Exploring Arterial Dysfunction in C57BL/6J Mice through Histological Examination

Mattia Reformato-Santoro, Reagan Stern, Mostafa Sobouri M.S., Xiangyu Zheng M.S., Daniel R. Machin PhD
Florida State University, College of Education, Health, and Human Sciences

Background

C57BL/6J mice are prized for their genetic uniformity and similarity to human physiology. This study focuses on unraveling the intricate relationship between diet, and arterial dysfunction—a precursor to cardiovascular disease (CVD). The Western Diet, prevalent in the U.S., is associated with obesity and arterial dysfunction, prompting investigation into its effects. By utilizing these mice, which share genetic traits with humans, we aim to uncover the underlying mechanisms linking dietary habits and sedentary lifestyles to cardiovascular health.

Arterial dysfunction poses significant health risks, including hypertension and endothelial dysfunction. Given that CVD remains a leading cause of mortality worldwide, understanding the factors contributing to arterial dysfunction is paramount. Through meticulous examination of diet-induced effects on arterial health using C57BL/6J mice, this research aims to elucidate pathways involved in cardiovascular pathogenesis. Insights gained could inform targeted interventions and public health strategies to mitigate the burden of CVD. The preliminary results from both the trichrome and elastin stains show that as the mice age, the collagen around the aorta increases and the elastin tissue around the aorta decreases, which leads to greater aortic dysfunction.

Methodology

- Mice are fed a normal chow diet for 12 weeks, then the mice either stay on the normal chow diet (NC), or are switched to the 4% salt normal chow diet (NC4%), western diet (WD), or 4% salt western diet (WD4%)
- After 12 weeks on the second diet, the mice are sacrificed
- Tissues are weighed & aortas are stored at -80 degrees Celsius in optimal cutting temperature (OCT) compound
- Aorta is cut using a cryostat machine at 8 micrometers thick
- ½ of the slides for each mouse aorta will be elastin stained and the other ½ will be trichrome stained
- Slides are imaged by Nikon Eclipse TS100 and stitched together using Microsoft Composite Editor
- Stitched images are analyzed using Image J to determine the aorta wall thickness and area.

Elastin Protocol

- Slides are put into distilled water for 2 minutes
- Transfer slides to Working Elastin Stain solution for 15 minutes
- Rinse slides under running tap water for 10 minutes until there is no excess solution
- Dip slides into Differentiating solution 20 times
- Rinse slides for 1 minute under running tap water
- Set slides in Sodium Thiosulfate Solution for 1 minute
- Rinse under running tap water for 1 minute
- set in Van Geison's Solution for 3 minutes
- set in 95% EtOH (twice changed)
- Set in 100% EtOH for 1 minute
- set in Xylene for 3 minutes twice
- Finally mounted with xylene based mounting medium

Trichrome Protocol

- Slides are set in decreasing percentages of EtOH (95%, 75%, 50%, 25%) for 2 minutes each,
- Then set in distilled water for 2 minutes and set overnight in Bouin's solution
- Following day, the slides are put under running tap water until the yellow disappears
- Rinse with DI water, and set in Weigert's hematoxylin for 5 minutes,
- Wash under tap water for 10 minutes, rinse in DI water for 1 minute, set for 5 minutes in Beibrich Scarlet-Acid Fucshin Solution,
- Dipp in DI water for 3 minutes, set for 7 minutes in Phosphotungstic/phosphomolybdic Acid Solution, set for 4 minutes in Aniline Blue Solution
- Dip in DI water for 3 minutes, set in 1% acetic for 3 minutes,
- Dip in DI water for 3 minutes, then set in increasing percentages of EtOH (75%, 95%, 100%) for 4 minutes each, and set in Xylene for 9 minutes

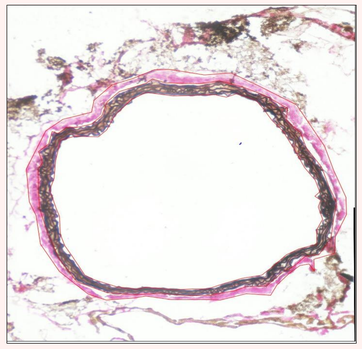
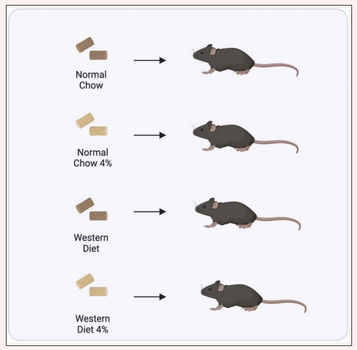


Figure 1: Elastin-stained aorta



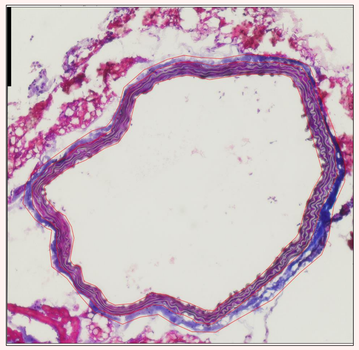


Figure 2: Trichrome-stained aorta

Discussion

We are comparing the average wall thickness, average whole aorta area, and average lumen diameter (inner-most aorta ring shown in figures **1** and **2** between the four different diets.

The graphs show the data produced by the elastin staining. We are not comparing measurements between the elastin staining and the trichrome (collagen) staining because it should be constant from both stains.

This research provides insight into how high salt diets, as well as aging, impact the tissues surround the aorta and lead to more arterial dysfunction.

References

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Preliminary Results

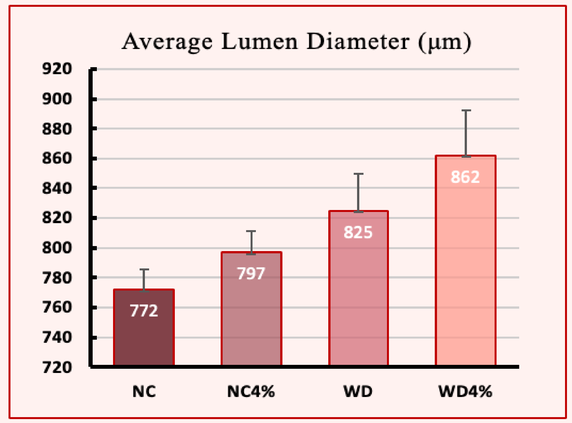

Figure 3: Comparisons between diet strains in the average lumen diameter; the inner-most part of the artery.

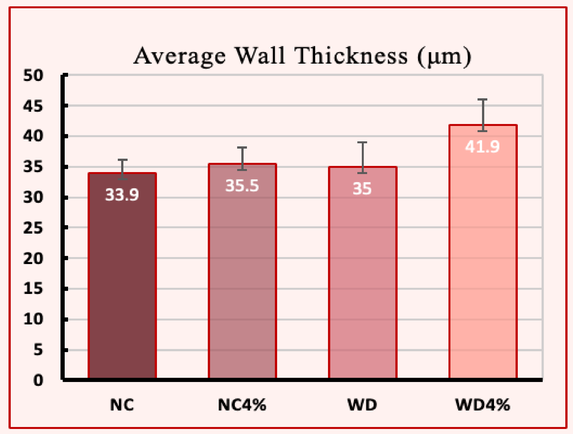

Figure 4: The graph demonstrates the average wall thickness between the four diet strains in the Elastin-stained aorta.

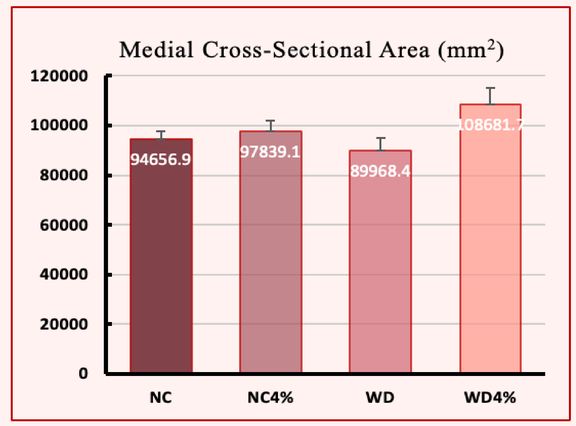

Figure 5: The graph demonstrates the average medial cross-sectional area (CSA) between the four diet strains in the Elastin-stained aorta.