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ORIGINAL RESEARCH

The Role of the Mechanical Characteristics and Microstructure of the Porcine Aortic Wall: Implications for Abdominal Aortic Aneurysm Rupture Risk

Adrian Vasile Mureșan[1,2], Emil-Marian Arbănași[1,2,3,4], Eliza Russu[1,2], Reka Kaller[2], Claudiu Constantin Ciucanu[1,2,3], Alexandru Petru Ion[5], Andrei Bogdan Cordoș[4,6], Marius Harpa[4,7,8], Eliza-Mihaela Arbănași[3]

[1] Department of Vascular Surgery, "George Emil Palade" University of Medicine, Pharmacy, Science and Technology, Târgu Mureș, Romania
[2] Clinic of Vascular Surgery, Mureș County Emergency Hospital, Târgu Mureș, Romania
[3] Doctoral School of Medicine and Pharmacy, "George Emil Palade" University of Medicine, Pharmacy, Science and Technology, Târgu Mureș, Romania
[4] Regenerative Medicine Laboratory, Centre for Advanced Medical and Pharmaceutical Research (CCAMF), "George Emil Palade" University of Medicine, Pharmacy, Science and Technology, Târgu Mureș, Romania
[5] "George Emil Palade" University of Medicine, Pharmacy, Science and Technology, Târgu Mureș, Romania
[6] Veterinary Experimental Base, "George Emil Palade" University of Medicine, Pharmacy, Science and Technology, Târgu Mureș, Romania
[7] Department of Surgery, "George Emil Palade" University of Medicine, Pharmacy, Science and Technology, Târgu Mureș, Romania
[8] Emergency Institute for Cardiovascular Diseases and Transplantation, Târgu Mureș, Romania

ABSTRACT

Introduction: Abdominal aortic aneurysm (AAA) represents the increase of the diameter of the aorta by more than 50% in the absence of surgical or endovascular intervention. The risk of rupture and, therefore, mortality is increased significantly in AAA. The role of the mechanical characteristics of the AAA wall is poorly studied. The **aim** of this study was to determine the mechanical properties of each layer of the porcine abdominal aorta for a better understanding of the role of the microstructural elements of the arterial wall in the development and risk of AAA rupture. **Materials and methods:** In this study, eight tubular segments of the abdominal porcine aorta were examined. From these segments, we processed 13 × 13 mm square samples for biaxial analysis and 15 × 5 mm samples for uniaxial analysis. At the biaxial analysis, the intact wall and each layer (intima, media, and adventitia) were stretched by 25% at a speed of 1% per s and we determined the mechanical characteristics of the samples at the point of failure. **Results:** In the circumferential axis, we found the adventitia (0.233 MPa) to be stronger than the media (0.182 MPa, $p = 0.007$), intima (0.171 MPa, $p = 0.008$), and the intact wall (0.192 MPa, $p = 0.045$). In the longitudinal axis, the adventitia (0.199 MPa) was stronger than the intima (0.117 MPa, $p < 0.001$) and the intact wall (0.156 MPa, $p = 0.045$), but there was no statistically significant difference compared to the media. Additionally, the adventitia had a greater stiffness than the other two layers ($p < 0.05$ for both layers and axes) and the intact wall ($p < 0.05$ for both axes). Stretching until failure, the adventitia was the strongest

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compared to the other layers and the intact wall (p <0.001 for all), and it also presented better compliance, with the highest stretch ratio. **Conclusions:** The results indicate that the adventitia layer is the strongest and stiffest compared to the other two layers, being the last mechanical resistance structure of the arterial wall. It is crucial to avoid injuring and aggressively manipulating the adventitia during surgery to maintain the vascular wall's resistance structure. By taking the measures mentioned above, it is possible to prevent postoperative complications like anastomotic pseudoaneurysm and anastomotic rupture.

Keywords: aortic wall, biomechanical profile, mechanical characteristics, abdominal aortic aneurysm

CORRESPONDENCE

Claudiu Constantin Ciucanu
Str. Gheorghe Marinescu nr. 50
540136 Târgu Mureș, Romania
Tel: +40 734 134 044
Email: claudio.ciucanu@gmail.com

INTRODUCTION

Aneurysmal disease is a condition in which the arterial wall bulges, causing the diameter to increase by more than 50% compared to the proximal vessel.[1] This can occur in various areas such as extracranial arteries,[2] visceral arteries,[3] arteriovenous fistulas for dialysis,[4,5] and most commonly in the thoracic and abdominal aorta.[6,7] The guideline of the European Society of Vascular and Endovascular Surgery (ESVS) states that surgical or endovascular treatment is required for an abdominal aortic aneurysm (AAA) with a diameter greater than 5 cm in women and 5.5 cm in men.[1] Deprived of surgical or endovascular intervention, the risk of rupture and, therefore, mortality increase significantly.[8–10]

Numerous studies have been recently published on the porcine aorta[11–13] and a limited number of human aortic tissues harvested from the deceased or during surgery to better understand the biomechanical process behind the onset and development of AAA, as well as the risk of rupture.[14,15] Histologically, the arterial wall consists of three layers: intima, media, and adventitia, with different structures and functions.[16] Mechanically, the intima only becomes significant with age,[17] whereas the media primarily consists of smooth muscle cells and has a pivotal role in the wall's mechanical characteristics.[16] Finally, the adventitia envelops the vessel and provides resistance to high pressure, preventing rupture.[18] Niestrawska *et al.*[19] demonstrated that the development of AAA involves several stages from a histological and biomechanical perspective, including remodeling of collagen fibers, elastin, and smooth muscle cells, and deposition of adipose cells.

The modern management of aortic aneurysms, whether thoracic or abdominal, depends on the risk-to-benefit ratio, or whether the risk of rupture justifies the risks associated with surgical repair. The current surgical decision-making process is largely based on the maximum diameter of the aneurysm.[1] However, it is known that aneurysms with diameters smaller than the threshold specified in the current guidelines[20] can also lead to dissection or rupture, and they may represent an alarming 40–60% of cases.[21,22] In a recent study, Arbănași *et al.*[7] have shown that the ratio between the maximum diameter of an aneurysm and the diameter of the aorta at the level of the renal arteries or the celiac trunk is a better predictor of the risk of AAA rupture than the maximum diameter of the aneurysm alone. In contrast, Columbo *et al.*[23] found that for patients with an average age of 60 years, the optimal maximum diameter of the aorta to reduce the risk of postoperative mortality is 6.1 cm for women and 6.9 cm for men. Therefore, it is essential to develop more precise techniques to predict the rupture of an aneurysm, rather than relying on the aortic diameter, and more biomechanical analyses are necessary because aneurysm rupture occurs when the stresses on the vessel wall surpass the strength of the wall tissue.[24]

The aim of this study is to determine the layer-specific mechanical properties of the porcine abdominal aorta under physiological stress until failure for a better understanding of the role of the microstructural elements of the arterial wall in the development and risk of AAA rupture.

MATERIAL AND METHODS

In this study, eight tubular segments of the abdominal porcine aorta were taken from a local slaughterhouse from animals slaughtered exclusively for commercial purposes. Immediately after sampling, the porcine aorta segments were transported and stored at 4 °C, and they were processed and analyzed in less than 6 h.

ABDOMINAL AORTA SAMPLE PREPARATION

We cut each fragment along the longitudinal axis and carefully removed the lax tissue around the adventitia. Using a surgical scalpel, we prepared the following samples from each abdominal aorta:

- a 13 × 13 mm sample of the intact arterial wall, for the biaxial biomechanical analysis;

- a 15 × 5 mm sample of the intact arterial wall in the circumferential axis for the uniaxial biomechanical analysis of ultimate stress.
- a 15 × 5 mm sample of the intact arterial wall in the circumferential axis from which we meticulously detached the intima, the media, and the adventitia for the uniaxial biomechanical analysis of ultimate stress.

We evaluated the thickness of each sample using a digital caliper. We measured the thickness at the midpoint of each side and took the average of these measurements as the sample's thickness. Then, we submerged each piece in phosphate-buffered saline (PBS) at room temperature and subjected it to biomechanical analysis.

BIAXIAL BIOMECHANICAL ANALYSIS

We conducted the biaxial analysis using the BioTester 5000 from the Laboratory of Regenerative Medicine of the Advanced Medical and Pharmaceutical Research Center in Târgu Mureș, Romania. The BioTester is equipped with four actuators and two load cells of 23 N, and we used four BioRakes with 11-mm active parts to carry out the analysis. We set an initial distance of 11 × 11 mm and recorded the force-displacement graph for each sample. We stretched the sample by 25% at a speed of 1% per s for ten cycles. Each cycle was standardized to a period of 25 s of stretch followed by 25 s of recovery.

We used the values obtained during the last cycles for statistical analysis. First, we examined the intact aortic wall, then each of the three layers (intima, media, and adventitia) separately. We used the same biaxial biomechanical analysis protocol for both the intact wall and each layer. Using the data generated by the BioTester's LabJoy 2.0 software (CellScale), we calculated the Cauchy stress and Young's modulus, as described in the literature.[25–29]

UNIAXIAL BIOMECHANICAL ANALYSIS – ULTIMATE STRESS TESTING

With the same BioTester, using two metallic clamps positioned on two opposing actuators, we analyzed the circumferential axis (the aneurysmal growth axis) of the intact arterial wall, followed by each layer separately. We set an initial distance of 5 mm between the two clamps. The samples were handled by the same person to minimize any positioning bias. The analysis was preceded by a 50 mN preconditioning, followed by the stretch of the tissue until its rupture. Using the data generated by the BioTester's LabJoy 2.0 software (CellScale), we calculated the ultimate stress and the stretch ratio, as described in the literature.[25–29]

STATISTICAL ANALYSIS

Statistical analysis was carried out using SPSS 28.01.0 (IBM) for MacOS. Data are presented as medians and interquartile ranges, and differences between sets were compared using the Mann–Whitney U-test. The correlation between the thickness of the arterial wall and the ultimate stress and stretch ratio was analyzed using the Spearman correlation. A p value of <0.05 was considered statistically significant.

RESULTS

In this experimental study, we examined the biomechanical behavior of the porcine abdominal aorta using eight tissue samples from the anterior wall. We analyzed the mechanical characteristics of the tissues under physiological stress, as well as their ability to withstand stretching until failure. We recorded an average thickness of 1.82 ± 0.34 cm for the arterial wall, 1.13 ± 0.24 cm for the media, 0.43 ± 0.056 cm for the adventitia, and 0.12 ± 0.054 cm for the intima (Figure 1).

In the first part of the experiment, we applied a physiological stretch of 25% to each aortic wall sample. Then, we individually analyzed each layer using the same protocol. The results showed that in the circumferential axis (Figure 2A), the Cauchy stress of the adventitia (0.233 MPa) was higher than that of the media (0.182 MPa, $p = 0.007$), intima (0.171 MPa, $p = 0.008$), and the intact wall

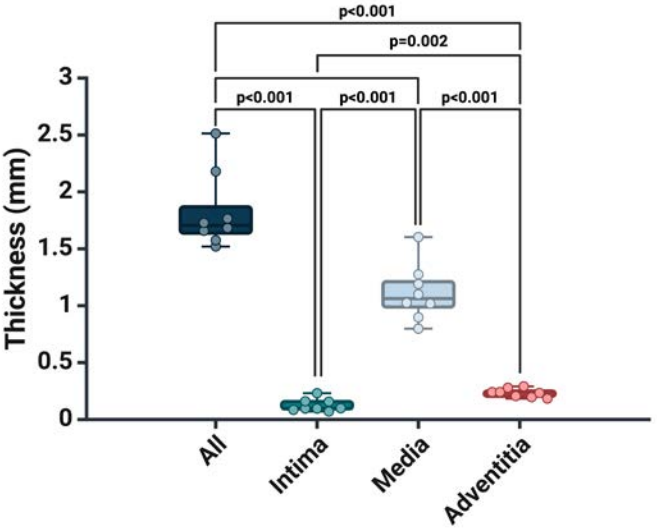

FIGURE 1. The thickness of the intact wall and of each layer

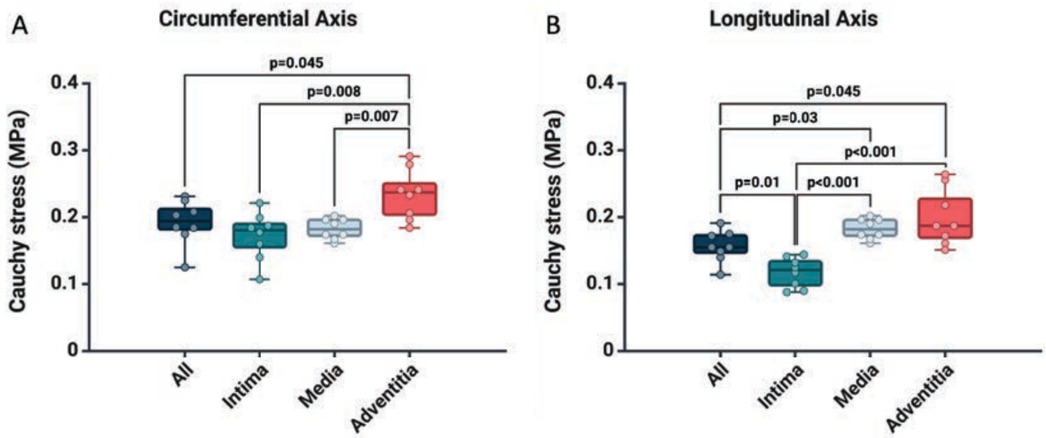

FIGURE 2. Cauchy stress for the circumferential axis (**A**) and the longitudinal axis (**B**) for the intact wall and for each layer at a stretch of 25%

(0.192 MPa, p = 0.045). In the longitudinal axis (Figure 2B), the adventitia (0.199 MPa) was stronger than the intima (0.117 MPa, p <0.001), and the intact wall (0.156 MPa, p = 0.045), but there was no statistically significant difference compared to the media. However, the intima exhibited the lowest Cauchy stress (0.117 MPa) compared to the intact wall (0.156 MPa, p = 0.01) and the media (0.142 MPa, p <0.001). Furthermore, the media was stronger than the intact wall (0.142 MPa vs. 0.156 MPa, p = 0.03).

Regarding the stiffness of each layer of the arterial wall, we found the same pattern for the circumferential axis. Thus, the adventitia had a greater stiffness than the other two layers (p <0.05 for both) and the intact wall (1.75 MPa vs. 1.32 MPa, p = 0.03). In the longitudinal axis, the adventitia showed the highest rigidity, but with the mention that the intima showed greater compliance than the media (1.02 MPa vs. 1.24 MPa, p = 0.014) and the intact wall (1.02 MPa vs. 1.32 MPa, p = 0.006). These findings suggest that the shear stress on the arterial wall caused by arterial pressure affects all three layers in physiological stress. Furthermore, the adventitia has a higher concentration of collagen fibers, which makes it more rigid than the other layers.

We analyzed 15 × 5 mm samples from the circumferential axis uniaxially and subjected them to stretching until failure. As seen in Figure 4A, the adventitia was the strongest compared to the other layers and the intact wall (p <0.001 for all), and it also presented better compliance with the highest stretch ratio, as seen in Figure 4B.

Next, we assessed the relationship between the thickness of the vascular tissue (for the intact wall and the three layers together, n = 32 samples) and mechanical characteristics at uniaxial analysis. The results showed a strong positive correlation between the thickness of vascular tissue and ultimate stress (r = 0.738, p <0.001). Additionally, there was a positive correlation between the thickness of vascular tissue and the stretch ratio (r = 0.422, p = 0.016) (Figure 5).

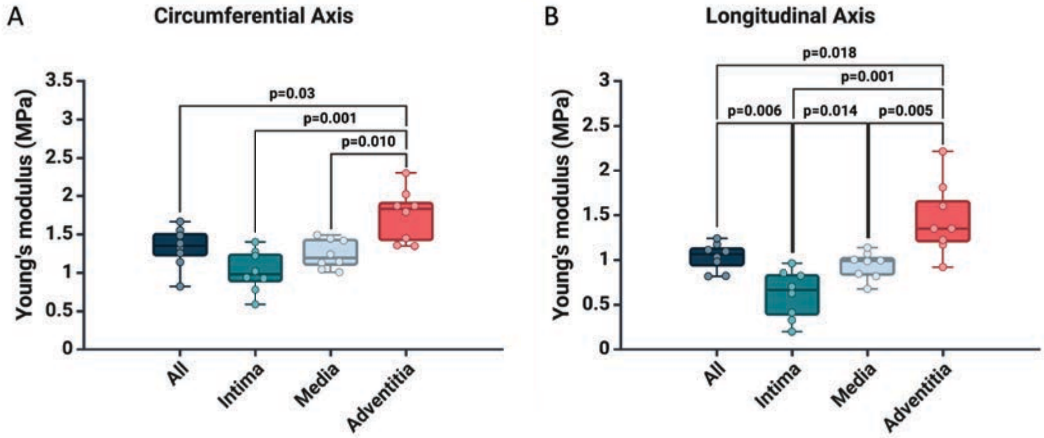

FIGURE 3. Young's modulus for the circumferential axis (**A**) and the longitudinal axis (**B**) for the intact wall and for each layer at a stretch of 25%

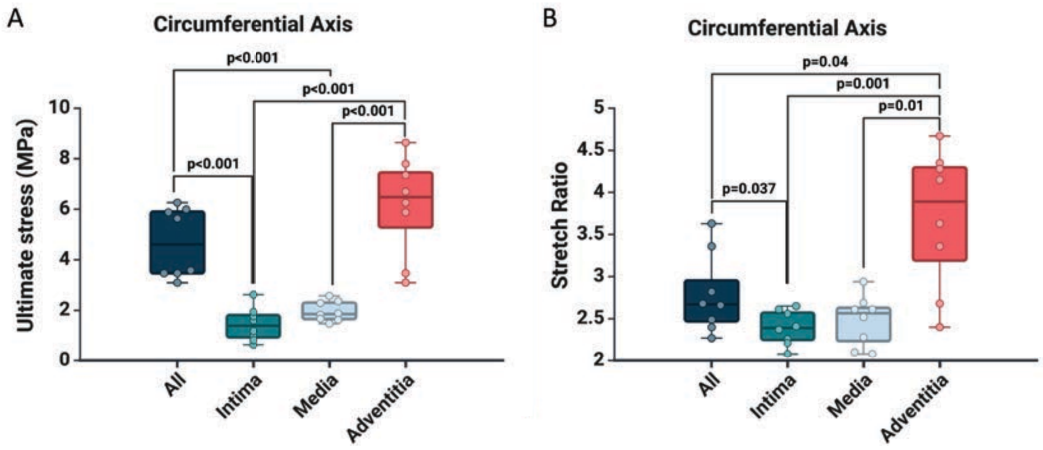

FIGURE 4. The mechanical characteristics of the porcine aortic wall and of each layer at uniaxial stretching in the circumferential axis until failure. **A**, Ultimate stress. **B**, Stretch ratio.

DISCUSSION

The main result of this experimental biomechanical study is the presentation of the biomechanical behavior of vascular tissue and its specific layers when subjected to physiological stretching until failure for a better understanding of the role of each layer of the vascular wall in the development and risk of AAA rupture.

Many previous studies have considered the aortic wall to be a uniform, consistent structure.[30–32] However, it is important to acknowledge that certain structural variations in biomechanical features exist between the longitudinal and circumferential directions, as well as between different regions of the aorta for all three layers of the arterial wall.[32,33] This was observed by Weisbecker *et al.*[32] and similar results were obtained by Noble *et al.*[33] The isotropic response of the intima and adventitia of the descending aorta contrasts with the similar anisotropic responses of intact wall and media samples, indicating that the mechanical response of the descending thoracic artery is primarily dominated by the behavior of the media.[34]

The aortic wall is a biological material comprised of elastin, collagen, and smooth muscle cells. According to Peña *et al.*,[35] the media is softer than the adventitia, regardless of their position along the aorta. Additionally, samples from the abdominal aorta are stiffer than those from the descending thoracic aorta, the most significant differences in their behavior being observed in the circumferential direction. This is consistent with our results and those of previous studies that have shown that aortic stiffness increases with the distance from the heart.[36–39]

Understanding the uniaxial and biaxial biomechanical behavior of the arterial wall can help in developing new therapeutic strategies to strengthen the early aneurys-

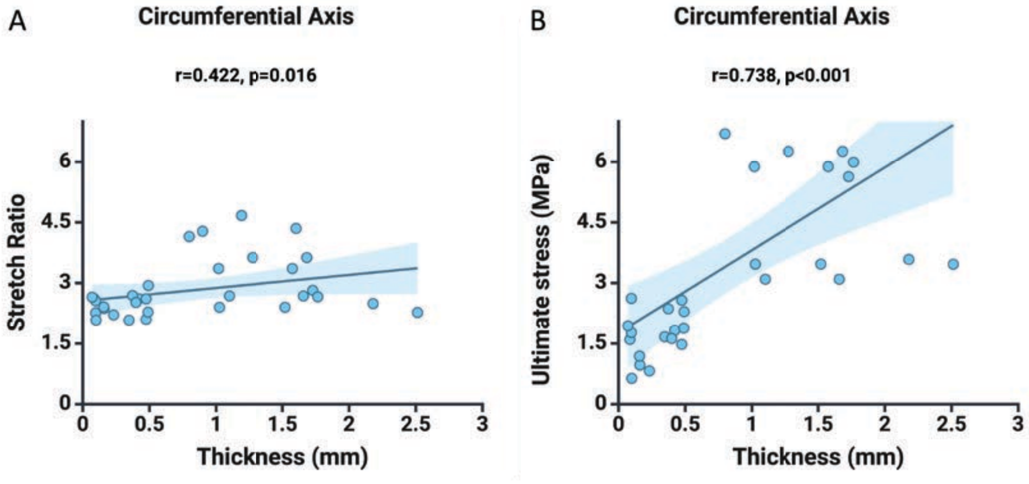

FIGURE 3. Spearman correlation between sample thickness and stretch ratio (**A**) and ultimate stress (**B**)

mal wall. Our team has recently proposed a new method for photocrosslinking adventitial collagen fibers. This involves exposing the wall to UV-A irradiation.[25–29] Chirila *et al.*[27] have presented preliminary results of this new therapeutic strategy, demonstrating an increase in strength ($p = 0.0015$) and stiffness ($p = 0.0012$) of the porcine aortic adventitia. Additionally, the same team has shown the effectiveness of exposure to UV-A in the case of enzymatically degraded aortic adventitia.[26,28] Most recently, our team has demonstrated that UV-A irradiation increases the mechanical characteristics of the vascular wall on samples of normal and aneurysmal human abdominal aorta.[25]

It is important to mention that the current study has some limitations. First, it did not include the histological analysis of the samples, and collagen and elastin fiber content was not measured. Second, it is important to consider that there are significant structural differences between porcine and human vascular tissue. Therefore, the results of this study cannot be extrapolated to human tissue. Last, the samples were not subjected to the same stress, which makes it difficult to compare the degree of anisotropy of each layer and the intact aortic wall.

CONCLUSIONS

The results of this study indicate that the porcine aortic wall presents the highest stiffness and strength in the adventitia when subjected to physiological stretch. The adventitia is also the strongest and most compliant layer in the uniaxial failure analysis, thus being the last mechanical resistance structure of the arterial wall. In addition, the samples' thickness is correlated with the mechanical characteristics of the tissue when stretched to failure. It is crucial to avoid injuring and aggressively manipulating the adventitia during surgery to maintain the resistance structure of the vascular wall and prevent post-operative complications like anastomotic pseudoaneurysm and anastomotic rupture.

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INSTITUTIONAL REVIEW BOARD STATEMENT

The present study has been approved by the Committee of Ethics in Scientific Research of the "George Emil Palade" University of Medicine, Pharmacy, Science and Technology of Târgu Mureș, Romania, decision no. 2653/15.12.2023.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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