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► **To cite this version:**

Valerie Deplano, Mourad Boufi, Vlad Gariboldi, Anderson Loundou, Xavier Benoit D'journo, et al.. Mechanical characterisation of human ascending aorta dissection. *Journal of Biomechanics*, 2019, pp.138-146. 10.1016/j.jbiomech.2019.07.028 . hal-02268507

HAL Id: hal-02268507

<https://hal.science/hal-02268507v1>

Submitted on 25 May 2020

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Mechanical characterisation of human ascending aorta dissection

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Abstract

Mechanical characteristics of both the healthy ascending aorta and acute type A aortic dissection were investigated using *in vitro* biaxial tensile tests, *in vivo* measurements via transoesophageal echocardiography and histological characterisations. This combination of analysis at tissular, structural and microstructural levels highlighted the following: *i*) a linear mechanical response for the dissected intimomedial flap and, conversely, nonlinear behaviour for both healthy and dissected ascending aorta; all showed anisotropy; *ii*) a stiffer mechanical response in the longitudinal than in the circumferential direction for the healthy ascending aorta, consistent with the histological quantifica-

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tion of collagen and elastin fibre density; *iii*) a link between dissection and ascending aorta stiffening, as revealed by biaxial tensile tests. This result was corroborated by *in vivo* measurements with stiffness index, β , and Peterson modulus, E_p , higher for patients with dissection than for control patients. It was consistent with histological analysis on dissected samples showing elastin fibre dislocations, reduced elastin density and increased collagen density. To our knowledge, this is the first study to report biaxial tensile tests on the dissected intimomedial flap and *in vivo* stiffness measurements of acute type A dissection in humans.

Keywords: Human ascending aorta, Acute type A dissection, Biaxial tensile test, *In vivo* measurements, Histological analysis

1. Introduction

2 An aortic dissection is a vascular pathology consisting in a tear of the
3 aortic wall intima layer which can propagate downstream or/and upstream
4 along the aorta, creating a false lumen through which blood flows. The Stan-
5 ford classification divides aortic dissections into two types: type A when the
6 initial tear is located along the ascending aorta and upstream of the left sub-
7 clavian artery, and type B when the tear is located on the descending aorta.
8 Acute type A dissection is characterised by rapidly developing severe com-
9 plications such as aortic rupture, whereas chronic dissection is not diagnosed
10 initially, being asymptomatic; patients remain stable. Acute type A dissec-
11 tion has a short- and long-term spontaneous mortality of 70% (Golledge et
12 al., 2008) and although its prognosis has been improved by surgical treat-

13 ment, post-operative mortality remains high. The tear initiates when the
14 aortic wall can no longer bear the wall stress exerted. While some biax-
15 ial tensile tests (Matsumoto et al. (2009), Haskett et al. (2010), Martin et
16 al. (2011), Azadani et al. (2012), Kamenskiy et al. (2014)), or pressurised
17 tests (Labrosse et al. (2009)) have been performed on healthy samples of
18 human ascending aorta, there are few existing studies on dissected samples
19 from acute type A dissection. To the authors' knowledge, only Babu et al.
20 (2015) assessed mechanical properties of entire wall fragments from type A
21 dissection. Studies using biaxial tensile or bulge inflation tests have gener-
22 ally focused on ascending thoracic aortic aneurysms (ATAA) (Okamoto et al.
23 (2002), Pham et al. (2013), Duprey et al. (2016)) rather than on dissection.
24 Moreover, there have been few *in vivo* investigations of mechanical behaviour
25 in dissections on the ascending aorta. Koullias et al. (2005) and Vitarelli et
26 al. (2006) used echocardiography measurements to assess structural charac-
27 teristics of ATAA and Marfan patients respectively. Shingu et al. (2009) were
28 the only authors to use an echo-tracking system to obtain distensibility mea-
29 surements, but on chronic dissection. Furthermore, the components of the
30 extracellular matrix (ECM), their concentration and their organization are
31 known to play an essential role in the mechanical behaviour of the human
32 aorta (Tsamis et al., 2013). Today, there is greater emphasis on quanti-
33 fying the arterial microstructure and microarchitecture using, for example,
34 microscopy image analysis (Koch et al., 2014) to corroborate macroscopic
35 mechanical response and enrich constitutive laws and thus numerical mod-
36 elling (Pasta et al. (2014), Thunes et al. (2018)).

37 Here, we sought insights into the mechanical behaviour of the ascending

38 aorta in acute type A dissection because of its high mortality risk. Three me-
39 chanical characterisations were therefore performed at different investigation
40 levels. *In vivo* measurements were performed on both healthy and patholog-
41 ical patients to obtain results at structure level. These measurements were
42 combined with appropriate *in vitro* biaxial tensile tests on both healthy and
43 dissected aortic samples to assess information at tissular level. Finally, qual-
44 itative and quantitative histological analyses were carried out on both types
45 of samples to link structural, tissular and microstructural level.

46 **2. Methods**

47 *2.1. Tissue preparation*

48 Healthy ascending aorta, *Haa*, tubular structures were harvested from un-
49 used aortic segments after lung transplantation. Dissected ascending aorta,
50 *Daa*, fragments were collected from patients after surgical repair. Informed
51 consent was obtained based on established research board protocol in each
52 hospital. All samples were stored, refrigerated at 4°C in 0.9% NaCl solution
53 and tested less than 50h after tissue extraction.

54 The *Haa* tubular structures (figure 1a) were longitudinally cut along the
55 curvature. Three types of samples were extracted from dissected fragments
56 (figure 1b) : a region without dissected layers, *WDaaL*, n=1, (figure 1d),
57 the adventitia layer alone, n=3, (figure 1e), and the media associated with
58 the intima layer called intimomedial flap, n=3, (figure 1f). Square 20X20mm
59 samples (a reproducible size) were subsequently obtained from healthy and
60 dissected samples, using a dedicated cutting device. Seven healthy samples
61 from 5 different donors of mean age 65.3 ± 4.9 years, with a mean thickness

62 of $2.26\pm 0.19\text{mm}$, were tested. The mean thickness and patient mean age
63 of the flap and adventitia samples were $1.98\pm 0.12\text{mm}$; 63.7 ± 9.2 years and
64 $1.06\pm 0.17\text{mm}$; 59.7 ± 8.3 years respectively. The thickness of *WDaaL* was
65 1.79mm and the patient was 57 years old. Thickness was measured via im-
66 age processing of the undeformed samples (Deplano et al. (2016)). Table A.1
67 Appendix A reported demographic data and risk factors of all *in vitro* tested
68 samples.

69

70 2.2. *In vitro* mechanical tests

71 The square samples were mounted using a home-made biaxial set-up de-
72 scribed in Deplano et al. (2016). Briefly, the circumferential, θ , and longitu-
73 dinal, L , directions of the sample were in line with the biaxial displacements.
74 A displacement-driven protocol was applied while the ratio, α , remained con-
75 stant. $\alpha = \frac{\lambda_\theta}{\lambda_L}$, noted $\alpha = \lambda_\theta : \lambda_L$, was the stretch ratio, λ_θ and λ_L being the
76 stretch in the θ , and L directions respectively.

77 Forces resulting from the displacements were measured using two load cells
78 ($10\text{N} \pm 0.0015\text{N}$, 31E10, Honeywell) located on each direction of displace-
79 ment. 3D displacement measurements (Dantec Q-400 software) were per-
80 formed using Stereoscopic Digital Image Correlation. Samples were sub-
81 merged in aqueous 0.9% NaCl solution maintained at 37°C during the ex-
82 periments.

83 The maximum stretch, λ_{max} , was set at 1.2 of the gripped undeformed
84 sample area, $X_L \times X_\theta$, where $X_L = X_\theta = 18\text{mm}$ were measured before
85 the protocol was applied. Twenty loading and unloading cycles of precon-
86 ditioning were first performed at λ_{max} and $\alpha = 1 : 1$ following by $k=7$

87 consecutive cycles, each one at different stretch ratios : 0.75:1, 1:0.75, 0.5:1,
 88 1:0.5, 0.25:1, 1:0.25, 1:1. This protocol was applied for a maximum stretch
 89 rate of $\lambda_{max} = 3.84 \times 10^{-2} s^{-1}$. Initial zero stress was assessed by tuning
 90 the position of the suture lines by 0.1mm steps at a very low displacement
 91 rate (0.1mm/s) until the load cell signals detected were above their noise
 92 level (2.4mV). A hybrid displacement/force control was implemented during
 93 unloading to avoid bending the sample with respect to its original dimensions.

94
 95 The first Piola Kirchhoff stress tensor, P , was determined using f_θ and
 96 f_L , the recorded force, X_θ^{k-1} , X_L^{k-1} and E_0 , the undeformed gripped sample
 97 dimensions and thickness. For each cycle performed during one protocol,
 98 X_θ^{k-1} and X_L^{k-1} correspond to the undeformed lengths of the sample at the
 99 end of the k-1 unloading path.

100 The components of P were expressed by $P_{\theta\theta}^k = \frac{f_\theta}{X_L^{k-1} E_0}$ and $P_{LL}^k = \frac{f_L}{X_\theta^{k-1} E_0}$.
 101 The components of the second Piola Kirchhoff stress tensor, S , were then
 102 obtained using $S_{\theta\theta}^k = \frac{P_{\theta\theta}^k}{\lambda_\theta}$ and $S_{LL}^k = \frac{P_{LL}^k}{\lambda_L}$, those of the Cauchy stress tensor,
 103 σ , using: $\sigma_{\theta\theta}^k = P_{\theta\theta}^k \lambda_\theta$ and $\sigma_{LL}^k = P_{LL}^k \lambda_L$. λ_i were calculated from the SDIC
 104 displacement measurements. The superscript k will be omitted in the rest of
 105 the paper.

107 2.3. Histological characterisation

108 Healthy and dissected segments (n=3 and n=4 respectively) were first
 109 fixed in 10% formalin (Appendix A details their characteristics). For each
 110 sample, 2 contiguous samples were cut along the axial and transversal artery
 111 axis. They were dehydrated in ethanol and embedded in paraffin. Four con-

112 tiguous sections 5 μm thick were cut and stained with orceid and Trichrome
113 masson for the visualisation of elastin and collagen fibres respectively. Calopix
114 software was then used for image processing and extraction of the relative
115 density of the stained structures. Density was defined as the ratio of stained
116 surface to total surface for a region of interest of the sample. For each sam-
117 ple, density was measured in 4 regions in media layers and in the entire wall,
118 and averages were calculated.

119 2.4. *In vivo* measurements

120 To determine the mechanical behaviour of both healthy and dissected
121 ascending aorta, *in vivo* transoesophageal echocardiography measurements
122 were performed on two new groups of patients not used for the *in vitro* tests
123 and histological characterisations. The control group (n=22, age 67 ± 9 years,
124 19 males) was composed of patients without any ascending aorta pathology,
125 while the second group (n=13, age 69 ± 10 years, 9 males) was composed of
126 patients admitted and treated for acute type A dissection. The mechanical
127 characterisation of both groups entailed determining the Peterson modulus,
128 E_p , and stiffness index, β . $E_p = \frac{\Delta P}{\epsilon}$, $\beta = \frac{\ln \frac{P_{sys}}{P_{dias}}}{\epsilon}$, where $\epsilon = \frac{\Delta D}{D_{dias}}$, ΔP and
129 ΔD were the difference between the diastolic and systolic pressure (P_{dias} ,
130 P_{sys}) and diameter (D_{dias} , D_{sys}) values respectively. Details of pressure and
131 diameter acquisitions as well as inclusion criteria for each group are given in
132 Appendix B.

133 3. Results

134 3.1. Constitutive modelling

135 Figures 2a) to d), which represent $S - \lambda$ curves for both Haa and $WDaaL$
136 samples, show nonlinear and rather anisotropic behaviour. These mechanical
137 responses were therefore modelled by a Holzapfel-Gasser-Ogden (HGO) form
138 of strain energy function, ψ , as per Babu et al. (2015).

139 $\psi = \psi_{iso} + \psi_{aniso}$ with $\psi_{iso} = \frac{C}{2}(I_1 - 3)$ and $\psi_{aniso} = \frac{k_1}{k_2}(e^{(k_2(I_4 - 1)^2)} - 1)$ consid-
140 ering that collagen fibres are symmetrically oriented in the arterial wall. C
141 and k_1 are positive dimensional (Pa) coefficients and k_2 is dimensionless. I_1
142 is the first invariant of the strain tensor and $I_4 = \lambda_\theta^2 \cos^2 \gamma + \lambda_L^2 \sin^2 \gamma$, where
143 γ is the angle between the fibre direction and the circumferential direction.

144

145 Figures 3 a) and b), which show $S - E$ curves for the Daa intimomedial
146 flap, highlight more or less linear and anisotropic behaviour. According to
147 these results, the mechanical response was described by a 3-parameter poly-
148 nomial strain energy function, $\psi = \frac{1}{2}(A_{\theta\theta}E_{\theta\theta}^2 + 2A_{\theta L}E_{\theta\theta}E_{LL} + A_{LL}E_{LL}^2)$. $E_{\theta\theta}$
149 and E_{LL} are the components of the Green-Lagrangian strain tensor. The co-
150 efficients $A_{\theta\theta}$ and A_{LL} describe the tissue stiffness in the θ and L directions
151 respectively and $A_{\theta L}$ is related to the interaction between the two directions.

152 Whatever the constitutive modelling, the components of the second Piola
153 Kirchhoff stress tensor can be derived from ψ according to $S = \frac{\partial \psi}{\partial E}$. The
154 coefficients C , k_1 , k_2 and γ angle for the HGO model, and $A_{\theta\theta}$, A_{LL} and
155 $A_{\theta L}$ for the linear model were thus obtained using an optimization procedure
156 (using `fmincon` function in Matlab). Part of the mechanical data from the
157 biaxial protocol was simultaneously fitted to the constitutive model. For

158 *Haa* and *WDaaL*, mechanical data from $\alpha= 0.5:1; 1:0.5$, and $1:1$ were used.
 159 For the *Daa* flap, mechanical data from $\alpha= 0.75:1; 1:0.75, 0.5:1, 1:0.5$ were
 160 considered.

161 The method involved minimizing the sum of the error χ_α (equation 1), e.g.
 162 $\sum_\alpha \chi_\alpha$ where α was the stretch ratio and m the number of experimental data
 163 recorded during loading (e.g $m=60$).

$$\chi_\alpha = \sum_{k=1}^m (S_{\theta\theta}^{exp} - S_{\theta\theta}^{mod})_k^2 + (S_{LL}^{exp} - S_{LL}^{mod})_k^2 \quad (1)$$

164 The superscript *exp* indicates the stress values from the experimental data,
 165 *mod* those predicted by the model.

166 Table C.1 Appendix C displays the C , k_1 , k_2 coefficients and γ angle for
 167 the mean data obtained from the healthy samples ($n=7$) and for the single
 168 *WDaaL* sample tested. Table C.2 Appendix C presents the A_{ij} coefficients
 169 obtained through the optimization procedure for each *Daa* flap sample and
 170 the mean data obtained from all flap samples. For each constitutive mod-
 171 elling process, the coefficients obtained at the end of optimization procedures
 172 converged towards the same values, whatever the initial guesses tested.

173

174 For mean data obtained from *Haa* samples and *WDaaL* data, the stress
 175 components derived from the strain energy function fitted the experimental
 176 data with average determination coefficients $\overline{R_L^2}$ of 0.811 and 0.911 respec-
 177 tively and $\overline{R_\theta^2}$ of 0.861 and 0.902 respectively (Table C.1 Appendix C). For
 178 mean data obtained from the flap samples, we found $\overline{R^2}$ of 0.991 and 0.995
 179 in the longitudinal and circumferential direction respectively (Table C.2 Ap-
 180 pendix C). Figures 3a) and b) underline the good fit between experimental

181 data and constitutive modelling for flap mean data. This is further illustrated
182 by figures 4a) and b), which show that the model's prediction of $S - E$ be-
183 haviour for $\alpha = 1$ is very close to the experimental behaviour, even though
184 this latter dataset was not used in the identification procedure.

185 *3.2. Mechanical behavior of healthy and dissected ascending aorta*

186 *3.2.1. In vitro mechanical tests*

187 None of the tensile tests performed on the adventitia *Daa* samples could
188 be exploited, due to systematic tearing under stress.

189 To compare the mechanical behaviour of *Haa* with that of *WDaaL*, σ - λ
190 curves for $\alpha=1$ in both longitudinal and circumferential directions were plot-
191 ted using an HGO model (figure 5). As mentioned, both samples exhibited
192 nonlinear and anisotropic behaviour. The mechanical response of *WDaaL*
193 was stiffer than that of *Haa* and both their responses were stiffer in the
194 longitudinal than in the circumferential direction. Although rather linear,
195 the mechanical behaviour of *Daa* flap samples (figures 3a) and b) was also
196 anisotropic ; however their $A_{\theta\theta}$ coefficient was greater than A_{LL} one, 277.653
197 versus 232.861 kPa, (Table C.2 Appendix C).

198 To compare anisotropy characteristics of *Haa* and *WDaaL*, stress levels
199 at 80mmHg and 120mmHg were first computed using Laplace law $\sigma_{\theta} = \frac{Pr}{h}$;
200 P and r being P_{dias} and $\frac{D_{dias}}{2}$ or P_{sys} and $\frac{D_{sys}}{2}$ respectively (Table 2); h being
201 the wall thickness. Using the experimental $\sigma - \lambda$ curves, values of longitu-
202 dinal and circumferential stretches were then inferred for σ_{θ} Laplace values
203 at 80 and 120mmHg using interpolation. The assessment of anisotropy at
204 diastole, Ani_{80} , and systole, Ani_{120} were then defined (Kamenskiy et al.,
205 2014) as the difference in longitudinal and circumferential stretches divided

206 by their average value. Whatever the stress level considered, the negative
 207 anisotropy values confirm that mechanical response is stiffer in the longitu-
 208 dinal direction for both healthy and *WDaaL* samples (Table C.1 Appendix
 209 C). Moreover, these high values show that anisotropy increases when there
 210 is dissection, $Ani_{80}=-0.017$, $Ani_{120}=-0.03$ for *Haa* versus -0.025 and -0.037
 211 respectively for *WDaaL*. Finally, for *Daa* flap samples the mean anisotropic
 212 level, defined as $Ani = 2\frac{A_{LL}-A_{\theta\theta}}{A_{LL}+A_{\theta\theta}}$, is 0.175 (Table C.2 Appendix C). While
 213 *Haa* and *WDaaL* show a stiffer response in the longitudinal direction, flap
 214 samples behave in the opposite way.

215

216 3.2.2. Histological characterisation

217 Figures 6 and 7 illustrate histological transversal and axial cuts of *Haa*
 218 and *Daa* flap samples respectively. A sketch of both cuts (figure 6e) shows
 219 that the transversal cut (TC) and axial cut (AC) generate faces that will
 220 be stretched in the longitudinal and circumferential direction respectively.
 221 Observations of the *Haa* TC reveal less elastin and more collagen fibres than
 222 for the AC (figures 6a), b) and c), d) respectively). The fibre areal densities
 223 (Table 1) quantitatively confirm these qualitative data. The ratio of collagen
 224 to elastic areal density within the *Haa* TC total wall is greater than for AC,
 225 3.61 versus 1.68.

226

227 Qualitative and quantitative comparison of histological cuts of *Haa* and
 228 dissected samples (figures 6, 7 and table 1) highlights lower elastin fibre den-
 229 sity and higher collagen fibre density for the dissected samples within the
 230 AC of the media layer compared to healthy samples (20 ± 2 versus 30 ± 6 and

fibres	Healthy samples n=3		Dissected samples n=4	
	Axial cut	Transversal cut	Axial cut	Transversal cut
Within total wall				
Elastin (%)	30±5.5	19.5±3	NA	NA
Collagen (%)	50.5±4	70.5±14	NA	NA
Within media layer				
Elastin (%)	30±6	18±2	20±2	24±3.5
Collagen (%)	54±4	66±8	60±4.5	45±6

Table 1: Average values in % of the elastin and collagen fibres areal density for both healthy and dissected samples.

231 60±4.5 versus 54±4 respectively). Moreover, figures 7b) and a) clearly show
232 areas with broken elastin fibres and dislocations, when flap media are com-
233 pared with *Haa* media.

234

235 3.2.3. *In vivo* measurements

236 Comparison of E_p and β parameters (Table 2) between the two groups of
237 patients shows that those with dissection have a significantly higher stiffness
238 index than control patients, with 20.7±24 versus 6.1±2.8, p=0.013. In addi-
239 tion, mean E_p in the dissection group is 436±393 kPa, compared to 198±107
240 kPa in the control group (p=0.041).

241 The systolic and diastolic aortic diameters are significantly larger in the
242 dissection group (p<0.001 for both) and no correlation is found between sys-
243 tolic ascending aorta diameter and stiffness index (p=0.87) and E_p (p= 0.82).

244

	Control group (n=22)	Dissection group (n=13)	p
Systolic diameter (mm)	34.8±4.9	47.7±5.5	<0.001
Diastolic diameter (mm)	33.4±4.9	44.3±5.2	<0.001
Systolic pressure (mmHg)	137±25	110±22	0.06
Diastolic pressure (mmHg)	79±14	57±17	0.01
E_p (kPa)	198±107	436±393	0.041
β	6.1±2.8	20.7±24	0.013

Table 2: Systolic and diastolic diameter and pressure values from *in vivo* measurements. Resulting Peterson modulus and stiffness index values for control and dissection groups. Data were expressed as mean \pm standard deviation. Comparisons were performed using the Mann-Whitney U test. The statistical significance was defined as $p < 0.05$

245 4. Discussion and conclusion

246 The first question that merits discussion is whether the behaviour of the
247 healthy aortic ascending wall is anisotropic or isotropic. This suggests the
248 need to link analyses performed at different scales to enhance understanding.
249 Results in the literature differ depending on the donors' age. Azadani et al.
250 (2012) and Martin et al. (2011) showed isotropic behaviour for both relatively
251 young (mean age 47) and very old (aged from 81 to 98) donors. Labrosse et
252 al. (2009) and Haskett et al. (2010) observed anisotropic behaviour with a
253 stiffer mechanical response in the circumferential direction than in the lon-
254 gitudinal direction for patients aged from 31 to 71. However, Haskett et al.
255 (2010) also observed that the longitudinal direction tended to stiffen with age
256 more than the circumferential direction, with donors over 61 showing lower
257 longitudinal than circumferential peak strain. In addition, Kamenskiy et al.
258 (2014) reported that 7 patients out of 8, with a mean age of 54, had a more
259 compliant mechanical response in the circumferential direction than longi-

260 tudinally. The general mechanical behaviour of our donors, 58 to 69 years
261 old, nonlinear and anisotropic, is consistent with behaviours observed in the
262 literature for the same age (figure 8). In our case, the mechanical response
263 is stiffer in the longitudinal direction. In parallel, the ratio of collagen to
264 elastic areal density is greater for a sample face stretched in the longitudinal
265 direction. As collagen fibres contribute to stiffening whereas elastic fibres
266 increase distensibility, the histological analysis confirms the results obtained
267 using biaxial tensile tests.

268 Several components of the extra-cellular matrix may be involved in dissection
269 and the associated mechanisms are not yet fully understood. Dysfunction in
270 the contractile apparatus within smooth muscle cells may place the tho-
271 racic aorta at increased risk (Emmott et al., 2016). Humphrey (2013) or
272 Roccabianca et al. (2014) have, meanwhile, revealed that pooled glysamino-
273 glycans/proteoglycans can induce stress concentration and pressure swelling
274 within the aortic wall, and thus contribute to dissection. Nevertheless, elastin
275 and collagen fibres are obviously implicated in arterial wall elasticity, tensile
276 stiffness and strength, and anisotropy with stiffer mechanical response in the
277 longitudinal direction could also play a role in dissection.

278 Few mechanical characterisations have been performed on dissected ascend-
279 ing aortic walls; it is therefore important to compare healthy and dissected
280 samples. Like *Haa*, *WdaaL* presents a stiffer mechanical response in the
281 longitudinal direction. Although the number of samples we tested is a limi-
282 tation, this result is in agreement with the only published work using biaxial
283 tests to characterise the mechanical behaviour of *WDaal*: Babu et al. (2015)
284 observed the same anisotropic feature for patients over 50 years old with a

285 dissection. Complementarity between *in vitro* and *in vivo* measurements, a
286 strength of our method, enables us to further the analysis. By subjecting
287 excised aorta samples to mechanical solicitation via *in vitro* biaxial tensile
288 tests, anisotropy, nonlinearity and stress are assessed at different stretch
289 values; Young's modulus can also be inferred within a specific range of so-
290 licitations. *In vivo* measurements enable aorta radial strain and pressure to
291 be assessed at systolic and diastolic instants, thereby revealing aorta disten-
292 sibility (β or E_p). These two supplementary measurements allow arterial
293 stiffness to be assessed from a different perspective. Here, *WDaaL* showed
294 greater rigidity than *Haa*. Only one *WdaaL* was tested, but this *in vitro*
295 assessment is confirmed by *in vivo* measurements performed on 13 patients
296 with dissection and 22 without. Patients with dissection had a significantly
297 higher stiffness index and E_p values than control. Moreover, as the *in vivo*
298 measurements were performed on patients of comparable ages, the dissection
299 stiffening cannot solely be associated with ageing. The relevance of our *in*
300 *vivo* measurements is underlined by the fact that we found a mean E_p value
301 in the control group, consistent with the literature values based on normal
302 populations similar to ours in age and vascular risk factors (Hirata et al.
303 (1991) and Stefanadis et al. (1990)).

304 Moreover, *in vivo* measurements can challenge assumptions in the clinical
305 indexes used to evaluate dissection risk. The aortic diameter is often consid-
306 ered an important parameter to assess dissection evolution. Here, although
307 both systolic and diastolic diameters were significantly larger in the dissec-
308 tion group, no correlation appears between systolic diameter and β or E_p . As
309 dissection is clearly associated with stiffening, diameter does not therefore

310 appear to be relevant to predict the evolution of dissection.
311 More work should be focused on better evaluating dissection evolution risk.
312 Not only is there a lack of mechanical characterisations of dissected aorta
313 samples, but there is also a pressing need to couple such studies with mi-
314 crostructural analysis, so as to link overall mechanical response to microstruc-
315 tural modification and focus on local changes in wall integrity.

316

317 Furthermore, fluid structure numerical modelling can add important in-
318 formation on the quantification of hemodynamic loads at the wall and which
319 are thus transmitted/perceived by the arterial wall cells. To date, few nu-
320 merical studies have used FSI modelling and appropriate arterial wall consti-
321 tutive laws (Alimohammadi et al., 2016). The results obtained in this work
322 on flap and dissected ascending aorta wall stiffness could be used to perform
323 such FSI numerical simulations, thereby contributing to the understanding
324 of hemodynamic stimuli. This would improve modelling of the aortic cell
325 response to such hemodynamic solicitations.

326

327 To conclude, this work used *in vitro* biaxial tensile tests, *in vivo* mea-
328 surements via transoesophageal echocardiography and histological character-
329 isation for ascending aorta mechanical characterisation, thereby combining
330 tissular, structural and microstructural levels in an original approach. To
331 our knowledge, this is the first study reporting biaxial tensile tests on the
332 intimomedial flap, and our *in vivo* investigations of mechanical properties on
333 acute type A dissection are the first in humans. Our major findings are : *i*)
334 The mechanical response of the intimomedial flap shows linear behaviour up

335 to stretch values of 1.2. Conversely, Haa and $WDaaL$ behave in a nonlinear
336 way. However, all show anisotropy. *ii*) The mechanical response of Haa in
337 the longitudinal direction is stiffer than in the circumferential direction. *iii*)
338 Dissection promotes ascending aorta stiffening.

339

340 The present work has some limitations. A larger number of samples would
341 be preferable, especially for biaxial tensile tests; we were mainly limited by
342 the logistics of testing fresh tissue. The tensile test protocol was established
343 in such a way as to be relevant to both healthy and dissected samples. In
344 particular, a rather low value of $\lambda_{max}=1.2$ was chosen to test the dissected
345 adventitia layer because of its fragility. Unfortunately, we did not manage
346 to test this specific sample, which would have permitted us to increase λ_{max}
347 for the other mechanical tests. Finally, the quantitative analysis of colla-
348 gen results contained in the histomorphetric data should be considered with
349 caution, because of the nonspecific nature of Trichrome Masson staining. De-
350 spite these limitations, which are currently the subject of ongoing studies,
351 our combination of *in vitro*, *in vivo* and histological results provides new
352 insights into the mechanical characteristics associated with ascending aorta
353 dissection.

354 5. Acknowledgment

355 The authors thank the Labex MEC ANR-11-LABX-0092 for financial
356 support. We thank Marjorie Sweetko for English language revision.

Conflict of interest statement

All authors declare that there are no conflict of interest.

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Appendix A. Table of subject characteristics

Table A.1 details the subject characteristics of all the samples tested using the biaxial tensile set-up.

Donor(D)ID and Sample(S)ID	Age(yr)	Gender	Risk factors (Rf)
D1S1	69	M	HBP
D2S1, D2S2, D2S3	69	M	no Rf
D3S1	58	M	smoking
D4S1	59	F	no Rf
D5S1	64	M	HBP and smoking
Flap(F)ID and Sample(S)ID	Age(yr)	Gender	Risk factors
F1S1, F1S2	69	M	HBP
F2S1	53	M	no Rf
WDaaL(W)ID and Sample(S)ID	Age(yr)	Gender	Risk factors
W1S1	57	M	HBP

Table A.1: Donors and dissected subject characteristics. M for male, F for female, HBP for High Blood Pressure.

The number of healthy samples (n=3) histologically characterised, was a subset of those tested on the biaxial tensile device. Two out of four dissected samples, which have been histologically characterised, were also tested on the biaxial tensile device. The 2 dissected samples, that were not tested *in vitro*, came from patients with similar risk factors as those tested.

Appendix B. Methodology of *in vivo* measurements

To determine the ascending aorta Peterson modulus and stiffness index for healthy and pathological cases, a standardized protocol using available TEE system (Philips EPIQ7 Ultrasound) with 4-7 MHz multiplane transoesophageal probe was used. Aortic diameters were measured 3 cm above the aortic valve location. Systole was detected by the full opening of aortic valve and end-diastole by the QRS wave peak on the simultaneously registered electrocardiogram. Pressure measurements were performed by sphygmomanometer for the control group and invasively by radial artery cannulation for the dissection group. A mean of two pressure measurements was retained for each patient. The dissection group was composed of patients aged from 50 to 85 years, with aortic dissection involving ascending aorta independently of the entry tear location occurring less than 2 weeks after the onset of symptoms. It is important to note that aortic dissection related to connective tissue genetic disorder (Marfan's or Ehlers-Danlos syndromes), iatrogenic dissection, type A intramural hematoma or dissection occurring in patients with bicuspid aortic valve were excluded of this pathological group. In the control group, patients were selected to match those in dissection group for age and vascular risk factors. They displayed no severe ascending aorta atherosclerosis, aortic-valve disease nor ascending aorta aneurysm.

Appendix C. Tables of results: coefficients of constitutive modelling, $\overline{R^2}$ and anisotropy levels

Table C.1 shows C , k_1 , k_2 coefficients and γ angle for the mean data obtained from the healthy samples (n=7), \overline{Haa} , and for the only one tested sample of Daa wall without dissected layers. $\overline{R^2}$ values obtained during the identification procedure and anisotropy level are also displayed. $Ani_{80} = 2 \frac{\lambda_L^{80} - \lambda_\theta^{80}}{\lambda_L^{80} + \lambda_\theta^{80}}$ and $Ani_{120} = 2 \frac{\lambda_L^{120} - \lambda_\theta^{120}}{\lambda_L^{120} + \lambda_\theta^{120}}$

	C (kPa)	k_1 (kPa)	k_2	γ	$\overline{R_\theta^2}$	$\overline{R_L^2}$	Ani_{80}	Ani_{120}
\overline{Haa}	29.828	10.369	20.93	0.922	0.861	0.811	-0.017	-0.03
Daa wall	13.950	62.056	12.01	0.879	0.902	0.911	-0.025	-0.037

Table C.1: Coefficients of the HGO constitutive modelling, $\overline{R^2}$ values and anisotropy level obtained through the optimization procedure for \overline{Haa} , mean data obtained from the n=7 healthy samples and for Daa wall without dissected layers.

Table C.2 shows the A_{ij} constitutive modelling coefficients of Daa flap samples, the $\overline{R^2}$ values obtained during the identification procedure and the anisotropy level, $Ani = 2 \frac{A_{LL} - A_{\theta\theta}}{A_{LL} + A_{\theta\theta}}$.

Flap(F)ID Sample(S)ID	$A_{\theta\theta}$ (kPa)	$A_{\theta L}$ (kPa)	A_{LL} (kPa)	$\overline{R_\theta^2}$	$\overline{R_L^2}$	Ani
F1S1	299.375	111.499	251.451	0.988	0.992	0.174
F1S2	265.494	96.506	212.808	0.978	0.991	0.220
F2S1	293.325	114.408	236.580	0.980	0.976	0.214
Mean data	277.653	114.350	232.861	0.995	0.991	0.175

Table C.2: A_{ij} coefficients, anisotropy index and $\overline{R^2}$ values obtained through the optimization procedure for each flap sample and for their mean data.

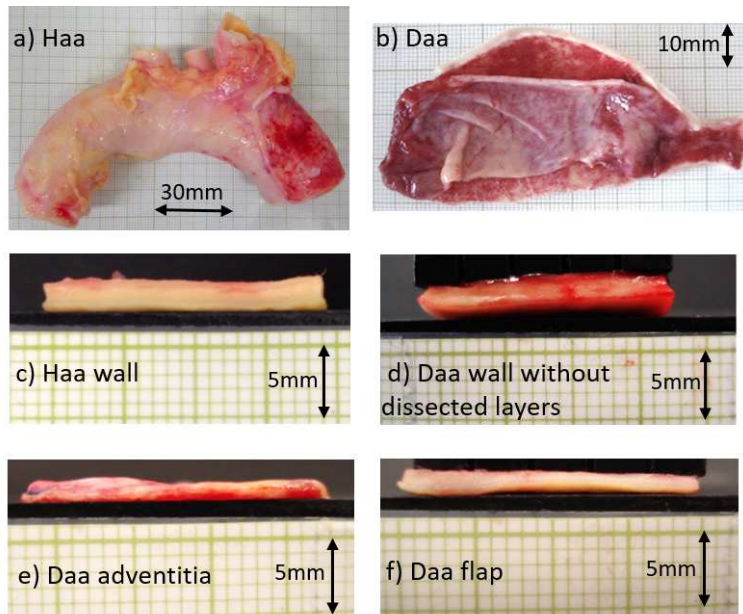


Figure 1: a) Representative healthy human ascending aorta, *Haa*. b) Representative fragment of dissected aorta, *Daa*. Side view of representative wall thickness of c) *Haa*, d) *Daa* without dissected layers, e) adventitia layer of *Daa*, f) intimomedial flap of *Daa*.

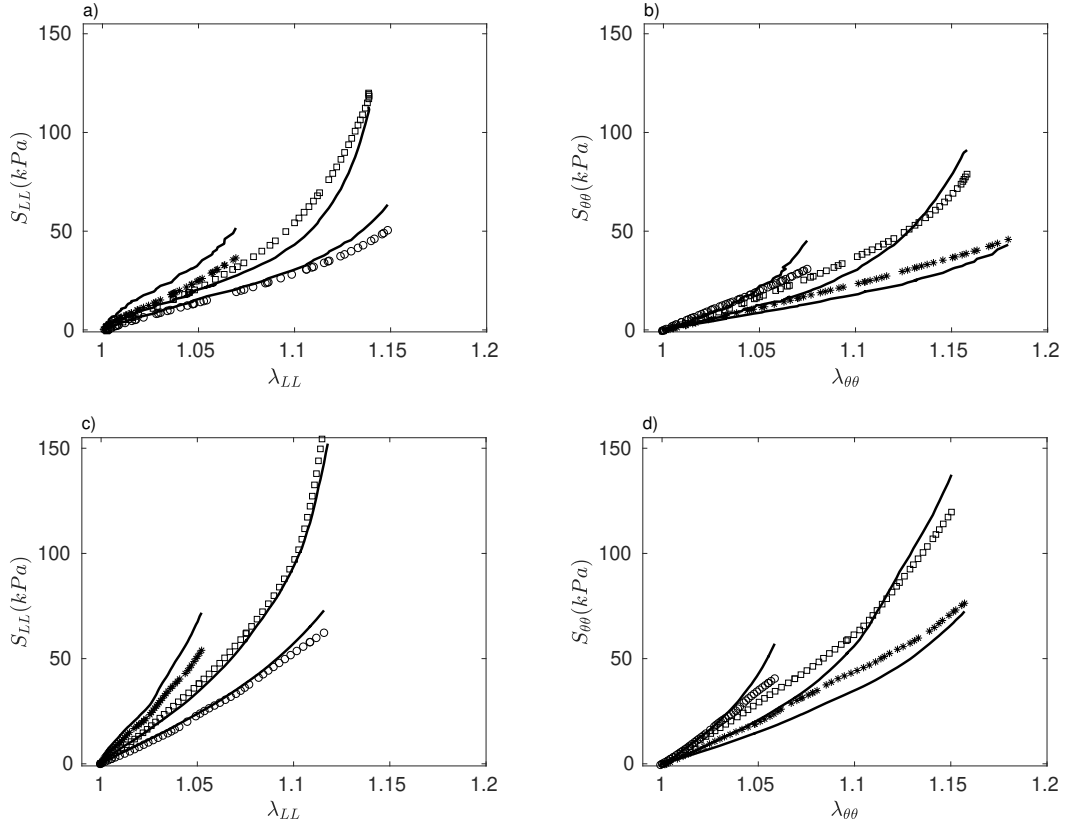


Figure 2: S- λ curves. a) & b) Mean data obtained from *Haa* samples. c) & d) *Daa* wall without dissected layers. $\alpha=0.5$ (circle symbol), $\alpha=2$ (star symbol) and $\alpha=1$ (square symbol). Symbols are used for experimental data and solid lines for constitutive modelling. a) and c): *L* direction. b) and d): θ direction

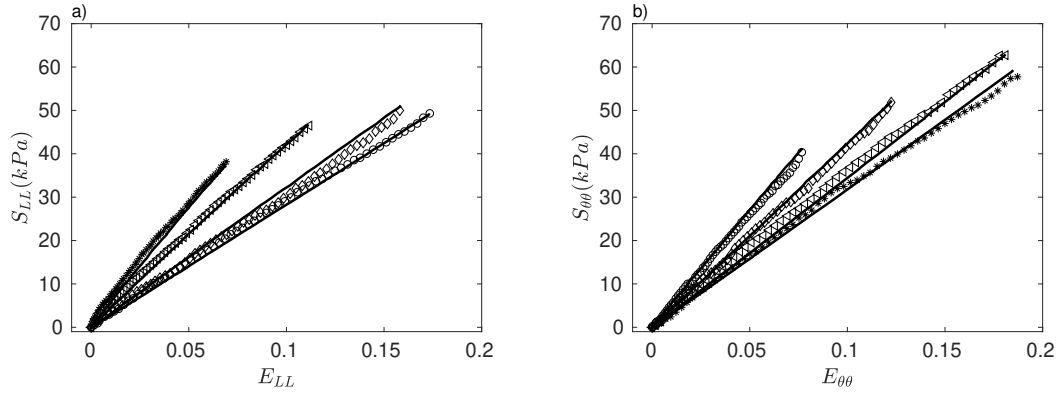


Figure 3: S-E curves plotted for the mean data obtained from the *Daa* intimomedial flap samples. $\alpha=0.75, 1.33, 0.5,$ and 2 (diamond, triangle, circle and star symbols respectively). Symbols are used for experimental data and solid lines for constitutive modelling. a) L direction. b) θ direction

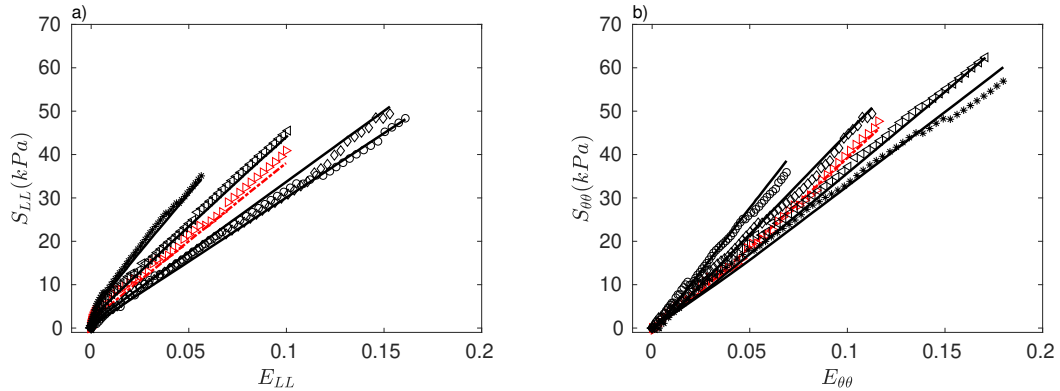


Figure 4: S-E curves illustrating a *Daa* intimomedial flap sample for $\alpha=0.75, 1.33, 0.5,$ and 2 (diamond, triangle, circle and star symbols respectively). Symbols are used for experimental data and solid lines for constitutive modelling. Red curves are for $\alpha=1$ that were not used for parameters identification. a) L direction. b) θ direction

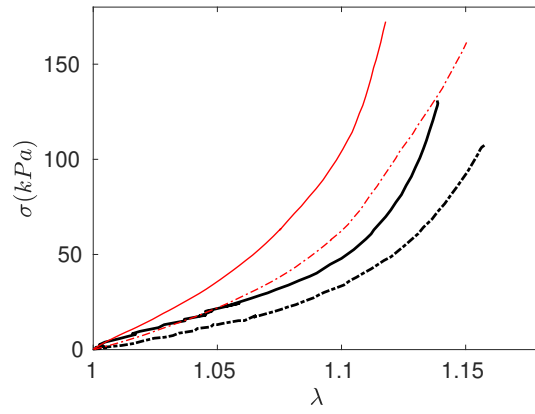


Figure 5: Comparison of $\sigma - \lambda$ curves between *Daa* wall without dissected layers sample and *Haa* mean sample (red thin and black thick lines respectively). Curves are plotted using constitutive modelling and $\alpha=1$. Solid and dashed lines are used for the L and θ direction respectively.

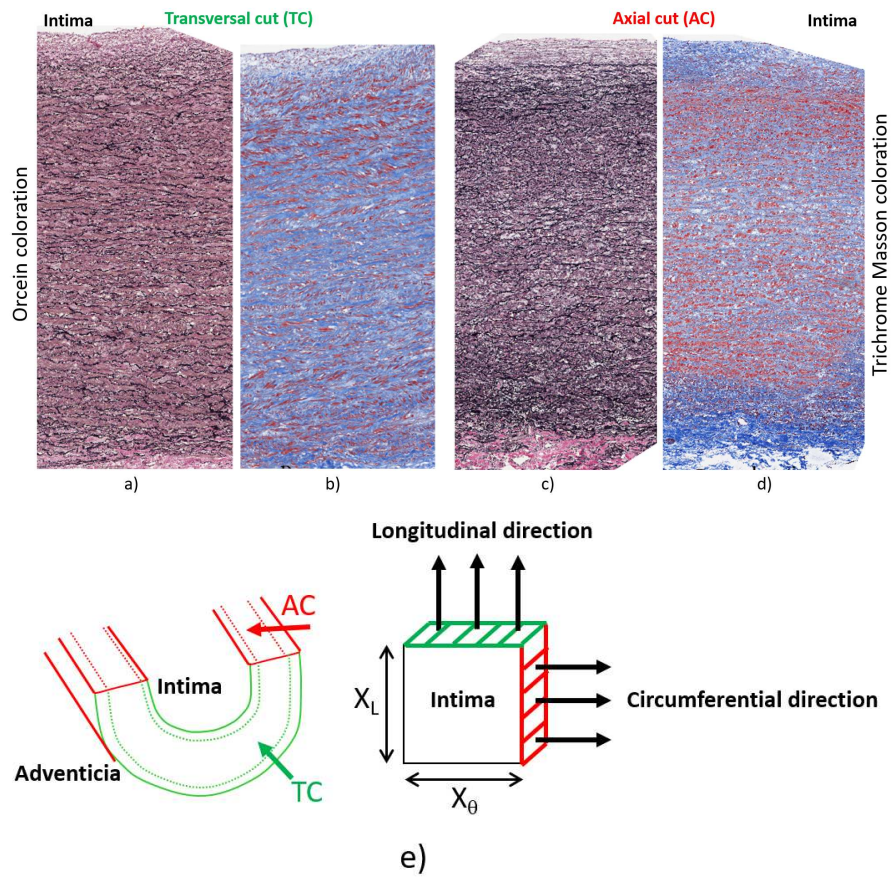


Figure 6: Transversal (a and b) and axial (c and d) histological cuts of *Haa*. Elastin fibres are colored in black a) and c) (orceid staining). Collagen fibres are colored in blue b) and d) (Trichrome masson staining). e) Scheme of arterial cuts and their corresponding stretching directions.

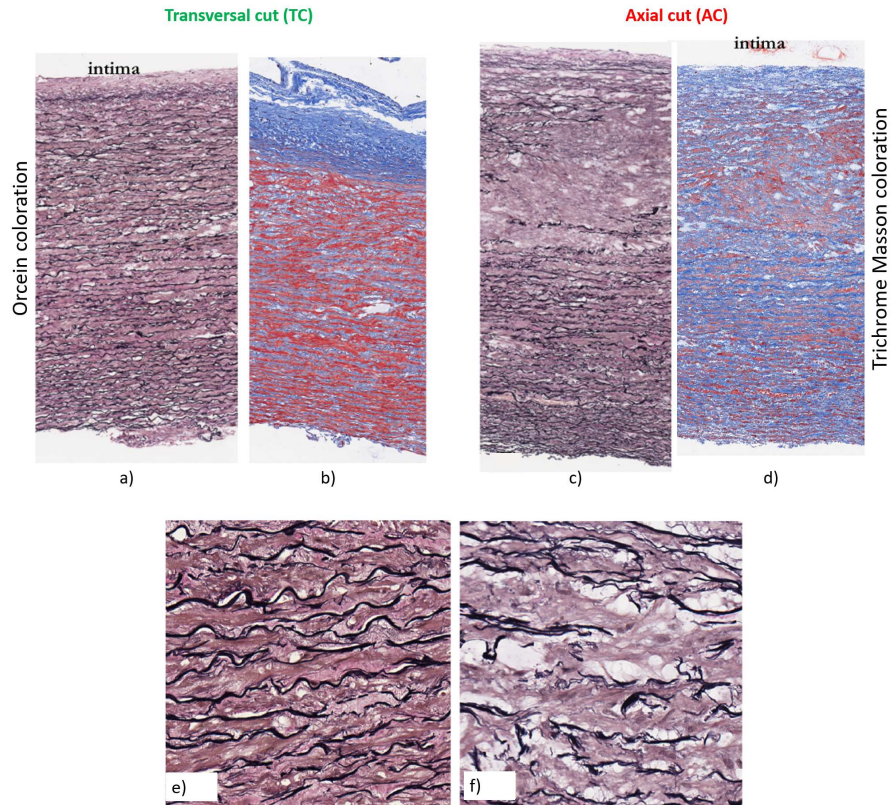


Figure 7: Transversal and axial histological cuts of *Daa* flap. Elastin fibres are colored in black a) and c) (orceid staining). Collagen fibres are colored in blue b) and d) (Trichrome masson staining). Zoom of the media layer histological transversal cut stained with orceid. e) Haa, f) Daa flap.

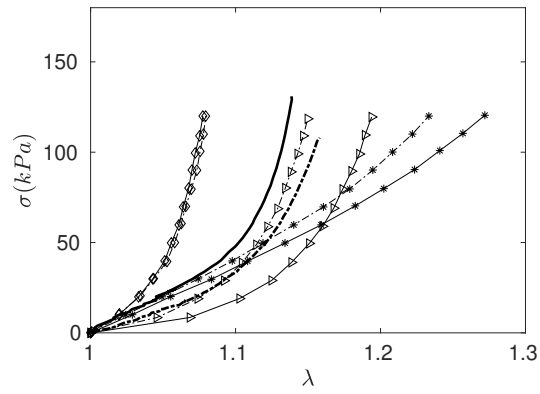


Figure 8: Comparison of $\sigma - \lambda$ curves between mean values of our Haa samples (without symbol) and Haa of literature (diamond, triangle and star symbols for Martin et al. (2011) Labrosse et al. (2009) and Haskett et al. (2010) respectively; extracted from (Roccabianca et al., 2014)). Our curves are plotted using constitutive modelling and $\alpha=1$. Solid and dashed lines are used for the L and θ direction respectively.