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In Vitro Characterisation of Physiological and Maximum Elastic Modulus of Ascending Thoracic Aortic Aneurysms Using Uniaxial Tensile Testing

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Abstract

Objective
Ascending thoracic aortic aneurysms (ATAA) are a life-threatening condition due to the risk of rupture or dissection. This risk is increased in the presence of a bicuspid aortic valve (BAV). The purpose of this study was to provide data on the elastic modulus of aortic wall of ATAA using uniaxial tensile testing in two different areas of the stress–strain relationship: physiological and maximum range of stresses. The influence of tissue location, tissue orientation and valve type on these parameters was investigated.

Materials and methods
Tissues freshly excised from ATAA with bicuspid or tricuspid aortic valve were obtained from greater and lesser curvature (GC and LC) and the specimens were tested uniaxially in circumferential (CIRC) and longitudinal (LONG) orientation. Maximum elastic modulus (MEM) was given by the maximum slope of the stress–strain curve before failure. Physiological modulus (PM) was derived from the Laplace law and from ranges of pressure of 80–120 mmHg. Means of each group of specimen were compared using Student’s t-test to assess the influence of location, orientation and valve type on each mechanical parameter.

Results
PM was found to be significantly lower than the MEM (p < 0.001). The MEM and PM were significantly higher (p < 0.01) in the CIRC (n = 66) than in the LONG orientation (n = 42). The MEM was higher in the circumferential orientation in the BAV group (p < 0.001 in GC and p < 0.05 in LC). MEM and PM in GC specimens were higher in the longitudinal orientation than the LC specimens (p < 0.05).

Conclusion
This study demonstrates the anisotropy of the aortic wall in ATAA and provides data on the mechanical behaviour in the physiological range of pressure.

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I. Introduction

The biomechanical properties of the aorta have been extensively studied over the past decades [1], [2], [3], [4], [5], [6], [7], [8], [9] and [10] and are still the subject of a growing interest to provide a better understanding of aortic dissection and aneurysm and predict the behaviour of stent grafts. These aortic diseases are associated with changes in the mechanical properties of the aortic wall. The characterisation of the biomechanical behaviour of the aortic wall is a potential tool for the prediction of growth and rupture of aneurysms and consequently may help clinicians in their decision-making process. In addition, the accuracy of in vitro experiments and computational analysis depends significantly on the mechanical properties assigned to the aortic wall. Mechanical testing of tissue specimen is one of the most widespread methods to gain this knowledge.

Ascending thoracic aortic aneurysms (ATAA) are a life-threatening condition that could benefit from such a prediction tool. They affect approximately 59 of 100,000 people in the United States every year [11]. Biomechanical properties of ATAA have been studied by Choudhury et al. [1]. Okamoto [4], Vorp [8] and Peterson [12] A large panel of different mechanical parameters, such as tensile strength, maximum elastic modulus, incremental modulus and others, has been investigated in the literature. Similarly, different stress–strain curve definitions have been proposed in the literature to study the elasticity of the aneurysmal wall. For example, Raghavan et al. [13] determined the mechanical properties of the abdominal aortic aneurysm as compared to non-aneurysmal aorta using uniaxial tensile testing and the stress–strain curve was plotted using true stress versus engineering strain. This method for the calculation of the elastic modulus was used in many other publications [8], [14], [15] and [16] although none of them explained the rationale for this choice. Another combination – engineering stress versus engineering strain [1] and [7] – has also been used where only the initial dimensions of the tissue at rest are considered. Given that specimens undergo large deformation in uniaxial testing, the initial dimensions are only remotely related to the range of strain where tissue failure occurs. We propose that the true stress–true strain relationship is the only method that truly takes into account the actual variation of the dimensions of the specimen during tensile testing. This method that has never been used in the bioengineering literature is proposed here as the method of reference.

Another issue found in the biomechanical engineering literature is that most studies deal with the failure behaviour of the aorta. The mechanical parameter given by this particular range of the stress–strain relationship is often referred to as the maximum elastic modulus. The pitfall of such analysis is the lack of data regarding the physiological range of the stress–strain relationship. Thus, it is possible to identify two areas of interest from the stress–strain relationship, which correspond to the physiological and failure range of the aorta. The purpose of this study was to determine the elastic modulus of the wall of ascending aortic aneurysms in these two areas using the true stress–true strain definition. Results are then compared between specific tissue locations (greater vs. lesser curvature) and tissue orientations (longitudinal vs. circumferential). The impact of a bicuspid aortic valve (BAV) which is thought to correlate with abnormal wall elasticity was also investigated.

II. Materials and methods

II.1. Human aortic tissue specimens

All procedures were carried out in accordance with the guidelines of the Institutional Review Board of the University of Michigan. A segment of aortic wall was excised from surgical specimens obtained from patients undergoing elective surgical repair of their ATAA. These segments were cut with custom-designed tissue cutters and multiple test specimens were
available when the surgical aortic specimen was large enough. Samples were obtained from two different locations, greater curvature (GC) and lesser curvature (LC). The maximum diameter of ATAA was known from the patient's preoperative Computed Tomography Angiography (CTA). The tissue specimens were stored in gauze wetted with saline and refrigerated at 4 °C. Tissue-testing was performed within 48 h. After equilibration at room temperature, connective and adipose tissue were removed from the surface of the adventitia and the samples were cut along either circumferential (CIRC) or longitudinal (LONG) orientation (Figure 1). The original thickness and width of the sample were measured at zero stress state with a digital caliper.

II.2. Uniaxial tensile testing

Experiments were carried out using a tensile testing machine (Instron® model 5542, Norwood, MA, USA). To avoid damaging the tissue fine grit adhesive sandpaper was placed on the surface of the pneumatic grips to prevent slippage during the test. To ensure a free shear deformation and local narrowing of the tissue between the grips, it was found during preliminary testing that an aspect ratio of at least 2 was required (the aspect ratio is defined as the ratio of gauge length divided by the width of the specimen). Once the tissue was in place, the pressure line operating the grips was slowly raised (to prevent crushing the sample) to 20 psi. The tissue was kept wet by a spray of phosphate buffering solution. Each specimen was preconditioned by applying two cycles of a 1 N load at 10 mm min$^{-1}$ to decrease the effect of relaxation in the mechanical response. Then the tensile testing was performed at 10 mm min$^{-1}$ until failure. Load and stretch were continuously recorded by the data acquisition software Merlin® provided by Instron®.

II.3. Data analysis

The load–stretch curve was derived to obtain the true stress–true strain relationship, which was considered as our reference method. This choice of method takes into account the large deformation of the specimen and subsequently the actual change of dimension of the specimen during testing. The aortic wall was assumed to be an incompressible material [17]. In order to calculate the true stress and true strain, we need to define engineering stress and engineering strain which depend on the initial dimensions of the tissue. The engineering stress is:

$$\sigma_E = \frac{F}{A_0}$$

where $F$ is the load and $A_0$ is the initial cross-sectional area.

The engineering strain is:

$$\varepsilon_E = \frac{\lambda}{L_0}$$

where $\lambda$ is the stretch and $L_0$ the initial length.

Then we define the true stress:

$$\sigma = \frac{F}{A}$$

where $A$ is the current cross-sectional area.

The true strain is defined by:

$$d\varepsilon_T = \frac{dL}{L}$$

where $dL$ is the instantaneous stretch and $L$ the current length of the specimen. The assumption of the arterial incompressibility implies a zero change of volume during the tensile testing:

$$A x L = A_0 x L_0$$

and then

$$\sigma_T = \frac{F}{A} = \frac{FL}{A_0 L_0} = \sigma_E x \frac{L_0 + \lambda}{L_0} = \sigma_E (1 + \varepsilon_E)$$
The true strain is defined as the sum of all the current engineering strains. Then
\[ \varepsilon_T = \int d\varepsilon = \int_0^1 \frac{dL}{L} = \ln \frac{L}{L_0} = \ln \frac{L_0 + \lambda}{L_0} = \ln(1 + \varepsilon_e) \]

To characterise the physiological modulus (PM) of the specimen, the Laplace law was derived to obtain the circumferential stress \( \sigma_\theta \), assuming the aneurysm to be of nearly cylindrical shape:
\[ \sigma_\theta = \frac{P \times R}{t} \]
where \( P \) is the pressure, \( R \) the radius of the aneurysm and \( t \) the thickness of the specimen. Similarly, the longitudinal stress \( \sigma_l \) was given by
\[ \sigma_l = \frac{P \times R}{2t} \]
Pressure range of 80–120 mmHg was chosen as the physiological range (Figure 2). Considering that the stress–strain curve is nearly linear within this short range, a linear fitting was applied yielding the PM calculated as the slope of the fitted line. The maximum elastic modulus (MEM) was taken from each curve as the maximum slope prior to failure (Figure 2).

Data processing was performed on Excel 2007® (Microsoft Corporation). Statistical analysis was performed on SPSS Statistics 17.0® (SPSS Inc., Chicago, USA and supplied by the University of Michigan, USA). Results of each testing were averaged on a per patient basis. Results of each group of specimens were given as the mean of the patients' data in order to account for the different number of testings from one patient to another. Student's t-test was performed to assess the influence of location, orientation and valve type on each mechanical parameter. Significance was assumed for a \( p \) value less than 0.05.

III. Results
One hundred and eight specimens were obtained from 12 patients with ATAA. Six patients had a BAV. Table 1 summarises the patient characteristics. Sixty-five testings were performed in GC and 43 in LC. Sixty-six specimens were tested circumferentially and 42 longitudinally. In the tricuspid aortic valve (TAV) group, specimens were significantly thicker in LC than GC. Tissues from BAV were significantly thicker than TAV in GC location (Table 2). No outlier was found in each set of testing for each patient and each group (location, orientation and valve type), demonstrating the consistency of the results. In 26 cases, the data could not be processed for the calculation of the mechanical parameters because the failure point was not reached for mechanical reasons such as slippage of the tissue. Results are shown in Table 3.

The results of this study showed that the effect of location (GC vs. LC) on the MEM results was not significant in the CIRC orientation group. In the LONG orientation, the MEM was found significantly higher in GC than LC (\( p < 0.05 \) in the BAV group and \( p < 0.01 \) in the TAV group). Similarly, the PM was significantly higher in GC than LC in the LONG orientation group (\( p < 0.01 \) in the BAV group and \( p < 0.05 \) in the TAV group). The PM was also found to be higher in GC than LC circumferentially in the BAV group with a \( p = 0.045 \).

When comparing orientations, the MEM was found to be significantly higher in the CIRC than in the LONG group (\( p < 0.01 \)) ([Figure 3] and [Figure 4]). Similarly, the PM was significantly higher circumferentially regardless of location and valve type (\( p < 0.01 \)) ([Figure 5] and [Figure 6]). The MEM was compared between the BAV and TAV groups in both locations and both orientations. In the CIRC group, the MEM was significantly higher in the BAV than in the TAV group (\( p < 0.001 \) in the GC group and \( p < 0.05 \) in the LC group). The PM was compared between the valve types as well and the only significant difference was found in the LC LONG group where TAV specimens were stiffer than their BAV counterparts (\( p < 0.05 \)).

The MEM and PM results were compared in each group of tissue. The MEM values were significantly higher in every group with \( p < 0.001 \).
IV. Discussion

Cardiovascular diseases are one of the leading causes of death in the modern world. ATAA is one of the most serious conditions as it can cause death by rupture or dissection. Since the advent of powerful computational software to perform finite element studies, the aortic diameter criterion has become insufficient to accurately determine patients eligible for surgical repair, especially to predict the likelihood of rupture of smaller aneurysms. Computational assessment of aneurysms on a patient-specific basis is one of the major challenges in the field of vascular surgery. This technology can be applied to the evaluation of new surgical devices such as aortic endografts. Knowledge of aortic wall behaviour is critical in improving the accuracy of computational analysis.

The mechanical parameters of the aortic wall have been studied by many researchers using both uniaxial and biaxial tensile testing. The common calculation methods found in the literature are the true stress versus engineering strain relationship and the engineering stress versus engineering strain relationship. These methods are relevant only for strain lower than 10%. Aortic specimens in uniaxial testing commonly undergo much larger strain, up to 60–70%. The problem with these methods is that they do not give a true indication of the deformation characteristics of the human tissue since they are based entirely on the original dimensions of the specimen and these dimensions change continuously during the test. The actual variation of the current cross-sectional area and length of the tissue must be taken into account and hence, under the assumption of the incompressibility of the tissue, the true stress–true strain relationship was introduced in the present study. The results presented in this study highlight many points that are discussed ahead.

IV.1. Effect of the location

The comparison of MEM between GC and LC locations showed no difference in the CIRC orientation whereas the difference was significant in the LONG group, where the GC specimens were stiffer than their LC counterpart. The effect of the location in MEM values of ATAA has been studied by Choudhury et al. [1] who reported that the MEM was stiffer in the GC than LC for both orientations but without significance. Our results confirm this trend in the longitudinally oriented specimens. Thubrikar [16] studied yield stress and strain of abdominal aortic aneurysms and observed regional variation in wall stiffness. Our results suggest that theoretically, under the same pressure, the LC is sustaining more elongation than its GC counterpart. Perhaps the 3D movement of the aorta from the root to the left subclavian artery is accounting for the local variation of the elasticity. As was proposed by Prehn [18], the dynamic cine-CTA seems a promising technique to investigate the complex 3D movement of the ascending aorta. Clinical observations show that aortic dissection is usually present along the greater curvature of the ascending aorta. This may be explained by our finding that the GC has a higher MEM than the LC in longitudinal direction. As such, the GC will experience higher stresses than the LC and consequently will be a potential site for rupture or dissection.

IV.2. Comparison between BAV and TAV

BAV is commonly related to abnormalities of the thoracic aorta, such as dilated aortic root, dissection and aortic coarctation [19]. Aortic stiffness was found to be increased and distensibility decreased in BAV patients assessed by echocardiography [20]. The MEM was found significantly higher in the BAV group in the CIRC orientation and both locations. The difference in mechanical properties between BAV and TAV was studied by Choudhury et al. [1] and their results showed that the BAV specimens were stiffer, as was observed in our study in the CIRC group.

IV.3. Effect of the orientation

The anisotropy of the aortic wall is still a debated issue and conflicting results are found in the literature. In the present study, both MEM and PM were found to be significantly higher in the CIRC group than the LONG one, suggesting that the aortic wall in ATAA is an anisotropic
material. Vorp [8] studied ATAA using uniaxial tensile testing and the true stress–
engineering strain definition. The MEM was 4.67 ± 0.42 MPa and 4.48 ± 0.59 MPa in the
CIRC and LONG orientations respectively. Using the same stress–strain definition, we
observed the same range of values in our study in the CIRC group. However, the MEM was
significantly lower in the LONG group, regardless of the location and valve type. Peterson
[12]s studied ATAA using biaxial testing and reported one of six patients with stiffer specimen
in the circumferential orientation. Tissues were found isotropic in biaxial testing performed
by Choudhury et al. [1] Okamoto et al. [4] also studied ATAA using biaxial testing and found
the tissue 'somewhat anisotropic'. Healthy thoracic aorta has been studied by Vorp [8] and no
difference was found between the two orientations. Ferraresi [21] performed uniaxial testing
of porcine aortic root tissue and reported the CIRC-oriented tissues to be stiffer than their
LONG counterpart. The issue of arterial wall anisotropy has been thoroughly investigated by
Zhou and Fung [22] They performed biaxial tensile testing on canine thoracic aorta and
demonstrated a significant anisotropy. This issue has been addressed in abdominal aortic
aneurysms (AAA) as well. Thubrikar [16] studied AAA using uniaxial testing in both
orientations and reported that the CIRC-oriented specimens were stiffer than their LONG
counterpart. Conversely, AAA were found isotropic by Raghavan [13]. Healthy abdominal
aorta demonstrated different MEM according to Xiong, [23] but no statistical analysis was
available to assess the significance.

The histological structure of the wall, especially the orientation and integrity of elastin and
collagen fibres, is related to the question of anisotropy and was studied by He and Roach [15]
in aneurysmal and healthy abdominal aortas. They found that in a healthy aorta, the media is
organised in lamellar units composed of elastin layers while in AAA the media lamellar units
were damaged and the elastin fragmented. These findings suggest that healthy tissue is
anisotropic and that, theoretically, the anisotropy is partially or fully lost in aneurysmal

tissue. Fibres are known to be arranged circumferentially mainly [21]. Orientation of collagen
fibres has been investigated in uniaxially stretched arterial tissue. Holzapfel [5] found that
collagen fibres have angles with the circumferential axis of 18.8°, 37.8° and 58.9° in the
intima, media and adventitia respectively. Using X-ray scattering technique to measure fibre
orientation in the adventitia, Schmid [24] observed a predominant circumferential alignment
of collagen fibres implying an increased circumferential stiffness. In our study, the aortic wall
was significantly anisotropic and the tissue was stiffer circumferentially. As described earlier,
our results are consistent with many of the previous studies. From a mechanical point of view,
the predominant deformation of aortic wall in physiological condition is taking place
circumferentially whereas longitudinally, the wall is pre-stretched and tethered and the
elongation under pressure change is low. In addition, if the aorta is considered as a cylindrical
shell of incompressible wall material, the derived Laplace law yields a longitudinal stress,
which is half of the circumferential stress. It can then be considered that the anisotropy of the
aortic wall meets these mechanical requirements.

IV.4. Physiological elastic modulus

It is widely admitted that the arterial wall is non-linear material. Besides the failure
conditions, the elasticity of the aortic wall has to be studied in an additional region of interest

corresponding to the physiological range of stress. The proposed method represents an
attempt to better understand the mechanical behaviour of ATAA wall in the physiological
range and aims to provide an actual value of elastic modulus for computational analysis and
hence, a gain of accuracy. It can also help in the choice of the appropriate material when
designing aortic bench-top models. Sokolis [7] studied pig aortas using uniaxial tensile testing
and the engineering stress–engineering strain definition. The stress–strain curve was divided
into three parts where part II was considered as the physiological range, corresponding to the
slope transition. The physiological range was also observed in the same region of the curve in
our results. This nearly linear part was then fitted as was done in the present study. However,
the physiological range of stress was set at 20–100 kPa, which is well below physiological
aortic pressures. Our study calculated the stress taking into account the dimensions of the tissue and the aneurysm and a physiological variation of pressure. These results can be compared to the values obtained from the incremental modulus method, as described by Thubrikar [16]. In his study, the incremental modulus was defined as the differentiate function of the stress–strain relationship. A pressure of 100 mmHg was then chosen to calculate the elastic modulus in both orientations, yielding values of 4 MPa in the CIRC and 1.5 MPa in the LONG. These results are in the same order of magnitude as the values found in our study.

IV.5. Limitations

One of the issues associated with in vitro characterisation of biomechanical behaviour of human tissue is the effect of the strain rate on the tensile properties of tissues. To date, this study and the majority of experimental investigations on arterial mechanics have used non-physiological strain rates (i.e., 10 mm min$^{-1}$) when determining the mechanical properties of human tissues in a tensile testing machine. For better assessment of the mechanical properties, tensile stress machines should be able to operate at a much higher strain rate corresponding to the physiology of aortic wall deformation.

The second problem with in vitro tissue-testing is the absence of the internal pressure of the human body surrounding the vessel and the simplification of the forces being applied to the tissue in uniaxial (generally) or biaxial (rarely) direction. Zanchi et al. [25] showed that the mechanical properties of the rat carotid artery substantially differ in vivo, in situ and in vitro.

Another limitation in the present study is the small sample size. No comparison was possible on a per patient basis. In order to achieve statistical power, it was necessary to pool data from different patients within the same group. Specimen slippage is the main cause of data loss as 24% of testing failed because of tissue slippage. Tensile mechanical testing technique has to be improved in the future to allow data comparison within one particular patient in order to minimise this bias. In addition, slippage of the tissue was detected visually or on the load–stretch relationship. This method appeared to us as efficient but the lack of a video tracking device can be considered as a bias in detecting slippage.

V. Conclusion

We studied the physiological and MEM of the aortic wall of ATAA using uniaxial tensile testing. We proposed the use of the true stress–true strain definition for the calculation of the elastic modulus in human aortas: it takes into account the changes in cross-sectional area and length that occur during tensile testing. The aortic wall was significantly anisotropic with the CIRC-oriented specimens being the stiffest. Interestingly, the aorta was stiffer longitudinally in the GC than in the LC. This observation can correlate with the physiological movement of the arch. The tissues from BAV were stiffer than in the TAV group in the CIRC orientation.

This study provides data on the mechanical behaviour in the physiological range for more accurate computational and experimental analysis.

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References


Figures

Figure 1: Example of circumferentially-cut strips of aortic tissue.

Figure 2: Principles of calculation of the physiological and maximum elastic modulus.
Figure 3: Results of maximum elastic modulus in the greater curvature. BAV: bicuspid aortic valve; TAV: tricuspid aortic valve; CIRC: circumferential; LONG: longitudinal. Bars are mean values. Error bars are SD.

Figure 4: Results of maximum elastic modulus in the lesser curvature. BAV: bicuspid aortic valve; TAV: tricuspid aortic valve; CIRC: circumferential; LONG: longitudinal. Bars are mean values. Error bars are SD.
Figure 5: Results of physiological elastic modulus in the greater curvature. BAV: bicuspid aortic valve; TAV: tricuspid aortic valve; CIRC: circumferential; LONG: longitudinal. Bars are mean values. Error bars are SD.

Figure 6: Results of physiological elastic modulus in the lesser curvature. BAV: bicuspid aortic valve; TAV: tricuspid aortic valve; CIRC: circumferential; LONG: longitudinal. Bars are mean values. Error bars are SD.
Tables

Table 1: Patient characteristics.

<table>
<thead>
<tr>
<th></th>
<th>BAV (n = 6)</th>
<th>TAV (n = 6)</th>
<th>ns</th>
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</thead>
<tbody>
<tr>
<td>Median age (range)</td>
<td>51 years (39–72)</td>
<td>64.5 years (55–76)</td>
<td></td>
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<tr>
<td>Median aneurysm diameter (range)</td>
<td>53 mm (50–57)</td>
<td>49 mm (45–67)</td>
<td></td>
</tr>
</tbody>
</table>

BAV: bicuspid aortic valve; TAV: tricuspid aortic valve; ns: non significant.

Table 2: Average specimen thickness.

| Location  | Valve type | BAV (n = 6) | TAV (n = 6) | Range is specified in brackets.
<table>
<thead>
<tr>
<th></th>
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<tbody>
<tr>
<td>GC (n = 65)</td>
<td>1.83 (1.4–2.3) 0.32</td>
<td>1.64 (1.2–1.9) 0.17</td>
<td>p &lt; 0.01</td>
<td></td>
</tr>
<tr>
<td>LC (n = 43)</td>
<td>1.98 (1.4–2.6) 0.30</td>
<td>1.97 (1.7–2.3) 0.20</td>
<td>ns</td>
<td></td>
</tr>
</tbody>
</table>

BAV: bicuspid aortic valve; TAV: tricuspid aortic valve; GC: greater curvature; LC: lesser curvature; ns: non significant.

Standard deviation is specified in green.
Table 3: Mean of PM and MEM for each patient and each group of specimen.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Diameter (mm)</th>
<th>PM</th>
<th>MEM</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>GC CIRC</td>
<td>GC LONG</td>
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<tr>
<td>BAV</td>
<td>1</td>
<td>72</td>
<td>53</td>
<td>4.38</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>57</td>
<td>55</td>
<td>–</td>
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<tr>
<td></td>
<td>3</td>
<td>39</td>
<td>52</td>
<td>2.90</td>
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<td>–</td>
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<td>52</td>
<td>–</td>
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<tr>
<td></td>
<td>6</td>
<td>50</td>
<td>57</td>
<td>2.29</td>
</tr>
<tr>
<td>TAV</td>
<td>7</td>
<td>61</td>
<td>51</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td>8</td>
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<td>48</td>
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<td>10</td>
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<td>67</td>
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<td></td>
<td>11</td>
<td>61</td>
<td>45</td>
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<td></td>
<td>12</td>
<td>55</td>
<td>50</td>
<td>2.5</td>
</tr>
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</table>

PM: physiological modulus; MEM: maximum elastic modulus; BAV: bicuspid aortic valve; TAV: tricuspid aortic valve; GC: greater curvature; LC: lesser curvature; CIRC: circumferential; LONG: longitudinal.

Number of specimens is specified in brackets. All values are in MPa.

Standard deviation is specified in green text.