Marfan syndrome (MFS) is an autosomal dominant connective-tissue disorder caused by heterozygous mutations in fibrillin-1 gene (FBN1). In a mouse model of Marfan syndrome, conventional Verhoeff-Van Gieson staining displays severe fragmentation, disorganization and loss of the aortic elastic fiber integrity. However, this method involves chemical fixatives and staining, which may alter the native morphology of elastin and collagen. Thus far, quantitative analysis of fiber damage in aorta and skin in Marfan syndrome has not yet been explored. In this study, we have used an advanced noninvasive and label-free imaging technique, multiphoton microscopy to quantify fiber fragmentation, disorganization, and total volumetric density of aortic and cutaneous elastin and collagen in a mouse model of Marfan syndrome. Thoracic aorta and dorsal skin samples were harvested from Marfan and control mice aged 3-, 6- and 9-month. Elastin and collagen were identified based on two-photon excitation fluorescence and second-harmonic-generation signals, respectively, without exogenous label. Measurement of fiber length indicated significant fragmentation in Marfan vs. control. Fast Fourier transform algorithm analysis demonstrated markedly lower fiber organization in Marfan mice as compared with control. Significantly reduced volumetric density of elastin and collagen and thinner skin dermis were observed in Marfan mice. Cutaneous content of elastic fibers and thickness of dermis in 3-month Marfan resembled those in the oldest control mice. Our findings of early signs of fiber degradation and thinning of skin dermis support the potential development of a novel non-invasive approach for early diagnosis of Marfan syndrome.

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Fig. 1: Measurements of aortic elastin & collagen volume and fiber organization using multiphoton microscopy and Fast Fourier analysis. A) 3-D reconstruction of aortic segments from 6-month old control and MFS mice (Elastin, green; collagen, purple). B) Analysis of the elastic fiber orientation using two-dimensional fast Fourier transform algorithm.