Differential Tensile Strength and Collagen Composition in Ascending Aortic Aneurysms by Aortic Valve Phenotype

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Background. Ascending thoracic aortic aneurysm (ATAA) predisposes patients to aortic dissection and has been associated with diminished tensile strength and disruption of collagen. Ascending thoracic aortic aneurysms arising in patients with bicuspid aortic valve (BAV) develop earlier than in those with tricuspid aortic valves (TAV) and have a different risk of dissection. The purpose of this study was to compare aortic wall tensile strength between BAV and TAV ATAAs and determine whether the collagen content of the ATAA wall is associated with tensile strength and valve phenotype.

Methods. Longitudinally and circumferentially oriented strips of ATAA tissue obtained during elective surgery were stretched to failure, and collagen content was estimated by hydroxyproline assay. Experimental stress-strain data were analyzed for failure strength and elastic mechanical variables: $\alpha$, $\beta$, and maximal tangential stiffness.

Results. The circumferential and longitudinal tensile strengths were higher for BAV ATAAs when compared with TAV ATAAs. The $\alpha$ and $\beta$ were lower for BAV ATAAs when compared with TAV ATAAs. The maximal tangential stiffness was higher for circumferential when compared with longitudinal orientation in both BAV and TAV ATAAs. The amount of hydroxyproline was equivalent in BAV and TAV ATAA specimens. Although there was a moderate correlation between the collagen content and tensile strength for TAV, this correlation is not present in BAV.

Conclusions. The increased tensile strength and decreased values of $\alpha$ and $\beta$ in BAV ATAAs despite uniform collagen content between groups indicate that microstructural changes in collagen contribute to BAV-associated aortopathy.


Bicuspid aortic valve (BAV) is the most common congenital heart malformation occurring in 1% to 2% of the population and is associated with ascending thoracic aortic aneurysm (ATAA) formation and a significantly greater risk of aortic valve disease than the general population [1, 2]. Ascending thoracic aortic aneurysms progress to aortic dissection, rupture, and sudden death. Current expert surgical consensus concludes that patients with ATAA should undergo prophylactic surgery when the maximal orthogonal diameter is 48 to 55 mm, depending on several risk factors [3]. In certain high-risk populations, such as patients with BAV, ATAAs have been known to dissect at smaller diameters [4], suggesting that diameter may not be the best predictor of aortic catastrophe. Alternatively, because aortic dissection or disruption is a biomechanical phenomenon, mechanical models should be developed to better predict risk of aortic catastrophe.

Although the mechanical properties of abdominal aortic aneurysm have been widely studied, data for ATAA are significantly more limited. Okamoto and colleagues [5] demonstrated nonlinear behavior of ATAA under biaxial testing and a decrease in distensibility with age. Our laboratory previously reported directional strength differences of ATAA compared with normal nonaneurysmal aorta [6]. Iliopoulos and associates [7] reported both regional and directional variations of strength and stiffness in ATAAAs. Choudhury and co-workers [8] reported local stiffness changes from biaxial tests for ATAAAs. To our knowledge, no information on aortic wall mechanical strength of ATAAAs with respect to valve phenotype has been reported.

Mechanical behavior of the aortic wall depends on extracellular matrix (ECM) structure and composition. Elastin and collagen determine the mechanical properties of the aortic wall, with distensibility primarily depending on elastin and peak aortic stiffness and tensile
strength provided by collagen [9]. The role of collagen content in ATAA development remains unclear; some investigators have reported no differences in the elastin and collagen content of aneurysmal wall tissues between patients with BAV and patients with tricuspid aortic valves (TAV) [10, 11], whereas others have observed regionally reduced collagen expression [12]. No previous studies examined the relationship between the ECM and the mechanical properties of ATAAas, or whether there is difference caused by aortic valve morphology.

The purpose of this study was (1) to determine whether there is a difference in wall tensile strength between BAV and TAV ATAAas and (2) to determine the relative collagen content within the ATAA wall and its relationship to mechanical properties of the two aortic valve phenotypes.

Material and Methods

Tissue Harvest

Whole, fresh, nondissecting ascending aortic specimens were harvested as an intact tubular structure (Fig 1A) from patients with BAV (age, 54 ± 4 years; diameter, 50 ± 5 mm, mean ± standard deviation; n = 23) and TAV (age, 66 ± 11 years; diameter, 57 ± 14 mm; n = 15) undergoing elective surgery. All tissues were excised after obtaining informed patient consent in accordance with a study protocol approved by our institutional review board. The tissue was harvested from above the sinuses of Valsalva with proximal and distal orientation noted by the surgeon, immersed in saline solution, and kept refrigerated at 4°C until testing. Patient demographics were obtained from clinical records. The maximal diameter of each ATAA was determined by means of computed tomography.

Tensile Testing

Ascending thoracic aortic aneurysm tissues were tested within 48 hours of harvest. The ATAAas were prepared by first removing the adipose tissues and then dissecting circumferentially (CIRC) and longitudinally (LONG) oriented strip specimens (Fig 1B). Specimen width and thickness were measured at three different locations using a caliper (Scienceware, Wayne, NJ) and then averaged. Both ends of each strip were sandwiched between the clamps using sandpaper and cyanoacrylate glue. The clamps were then mounted in a custom-built uniaxial tensile testing machine [13] with a 25-pound load cell and amplifier (Transducer Techniques, Temecula, CA), and LabVIEW 7.1 (National Instruments, Austin, TX) was used to record measurements. The specimen was slowly stretched until a small increase in load was observed, and the initial specimen length was noted. The specimens were preconditioned and then stretched until specimen failure as described previously [13]. The applied force and corresponding displacement were collected synchronously and continuously at the sampling rate of 10 Hz until specimen failure.

Biomechanical Data Analysis

As described previously [13], stress and strain were calculated from force-displacement data and initial specimen dimensions. Briefly, the Cauchy stress (T) within the specimen was calculated as the applied force normalized by deformed cross-sectional area, and the strain (ε) was calculated as deformation normalized by original specimen length. The maximal tangential stiffness (MTS) was taken as the maximal slope (ie, maximal resistance to deformation), and the tensile strength was taken as the peak stress obtained before failure from the stress-strain curve [6]. We used our prior model to further characterize the biomechanical response of the aneurysm wall [14]. The model relates the stress in a uniaxial loaded specimen to the stretch (λ = ε + 1) through the following equation:

\[
T = \left[ 2\alpha + 4\beta \left( \lambda^2 + 2\lambda^{-1} - 3 \right) \right] \left[ \lambda^2 - \lambda^{-1} \right]
\]  

(1)

where α and β are model parameters indicative of the mechanical properties of the tissue. The mathematical
model was fit to the elastic region of each experimentally
derived T - λ data sets (ie, before yield) using the
Marquardt-Levenberg least squares nonlinear regression
algorithm in Sigma Plot 11.0 (Systat Software, Inc, San
Jose, CA). A representative model was fit for stress-
stretch data (Fig 2).

Collagen Content Assessment

TRICHROME STAINING. Aortic specimens (approximately 1 x
0.5 cm²) were fixed in 10% buffered formalin and
embedded in paraffin. Four-micrometer sections were
stained using Masson’s trichrome (Research Histology
Services, University of Pittsburgh Thomas E. Starzl
Transplantation Institute, Pittsburgh, PA) to qualitatively
assess collagen composition. Slides were visualized using
a Nikon TE-2000-E inverted microscope and captured
using a Nikon DS-Fi1 5MP color camera and NIS Ele-
sments Software (Nikon, Melville, NY).

HYDROXYPROLINE. Total collagen content was determined
for a subset of samples from each group using a modifi-
cation of a hydroxyproline (HYP) assay [15, 16]. After
tensile testing, a small portion of flanking tissue (50 to 100
mg) from both sides of the failure region was minced
(approximately 1 x 1 mm) specimens (n = 49 for BAV;
n = 26 for TAV) and lyophilized. Specimens were flame-
sealed in 10-mm Pyrex tubes with 100 µL of 6N HCl
containing 0.5% (v/v) phenol, incubated at 110°C for
24 hours, and dried under vacuum. The dried sample was
resuspended with 1% (v/v) HCl, incubated at 50°C, and
then neutralized with an equivalent amount of NaOH.
Chloramine T reagent (0.056 mol/L) was added, followed
by incubation at room temperature for 25 minutes.
Freshly prepared Ehrlich’s reagent (1 mol/L) was added,
followed by incubation at 65°C for 20 minutes. Absor-
bance of replicates was measured at 550 nm in a
spectrophotometer (SpectraMax M2, model D02667, Mo-
olecular Devices, LLC, Sunnyvale, CA). Experimental HYP
levels were normalized to the mass of dry tissue.

Statistical Analysis

All data are presented as mean ± standard error of the
mean. Student’s t test was performed to compare the
mean values between the groups using Sigma Plot 11.0
(Systat Software Inc, Chicago, IL). Significance was
assumed for a probability value of less than 0.05.

Results

A total of 23 ATAAs with BAV and 15 ATAAs with TAV
were included in this study. The average maximal
orthogonal diameter of the aorta was 50 ± 5 mm for BAV
versus 57 ± 14 mm for TAV (p = 0.15). The age discrep-
ancy noted is consistent with the clinical observation that
BAV ATAAs present 10 to 20 years earlier than patients
with TAV and ATA [17]. From these ATAAs, 178 sam-
ples were tensile tested; 15 of those were either slipped or
broke at the clamp, and hence were discarded from data
analysis. There was no significant difference between the
tensile test data normalized per patient and the non-
normalized data of all specimens averaged for the study
group. Thus, nonnormalized values averaged for the
whole group are reported here.

For BAV tissues, the CIRC and LONG tensile strengths
were 165.6 ± 9.8 N/cm² and 69.8 ± 3.1 N/cm², respectively
(Table 1; Fig 3). For TAV, these were 96.1 ± 6.1 N/cm² and
54.0 ± 3.7 N/cm², respectively. In both BAV and TAV, the
CIRC oriented samples were stronger than LONG ori-
tented samples (p < 0.05), and the strength of BAV was
stronger than TAV in both orientations (p < 0.05). The
average percentage of failure strain for BAV in CIRC and
LONG orientations is shown in Table 1.

The MTS of BAV appeared to be higher when
compared with TAV CIRC specimens and lower than
TAV in LONG specimens (Table 1). In BAV the MTS was
350.4 ± 16.0 N/cm² and 191.6 ± 9.6 N/cm² for CIRC and
LONG specimens (p < 0.05), respectively. Similarly, in
TAV the MTS was 335.1 ± 22.2 N/cm² and 220.7 ± 20.3
N/cm² for CIRC and LONG specimens (p < 0.05), respec-
tively.

The average closeness-of-fit of the biomechanical model
described in Equation 1 to the experimental stress-stretch
data was found to be greater than 99% (r² > 0.99 ± 0.001).
The mean value of α was not significantly different
in LONG and CIRC specimens for either BAV or TAV
(Table 1; TAV, 6.7 ± 2.3 N/cm² versus 6.2 ± 0.8 N/cm²;
BAV, 4.8 ± 0.3 N/cm² versus 4.5 ± 0.4 N/cm²). However, α is
significantly lower in BAV LONG specimens compared
with TAV LONG (p < 0.05). The β is not significantly
different between CIRC and LONG specimens in TAV
(Table 1; 71.0 ± 22.2 N/cm² versus 120.1 ± 31.5 N/cm²; p =
0.1), but was significantly different within BAV (12.3 ± 1.1
N/cm² versus 18.1 ± 1.8 N/cm²; p < 0.005). In addition, the β
is significantly lower in BAV compared with TAV in both
orientations (p < 0.005).

Trichrome staining of ATAAs revealed cystic media
degeneration, elastin fragmentation, and a dispropor-
tionate distribution of collagen among BAV and TAV,
suggesting increased collagen in BAV (Fig 4). However,
there was no difference in HYP for BAV and TAV (18.9 μg/mg ± 1.9 versus 19.0 ± 1.5, respectively; \( p = 0.17 \); Table 1). For TAV, a moderate correlation between the collagen content and tissue strength was demonstrated (\( r = 0.359 \); Fig 5), but this correlation was not seen in BAV tissues (\( r = 0.014 \)).

**Comment**

Aortic dissection or rupture represents a mechanical failure of the aortic wall, which can occur when aortic wall integrity is altered. Despite a higher percentage of patients with BAV compared with TAV among the aortic dissection population than among the general population [18], no investigation has been done, to our knowledge, on the condition of ATAA wall tensile strength with respect to valve phenotype. This study found differences in the wall strength between ATAAs from BAV and TAV patients. The tensile strength in the CIRC orientation was higher than in the LONG orientation for both BAV and TAV ATAAs. Tensile strength of BAV ATAAs was higher when compared with that of TAV ATAAs for both the CIRC and LONG orientations. The directional differences in the tensile strength of the ATAA wall are consistent with the demonstrated anisotropic nature of the ATAA wall as previously reported by our group and others and is consistent with lower tensile strength of abdominal aortic aneurysms (AAAs) and ATAAs when compared with normal aortas [5–7, 19, 20]. Moreover, Iliopoulos and associates [7] reported spatial variation in the material variables such as strength and stiffness of ATAAs.

Our qualitative, histologic observation of increased collagen in BAV compared with TAV (Fig 4) agrees with a prior report from our team that demonstrated elevated type I collagen mRNA [21], and from data reported by Choudhury and colleagues [8]. Further, Wang and coworkers [22] demonstrated increased collagen deposition in aortic dissection. Iliopoulos and colleagues [19] documented higher levels of collagen histologically in ATAAs when compared with normal aortas. On the other hand, our quantitative assessment of collagen content by HYP assay showed no difference between BAV and TAV, which is consistent with reports by Fedak and associates [10] and LeMaire and coworkers [11]. Collectively, these data suggest that differences in mechanical

<table>
<thead>
<tr>
<th>Variable</th>
<th>α (g/mg)</th>
<th>β (g/mg)</th>
<th>MTS (kPa)</th>
<th>Failure Strain (%)</th>
<th>Tensile Strength (kPa)</th>
<th>HYP (μg/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TAV CIRC</td>
<td>6.8 ± 2.3</td>
<td>71.0 ± 22.2</td>
<td>335.1 ± 22.2</td>
<td>60.7 ± 4.1</td>
<td>96.1 ± 6.1</td>
<td>19.0 ± 1.5 (all TAV)</td>
</tr>
<tr>
<td>TAV LONG</td>
<td>6.2 ± 0.8</td>
<td>120.0 ± 31.5</td>
<td>220.7 ± 20.3</td>
<td>46.9 ± 3.1</td>
<td>54.0 ± 3.7</td>
<td>(all TAV)</td>
</tr>
<tr>
<td>BAV CIRC</td>
<td>4.8 ± 0.3</td>
<td>12.3 ± 1.1</td>
<td>350.4 ± 16.0</td>
<td>91.7 ± 3.7</td>
<td>165.6 ± 9.8</td>
<td>18.9 ± 1.9 (all BAV)</td>
</tr>
<tr>
<td>BAV LONG</td>
<td>4.5 ± 0.4</td>
<td>18.1 ± 1.8</td>
<td>191.6 ± 9.6</td>
<td>63.4 ± 2.2</td>
<td>69.8 ± 3.1</td>
<td>(all BAV)</td>
</tr>
</tbody>
</table>

\( * p < 0.05 \) TAV CIRC compared with TAV LONG. \( * * p < 0.05 \) TAV LONG compared with BAV LONG. \( * * * p < 0.05 \) BAV CIRC compared with TAV CIRC. \( * * * * p < 0.05 \) BAV LONG compared with BAV CIRC.

BAV = bicuspid aortic valve; CIRC = circumferential; HYP = hydroxyproline; LONG = longitudinal; MTS = maximal tangential stiffness; TAV = tricuspid aortic valve.
properties are not attributable to absolute collagen content, but may be accounted for by microstructural changes in the collagen framework within the ECM (ie, collagen architecture) as we recently revealed in native aortic specimens [23].

Although the tensile strength of BAV is significantly stronger when compared with TAV in both CIRC and LONG orientations, the total HYP was equivalent in both groups. The finding of differential correlation between collagen content and tensile strength in TAV ($r = 0.359$) compared with BAV ($r = 0.014$) may indicate distinguishable architecture. Our finding suggests the proportional differences in tensile strength between BAV and TAV cannot be explained by alterations in total collagen content.

The aortic walls of BAV and TAV exhibit differences in mechanical behavior as characterized by our nonlinear elastic biomechanical model. The material variables ($\alpha$, $\beta$) represent the initial stiffness of the material in the nonlinear stress-stretch curve in the elastic region up to MTS for each specimen tested: $\alpha$ represents the shear modulus for infinitesimal deformation of structural fibers, and $\beta$ is a stiffening variable or bulk modulus of the tissue. We tested this model to predict which of these two variables influences the stress-stretch curves with one of the variables kept constant while varying the other variable. We found that $\beta$ influences the model 12 times more than $\alpha$ when the difference in simulated stress for incremental $\beta$ increase was divided by the difference in stress for the corresponding incremental $\alpha$ increase at the maximal failure stretch of 1.9. For BAV, $\alpha$ is lower by 28% and 26%, in the CIRC and LONG orientations, respectively, and $\beta$ is lower by 83% and 85%, respectively, when compared with TAV. The fact that $\alpha$ and $\beta$ were much lower with BAV when compared with TAV indicates that fewer structural fibers were engaged to bear the initial stretch load in BAV. This low $\alpha$ and $\beta$ in BAV suggests that collagen may be more coiled or disrupted as a result of microstructural changes of the collagen network within the ECM. $\beta$ is higher in LONG when compared with CIRC for BAV and TAV. A significantly lower value of $\beta$ in CIRC for BAV indicates a possibility of immature organization of collagen fibers in CIRC. Furthermore, $\beta$ within TAV between CIRC and LONG oriented specimens does not vary.
ADULT CARDIAC

with TAV. Previously we reported no difference in

ables suggests there are distinct architectural

total collagen material property characteristics are strongly associ-

cation. The current data suggest that the ATAA wall

TAV compared with the average for all ATAA re-

longer MTS of LONG indicates that BAV ATAA became stiffer after initial stretching in the
circumferential direction, and lower MTS of LONG in-

tinctions in the ECM in the two phenotypes that

Previously, we observed increased MTS in ATAA when compared with normal aortas [6]. In the current

highly compliant in the longitudinal direction. The higher difference between

collagen content despite different biomechanical material vari-

Fig 5. Collagen content, as estimated by hydroxyproline (HYP), as a

function of tensile strength for each tricuspid aortic valve (TAV; A)
and bicuspid aortic valve (BAV; B) specimen tested. Note a moderate

correlation (r = 0.359) between the collagen content and strength for
tricuspid aortic valve, but not for bicuspid aortic valve (r = 0.014).

significantly, suggesting a more mature organization of
collagen fibers in both orientations for TAV, which is

consistent with our recent observation of increased mature collagen in TAV versus BAV ATAA [23].

Okamoto and associates [5] reported differences in the

properties of BAV and TAV using an exponential

constitutive model in biaxial testing, which were

consistent with reduced α and β in BAV compared with

TAV. Previously we reported no difference in α

and β between LONG and CIRC of ascending

aortic aneurysms, but ATAA showed a difference

between LONG and CIRC for β [6, 13]. Both α and β of

ATAA were reduced when compared with ascending

aortic aneurysms, and for ATAA β is higher in LONG

(53 N/cm²) than in CIRC (17 N/cm²). A similar trend is

now observed in BAV and TAV as shown in Table 1.

However, the value of β is lower in BAV and higher in

TAV compared with the average for all ATAA re-

ported earlier in both LONG and CIRC oriented

direction. The current data suggest that the ATAA wall

material property characteristics are strongly associ-

ated with valve phenotype. Equivalent total collagen

content despite different biomechanical material vari-

ables suggests there are distinct architectural

differences of the ECM in the two phenotypes that

impart the mechanical property differences.

Previously, we observed increased MTS in ATAA when compared with normal aortas [6]. In the current study, we showed that the MTS measured in the failure region is higher in CIRC than in LONG for both TAV and BAV, which seems contrary to the stiffness measured in the elastic region in both cases. This may be because at higher stretch, all the structural fibers of the tissue may be engaged or aligned in the preferred direction to resist deformation. Circumferential MTS of BAV was the highest and LONG MTS of BAV was the lowest when compared with the corresponding orientation of TAV. Higher MTS of CIRC indicates that BAV ATAA become stiffer after initial stretching in the circumferential direction, and lower MTS of LONG indicates that BAV ATAA become more compliant in the longitudinal direction. The higher difference between CIRC and LONG stiffness of BAV implies that BAV ATAA are more anisotropic when compared with TAV ATAA. The increased anisotropic nature of BAV could explain why BAV ATAA tend to develop in the characteristically asymmetric pattern that they do along the greater curvature of the proximal ascending aorta. To date, our observations of aortic wall biomechanics including alterations in tensile strength, elastic constitutive material modeling, and tangential stiffness all support our overarching hypothesis that aneurysm formation in BAV patients is caused by alterations in ECM architecture rather than collagen content.

A potential limitation of the study is the age discrepancy between patients with BAV and TAV ATAA, such that aging may compound architectural changes [4, 17]. However, we have previously shown that patient age and aneurysm diameter do not impact the delamination strength of the aortic media [24]. We proposed that reduced medial delamination strength could contribute to a greater risk of dissection in BAV compared with TAV ATAA patients [24]. In this study, we linearly extrapolated the strength of BAV to the corresponding age of TAV. When the tensile strength of BAV was extrapolated to that of the age of TAV, strength of BAV remained higher than TAV (Fig 6).

This study is the first to our knowledge to compare material property, directionally dependent strength, and collagen content in the same ATAA specimens between BAV and TAV ATAA. We showed that BAV ATAA have greater tensile strength in the CIRC and LONG directions and greater failure strain than TAV ATAA. These data suggest that absolute collagen content does not explain the differences found in the mechanical properties tested for BAV and TAV ATAA. Rather, given the marked differences in both strength and failure strain, these biomechanical differences support the notion that microstructural differences arise in the collagen framework of the ECM in BAV compared with TAV. Ongoing investigations are focused on the role that matrix architecture plays in region-specific biomechanical strength of the BAV aorta and its pathologic propensity.
The authors thank Benjamin Green and Michael Eskay for their generous help in tissue collection and transportation and Kristin Valchar, Michelle Shirey and Erica Butler for their assistance in IRB procedures and informed patient consent. This research work was supported by grants R01-HL060670-09 (D.A.V.), R01-HL086418-04 (D.A.V.) and R01-HL09132-01 (T.G.G.) from the National Heart, Lung, and Blood Institute of the National Institutes of Health. We certify that this manuscript has not been previously published and is not being considered in whole or in part in any other scientific journal. All authors are aware of and agree to the content of the manuscript. None of the authors have any conflict of interest to declare.

References


INVITED COMMENTARY

Pichamuthu and colleagues [1] have reported an elegant biomechanical study that reveals significant differences in tensile strength of ascending thoracic aortic aneurysms (ATAAs) associated with bicuspid (BAV) compared with tricuspid (TAV) aortic valves. For the first time, BAV ATAAs were shown to have surprisingly higher tensile strength than TAV ATAAs in both the circumferential and longitudinal directions. The circumferential BAV ATAA tensile strength reported here (165.6 ± 9.8 N/cm²) is similar to that reported previously for normal ascending aorta (180 ± 24 N/cm² [2] and ~165 N/cm² [3]), suggesting BAV aneurysms may not be weaker than normal in the circumferential direction. However, TAV ATAA tensile strength is much weaker than normal in the circumferential direction (96.1 ± 6.1 N/cm² TAV).

Data from the International Registry of Acute Aortic Dissection [4] showed that BAVs accounted for about 2.6% of ATAAs that dissected with a diameter of less than 5.5 cm, increasing to 6.5% for 5.5 cm or larger, but not statistically significant. Other groups also suggest that BAV ATAA dissect and rupture at sizes comparable to non-BAV ATAAs [5, 6].

Pichamuthu and colleagues also demonstrate another important finding: the wall strength in the circumferential direction is substantially greater than that in the longitudinal one for both BAV and TAV ATAAs. These results agree well with the study of Iliopoulos and colleagues [3], where circumferential strength was significantly greater than longitudinal strength for ATAA and normal controls. A lower longitudinal tensile strength reconciles well with clinical findings where primary tears of aortic dissection most frequently occur along the transverse direction [7]. If circumferential tensile strength were similar in both circumferential and longitudinal directions, then more tears in the longitudinal direction would be observed. Of note, BAV and TAV longitudinal tensile strength are both much weaker than normal (69.3 ± 3.1 N/cm² BAV, 54.0 ± 3.7 N/cm² TAV vs 171 ± 14 N/cm² normal [2] and ~105 N/cm² normal [3]). Lower longitudinal strength of both BAV and TAV ATAAs compared with normal may theoretically account for their increased risk of dissection. However, the findings by Pichamuthu and colleagues are at odds with a prior report from Vorp and colleagues [2] showing similar strength in both longitudinal and circumferential directions for ATAAAs and normal controls. Likely, greater numbers and further tests will be required to verify the findings.

Understanding the biomechanics of aortic dissection or rupture will require further study combining wall strength assessment with patient-specific finite element models of ATAA wall stress to determine when wall stress exceeds wall strength.

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