Does Freezing Change the Mechanical Behavior of Aortic Tissue?

Kunal Shanbhag, YSP Student, *North Andover High School*
Anjali Misra, YSP Student, *Weston High School*
Guadalupe Garcia, Department of Mechanical and Industrial Engineering, *Northeastern University*
Sean Harrington, Department of Mechanical and Industrial Engineering, *Northeastern University*
Rouzbeh Amini, Department of Mechanical and Industrial Engineering, *Northeastern University*

Northeastern University **College of Engineering**

Northeastern University **Michael B. Silevitch and Claire J. Duggan Center for STEM Education**

Abstract

The aorta, the body's main artery, must stretch and recoil with every heartbeat to maintain healthy blood flow. Its mechanical behavior, including stiffness, flexibility, and strength, is important for understanding cardiovascular function, and conditions such as aneurysms and dissections. In soft tissue biomechanics research, aortic tissue is often tested outside the body (ex-vivo), but fresh samples are not always available. Due to this, tissue is commonly cryopreserved at subfreezing temperatures and tested later. This project determines whether short-term storage at -80°C changes the mechanical properties of descending thoracic aortic tissue. To test this, we compared fresh and frozen porcine aortic samples using biaxial mechanical testing, which stretches tissue in both circumferential and axial directions to mimic physiological loading. By using paired samples from the same tissue sources and consistent suture distances, we reduced variability and more directly measured the effect of freezing. We compared key mechanical outcomes such as stretch and stiffness, while also evaluating stress-strain behavior. Using the data from the fresh and frozen cycles, we compared mechanical characteristics like stiffness, stretch, and stress helping us understand whether frozen tissue behaves similarly to fresh tissue. In the end, our results indicated that there was no significant difference in the mechanical behavior between fresh and frozen descending thoracic aortic tissue, suggesting that short-term storage at -80°C preserved the mechanical properties of the tissue. Future work should test larger sample sizes, longer storage times, and different aortic regions to better define when frozen tissue can reliably replace fresh samples.

Background

The aorta is a extremely vital organ in the cardiovascular system, as it is responsible for distributing oxygenated blood from the heart throughout the body. It is therefore imperative that researchers can reliably study the pathogenesis of cardiovascular diseases (CVD) like aneurysms and dissections: diseases that can obstruct the functionality of the aorta which then disrupts the rest of the body.

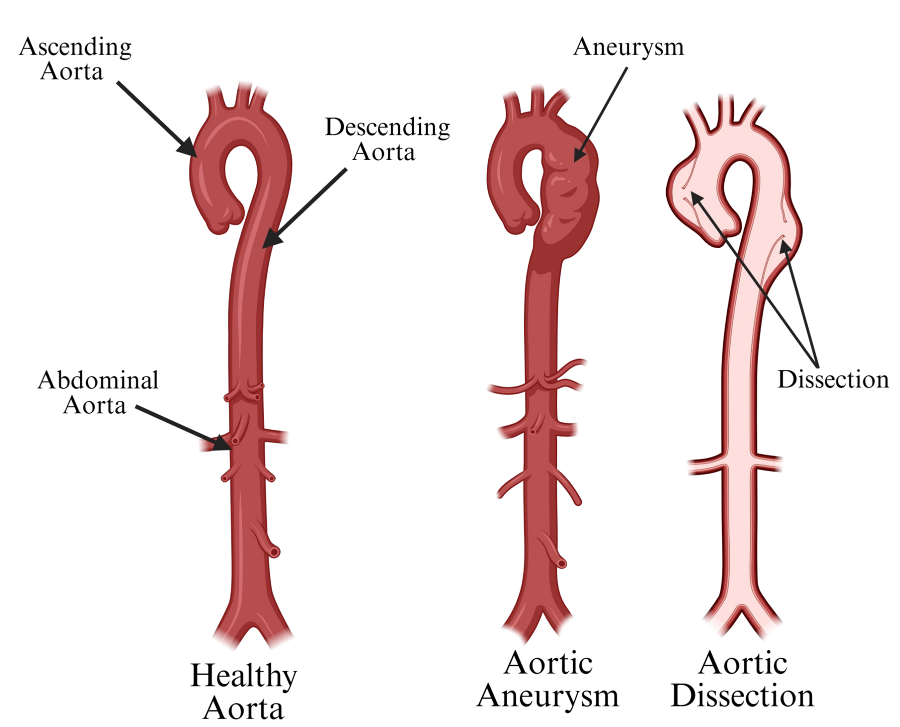

Figure 1. Diagram of healthy aorta, Aortic aneurysms, and aortic dissections

Because immediate mechanical testing of ex-vivo tissue is not always possible after harvest, researchers commonly cryopreserve samples at -80°C. When researchers utilize frozen aortic samples it is necessary that they share similar mechanical behaviors as fresh tissue to insure accurate representation of the aorta.

Previous labs have researched the effect of cryopreservation on other soft tissue: heart valves, tendons, arterial tissue. These findings suggest that the effect of freezing is tissue dependent; therefore, it is necessary to test individual soft tissue variants. Additionally, a study conducted in 2014 determined that cryopreservation at -20°C had negligible distinction between the mechanical behaviors of the fresh and frozen aortic tissue samples. Although the research proved conclusive that -20° C shows no change in property, the standard cryopreservation used in most research labs are at -80°C. Further testing is necessary to ensure that current lab cryopreservation is viable for proper research.

In accordance to past Lab assertions, we hypothesize that there are negligible differences between the mechanical properties of the aorta when fresh and post-frozen.

Experimental Methods

Experimental Procedure

I. Whole heart with aorta is harvested from pig and shipped on ice overnight
II. Aorta section is isolated and cut out, noting orientation with a mark in the upper right corner
III. Sutures are attached and fundical markers are glued on
IV. Samples, either fresh or frozen, are then mounted onto the biaxial machine for testing

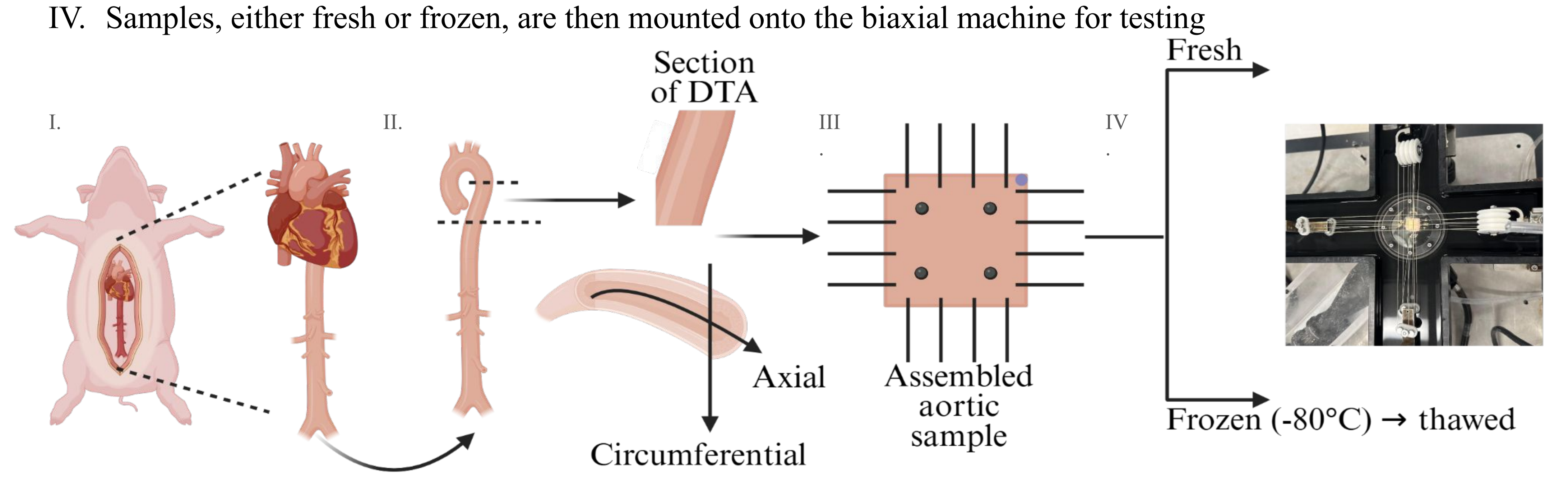

Figure 2. Schematic of experimental procedure

Freeze-Thaw Protocol

I. Samples are stored at -80°C
II. 5 days after freezing, samples are transferred to -20°C for 8-12 hours
III. Samples are then transferred to 4°C
IV. 3hr before testing, samples are transferred to room temperature PBS

Results

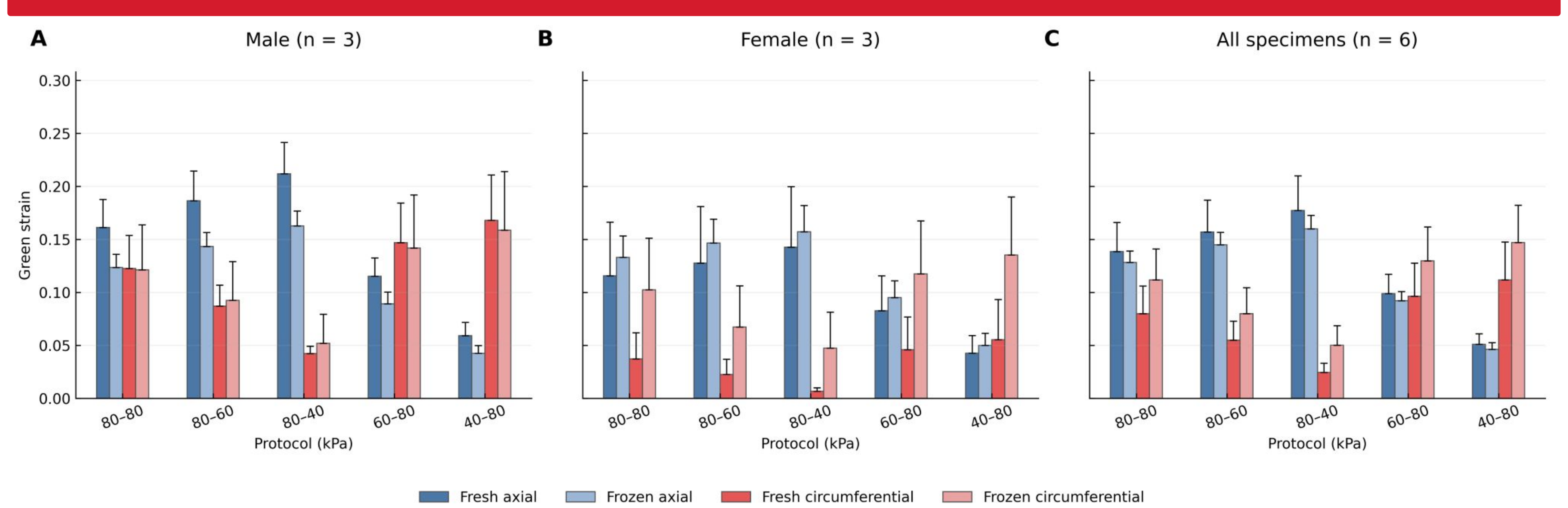

Figure 3. Three bar-charts which show the strain of test samples at different conditions and stress values: chart on the left, male samples; middle chart, female samples; and chart to the right, both female and male samples.

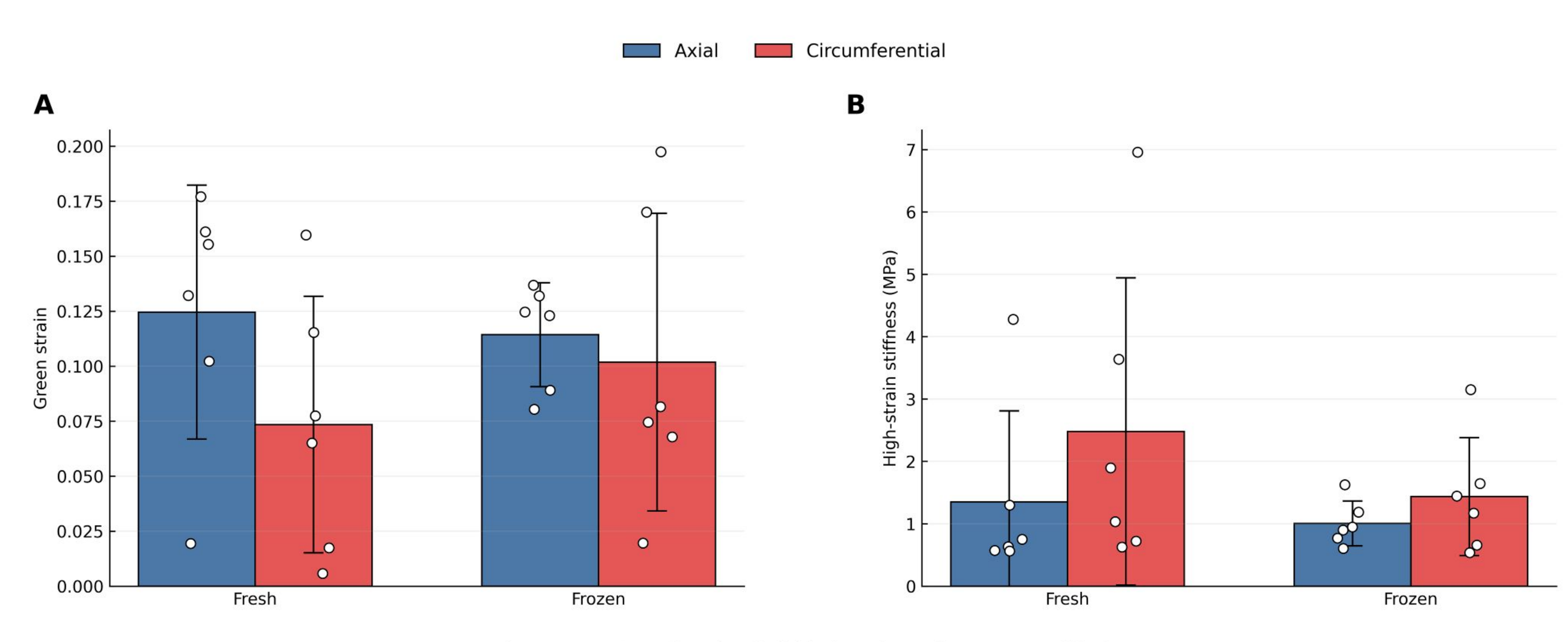

Figure 4. Chart A and B show data of the mechanical properties of the tissue when fresh and frozen: chart **A** depicts the green strain of Axial and Circumferential axis and chart **B** compares the stiffness of the axes when they were fresh and frozen.

Conclusion and Future Steps

Conclusion:

Overall, short-term freezing at -80°C did not produce a statistically significant difference in the mechanical behavior of fresh versus frozen descending thoracic aortic tissue. This suggests that, under our current protocol, frozen aortic tissue may be a viable substitute for fresh tissue. However, because our sample size was on a smaller scale, we cannot conclude whether sex-specific differences exist. Some trends may be present, but more data would be needed before making a strong claim.

Next Steps:

Future work should utilized a larger sample size to improve statistical conclusions, especially for comparing male and female tissue responses. It would also be useful to examine whether longer-term storage at -80°C changes tissue mechanics yields a different output versus short-term freezing. Additionally, future studies could compare different regions of the aorta, such as the ascending thoracic aorta versus the descending thoracic aorta, as mechanical behavior may vary by anatomical location.

References

Salinas, S. D., Clark, M. M., & Amini, R. (2020). The effects of −80 °C short-term storage on the mechanical response of tricuspid valve leaflets. Journal of Biomechanics, 98, 109462. https://doi.org/10.1016/j.jbiomech.2019.109462

Vargas, A. I., Tarraf, S. A., Fitzgibbons, T. P., Bellini, C., & Amini, R. (2023). Biomechanical remodeling of the murine descending thoracic aorta during late-gestation pregnancy. Current Research in Physiology, 6, 100102. https://doi.org/10.1016/j.crphys.2023.100102

Vargas, A. I., Tarraf, S. A., Jennings, T., Bellini, C., & Amini, R. (2024). Vascular remodeling during late-gestation pregnancy: An in-vitro assessment of the murine ascending thoracic aorta. Journal of Biomechanical Engineering, 146(7). https://doi.org/10.1115/1.4064744

O'Leary, S. A., Doyle, B. J., & McGloughlin, T. M. (2014). The impact of long term freezing on the mechanical properties of porcine aortic tissue. Journal of the Mechanical Behavior of Biomedical Materials, 37, 165-173. https://doi.org/10.1016/j.jmbbm.2014.04.015

Acknowledgements

Soft Tissue Biomechanics Lab
Rouzbeh Amini, Professor
Guadalupe Garcia, PhD Student
Sean Harrington, PhD Candidate
Center for STEM Education
Claire Duggan, Executive Director
Jennifer Love, Associate Director
Kenneth Rene Santizo Carbajal,
Frances Corcell & Ahmed Othman, YSP Coordinators
Nicolas Fuchs, Program Manager
Mary Howley, Administrative Officer

This poster contains unpublished confidential information

This work was supported by the National Science Foundation