/diastolic pressure)/aortic strain [1]. Aortic strain was calculated as follows: [systolic aortic diameter (Ds)−diastolic aortic diameter (Dd)]/Dd. After baseline hemodynamics and recordings of thoracic and abdominal aortic diameter were stable, phenylephrine (100-150μg/kg IV) was administered to increase arterial pressure; the changes of arterial pressure and aortic diameters were recorded continuously during the drug response. The thoracic aortic stiffness was measured in the descending aorta and the abdominal aortic stiffness was measured between the renal artery and the iliac artery bifurcation.

Histology Histology samples were collected adjacent to the locations in the aorta where stiffness was measured. Samples were preserved in 10% buffered formalin. Aortic cross-sectional slices were cut and mounted onto glass slides. Masson's Trichrome staining was used to visualize the overall aortic morphology and architecture. Medial thickness was calculated from the medial area and a known length. Picrosirus red staining under circularly polarized light was used to visualize collagen, and aldehyde fuchsin staining was used to visualize elastin within the aortic wall. Collagen and elastin density were determined within each sampled field (15 fields per ring) by direct pixel quantification using custom MATLAB image analysis scripts.

Collagen and Elastin Disarray: The disarray of elastin and collagen networks in the medial layer, was determined by loss of parallel orientation and increased dispersion/randomness of fibers, and was graded from level 1-10 among all the aortic rings of both young and old monkeys under microscopic 10x magnification (See Table 1). Level 1 reflected almost all collagen and elastin fibers throughout the aortic ring evenly distributed and aligned in parallel. Level 10 was graded for samples with 95-100% of elastin or collagen fibers disrupted and distorted or aggregated. Examples of levels 1, 5 and 10 are shown in Figure 4C. Grading of samples was performed blinded by two different histologists.

Table 1: Collagen and Elastin Disarray; Percentage of Fibers Not in Parallel Orientation, Broken With Increased Dispersion and Randomness and Aggregated

Disarrray Level	1	2	3	4	5	6	7	8	9	10

%	0-14	15-24	25-34	35-44	45-54	55-64	65-74	75-84	85-94	95-100

Single Smooth Muscle Cell Stiffness Measurement Single smooth muscle cell stiffness was measured using cell nano-indentation with atomic force microscopy (AFM) as previously described [2-4]. Briefly, the AFM probes repeatedly approached and retracted from the same surface site on isolated vascular smooth muscle cells [4] with different forces in order to generate indentation depths of 100-600nm. With similarly generated indentation depths, the required force was compared between age groups and between thoracic and abdominal aorta.

Statistical Analysis Data are reported as mean ± standard error. Statistically significant differences were calculated by a 2-way ANOVA, two-tailed Mann-Whitney U-Test and Kruskal–Wallis test. A value of $P<0.05$ was considered significant for all analysis. The 2-way ANOVA contained an interaction term; no interactions were found significant for any of the data. A non-parametric statistical test, the two-tailed Mann-Whitney U-Test and Kruskal–Wallis test, were used to compare disarray differences between age groups and between thoracic and abdominal aorta.

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