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Original Article

Loss of mechanical directional dependency of the ascending aorta with severe medial degeneration

Jennifer Chung [a], Kevin Lachapelle [a], Raymond Cartier [b], Rosaire Mongrain [c], Richard L. Leask [d,*]

[a] Division of Cardiac Surgery, McGill University Health Centre
[b] Department of Cardiac Surgery, Montreal Heart Institute
[c] Department of Mechanical Engineering, McGill University
[d] Department of Chemical Engineering, McGill University

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ABSTRACT

Biomechanical characterization of the aortic wall may help risk stratify patients with aneurysms. We investigated the degree of anisotropy, the directional dependency of mechanical properties, in control and aneurysmal ascending aortic tissue. We hypothesized that medial degeneration and aortic wall remodeling as found in aneurysmal tissue alter energy loss in both the circumferential and longitudinal directions, thereby reducing anisotropy. Aneurysmal and control ascending aortic tissue excised during surgery was subjected to biaxial tensile testing. Stress–strain relationships were collected in the circumferential and longitudinal directions; from these data, the mechanical properties of energy loss and the apparent modulus of elasticity were derived, and the associated anisotropy indices were calculated. Movat pentachrome histological staining was performed, and aortic wall medial degeneration was quantified. Energy loss was greater in the circumferential than the longitudinal direction, demonstrating significant anisotropy in both normal and aneurysmal aortas ($P<.0001$). This directional dependency diminished in (a) larger aortas ($r^2=0.15$, $P=.01$), especially when indexed to body surface area ($r^2=0.29$, $P=.002$); (b) aortas with greater overall energy loss ($r^2=0.44$, $P<.0001$); (3) aortas associated with tricuspid valves ($P=.004$); and (4) higher collagen-to-elastin ratio ($r^2=0.29$, $P=.001$). Aortas with collagen-to-elastin ratios greater than 2 were uniformly isotropic. Furthermore, the greatest energy loss anisotropy was found on the inner curvature of the aorta ($P=.01$). Energy loss demonstrates the directional dependency of aortic tissue. Energy loss isotropy is associated with medial degeneration, indicating that microstructural changes can be captured by global biomechanics, thereby identifying it as a marker of disease severity.

1. Introduction

Current methods of identifying ascending aortas at risk of complications are imperfect and miss many aortas that dissect and rupture while exposing other patients to unnecessary surgical risk [1,2]. The study of aortic biomechanics has the potential to provide insight into the pathogenesis of aortic complications and improve risk stratification. Developments are being made in quantifying biomechanical metrics through medical imaging, enabling practical avenues for screening patients [3]. However, prior to clinical application, characterization of normal and aneurysmal aortic biomechanics is needed alongside examination of how various biomechanical metrics reflect aortic function and structure. Anisotropy is one such example of a biomechanical metric.

Studies usually characterize aortic aneurysms in a single axis focusing on circumferential growth despite the fact that ascending aortas experience stress in a three-dimensional manner and aneurysms grow in the circumferential, longitudinal, and radial directions. Materials that behave mechanically different to stresses depending on the direction of measurement are described as “anisotropic,” and the absence of directional dependency is referred to as “isotropy.” Underlying tissue microstructure is responsible for directional dependency. The aortic media is organized in concentric elastin lamellar sheets and cross-linked collagen bundles helically arranged at different angles with embedded layers of smooth muscle cells and mucopolysaccharides [4]. How this carefully organized tissue structure responds to stress in different axes is not well defined, and how medial degeneration found in aneurysmal disease affects tissue anisotropy is poorly understood. In the literature, there have been conflicting reports regarding the existence of anisotropy in ascending aortas [5–12]. The lack of clarity may be because the apparent modulus of elasticity (stiffness) is usually the mechanical metric used to compute anisotropy, and it is fraught with many important limitations [3].

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* Corresponding author at: Department of Chemical Engineering, McGill University, 3610 University St., Montreal, Quebec, Canada, H3A 2B2.
E-mail address: richard.leask@mcgill.ca (R.L. Leask).

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"Energy loss," related to the Windkessel function of the aorta, is a measure of mechanical performance of a material over cyclical loading such as the cardiac cycle [13]. Ascending aortas are dynamic, storing energy during systole and returning a proportion to the circulation during diastole [14]. The proportion of energy absorbed by the tissue is attributed partly to the viscous component of the aortic wall and is "energy loss." Previously, we demonstrated that energy loss in the longitudinal direction increased with aortic aneurysm size and exhibited a hinge-point rise in aortas greater than 5.5 cm [13]. Moreover, longitudinal energy loss correlated strongly with histological changes found in aneurysmal aortas and could be used to identify histological outliers. Energy loss was found to be a more robust measure of tissue remodeling than the apparent modulus of elasticity [13].

In this study, we aim to characterize the directional dependency of ascending aortas in our patient population using both energy loss and the apparent modulus of elasticity. We hypothesize that patient factors and aneurysm disease-related remodeling of the aortic wall microstructure may differentially affect the longitudinal versus circumferential direction, thus translating into changes in anisotropy.

2. Material and methods

Excised aneurysmal ascending aortic tissue was obtained at the time of surgery from July 2012 to May 2013 at McGill University Health Centre and Montreal Heart Institute with informed consent and research ethics board approval. At the time of tissue collection, the patient chart was reviewed for relevant clinical details. Control ascending aortic tissue was obtained from heart transplant donors and autopsy patients without heart or aortic disease.

2.1. Mechanical testing

Testing was done within 24 h of tissue collection, and specimens were kept on ice until then. The autopsy specimen were collected within 24 h of death and were tested as soon as possible. All samples were collected as rings of tissue with orientation marked by a single clip. Aneurysmal and control tissues were tested the same way. Four 1.5-cm×1.5-cm squares were collected along the widest part of the aneurysm in prespecified quadrants: the inner curvature, anterior, outer curvature, and posterior areas (Fig. 1a). The average of the four quadrants was used for data analysis except when analyzing biomechanical variation depending on location around the aortic ring (i.e., regional variation). The thickness of each sample was evaluated using an extreme-precision light-touch indicator in quintuplicate (Litematic VL-50A; Mitutoyo, Mississauga, Canada).

Each square underwent equibiaxial tensile testing at 37°C in a Ringer's lactate solution to a maximum of 60% strain (Fig. 1b, EnduraTEC ELF 3200; Bose Co., Framingham, MA, USA). Ten preconditioning loops were completed prior to the test loops, which were done in triplicate at a strain rate of 0.1 mm/s to approximate steady-state conditions. Zero strain was set to a stress of 0.050 N.

2.2. Histology

Samples from each quadrant were stored in 10% formalin and then sectioned and stained with Movat pentachrome. Images were taken in 3 different areas per slide, giving a total of 12 images per aorta. All images were renamed and analyzed in a blinded fashion. The percentages of collagen, elastin, and mucopolysaccharides were quantified using ImageJ 1.46r (National Institutes of Health, Bethesda, MD, USA). Color thresholds were adjusted manually to select "yellow" for collagen and "blue" for mucopolysaccharides, and the percent areas for each selection were recorded. To select "black" for elastin, we manually adjusted thresholds of 8-bit versions of each image to obtain the percent area of black.

2.3. Data analysis

Analysis was performed using MatLab R2012a (MathWorks, Natick, MA, USA). The engineering stress–strain curves during both loading and unloading in the longitudinal and circumferential directions were fit using polynomial functions. We measured the percent of energy lost between the loading and unloading curves, the "energy loss," to quantify the viscoelastic nature of the aorta [13]. The percent energy loss was calculated as follows (ε_1=final strain, σ_{loading}=polynomial model for loading curve, ε=strain, $\sigma_{\text{unloading}}$=polynomial model for unloading curve):

$$\text{Energy loss} = \frac{\int_0^{\varepsilon_1}\left[\sigma_{\text{loading}}(\varepsilon)-\sigma_{\text{unloading}}(\varepsilon)d\varepsilon\right]}{\int_0^{\varepsilon_1}\sigma_{\text{loading}}(\varepsilon)d\varepsilon} \quad (1)$$

The apparent modulus of elasticity was calculated as the slope of the loading curve at 40% and 50% strain. When variables were normalized to body surface area (BSA), the BSA was calculated using the Dubois formula.

In order to quantify directional dependency, the anisotropy index was calculated using the formula below (AI, anisotropy index; BV, biomechanical variable; L, longitudinal direction; C, circumferential direction), consistent with previous work [8,15]. A value of zero indicates complete isotropy or lack of directional dependency, whereas the

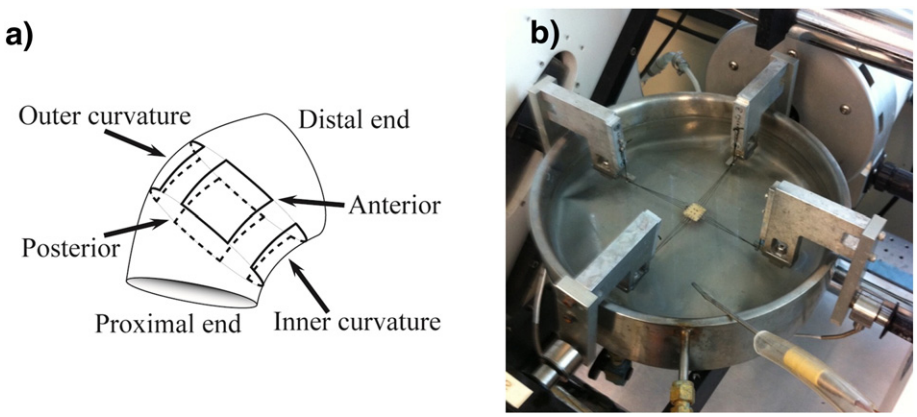

Fig. 1. Experimental setup. (a) For each aorta in this series, four squares are sampled from these prespecified quadrants. (b) Each square is then subjected to biaxial tensile testing.