

Biomechanics in ascending aortic aneurysms correlate with tissue composition and strength



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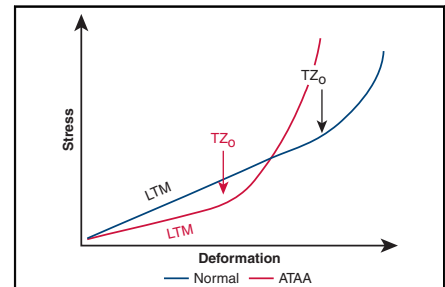
ABSTRACT

Objective: This study correlates low strain tangential modulus (LTM) and transition zone onset (TZo) stress, biomechanical parameters that occur within the physiological range of stress seen in vivo, with tissue strength and histopathologic changes in aneurysmal ascending aortic tissue.

Method: Ascending aortic aneurysm tissue samples were collected from 41 patients undergoing elective resection. Samples were subjected to planar biaxial testing to quantify LTM and TZo. These were then correlated with strength assessed from uniaxial testing and with histopathologic quantification of pathologic derangements in elastin, collagen, and proteoglycan (PG).

Results: Decreased LTM and TZo were correlated with reduced strength ($P < .05$), PG content ($P < .05$), and elastin content ($P < .05$). Reduced TZo also was correlated with increased elastin fragmentation ($P < .05$).

Conclusions: LTM and TZo are correlated with common biomechanical and histopathologic alterations in ascending aortic aneurysm tissue that are thought to relate to the risk of acute aortic syndromes. LTM and TZo are measured under conditions approximating in vivo physiology and have the potential to be obtained noninvasively using medical imaging techniques. Therefore, they represent parameters that warrant future study as potential contributors to our growing knowledge of pathophysiology, disease progression, and risk stratification of aortic disease. (JTCVS Open 2022;9:1-10)



Biomechanics that can be measured within physiological stress-strain levels may reflect aortic pathophysiology.

CENTRAL MESSAGE

Low strain modulus and transition zone onset stress can provide the necessary link between the ex vivo and in vivo avenues of research into the pathogenesis and risk stratification of aortic disease.

PERSPECTIVE

Previous biomechanical assessments of aortic tissue generally have focused on measures that are obtained experimentally at supraphysiologic levels of stress. Low strain modulus and transition zone onset stress are measured in the in vivo range of stress (and thus can be obtained noninvasively through imaging techniques) and correlate with common histopathologic alterations in ascending aortic aneurysm tissue.

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Data supporting the findings of this study are available from the corresponding author on reasonable request.

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Abbreviations and Acronyms

ATAA	= ascending thoracic aortic aneurysm
BAV	= bicuspid aortic valve
ECM	= extracellular matrix
HTM	= high-strain tangential modulus
LTM	= low-strain tangential modulus
PG	= proteoglycan
TAV	= tricuspid aortic valve
TZe	= Stress at the end of the transition zone
TZo	= onset stress of the transition zone

Our understanding of the pathogenesis of ascending thoracic aortic aneurysms (ATAAs) has grown significantly over the past decade, including a deeper understanding of the contributions of cellular and noncellular components, genetics and epigenetics, mechanotransduction, blood flow properties, and a variety of often-interrelated pathways (metabolic, inflammatory, and immune).¹⁻⁴ The complex interplay of these mechanisms results in microstructural changes in the aortic wall extracellular matrix, with corresponding alterations in histologic examination and biomechanical properties of excised tissue.^{5,6}

Starting in the early 2000s, there has been significant research into the pathophysiology of ATAAs. Previous studies have examined excised aneurysmal aortic tissue to correlate disease-related histopathologic changes with ex vivo uniaxial biomechanical testing of stiffness and strength parameters, or both.^{7,8} This approach has provided valuable insight into the biologic and mechanical processes underlying aortic disease, as well as their interrelationship and clinical implications. However, this approach is ultimately limited by (1) the need for excised tissue, (2) a reflection of changes that occur at the end stage of the disease process, and (3) a reliance on biomechanical measures of stiffness and strength derived under supraphysiologic loading conditions with limited in vivo translation. These limitations hinder our understanding of the full spectrum of disease epidemiology, pathophysiology, and temporal disease progression. They also may prevent the optimal application of findings into a multifaceted aortic risk stratification model.

If pathophysiologic changes in aneurysm tissue composition manifest as changes in aortic mechanical behavior, then enhancing our biomechanical assessments should improve efforts to study disease progression and prognosis. One area of improvement would be to expand our research into in vivo biomechanical parameters. Benchtop biaxial mechanical testing results in a nonlinear stress–strain behavior, in which the material property of the tissue is

dependent on which portion of the curve is being measured (Figure 1, A). Only a portion of the stress–strain curve produced by standard biaxial testing corresponds to loading conditions present in vivo under normal physiologic loading conditions (Figure 1, B). Focusing on benchtop measures obtained within a physiologic range of loading conditions could provide insight into tissue material properties in vivo and identify candidates for noninvasive biomechanical assessments. Low-strain tangential modulus (LTM; Figure 1, C) and low-stress onset transition zone (TZ_O; Figure 1, D) are measured in the early phase of the biaxial stress–strain response, which approximates loading conditions in a typical aortic pulse wave. In contrast, more commonly reported measures, such as high-strain tangential modulus (HTM; Figure 1, C), energy loss (Figure 1, E), and elastance (Figure 1, F), are measured in the late phase or across the entire response, partially outside of normal physiological conditions.

To date, there have been no studies comparing LTM and TZ_O with known histopathologic changes in excised ATAA tissue or with the results of biaxial mechanical tests (energy loss, elastance, and strength) commonly reported in the literature. The present exploratory study was conducted to help determine the potential utility of LTM and TZ_O as ex vivo biomechanical measures—with plausible noninvasive in vivo equivalents—for studying the pathophysiologic alterations of wall properties in aortic disease.

METHODS

Study Population

Consecutive patients undergoing elective aortic replacement surgery at Foothills Medical Centre in Calgary were recruited for this study between February 2015 and August 2017. Inclusion criteria included age >18 years, tricuspid aortic valve or bicuspid aortic valve, no known or suspected connective tissue disorders, and no previous aortic dissections or ruptures. This study was approved by the Conjoint Faculties Research Ethics Board at the University of Calgary (REB14-0084; October 2014), and written informed consent was obtained from all participants. Clinical data, including age, sex, valve morphology, and aneurysm size, were collected.

Specimen Preparation

The anterior region of the aorta from each patient was included in this study, with 1 sample obtained per patient. The anterior region was chosen for consistency across the samples. Adjacent samples on the anterior specimen were chosen for biaxial, uniaxial, and histopathologic testing to limit the degree of heterogeneity across the tissue samples for the different tests.

Mechanical Properties

Ex vivo testing of tissues was performed as described previously⁹; details are provided in Appendix E2. Each biaxial sample was cut into a square of approximately 10 mm per side and tested on a planar biaxial device with 2 load cells (22N; ElectroForce System; TA Instruments, Springfield, Mo). Five dots were placed on the center of each sample to allow optical tracking of local deformation. The samples were mounted onto the machine by surgical sutures attached to 16 hooks (4 per edge) and submerged in a PBS bath at 37 °C to simulate the in vivo environment. All

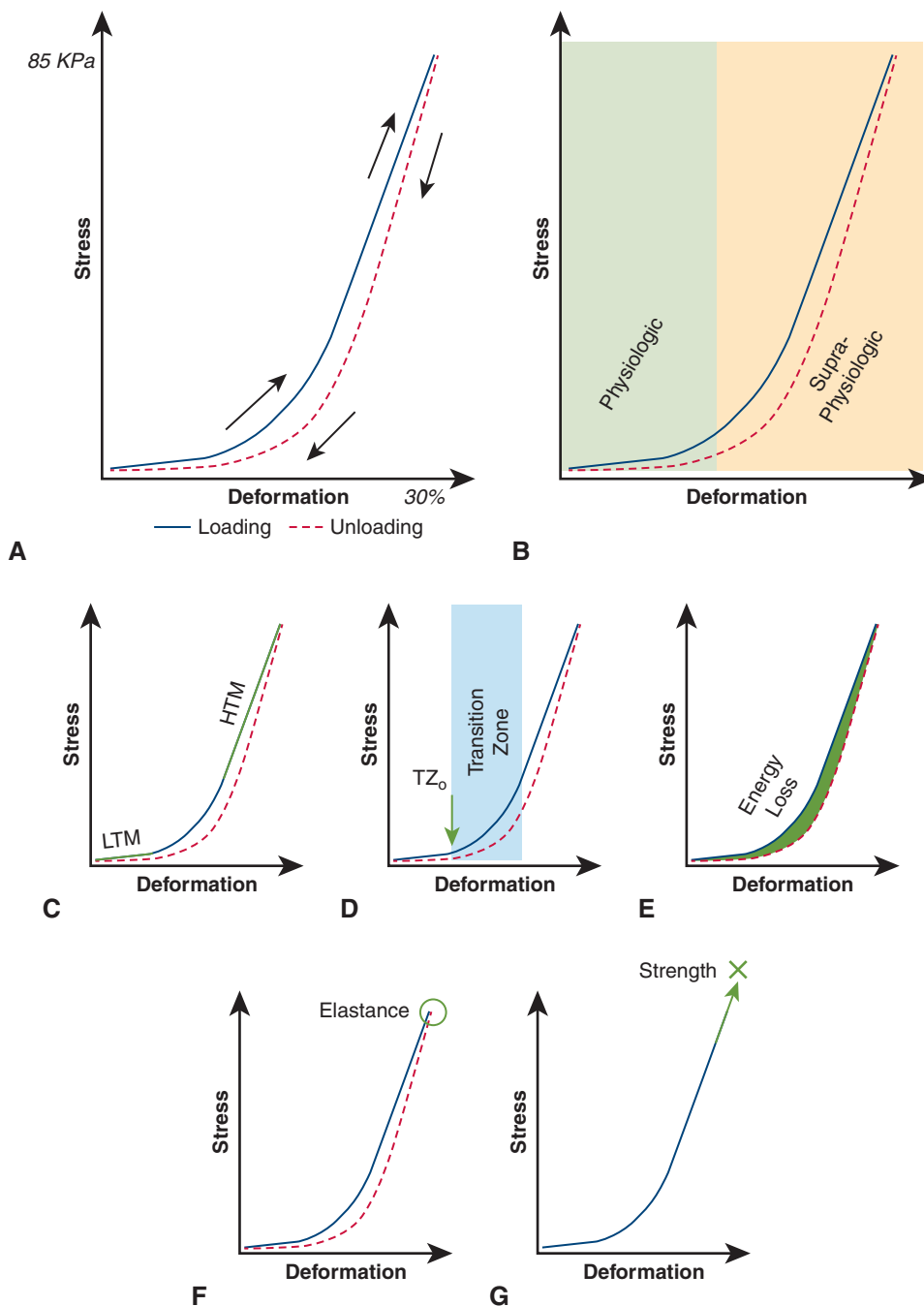


FIGURE 1. A, Typical loading and unloading curves for aortic aneurysm tissue. B, Loading and unloading curve showing typical deviation between physiological and supra-physiological strain. C, Illustration showing how low-strain tangential modulus (*LTM*) and high-strain tangential modulus (*HTM*) were derived from the biaxial mechanical response. D, Stress at onset of transition zone (*TZ_o*) as derived from the mechanical behavior. E, Energy loss is defined as the %difference between the loading and unloading mechanical behavior of the tissue (depicted by the area in *green* between the loading and unloading curves). Elastance (F) and strength (G) as derived from the mechanical response.

samples were subjected to a preloading of 0.05 N on each axis to remove slack and bending effects and then to 10 preconditioning cycles to ensure repeatable stress-strain behavior. For this study, a displacement-controlled protocol with a maximum displacement of 60% of the hook-hook length for both the circumference and axial direction was used. The mechanical behavior of the tissue was characterized by the second

Piola-Kirchhoff stress and the Green strain both locally at the center of the specimen (through optically measured dot displacement) and globally (through hook-hook displacement through the testing device) in both directions via a MATLAB program. Further information on calculating the second Piola-Kirchhoff stress and Green strain is provided in the [Appendix E2](#). Aortic mechanical behaviors were determined under the

assumption of homogeneity and incompressibility. A combination of testing methods and postprocessing data selection ensured that the shear stress on tested samples was low and could be considered negligible in our analysis.

The mechanical properties for both the circumferential and axial directions were determined from the resulting local stress–strain curves. Tangential modulus, a parameter most closely resembling the colloquial stiffness term, was determined from the biaxial mechanical responses. The low-strain tangential modulus (LTM) was calculated from the first linear region of the mechanical response. If there was a second linear region after the nonlinear transition zone, then a high tangential modulus (HTM) was calculated (Figure 1, C). The LTM and HTM regions were determined by assessing the second Piola–Kirchhoff stress–Green strain curve for deviations from linearity. The stresses corresponding to the onset and end of the nonlinear transition zone (TZ_o and TZ_e) were recorded (Figure 1, D). Global (from the hook–hook displacement) energy loss was calculated as the area between the loading and unloading of the tissue as described previously¹⁰ (Figure 1, E). Elastance (the reciprocal of compliance) was defined as the maximum stress during deformation (Figure 1, F). A uniaxial sample was also obtained and cut into an 8 mm × 2 mm rectangle and then tested on a linear motor uniaxial testing system (22N; ElectroForce System 3200, TA Instruments, Eden Prairie, Minn). An initial set of 10 preconditioning cycles to 15% strain based on initial grip–grip length was performed, and then the tissue was pulled until failure (Figure 1, G). Strength, or failure stress, was defined as the first local maximum in the uniaxial stress–strain graph and was determined in both circumference and axial direction.

Histopathologic Analysis

A subset of samples was formalin-fixed, paraffin-embedded, and sectioned at 5 μm. Sections were stained with Musto–Movat pentachrome and picrosirius red. The slides were digitized using Aperio ImageScope digital scanning (Aperio ImageScope, version 12.4.3.5008; Leica Biosystems, Nussloch, Germany). A colorimetric pixel algorithm was applied to the media of the aortic sections to quantify the relative proportions (content) of elastin (black) and proteoglycan (PG; cyan) on Musto–Movat staining. The sections were scored for elastin fragmentation, fragmentation distribution, collagen alteration (an increased presence of collagen in the media), and alteration distribution on both Musto–Movat and picrosirius red (assessed under polarizing light) staining in accordance with the consensus statement for evaluation of noninflammatory aortopathy from the Society for Cardiovascular Pathology.¹¹

Statistical Analysis

The statistical analysis was completed using a custom software pipeline developed using the SciPy statistical package written in the programming language Python.¹² The significance threshold for all tests was set to 0.05. The normality of continuous variables was assessed through the Shapiro–Wilk test. The Pearson correlation (normal distribution) or Spearman rank correlation (nonnormal distribution) were used to assess the relationships between continuous variables. Categorical variables were assessed for equivalence of variance using the Levene test. ANOVA (normal distribution), the Mann–Whitney *U* test (nonnormal, 2 categories), or the Kruskal–Wallis test (nonnormal, >2 categories) was used to assess the relationships between categorical and continuous/categorical variables. If categorical significance was determined, a Tukey post hoc test was used to determine significance between individual category level for normal data for categories >2. For nonnormal data, the Mann–Whitney *U* test was used to compare individual levels. The descriptive statistics presented in Results are in the form of mean ± SD and were determined using the SciPy package functions for mean and SD. The descriptive statistics visualizations shown in the boxplots (eg, median, upper quartile) were

determined through the boxplot function from the Python data visualization package Plotnine.

RESULTS

Study Cohort

Tissue samples were collected from 41 patients. Patient demographics and preoperative characteristics are summarized in Table 1. All samples were mechanically tested on the planar biaxial device. Uniaxial tensile strength testing was performed in both the circumferential and axial directions (n = 20) or in the circumferential direction alone (n = 14). Twenty-eight samples underwent histopathological analysis.

Mechanical properties and histopathological analysis.

The biaxial mechanical properties LTM, TZ_o, energy loss, and elastance were found to be variably correlated with histopathologic results (Table 2). Lower LTM and TZ_o were correlated with decreased PG and elastin content (Figure 2, A–D), with lower TZ_o also associated with increased elastin fragmentation (Figure 2, E). Neither LTM nor TZ_o had any significant correlations to abnormalities in collagen. Energy loss correlated well with most abnormalities in PG, elastin, and collagen (Figure 2, F–H), whereas elastance correlated poorly with all histopathologic features except the collagen alteration score (Figure 2, I). TZ_e and HTM did not correlate with any histopathologic properties in either direction.

Mechanical properties and strength. The results of the correlation of biaxial mechanical properties with uniaxial tensile strength testing results are presented in Table 3. Reduced LTM in the axial direction correlated with reduced strength in both directions, whereas LTM in the circumferential direction had a nonsignificant trend toward correlation with reduced strength. Low TZ_o correlated with reduced circumferential strength (Figure 2, J) but not with axial strength. Axial energy loss correlated with reduced strength in both directions, whereas circumferential energy loss correlated only with reduced circumferential strength (Figure 2, K). Finally, lower circumferential elastance correlated with reduced strength in both directions (Figure 2, L for circumference), whereas axial elastance correlated only with reduced axial strength. TZ_e

TABLE 1. Patient demographics and preoperative characteristics

Characteristic	Total (n = 41)	BAV (n = 24)	TAV (n = 17)
Hypertension, %	49	37	65
Smoking, %	36	46	23
Diabetes, %	5	4	6
Sex, male/female, n	27/14	17/7	10/7
Age, y, mean ± SD	61.58 ± 11.5	57.12 ± 9.64	67.88 ± 11.20
Aortic diameter, cm, mean ± SD	5.31 ± 0.75	5.06 ± 0.36	5.66 ± 1.0

BAV, Bicuspid aortic valve; TAV, tricuspid aortic valve.

TABLE 2. Histopathologic analysis versus mechanical properties

Parameter	PG content, <i>P</i> value (correlation ρ when available)	Elastin			Collagen	
		Content	Fragmentation	Fragmentation distribution	Alteration	Alteration distribution
Circumferential direction						
LTM	.038 ($\rho = 0.39$)	.041 ($\rho = 0.39$)	.089	.082	>.1	>.1
TZ _O	.015 ($\rho = 0.45$)	.0095 ($\rho = 0.48$)	.047	.081	>.1	>.1
Elastance	>.1 ($\rho = 0.20$)	>.1 ($\rho = 0.12$)	>.1	>.1	.045	>.1
Energy Loss	7.0×10^{-4} ($\rho = -0.60$)	.087 ($\rho = -0.33$)	.0045	>.1	.0035	.0011
Axial direction						
LTM	>.1 ($\rho = 0.23$)	>.1 ($\rho = 0.29$)	>.1	>.1	>.1	>.1
TZ _O	.028 ($\rho = 0.41$)	>.1 ($\rho = 0.32$)	.091	>.1	>.1	>.1
Elastance	>.1 ($\rho = 0.02$)	>.1 ($\rho = -0.09$)	>.1	>.1	>.1	>.1
Energy Loss	.0073 ($\rho = -0.50$)	>.1 ($\rho = -0.31$)	.0053	>.1	.031	.0116

PG, Proteoglycan; LTM, low-strain tangential modulus; TZ_O, stress at onset of transition zone.

and HTM did not correlate with strength in either direction.

DISCUSSION

The media/middle layer of the aortic wall, considered the main contributor to overall mechanical properties,¹³ is composed of equally spaced elastin lamellae, with each lamella perpendicularly connected by elastin as well.¹⁴ Smooth muscle cells and collagen are interspaced between the elastin layers.¹⁴ The elastin lamellae structure is supported by ground substance consisting mostly of mucopolysaccharides (usually referred to as PGs).¹⁵ Changes in the nonstructural proteins, both cellular and extracellular, will result in observable changes in biomechanical parameters through their impact on elastin and collagen.^{14,15}

Elastin is associated with elastic mechanical behavior and a wide range of reversible deformation and is the dominant aortic tissue component characterizing the behavior of healthy aortic tissue. Collagen, which remains crimped or wavy during low loading conditions, is responsible for reinforcing the tissue, normally engaging exclusively at high loads.^{16,17} The combination of the biomechanical properties of these 2 extracellular matrix (ECM) materials results in a nonlinear tissue stress–deformation curve indicative of the relative contributions of elastin and collagen along the range of loading conditions (ie, wall stress) (Figure 3, A).¹⁶ At low stress/deformation, the curve is mostly linear, represents almost exclusively elastin function, and correlates with in vivo aortic wall stress seen early in the systolic pulse wave. This portion of the curve was quantified by the low-strain tangential modulus (LTM) (Figure 1, A and C). At high stress/deformation, the curve is also linear but has a steeper slope/higher stiffness and represents fully engaged collagen. This section of the curve (quantified by HTM) normally occurs at supraphysiologic loading conditions (Figures 1, A, B and 1, C), with only the early

component correlating to the brief end-systolic peak of the typical aortic pulse wave (if at all).¹⁸ In between the LTM and HTM is a nonlinear transition zone as the predominant contributor to the mechanical properties in the switch from elastin to collagen, with a period of mutual contribution and overlap in function (Figure 1, A and D).¹⁷

In ascending thoracic aortic aneurysms, elastin is often fragmented and depleted, leading to increased collagen in the media and possibly premature collagen engagement during lower loading conditions.¹¹ Elastin fragmentation and content have been established as signs of aneurysm disease progression.^{8,19}

Given the role of elastin, the effect of its pathologic remodeling on mechanical properties of the aortic wall should be reflected primarily in the low-stress low-deformation region of biaxial testing. Loss of functional elastin should result in decreased LTM and onset of the transition zone at lower stress/deformation (TZ_O) (Figure 3, B). The preliminary findings of our study support this theory, with lower LTM correlating with histological changes in elastin, in both fragmentation and overall content.

An increase in collagen in the media has been recognized as a part of the remodeling process as the tissue compensates for the loss of elastin by replacing it with collagen.¹¹ Neither LTM nor TZ_O was correlated with abnormalities in the collagen histology in our study; however, a lower TZ_O (indicative of collagen engagement) was significantly associated with a decrease in elastin content. This change in the biaxial mechanical response could be representative of the collagen replacing the elastin in terms of structural support at lower levels of stress, possibly with earlier activation to compensate for the degraded elastin. This theory is further reinforced by the significant association between altered collagen in media and increased energy loss, representing the tissue transitioning into a material that more closely resembles the properties of collagen. TZ_O appears to capture this transition.

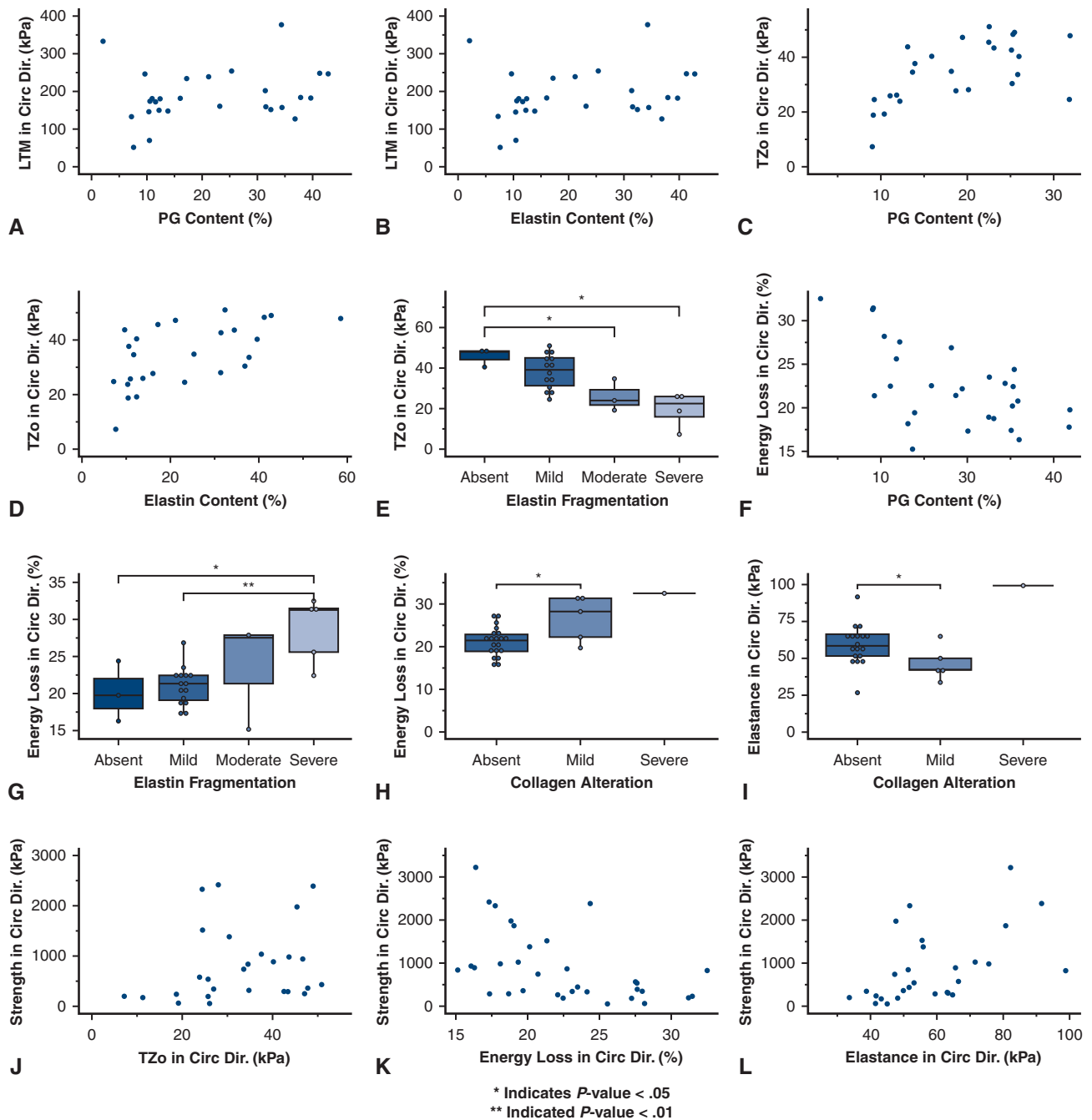


FIGURE 2. Scatterplots and boxplots showing the significant associations between the mechanical and histopathological properties in the circumference direction. For the boxplots, the *middle horizontal line* represents the median value and the *upper and lower borders of the box* represent the upper and lower quartiles, respectively. The *upper and lower whiskers* represent the values at the upper/lower quartile ± 1.5 times the interquartile range (height of the box). *Extra dots* are considered outliers outside of this range. A, Low-strain tangential modulus (LTM) and proteoglycan (PG) content. B, LTM and elastin content. C, Stress at onset of transition zone (TZo) and PG content. D, TZo and elastin content. E, TZo and elastin fragmentation. F, energy loss and PG content. G, Energy loss and elastin fragmentation. H, Energy loss and collagen alteration. I, Elastance and collagen alteration. J, TZo and strength. K, Energy loss and strength. L, Elastance and strength.

Uniaxial testing is currently the method most closely simulating tissue rupture. Although it is dangerous to make false equivalencies, information from these tests can

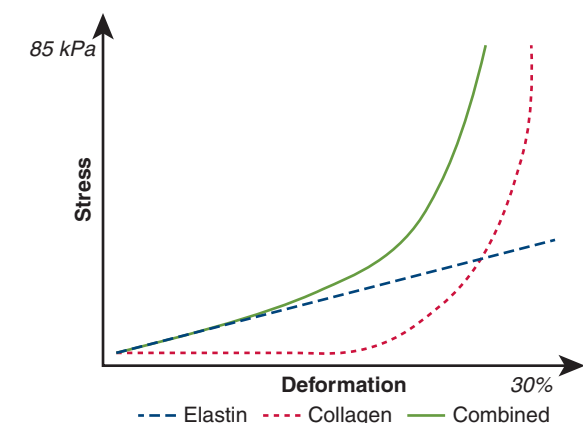
provide insight into in vivo mechanical behavior, such as the ability of a tissue to resist failure/tears. Strength, defined as the point at which aortic tissue fails, is also the

TABLE 3. Strength testing versus mechanical properties

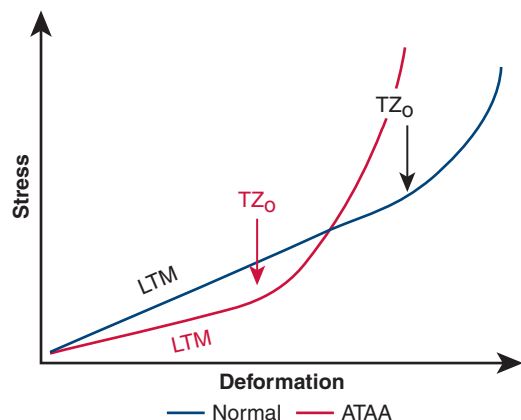
Parameter	Strength	
	Circumferential	Axial
Circumferential direction		
LTM	.067 ($\rho = 0.31$)	.059 ($\rho = 0.43$)
TZ _O	.033 ($\rho = 0.37$)	>.1 ($\rho = 0.33$)
Elastance	.00073 ($\rho = 0.55$)	$P = .02$ ($\rho = 0.51$)
Energy loss	.0014 ($\rho = -0.53$)	>.1 ($\rho = -0.26$)
Axial direction		
LTM	.013 ($\rho = 0.42$)	.017 ($\rho = 0.53$)
TZ _O	.024 ($\rho = 0.388$)	>.1 ($\rho = 0.30$)
Elastance	>.1 ($\rho = 0.33$)	.01 ($\rho = 0.56$)
Energy loss	.00035 ($\rho = -0.58$)	.033 ($\rho = -0.48$)

LTM, Low-strain tangential modulus; TZ_O, stress at onset of transition zone.

mechanical property traditionally used to correlate histopathologic changes with mechanical weakening.^{7,8} Decreases in both LTM and TZ_O generally correlated well with reduced tissue strength.



A



B

FIGURE 3. A, Typical aortic tissue behavior illustrating the contribution of the elastin and collagen fibers for one direction. B, Change in biomechanical behavior in healthy versus ascending thoracic aortic aneurysm (ATAA) tissue. LTM, Low-strain tangential modulus; TZ_O, stress at onset of transition zone.

Energy loss is a biomechanical parameter of recently increased interest as it is thought to reflect viscoelastic properties (and possibly endothelial function), deformation irreversibility, and other mechanical properties related to elastin function.¹⁰ With the elastic material effect/reversibility no longer maintained owing to the fragmented elastin, energy loss increases, as measured in the increased difference between the loading and unloading tissue responses. In our study, energy loss outperformed LTM and TZ_O in some measures, including PG content, elastin fragmentation, and circumferential strength. In other measures, such as elastin content, distribution of fragmentation, and axial strength, it was inferior. This suggests that LTM, TZ_O, and energy loss can be viewed as complementary measures, possibly reflecting structural alterations of ATAA tissue in a more comprehensive fashion when combined.

Although data correlating ex vivo biomechanical parameters with histologic alterations and weakening of aortic tissue in ATAA has been published previously, few of the biomechanical parameters were measured from biaxial loading.^{10,18,20} Among these biaxial studies, only one—“stiffness” or tangent modulus, equivalent to the HTM parameter, calculated around 30%-60% of global (ie, hook–hook) strain—was frequently presented. This level of strain is thought to be in the nonphysiological range. LTM is more representative of in vivo strain conditions, as many of the aortic tissue behavior curves (17 of 42) did not reach the HTM linear region by the maximum in vivo strain. In the few biaxial studies reporting LTM, the parameter was determined from global or hook–hook strain and thus does not produce as accurate a measurement as the dot displacement strain, which mitigates the effects of stress/strain concentrations at the hook sites.¹⁸ Parameters related to transition zone are even rarer than LTM; when reported, they are determined as the intersection point of the LTM and HTM, whereas TZ_O is determined directly from tissue behavior and thus is more representative of what is occurring in the tissue response and has physical meaning.²¹

Ex vivo biomechanical parameters can be obtained under conditions that mimic physiologic or nonphysiologic conditions. LTM and TZ_O are examples of the former, and traditional measures, such as elastance and strength, represent the latter. Because energy loss is defined by the entire curve, it can be technically physiologic in nature if the biaxial testing is performed with a maximum displacement or load similar to that seen under physiologic conditions. However, standard biaxial displacement protocols, including supraphysiologic displacement/load, are often used when testing tissue ex vivo.

Although many parameters correlate with known changes in the ECM and cellular wall components and thus are valuable in the study of aortic disease, supraphysiologic measures limit the ability to perform comparisons

with equivalent biomechanical properties noninvasively *in vivo*. They also limit the scope of studies to tissues that have been excised, owing to either a large diameter or tissue failure presenting as an acute complication, such as dissection, intramural hematoma, or rupture. Not only does this result in studying exclusively “end-stage” aortic disease, thereby hindering our understanding of disease progression, it also provides little help in identifying high-risk patients with aortic diameters below current recommended thresholds for surgical resection.²² Although accurately calculating stress *in vivo* remains elusive, given the difficulty in determining certain geometric properties such as thickness, *in vivo* stand-ins for LTM, TZ₀, and even energy loss derived from *in vivo* pressure–strain or membrane stress–strain data are possible. These stand-ins have the potential for clinical applications given their ability to be measured *in vivo*. For example, *in vivo* pressure–strain data can be derived from a speckle tracking analysis of images acquired with transesophageal echocardiography, as illustrated in Figure 4. This *in vivo* behavior contains a low-strain linear region in which a modulus similar to LTM can be determined. The same can be said for the TZ₀. In Figure 4, a nonlinear transition zone in which a starting pressure or strain can be extracted can be clearly defined. Previous work with ultrasound-derived cardiac cycle pressure modulus index in ATAAs is an example of how this approach can be feasible, practical, and informative.²³ The pressure modulus was seen to

correlate well with *ex vivo* biomechanical and histopathologic properties.²³

Consequently, we believe that both parameters should be considered for additional study and may assist in linking benchtop research with large-scale clinical applications.

Limitations

As an exploratory study, this work has several limitations, and our results should be viewed as preliminary and hypothesis-generating. A prospective power analysis was not performed to determine an appropriate sample size, but our small sample size likely makes the study underpowered, a conclusion supported by several nearly significant correlations. Many of the relationships among LTM, TZ₀, and histology had only moderate correlation coefficients. Perhaps a byproduct of the complex nature of tissue microstructure pathology and significant local heterogeneity of mechanical properties, this underscores the need for a multifaceted comprehensive strategy to studying aortic aneurysms.

No measurements were taken from normal aortic tissue to function as controls. Although *ex vivo* controls are generally limited to cadaver studies, LTM and TZ₀ could be obtained *in vivo*, and thus normal reference values should be determined in future studies. Although we included both BAV and TAV patients, we did not have enough samples to determine whether LTM and TZ₀ perform differently between the 2 patient populations. This will be an important aspect to properly delineate, as the differences between the 2 groups in tissue pathophysiology, histology, and biomechanical properties have been described.^{24–26} Finally, as with all *ex vivo* approaches, this study is limited to passive mechanical properties, missing any changes associated with the interactions of living cells either in the ECM (eg, smooth muscle cells) or in the endothelium, which are known to interact with the noncellular components and thus impact mechanical properties.²⁷

Future Study

Although caution is always recommended when interpreting the results of *ex vivo* testing and their *in vivo* implications, our preliminary data suggest what one might expect to observe during the pathophysiologic lifespan of aortic disease. If the mechanism of aortic aneurysm formation and risk of rupture are manifestations of changes in the tissue composition with progressive elastin dysfunction and early collagen activity, then progressively lower LTM and earlier TZ₀ would be expected. Perhaps a value of either parameter could be found that correlates with end-stage disease and/or an increased risk of acute aortic event. To fully explore the usefulness of LTM and TZ₀, future studies will need to include the following steps: (1) determine whether *in vivo* LTM and TZ₀ correlate with their *ex vivo* biaxial counterparts; (2) confirm that LTM and TZ₀ reliably measure changes in tissue histopathology in larger numbers

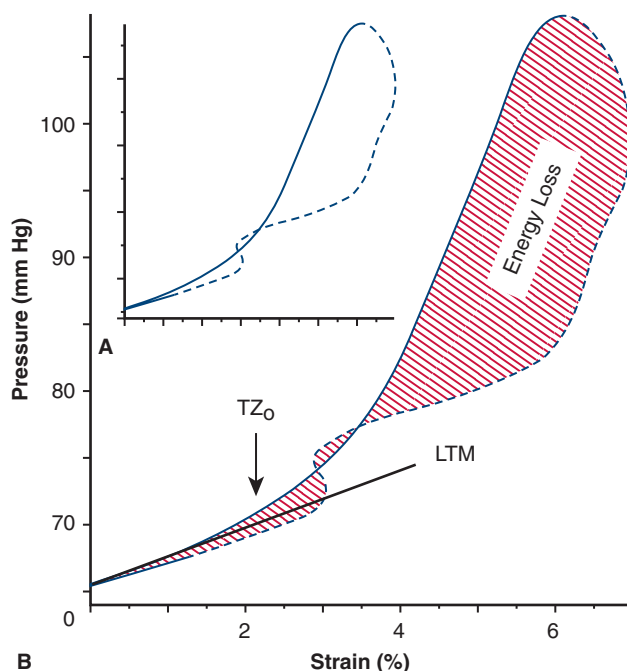


FIGURE 4. Illustration showing how low-strain tangential modulus (LTM), stress at onset of transition zone (TZ₀), and energy loss can be derived from a pressure–strain loop from transesophageal echocardiography and speckle-tracking imaging analysis.

and within different subgroups (eg, bicuspid vs degenerative vs inflammatory); (3) study the feasibility, reproducibility, and reliability when obtained from noninvasive in vivo measurements; (4) formulate noninvasive, in vivo reference values from patients with normal aortic tissue across age, ethnicity, and sex; and (5) quantify any additive benefit of LTM and TZ_O to patient outcomes along with other measures of interest, including size and growth rate, genetic and epigenetic markers, 4-dimensional flow and wall shear stress, and other biomechanical parameters.

CONCLUSIONS

The novel application of 2 biomechanical testing parameters, LTM and TZ_O, show promise as measures of aortic function and tissue properties under physiologic loading conditions. Both parameters correlate with traditional histopathologic, tensile strength, and common supraphysiologic mechanical parameters in patients with ascending aortic aneurysms. Future research will help determine the role of noninvasive biomechanical analysis in exploring the pathogenesis and risk stratification of aortic disease.

Conflict of Interest Statement

The authors reported no conflicts of interest.

The *Journal* policy requires editors and reviewers to disclose conflicts of interest and to decline handling or reviewing manuscripts for which they may have a conflict of interest. The editors and reviewers of this article have no conflicts of interest.

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APPENDIX E2. DETERMINING THE SECOND PIOLA-KIRCHHOFF AND GREEN STRAIN BEHAVIOR

The mechanical behavior of the tissue was characterized by the second Piola-Kirchhoff (P-K) stress and Green strain in the circumferential and axial directions. Under low shear and the assumption of homogeneity and incompressibility, the deformation gradient was calculated from the diagonal components both locally and globally,

$$F = \begin{bmatrix} F_{11} & F_{12} & & 0 \\ F_{21} & F_{22} & & 0 \\ 0 & 0 & \frac{1}{F_{11} F_{22}} & \\ & & F_{12} & F_{21} \end{bmatrix}$$

where F_{11} and F_{22} are the stretch ratios along the 2 axes in the circumferential and axial directions, respectively, and F_{12} and F_{21} are the in-plane shear components. The deformation gradient was determined for 4 triangles based on dot placement (Figure 1). The local deformation gradient was calculated as the average of these 4 gradients, with markers/triangles excluded in cases exhibiting high

shear stress. From the deformation gradient, the right Cauchy-Green strain tensor, $C = F^T F$, can be calculated and the Green strain, $E = \frac{1}{2}(C - 1)$, obtained. The first P-K stress tensor was calculated along the two axes as $P_{11} = \frac{f_1}{L_2 H}$ (circumferential) and $P_{22} = \frac{f_2}{L_1 H}$ (axial), where f_1 and f_2 are the forces measured by the biaxial load cells, L_1 and L_2 are the in-plane undeformed edge lengths, and H is the undeformed thickness. The second P-K stress tensor was calculated from the first P-K, and the deformation gradient, $S = P F^{-T}$, and the circumferential and axial components were determined.

Determining the LTM and HTM Linear Regions from the Aortic Tissue Behavior Stress-Strain Curve

The LTM and HTM regions were determined by assessing the aortic behavior for deviations from linearity. Convolution of the behavior with a 1-dimensional second-order Gaussian kernel (SOGK) provided a method to detect deviations from linearity. This approach has been used previously in image processing for the assessment of similarities and divergence in images.^{E1} The convolution was performed over a window of 101 strain data points, with a padding of data at the edge of the stress-strain curve equal to one-half the window width. Padding the edges of the data is necessary to avoid a loss of data at the edges and is standard in convolutional methods.^{E2} The result of the convolution produces a secondary description of aortic behavior where the curve converges on a value of 0 when the data within the window approaches linearity and diverges from 0 at points where the curve deviates from linearity. The result of the convolution was assessed within a subset of the data starting at 0.05% strain. Within the window, the first large deviation from 0 was identified as the onset of the transition zone. The end of the transition zone was identified as the convergence to 0 of the convolved curves closest to the end strain of the specimen. Following the identification of the transition zone, regions before and after the transition zone were fit to a first-order curve using linear regression to obtain the LTM, and HTM values were reported.

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