Title: The biaxial biomechanical behaviour of abdominal aortic aneurysm tissue

Abbreviated Title: Biaxial behaviour of abdominal aortic aneurysm

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ABSTRACT

Background

Rupture of the Abdominal Aortic Aneurysm (AAA) occurs when the local wall stress exceeds the local wall strength. Knowledge of AAA wall mechanics plays a fundamental role in the development and advancement of AAA rupture risk assessment tools. Therefore, the aim of this study is to evaluate the biaxial mechanical properties of AAA tissue.

Methods

Multiple biaxial test protocols were performed on AAA samples harvested from 28 patients undergoing open surgical repair. Both the Tangential Modulus (TM) and stretch ratio (λ) were recorded and compared in both the circumferential (Θ) and longitudinal (L) directions at physiologically relevant stress levels, the influence of patient specific factors such as sex, age AAA diameter and status were examined. The biomechanical response was also fit to a hyperplastic material model.

Results

The AAA tissue was found to be anisotropic with a greater tendency to stiffen in the circumferential direction compared to the longitudinal direction. An anisotropic hyperelastic constitutive model represented the data well and the properties were not influenced by the investigated patient specific factors however, a future study utilising a larger cohort of patients is warranted to confirm these findings.

Conclusions

This work provides further insights on the biomechanical behaviour of AAA and may be useful in the development of more reliable rupture risk assessment tools.

Key terms: Mechanical Properties, AAA, Anisotropy

INTRODUCTION

Abdominal aortic aneurysm (AAA) is a primarily asymptomatic, degenerative disease defined as a localised and irreversible dilatation of the distal aortic wall by more than 50%⁴². The prevalence of AAA increases with age and in the United States the estimated total number of people, aged between 50 and 84 years old, with AAA is 1.1 million (prevalence 1.4%)¹³. If untreated, AAA progressively expands until it ruptures. Rupture of AAA is responsible for approximately 6,000 to 10,000 deaths per year in the USA^{29, 33}. Considering, individual AAAs are treated based on the assessment of rupture risk, it is imperative that this assessment is accurate. Currently, preventative surgical treatment is recommended when the maximum diameter is greater than 5.5cm (5cm in women) and the growth rate is greater than 1cmyear⁻¹. However, reports of ruptured smaller AAAs²² (1.6%) and nonruptured larger AAAs⁷ (2.0%) have questioned the reliability of this criterion. Based on the hypothesis that rupture occurs when the induced wall stress exceeds the wall strength, many researchers have advocated that the finite element method (FEM) (determining the peak wall stress computationally) may aid in the prediction of AAA rupture¹⁰.

Considering the AAA material model chosen to describe the mechanics of the AAA wall *in vivo* is integral to the validity of wall stress computations²¹, it seems of crucial importance to fully understand, evaluate and describe the mechanics of the AAA wall accurately. The development of AAA is associated with a degradation of structurally important connective tissues such as elastin and collagen⁵ and therefore has a direct impact on the tissue's mechanical properties. Previous assessments of AAA biomechanics utilising uniaxial mechanical test methods have found that the AAA tissue is weaker and stiffer than non-aneurysmal aorta^{24, 32}, has regionally varying properties^{23, 34} and can vary significantly from patient to patient⁹. Also, some studies have reported similar properties in both the

circumferential and longitudinal directions^{24, 26}, while another found that the tissue tended to be more stiff in the circumferential than the longitudinal direction³⁴.

Considering AAA tissue is subjected to multi axial loading in vivo, biaxial testing is recommended. This mode of testing is essential for detecting anisotropy and determining the material coefficients for nonlinear constitutive models where multiple test protocols, spanning the entire range of physiological stresses, are required to ensure accuracy²⁸. These factors have been shown to have a direct influence on computationally derived AAA rupture risk assessment^{21, 39}. Despite this, there have been few studies which have investigated the biaxial properties of AAA tissue. Vande Geest *et al.*³⁸ reported that the AAA was anisotropic with a reduction in AAA extensibility and an increase in AAA stiffness in the circumferential direction compared to the abdominal aorta. In addition, this study demonstrated the differences in the strain energy derived from uniaxial and biaxial data, further underlining the superiority of the biaxial test mode. While in basic agreement with the previous work, Tong *et al.*³⁵ concluded that the age of the intraluminal thrombus (ILT) increased the mechanical anisotropy and decreased the strength of the underlying AAA wall.

Investigating the influence of patient specific factors such as sex, age, AAA diameter and AAA status, i.e. elective or emergency repair, on the AAA biomechanical properties may contribute to the understanding of which patients are more susceptible to rupture. Although some of these factors have been linked to AAA failure properties previously^{8, 25, 26, 32, 37}, their influence on the biaxial properties has not been well documented³⁶.

Knowledge of AAA wall mechanics plays a fundamental role in the development and advancement of AAA rupture risk assessment tools. However, considering endovascular repair (EVAR) is becoming increasingly popular, opportunities to harvest and conduct experimental analyses on these tissues may become rare. Therefore, although there already exists studies detailing the biaxial mechanical response of these tissue it is of crucial

importance that this database is expanded to include the properties of more patients due to the high inherent inter and intra patient variability, while the tissue is still available, and also to investigate if common patient-specific factors influence AAA biaxial mechanical properties. Thus, the aim of this study is to **(a)** evaluate the biaxial mechanical properties of AAA tissue, **(b)** estimate the material coefficients of a suitable constitutive model capable of describing the tissue's biomechanical response and finally, for the first time **(c)** investigate the influence of various patient specific factors on the biaxial mechanical properties of AAA.

MATERIALS AND METHODS

Tissue Specimens

This study was approved by the University Hospital Limerick and the University of Limerick Ethics Committees, patient consent was obtained in all cases and this work adheres to the Declaration of Helsinki. Tissue from the anterior region of the AAA wall was harvested from 28 patients undergoing open AAA repair at the University Hospital Limerick. All tissue was excised such that the longitudinal dimension was always larger than the circumferential dimension. Upon excision, all samples were stored frozen in isotonic saline at -20°C until further analysis¹⁹.

Tissue Preparation

AAA tissue samples were thawed at 4°C overnight, allowed to equilibrate at room temperature (~20°C), and then immersed in warm (37°C) isotonic saline. To prepare the samples for tensile testing the loose connective tissue was carefully removed from the abluminal side of the tissue. At least one 14 mm square sample was cut from each tissue piece using a custom made die orientated such that the longitudinal axis was parallel to the blood-flow. Care was taken to avoid atherosclerotic or calcified regions when cutting the samples. Using a digital thickness gauge (Mitutoyo, Series 547), the thickness of all samples was measured at five locations, repeated three times and averaged as described in a recent

study¹⁸. A light mist of white paint (enamel, rustoleum) was then applied to the surface of the tissue for use with the optical strain tracking system. Samples were allowed to dry (~5mins) and then immersed in isotonic saline (37°C) to avoid any adverse effects of tissue dehydration.

Biaxial Test Method

Specific details of the biaxial tensile test methods have been recently reported¹⁸⁻²⁰. Briefly, the square samples (14 x 14 mm) were mounted in a BioTester 5000 test system (Cellscale, Biomaterials Testing, Waterloo, Canada) using a set of four BioRakes (one rake per side). Each BioRake consists of five evenly spaced tungsten tines used to pierce and grip adjacent sides of the square tissue sample. Each specimen was mounted so that it was stretched in the circumferential and longitudinal directions. All samples were submerged in 37°C isotonic saline and tested using a series of circumferential to longitudinal displacement controlled ratios, i.e. $\lambda_{\Theta}:\lambda_L = 1:1, 0.75:1, 1:0.75, 0.5:1, 1:0.5, 0.25:1, 1:0.25, 1:1$. The 1:1 ratio was repeated at the end of the regime to ensure the tissue was not mechanically compromised as a result of test protocols. The BioRakes were set to displace by a maximum of 15% of the gripped area (8.9 mm x 8.9 mm) at a rate of 0.2 mms⁻¹. Therefore, a prescribed ratio of for example $\lambda_{\Theta}:\lambda_L = 0.75:1$ was equivalent to a circumferential stretch of 11.25% and a longitudinal stretch of 15%. Samples were preconditioned for 10 cycles for all protocols, with data from the final cycle used for subsequent analysis. As the tissue was stretched, the force was measured continuously by a 5N load cell (accurate to 10mN) and images of the tissue were taken by a charge coupled device (CCD) camera (1280 x 960 pixels at 15 frames per second). All strain measurements were computed using the particle tracking software provided with the BioTester and analysed from the central 25% of the tissues dimensions to avoid important edge effects. This method is fully described in O'Leary *et al.*¹⁸ and the associated Supplementary Material (Appendix B).

Data Analysis

The Cauchy stress was determined as follows: $\sigma_{\Theta\Theta}=F_{\Theta}\lambda_{\Theta}/TX_L$ and $\sigma_{LL}=F_L\lambda_L/TX_{\Theta}$, where, F is the measured load, λ is the stretch, X is the initial width, T is the averaged initial thickness of the tissue sample, subscripts Θ and L represent the circumferential and longitudinal directions, respectively and shear was considered negligible. Transverse forces due to time deflection were found to be small compared to the axial forces and therefore have been neglected in these analyses. Stretch was measured within the central region and was calculated as follows: $\lambda_{\Theta} = x_{\Theta}/X_{\Theta}$ and $\lambda_L = x_L/X_L$, where, x represents the final length in each direction.

Mechanical Parameters

The stretch and Tangential Modulus (TM) were recorded at a Cauchy stress of 80kPa and 150kPa for each tissue sample in both the circumferential and longitudinal directions. These values represent a range of stresses that the AAA is potentially exposed to in vivo and have previously been utilised by similar studies^{35, 36, 39}. The Tangential Modulus (TM) was calculated by determining the slope of the Cauchy stress-stretch curve at each stress level. The stretch and TM were compared in the circumferential and longitudinal directions, at both 80kPa and 150kPa, in order to identify and quantify anisotropy as a function of stress. To compare the degree of anisotropy present within the experimental data to that predicted by the constitutive model (described later), the following anisotropic index (AI) was calculated at 150kPa as follows: $AI_{exp} = \lambda_L/\lambda_{\Theta}$, where, subscript 'exp' relates to the experimental data and the material is considered isotropic when $AI=1$.

Constitutive Model

The AAA tissue was assumed to be a homogeneous³⁰, incompressible⁶, anisotropic^{35, 38}, hyperelastic material undergoing finite deformations. Based on these assumptions, a constitutive model originally proposed by Choi and Vito⁴, for canine pericardium, employed

by Sacks and Choung²⁷ for chemically modified bovine pericardium and most recently by Vande Geest *et al.*³⁸ for both aneurysmal and non-aneurysmal abdominal aortic tissue was chosen to fit the data presented here. The proposed strain energy function (SEF) takes the following form:

$$W = b_0 \left[e^{b_1 \frac{1}{2} \left(\frac{\lambda_\theta^2 - 1}{2} \right)^2} + e^{b_2 \frac{1}{2} \left(\frac{\lambda_L^2 - 1}{2} \right)^2} + e^{b_3 \left(\frac{\lambda_\theta^2 - 1}{2} \right) \left(\frac{\lambda_L^2 - 1}{2} \right)} \right]$$

Where, W is the strain energy and b_0 , b_1 , b_2 and b_3 are the material coefficients to be determined. Considering, $\sigma_i = \lambda_i (dw/d\lambda_i)$, the expressions for Cauchy stress are as follows:

$$\sigma_\theta = b_0 \left[\lambda_\theta^2 b_1 \left(\frac{\lambda_\theta^2 - 1}{2} \right) e^{b_1 \frac{1}{2} \left(\frac{\lambda_\theta^2 - 1}{2} \right)^2} + \lambda_\theta^2 b_3 \left(\frac{\lambda_L^2 - 1}{2} \right) e^{b_3 \left(\frac{\lambda_\theta^2 - 1}{2} \right) \left(\frac{\lambda_L^2 - 1}{2} \right)} \right]$$

and

$$\sigma_L = b_0 \left[\lambda_L^2 b_2 \left(\frac{\lambda_L^2 - 1}{2} \right) e^{b_2 \frac{1}{2} \left(\frac{\lambda_L^2 - 1}{2} \right)^2} + \lambda_L^2 b_3 \left(\frac{\lambda_\theta^2 - 1}{2} \right) e^{b_3 \left(\frac{\lambda_\theta^2 - 1}{2} \right) \left(\frac{\lambda_L^2 - 1}{2} \right)} \right]$$

The data from the test protocols 1:1, 0.75:1, 1:0.75, 0.5:1, 1:0.5 for each sample in the circumferential and longitudinal direction were then simultaneously fit to this model using a Marquart-Levenberg least-squares optimisation technique²⁷ and the material coefficients were obtained for individual samples. As previously suggested by Vande Geest *et al.*³⁸, constraints i.e. $b_1 > 0$, $b_2 > 0$ and $b_3 > 0$ were imposed to ensure that the parameters were physically sensible. In order to obtain a single set of material constants which represent the group of samples, data from each protocol was averaged to obtain a single dataset to which the material model could then be fitted to. Additional test protocols, $\lambda_\theta:\lambda_L = 0.25:1$ and $1:0.25$ were then used to assess the predictability of the model based on the derived material coefficients.

Based on the relative contribution of both material coefficients b_1 and b_2 to the strain energy, the following measure of anisotropy was calculated^{4, 27, 38} as follows: $AI_{mod} = \sqrt{b_1/b_2}$, where, subscript 'mod' relates to the material model and as before, the material is considered isotropic when $AI = 1$.

Statistical Analysis

All measurements are presented as a mean $\pm$ standard deviation. The Shapiro-Wilk normality test was performed prior to statistical tests which assumed a normally distributed sample. Paired t-tests were employed to detect differences between the mechanical properties in the circumferential and longitudinal direction of each tissue sample. Independent t-tests were utilised to assess differences in the tissue's properties grouped by patient specific factors, i.e. sex, age, AAA diameter and AAA status. Pearson's correlation coefficient was used to determine if a relationship existed between AI_{exp} and AI_{mod} . A two-sided p-value less than 0.05 indicated statistical significance for all paired and independent t-tests and also when determining a Pearson's correlation coefficient. All analyses were performed using IBM SPSS Statistics (IBM Corp. Released 2011. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp).

RESULTS

Sample Characteristics

To summarise patient sample characteristics; 22/28 of patients were male, 18/28 were elective repair, the average maximum AAA diameter, ϕ , was 6.65 ± 1.57 cm and the average patient age was 71.60 ± 6.67 years. In total, from the AAA wall tissue samples of 28 patients, 34 specimens were tested successfully. Samples which underwent structural damage ($n=7$) during testing evidenced by the final 1:1 testing ratio were excluded from the results. The main reason for structural damage was attributed to the tines pulling through the tissue when experiencing high force. Where more than one sample was tested from the same tissue piece

(patient no. = 5), the average mechanical properties of the specimens were chosen to represent the overall mechanical response for that patient.

Equibiaxial Response

As shown in Figure 1, the AAA tissue was found to be anisotropic, with higher average TM values and lower average λ values in the circumferential than in the longitudinal direction when evaluated at both 80kPa (i.e. TM_{Θ} vs TM_L : 4.2 ± 1.3 vs 3.7 ± 0.9 ($p = .04$) and λ_{Θ} vs λ_L : 1.09 ± 0.02 vs. 1.10 ± 0.02 ($p = .02$)) and 150kPa (i.e. TM_{Θ} vs TM_L : 8.1 ± 3.2 vs 6.6 ± 1.2 ($p = .01$) and λ_{Θ} vs λ_L : 1.11 ± 0.03 vs. 1.12 ± 0.03 ($p = .06$)). Therefore, in general the tissue is found to be stiffer and less extensible in the circumferential than in the longitudinal direction. The percentage difference between TM measured in each direction is shown to increase (9%) when measured at higher stresses while, the percentage difference between λ is shown to decrease (3%) at higher stresses.

Constitutive Model

From the average Cauchy stress-stretch response plots of the multiple test protocols in both the circumferential and longitudinal directions illustrated in Figure 2, it can be seen that the material model provided an excellent fit for the averaged group data ($R^2 = 0.98$) and also fit the individual samples very well ($R^2 = 0.96 \pm 0.03$). Validation of the models predictive capabilities based on the group material coefficients and using test protocols, 0.25:1 and 1:0.25 was also successful and the agreement between the model and the experimental data was high ($R^2 = 0.88$).

A positive correlation was found between the AI_{exp} and AI_{mod} values ($r = .85$, $p < .01$) which again shows good agreement between the model and the experimental data and also implies that either index can be employed to assess the degree of anisotropy (Figure 4). The AI_{mod} values indicate that the relative contribution of b_1 to the strain energy is higher than b_2 for

19/28 patients, lower than b_2 for 6/28 patients and approximately equal for the remaining 3 patients ($0.95 \leq AI \leq 1.05$).

Patient Specific Factors

Table 2 and Table 3 compare the measured mechanical properties based on various groupings such as male vs. female, AAA diameter: <5.5 cm vs. >5.5 cm, AAA status: elective vs. emergency repair (ruptured or highly symptomatic) and patient age: <70 years vs. >70 years. There was no significant difference found between the mechanical parameters measured for any of these groups. In addition, all groups were well matched for thickness (Supplementary Data).

DISCUSSION

In this study, the biaxial mechanical properties of AAA tissue from 28 patients undergoing open AAA repair were evaluated. In general, the AAA tissue was found to be anisotropic with a tendency to be stiffer in the circumferential than in the longitudinal direction. The constitutive relationship chosen to model the biomechanical response provided an excellent fit for the data and the material coefficients derived from the averaged model were shown to be capable of predicting physiologically relevant loading regimes accurately. A significant difference in mechanical properties as result of sex, age, AAA diameter or AAA status was not detected.

Equibiaxial Response

Comparing our results to the literature, our study concurs that AAA is anisotropic with preferential stiffening in the circumferential direction^{35, 38}. While, our reported values of TM at 150kPa are comparable to those of Tong *et al.*³⁵ at the same stress level, i.e. 8.1 ± 3.2 MPa and 6.6 ± 1.2 MPa vs. 8.7 ± 1.7 MPa and 6.6 ± 2.7 MPa in the circumferential and longitudinal directions respectively, the magnitude of the differences between our measured material properties in each direction is lower, i.e. 20% vs. 27%. Vande Geest *et al.*³⁸ reported

average values for TM in each direction that were larger than either of these studies, i.e. 11.7 ± 1.9 and 8.3 ± 1.2 MPa and also had more pronounced anisotropy (34% difference between TM in the circumferential and longitudinal direction).

Interestingly, larger deviances were reported for the values of stretch in both directions compared to previous studies, i.e. $\lambda_{\Theta} = 1.07 \pm 0.03$ and $\lambda_L = 1.11 \pm 0.04$ ³⁵, $\lambda_{\Theta} = 1.07 \pm 0.01$ and $\lambda_L = 1.09 \pm 0.01$ ³⁸ vs. $\lambda_{\Theta} = 1.11 \pm 0.03$ and $\lambda_L = 1.12 \pm 0.03$ (current study @150kPa) and also the average percentage difference between these directions is larger i.e. 44%³⁵ and 25%³⁸ vs. 9% (current study @150kPa). Considering our reported values for TM in the stiff region of the Cauchy stress–stretch response plots are similar, differences in the reported values of stretch indicate that there was an increase in extensibility in the toe region of the Cauchy stress–stretch response plot prior to stiffening compared to these studies. In a more recent publication by Polzer *et al.*²¹, who performed similar tests on the AAA tissue of a smaller group of patients (n=7), a similar trend was observed and these differences were potentially attributed to the use of different reference configurations i.e. stress-free (Polzer *et al.*, 21) vs. pre-stressed (Vande Geest *et al.*³⁸). Similar to this study, a pre stress was not prescribed here and therefore it is possible that these subtle differences in protocols may contribute to the differences in extensibility values reported in the literature.

There are a number of other possible reasons for deviations in the biomechanical parameters found between these studies. For example, the presence of high inherent inter and intra-tissue variability is always influential when comparing mechanical properties between different groups of patients. Furthermore, the presence of intraluminal thrombus is thought to be responsible for the proteolytic³¹ and hypoxic⁴¹ degradation of the wall resulting in a thinner wall of diminished strength. In addition, increasing thrombus age has also been associated with an increasing degree of anisotropy of the underlying AAA wall³⁵. Considering data detailing the thrombus age is unavailable for the current study and it is not clear whether the

AAA wall tested by both Vande Geest *et al.*³⁸ and Polzer *et al.*²¹ had previously been covered by thrombus, it is possible that the variation in stiffness, extensibility and degree of anisotropy may be associated with the presence, absence or age of the intraluminal thrombus.

Constitutive Model

Although we found that the degree of anisotropy (differences between the TM and λ in the circumferential and longitudinal direction) to be small at lower stresses (80kPa), it was shown to increase with stress (150kPa). Therefore, our decision to choose an anisotropic model to represent the biomechanical response of AAA tissue *in vivo* is justified. The anisotropic index associated with the constitutive model also corroborates the findings from the measured mechanical properties which indicated that the circumferential direction has a tendency to be stiffer than the longitudinal direction. Although the majority of the tissue specimens displayed preferential stiffening in the circumferential direction, variations in the preferred direction also existed within the test data. Due to the changes of the wall's structural components such as elastin and collagen as a result of aneurysm progression, anisotropy can be affected which has previously been observed in aortic aneurysmal tissues³⁸ and most other arteries¹². Comparing our material coefficients to those acquired in a similar study of AAA by Vande Geest *et al.*³⁸, our group values are comparable but lower, i.e. b_0 , b_1 , b_2 and b_3 = 0.44, 297.0, 219.9 and 184.3 vs. 0.14, 477.0, 416.4 and 408.3, which is not surprising considering the previously discussed differences in the mechanical properties between studies.

Patient Specific Factors

Sex

A recent biaxial study³⁵ and a previous uniaxial study³⁸ of the AAA biomechanical properties have concluded that men have AAA tissue that is stiffer, less extensible and has a higher tensile strength than women, which may explain why although, the occurrence of AAA is less

common in women¹⁴, the relative incidence of rupture is greater than their male counterparts¹. However, the current study, for which the ratio of male to females is comparable to these previous studies, i.e. 21:6³⁵, 24:10³⁸ and 22:6 (current study), did not find a significant difference between the AAA mechanical properties of men and women and on average the female properties were higher than males. This suggests that the difference in rupture risk observed between men and women may not be significantly influenced by their biomechanical properties and other reported factors such as an increased AAA growth rate in females¹⁶ may play a more dominant role.

Patient Age

It has been reported previously that the stiffness of the non-aneurysmal aorta increases with age¹⁷, which is thought to be linked to a decrease in the amount of elastin and an increase in the amount of collagen present in the aortic wall as it ages¹¹. It is also well known that the prevalence of AAA increases with age⁴⁰. Although no statistically significant difference was found between the measured properties of patients over and under 70 years of age the average stiffness was increased and extensibility remained unchanged for the older group, which indicates that the aortic wall even in the presence of AAA may increase in stiffness as it ages.

AAA Diameter

A larger diameter AAA is associated with an increase in rupture risk^{3, 15}. However, in the largest uniaxial study and histological assessment of AAA tissue to date³², the AAA tissue of diameter > 5.5cm (n=65) was found to have a higher failure strength and extensibility than the AAA tissue of diameter < 5.5cm (n=25). In contrast, this study which examined the biaxial properties of a smaller amount of patients (> 5.5cm = 20 vs. < 5.5cm = 8), although proportionally similar, did not detect a significant difference in the properties between these two groups. Reasons for increases in mechanical strength as the aneurysm increases in size have been attributed to wall remodelling which results in an increase in the absolute mass of

the collagen present. However, the exact pathogenesis of AAA is still not fully understood and the exact rate at which the AAA remodels is not known. This reason and possibly as a result of lower patients numbers, may help explain why this study did not detect a significant difference between the two groups.

AAA Status

It is hypothesised that AAA rupture occurs when the wall stress exceeds the wall strength, however, there have been no known studies reporting the difference in biaxial properties between the ruptured and nonruptured AAA tissue, presumably due to the life threatening nature of AAA rupture repair surgery. Interestingly our study did not detect a statistically significant difference in the mechanical properties between the two groups. Although not directly comparable due to differing test modes, the findings support the most recent uniaxial study of the AAA biomechanics of entire AAA structures where it was concluded that the regional properties of ruptured AAAs are not globally weaker than nonruptured AAAs²⁵. In a similar study⁸, it was found that the stiffness was unaffected by AAA status however, the failure stress was significantly decreased for ruptured AAAs vs. nonruptured AAAs.

Future Perspective

Patient specific factors such as sex, age, AAA diameter and AAA status, i.e. elective or emergency repair, have not been found to significantly influence AAA biomechanical properties and therefore, it may not be possible to differentiate those AAAs which are more susceptible to rupture than others, based on these factors. Although these findings need to be confirmed by examining their influence amongst a larger cohort of patients, it is likely that they do not play a key role in determining AAA rupture risk. Although other factors such as the presence of calcification have been shown to correlate with increased AAA rupture risk², their influence on AAA biomechanical properties has not been well documented and has rarely been included in computational rupture risk assessments. Therefore, future

examinations of the influence of calcification on AAA properties may further the understanding of AAA rupture risk.

Limitations

Due to the nature of AAA repair surgery, excised samples for the purposes of this study were restricted to the anterior region of the AAA sac. This is limitative, considering, AAA mechanical properties have been found to change regionally^{25, 34} and for the ruptured or highly symptomatic patient group, assessment of properties was not at the actual rupture site. Furthermore, considering the tissue specimens had to be at least 14mm x 14mm for successful biaxial testing, the size of the excised tissue limited the number of samples that could be prepared per patient. Unfortunately, excision of the complete AAA structure is extremely rare and can only be attained post mortem. As previously mentioned, at high stresses the tines had a tendency to pull through the AAA tissue sample; therefore, failure properties of the AAA tissue could not be assessed. Furthermore, considering that the displacement of the tines was controlled and not the strain of the tissue, the strain measured at the centre of the specimen did not always reach the exact amount of prescribed stretch, presumably due to the inability of the fibrous structure (which is only loaded at discrete points) to translate the entire load. Substantial effort was made to eliminate the effect of the gripping method on the derived mechanical properties and yet the response of the small tissue pieces may not fully represent the response of the complete AAA structure. Additionally, residual stresses and strains, if present, were not accounted for in this analysis. Furthermore, the evaluation of tensile stress may be subject to minor errors as a result of the instrument used to measure the sample thickness¹⁸.

CONCLUSION

The aim of this study was to both confirm the findings of similar studies utilising a separate patient sample and increase our understanding of the role of patient-specific factors in AAA

biomechanics. In general, biaxial tests confirmed that the AAA tissue is anisotropic with a greater tendency to stiffen in the circumferential direction compared to the longitudinal direction. An anisotropic hyperelastic constitutive model was found to represent the data well (although the average estimated material parameters were lower than a previous study) and implementation of this model in future finite element studies of AAA tissue may provide a more realistic assessment of AAA wall stress and rupture risk. No significant difference was found between any of the patient groups based on sex, age, AAA diameter and AAA status and their associated mechanical properties. Although these findings need to be confirmed by examining their influence amongst a larger cohort of patients, it may be likely that they do not play a key role in determining AAA rupture risk. It is possible that other factors such as the presence of calcification may have a greater influence on the AAA rupture risk (this will be reported in an upcoming publication).

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CONFLICT OF INTEREST STATEMENT

There are no conflicts of interest.

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TABLE 1. Material parameters derived from the previously described material model, calculated anisotropic index values and the material model correlation coefficients associated with individual patient's fit and also the averaged group fit.

Patient	b_0(kPa)	b_1	b_2	b_3	AI_{mod}	AI_{exp}	R^2
1	0.27	86.96	241.06	126.04	0.60	0.69	0.96
2	0.72	175.48	277.16	158.23	0.80	0.86	0.96
3	0.56	156.81	57.85	96.05	1.65	1.32	0.98
4	0.59	387.36	320.61	198.02	1.10	1.13	0.98
5	0.12	528.78	204.54	224.17	1.67	1.84	0.97
6	0.40	327.72	241.17	163.24	1.17	1.18	0.98
7	0.62	432.08	216.93	251.22	1.41	1.29	0.95
8	0.25	269.44	281.00	190.57	0.98	0.97	0.94
9	0.78	187.14	253.54	159.42	0.86	0.86	0.96
10	0.47	329.87	106.88	193.23	1.76	1.36	0.95
11	0.32	124.67	162.23	109.56	0.88	0.90	0.98
12	0.30	255.51	158.10	114.04	1.27	1.15	0.99
13	0.26	291.19	156.46	169.83	1.36	1.17	0.94
14	0.34	345.08	305.69	225.18	1.15	1.07	0.92
15	0.11	537.99	290.85	414.95	1.36	1.24	0.89
16	0.38	290.21	84.73	110.23	1.85	1.44	0.99
17	0.48	396.95	68.85	157.59	2.40	1.40	0.90
18	0.51	204.43	217.80	145.33	0.97	0.94	0.95
19	0.47	188.07	190.84	129.27	0.99	1.01	0.95
20	1.33	230.43	155.24	87.72	1.22	1.27	0.98
21	0.23	281.27	339.74	204.32	0.91	0.93	0.92
22	0.20	268.70	176.39	129.37	1.23	1.14	0.98
23	0.63	405.74	292.17	261.57	1.18	1.14	0.94
24	0.59	182.92	96.91	98.95	1.37	1.20	0.98
25	0.35	207.08	85.27	99.49	1.56	1.27	0.98
26	0.41	64.11	303.00	248.35	0.46	0.76	0.97
27	0.16	263.16	221.22	127.60	1.09	1.08	0.99
28	0.95	435.34	218.80	374.57	1.41	1.22	0.93
Group	0.44	306.83	213.34	218.71	1.20	1.09	0.98

TABLE 2. The average and standard deviation values of tangential modulus and stretch ratio evaluated at 80kPa in both the circumferential and longitudinal directions for patient specific factors patient sex, age, AAA diameter and status. A p-value less than 0.05 indicates a statistically significant difference between the properties.

Variable	n	λ @80kPa		TM (MPa) @80kPa		
			Θ	L	Θ	L
Sex	Male	22	1.10 ± 0.01	1.11 ± 0.03	4.07 ± 1.23	3.68 ± 1.08
	Female	6	1.08 ± 0.01	1.10 ± 0.03	4.69 ± 1.41	4.14 ± 0.64
	p		0.17	0.33	0.3	0.94
Age	≤ 70 years	12	1.09 ± 0.02	1.10 ± 0.02	3.89 ± 0.43	3.68 ± 0.72
	> 70 years	16	1.09 ± 0.02	1.10 ± 0.03	4.44 ± 1.62	3.84 ± 1.19
	p		0.67	0.69	0.26	0.69
Diameter	≤ 5.5 cm	8	1.08 ± 0.02	1.09 ± 0.03	4.11 ± 1.42	3.68 ± 0.79
	> 5.5 cm	20	1.09 ± 0.02	1.11 ± 0.02	4.23 ± 1.25	3.81 ± 1.09
	p		0.84	0.74	0.15	0.32
AAA Status	Elective	18	1.09 ± 0.02	1.11 ± 0.03	4.46 ± 1.46	6.92 ± 2.26
	Emergency	10	1.09 ± 0.02	1.10 ± 0.02	3.73 ± 0.65	6.41 ± 2.22
	p		0.84	0.74	0.15	0.74

TABLE 3. The average and standard deviation values of tangential modulus and stretch ratio evaluated at 150kPa in both the circumferential and longitudinal directions for patient specific factors patient sex, age, AAA diameter and status. A p-value less than 0.05 indicates a statistically significant difference between the properties.

Variable	n	λ @150kPa		TM (MPa) @150kPa		
			Θ	L	Θ	L
Sex	Male	22	1.11 ± 0.03	1.12 ± 0.03	7.74 ± 3.04	6.43 ± 2.15
	Female	6	1.10 ± 0.02	1.11 ± 0.03	8.96 ± 3.19	7.85 ± 2.29
	p		0.24	0.54	0.39	0.17
Age	≤ 70 years	12	1.11 ± 0.02	1.12 ± 0.02	7.82 ± 2.71	6.42 ± 1.39
	> 70 years	16	1.11 ± 0.03	1.12 ± 0.03	8.15 ± 3.37	6.98 ± 2.70
	p		0.97	0.52	0.78	0.52
Diameter	≤ 5.5 cm	8	1.10 ± 0.02	1.11 ± 0.03	7.15 ± 2.97	6.85 ± 2.19
	> 5.5 cm	20	1.11 ± 0.03	1.12 ± 0.03	8.35 ± 3.09	6.69 ± 2.28
	p		0.53	0.58	0.45	0.93
AAA Status	Elective	18	1.11 ± 0.02	1.12 ± 0.03	8.34 ± 3.15	6.92 ± 2.26
	Emergency	10	1.11 ± 0.03	1.12 ± 0.02	7.41 ± 2.92	6.41 ± 2.22
	p		0.54	0.61	0.45	0.57

FIGURE 1. The average tangential modulus (TM) and stretch ratio (λ) evaluated at 80kPa and 150kPa in the circumferential (Θ) and longitudinal (L) directions of the equibiaxial response curves. The percentage change in properties between the axes is illustrated and (*) indicates a statistically significant difference between the properties at a significance level of 0.05.

FIGURE 2. The averaged Cauchy stress-stretch response curves for the prescribed multiple biaxial test regimes ($\lambda_\Theta:\lambda_L = 1:1, 0.75:1, 1:0.75, 0.5:1, 1:0.5$) in both the circumferential (left) and longitudinal (right) directions and the material model fit (solid lines).

FIGURE 3. The predicted model fit based on group material coefficients for additional group averaged test protocols $\lambda_{\Theta}:\lambda_L = 0.25:1$ (left) and $1:0.25$ (right). These protocols were not used to derive the group material coefficients and therefore represent a validation of the model.

FIGURE 4. Correlation between the anisotropy indices associated with the experimental data ($AI_{exp} = \lambda_L/\lambda_\Theta$) and the material model ($AI_{mod} = \sqrt{b_1/b_2}$).