

**Cardiac motion simulation in cadavers and carotid artery
longitudinal wall behaviour**

by

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Author's Declaration

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

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Abstract

Carotid artery longitudinal motion (CALM) is a bidirectional, multiphasic motion of the intima-media complex of the common carotid artery (CCA) wall. This motion has exhibited changes with aging, elevated cardiovascular disease risk, and various diseased conditions. Understanding the determinants of this motion may position CALM as a diagnostic tool for arterial health. Retrograde wall displacement is the hallmark feature of this phenomenon and represents movement of the arterial wall in the opposite direction of blood flow, but its etiology remains undiscovered. While retrograde motion has been proposed to result from cardiac contraction due to ventricular-vascular coupling of the arterial tree and the heart, separating hemodynamic influences from cardiac contraction is not possible *in vivo*. This study explores simulated cardiac contraction in cadavers and the influence of longitudinal prestretch and material stiffness on carotid artery longitudinal wall behavior. Eight cadavers (5 males, 85 ± 7 years ($n = 7$) in addition to one 19-year-old donor ($n = 1$)) underwent a dissection and tissue testing protocol. The dissection protocol examined the relationship between caudal force application at the ascending aorta and resulting CCA longitudinal wall displacement. A repeated measures correlation revealed a moderate negative correlation for applied force and wall displacement ($r = -0.68$, $p < 0.001$, 95%CI [-0.76, -0.60]). Displacement was estimated for a standardized force of 6 N for each donor and artery. Longitudinal prestretch did not influence displacement at 6 N ($r_{LCCA} = -0.13$, $p = 0.78$; $r_{RCCA} = -0.63$, $p = 0.13$). There was no significant relationship found between displacement at 6 N and either longitudinal strain [$r_{LCCA} = -0.72$, $p = 0.070$; $r_{RCCA} = -0.27$, $p = 0.56$] or elastic modulus at 35 kPa [$r_{LCCA} = 0.50$, $p = 0.25$; $r_{RCCA} = 0.29$, $p = 0.53$] for either side. Circumferential strain [$r_{LCCA} = -0.068$, $p = 0.14$; $r_{RCCA} = 0.13$, $p = 0.79$] and elastic modulus [$r_{LCCA} = 0.58$, $p = 0.23$; $r_{RCCA} = -0.31$, $p = 0.50$] were not related with displacement at 6 N for either side. Differences between the LCCA and RCCA were identified for the slope of force-displacement line of best fit [$p = 0.0347$, Cohen's $d = 1.17$, $n = 6$], longitudinal strain at 35 kPa [$p = 0.020$, Cohen's $d = 1.37$, $n = 6$], and circumferential elastic modulus at 35 kPa [$p = 0.018$, Cohen's $d = 1.72$, $n = 5$],

suggesting increased wall displacement in the LCCA and increased stiffness of the RCCA. Our findings suggest that cardiac contraction is a primary determinant of CALM, and cardiac imaging should be conducted with CALM imaging in future studies to contextualize differences in motion between individuals and across acute interventions. Due to anatomical branching differences, images should be taken on the LCCA for a more direct influence from cardiac contraction. Our results support the ventricular-vascular coupling theory for retrograde motion in CALM and demonstrate greater wall displacement in the LCCA compared to the RCCA.

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List of Abbreviations:

CALM: carotid artery longitudinal motion
CCA: common carotid artery
CLSM: confocal laser scanning microscope
CPA: cryopreservation agents
CRF: controlled-rate freezer
CVD: cardiovascular disease
LCCA: left common carotid artery
LV: left ventricle
LWM: longitudinal wall motion
NCC: normalized correlation coefficient
NO: nitric oxide
PP: pulse pressure
PVAT: perivascular adipose tissue
PVC: premature ventricular contraction
RCCA: right common carotid artery
RMCORR: repeated measures correlation
ROI: region of interest
VSMCs: vascular smooth muscle cells

1.0 LITERATURE REVIEW

Cardiovascular disease (CVD) is an umbrella term including ischemic heart disease, heart failure, stroke, peripheral arterial disease, and various other cardiac and vascular conditions (1). CVD is one of the leading causes of global mortality (2, 3), and in Canada, it is the leading cause of premature death in women (4). Arterial health is a useful indicator for overall cardiovascular health and further understanding of arterial mechanics and behaviour may lead to improvements in CVD diagnostic applications. The dogmatic research focus has been centered on radial motion given the cyclic behaviour of blood pressure and resulting changes in circumferential diameter. However, recent studies have begun to examine movement of the artery axially (i.e., in the longitudinal plane), primarily given the improvements in ultrasound technology and tracking software (5, 6). Longitudinal motion is typically measured at the common carotid artery (CCA) (specified as carotid artery longitudinal wall motion (CALM)) and is the bidirectional motion of the intima-media complex of the arterial wall. This movement has been shown to be of a similar magnitude to that of radial expansion (5), and the waveform is reproducible beat-to-beat (5, 7). Furthermore, various studies have examined that this motion pattern has interindividual variability and have identified motion changes with aging, coronary artery disease, plaque, metabolic syndrome, insulin resistance, and those with elevated CVD risk (7–13). As it currently stands in the literature, there are various proposed determinants of longitudinal motion, albeit no confirmation due to confounding factors of material stiffness, hemodynamics, and cardiac contraction for studies *in vivo*. This review will examine arterial structure and explain the various theories proposed for the determinants of CALM.

1.1 Vascular Health and Structure

1.1.1 Artery structure

Each blood vessel is comprised of three tissue layers, with variations in layer size and structure between different sized vessels (14) as shown in Figure 1. The inner layer or intima consists of a single cell layer made up of elongated endothelial cells. The endothelial cells synthesize vasoactive factors such as nitric oxide (NO) to act on neighbouring vascular smooth muscle cells (VSMCs) to allow for vascular adaption to flow (15–17). This layer surrounds the lumen of the vessel and is connected to the basal lamina which lies between the endothelial cells and the middle layer called the media (14, 17). The media layer consists of an internal elastic lamina and one to two layers of VSMCs. The internal elastic lamina appears as a solid sheet with holes in it allowing for diffusion between the endothelial and VSMCs (17). The media layer also contains extracellular matrix components such as elastin and collagen (14, 18). Collagen largely supports the strength of the vessel while elastin is important for maintaining blood pressure, by stretching to accommodate blood flow following systole and recoiling during diastole (14, 17). The tunica media regulates vascular tone and provides structural integrity. Media thickness varies between vessels depending on the number of layers present which are made up of repeating discrete structural and functional units called musculoelastic fascicles (14, 18, 19). The media may also contain an external elastic lamina which further provides structural support to a blood vessel. The outmost layer is the adventitia, and it contains fibroelastic connective tissue, nerves, and vasa vasorum which are small arteries that supply nutrients to the blood vessel layers in larger arteries (14, 18). Perivascular adipose tissue (PVAT) surrounds the artery tethering it to

surrounding tissues and providing mechanical support in addition to secretion of vasoactive factors to modulate vascular tone (20, 21).

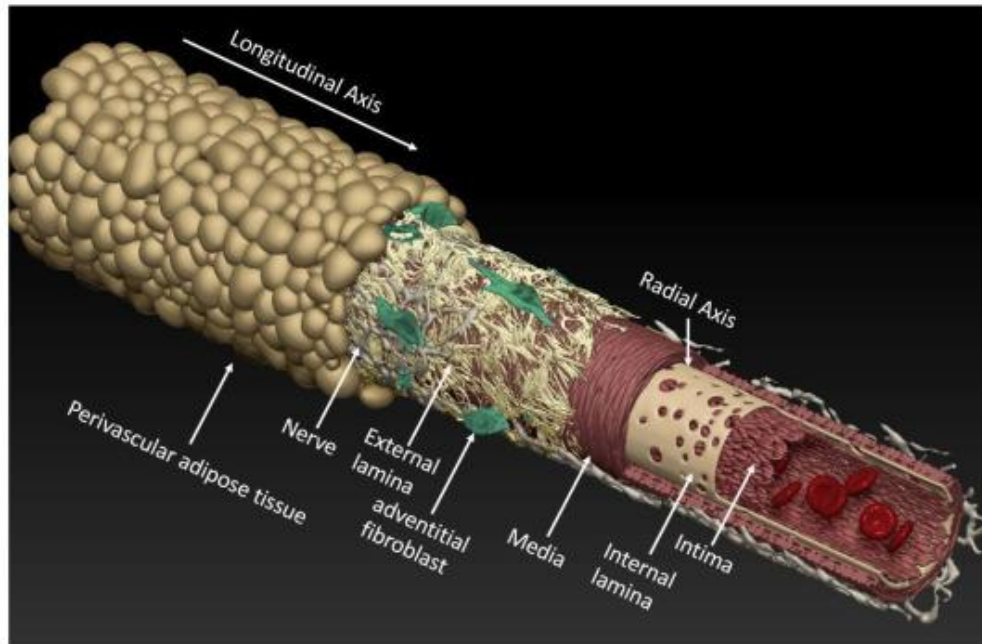


Figure 1: Image from (22) shows the structure of an artery including surrounding PVAT. Most structures shown are based on data from a confocal laser scanning microscope (CLSM).

Blood vessels that move large volumes of blood such as those close to the heart are called elastic or conducting arteries (18, 23). Conducting arteries have multiple layers of perforated elastic membranes and typically larger diameters and thus can accommodate large changes in blood volume. This is important in dampening large oscillations in blood flow for the smaller distal arterial branches (18). This dampening effect also allows for continuous outflow of blood during diastole as the elastic arteries compress which has been modelled as the Windkessel effect. This effect is observed as a decay in pressure during diastole, whereby the decay time slows later in diastole due to an increase in arterial compliance with lower pressure (15). However, the greatest population of arteries are muscular or distributing arteries to ensure rapid and complete

delivery of blood to tissues. These vessels are predominantly made up of VSMCs, however they do contain discontinuous elastic fibers (24). These differences are important to note as even within the same vessel there can be segmental differences in elastin, collagen, and VSMC distribution as seen in porcine aortas (25, 26).

Arrangement of cells, particularly VSMCs within the vessel wall is also an important consideration for understanding how the artery wall will respond to changes in blood pressure. Various studies have demonstrated a helical arrangement of VSMCs; in the mouse superior mesenteric artery (22), murine carotid artery (27) and porcine aorta (28). Specifically in murine carotid arteries this helical pattern was determined to be right-handed in both the right and left arteries (27). This arrangement is an important consideration as changes in wall thickness and lumen diameter can be altered due to a rearrangement of these VSMCs (22). A continuous fibrous helix is formed in the media layer consisting of VSMCs, collagen and elastin (19). As the artery distends, this helical orientation allows for relatively constant axial forces. A study in a porcine carotid artery found collagen to be oriented more circumferentially at the outer adventitia and progressively switch to more axial orientations moving towards the inner adventitia (29). Another study noted adventitial collagen to be wavy bundles which exhibit engagement following stretching of the tissue (30). Arteries contain both circumferential and axial oriented fibers for enduring different directional strains. Identifying arterial structure is integral to understanding its function and thus may improve our understanding of CALM.

1.2 Carotid Artery Longitudinal Motion

1.2.1 Origin and history

Research on arterial health historically has focussed on radial motion, often disregarding longitudinal motion. Various animal studies have measured dynamic arterial wall changes during the cardiac cycle (31–33), all noting changes in length along with radial distension which is now known to be due in part from helical arrangement of VSMCs (22, 27, 28). Patel and colleagues in 1961 demonstrated movement of the canine aorta in the same direction as blood flow during systole and a decrease in absolute displacement when compared to a location further down the vessel. The greatest vessel length changes were attributed to breathing (31, 33). Longitudinal motion was only first confirmed following a study using cinematography where reflective beads were sewn onto a pig CCA (34). All these previous studies were invasive which prevented the application for viewing *in vivo* arterial mechanics in humans. Additionally arterial wall motion was observed at the adventitial layer where longitudinal motion is limited. More recently, ultrasound techniques have allowed non-invasive estimation of longitudinal motion, with motion at the intima-media complex showing the greatest displacement (5, 6, 35). Earlier studies showed poorer resolution in the axial direction as wall tracking at the wall-lumen interface posed some challenges with aperture issues (36). It has since been demonstrated that CALM is of a similar amplitude to that of radial displacement (5, 6, 37, 38), leading to further research to explore the determinants behind it.

1.2.2 Defining carotid artery longitudinal motion

CALM has recently gained further interest in research. There is a lack of consensus by research groups on the terminology to describe CALM, with reports ranging from axial

motion, longitudinal wall motion (LWM) (39), longitudinal movement (LMov) (40), and longitudinal kinetics (LOKI) (41). For clarity as the majority of research is conducted on the CCA, CALM will be used to describe the wall motion in the literature review. CALM is defined as the bidirectional, multiphasic motion of the CCA along its length (5, 39, 42, 43). Motion in the same direction as blood flow is called anterograde motion, whereas retrograde motion is in the opposite direction of blood flow. The multiphasic pattern typically has three distinct phases: systolic anterograde motion, systolic retrograde motion and diastolic motion as seen in Figure 2 (5, 44, 45).

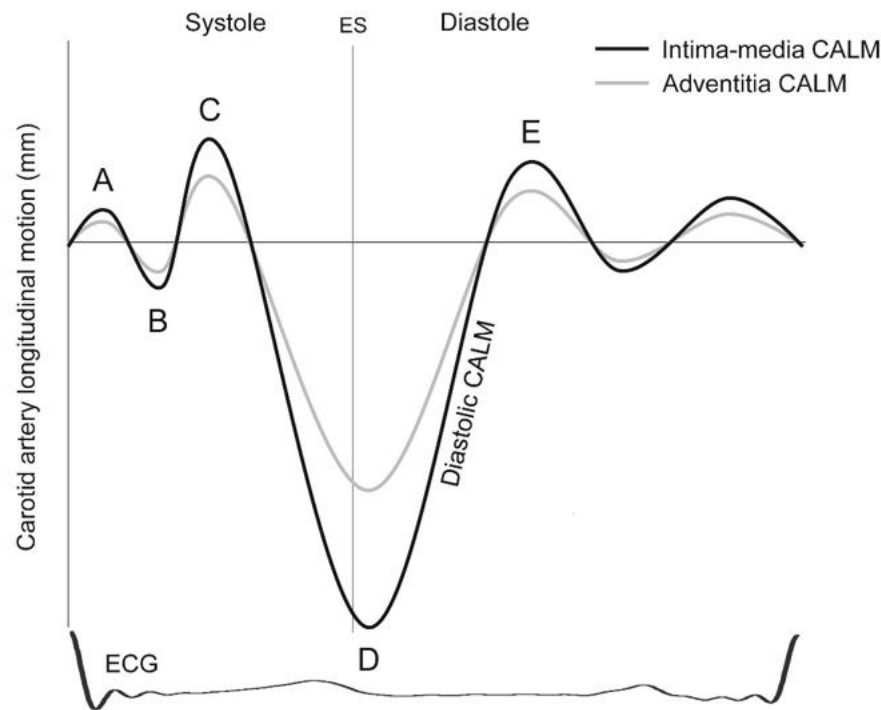


Figure 2: Image modified from (8) showing a schematic of CALM gated to the R spikes from an ECG trace. CALM is shown for the intima-media (black) and adventitia (grey) to demonstrate the difference in displacement amplitude.

The general motion pattern is as follows; point A seen in Figure 2 is described as an initial anterograde followed by a retrograde shift to B and then up to a peak anterograde

displacement at point C. Point B to C is systolic anterograde CALM. Point C to D shows maximal retrograde displacement and is representative of systolic retrograde CALM. Diastolic CALM is represented from D to E and an oscillating return to baseline can be seen following point E. Although there has been considerable interindividual differences noted as seen in Figure 3 (7, 11, 46).

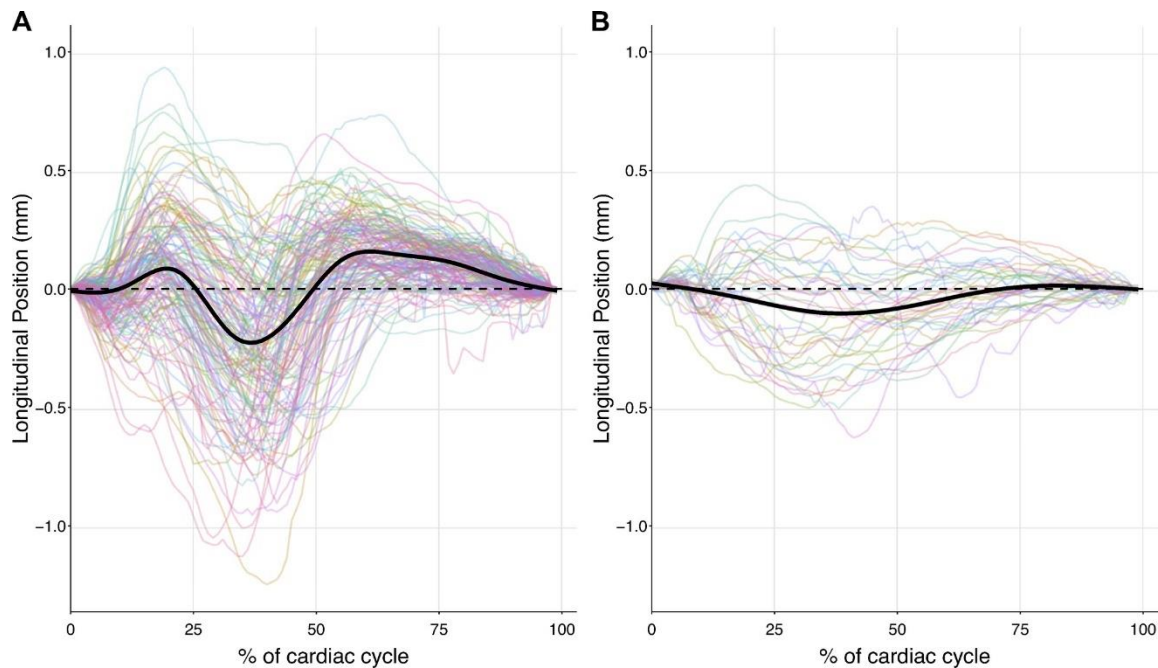


Figure 3: Image from (46). A: 161 young, healthy adults aged 23 ± 4 (117 males). B: 51 older, healthy adults aged 69 ± 6 (30 males). Coloured lines are representative of individual traces, with a group averaged trace represented in the solid black line. This demonstrates the large variability particularly in amplitude and duration of motion phases between individuals, and age-related diminished amplitude in older adults.

The variation in amplitude and curve profile observed in the traces of young, healthy individuals, demonstrates the need to investigate the determinants of CALM. Exploration into a central mechanism or physiological framework should be the primary focus, as anatomical and physiological differences would account for interindividual variability.

1.3 Theories and proposed determinants

Several theories have been proposed as determinants of CALM, with each attempting to explain a part of the curve profile. Wall shear stress has been previously associated with anterograde motion with forward blood velocity (8, 39, 42). Cardiac motion during systole has been associated with retrograde motion, with regulating influences from arterial wall mechanics (39, 42, 46). Each theory has considerable overlap provided the interconnectedness of the cardiovascular system both anatomically and physiologically with hemodynamic influences resulting from cardiac contraction. The current theories and available evidence will be discussed below.

1.3.1 Wall shear stress

During early systole the ejection of blood from the heart creates forward blood flow which has been associated with the timing of the systolic anterograde phase of CALM (39). As blood flows through an artery, it exerts force onto the wall which is represented as wall shear stress (6, 47). Shear stress is typically estimated via shear rate which does not consider velocity-varying blood viscosity. Previous work hypothesized forward local blood velocity acted as a pushing force leading to anterograde motion. However, studies manipulating shear rate via catecholamines, found no significant correlation between shear rate and CALM (40, 48). Even during spontaneous premature ventricular contractions (PVCs) where forward blood velocity was either nearly abolished or doubled due to compensatory increases in diastolic relaxation time, there was little change in anterograde displacement (49). Recent mathematical estimations of wall shear stress assuming a peak carotid shear stress of 2 Pa demonstrated length fluctuation to be only 0.15 mm which is similar to reported anterograde displacement values (46). This

calculation shows that there may be a small influence of wall shear stress; however, another study calculated the longitudinal elastic modulus of the intima-media complex *in vivo* assuming shear stress as the driving force and found it to be up to 1000 times lower than values seen in *ex vivo* studies (50). Thus, these calculations in conjunction with other studies that have manipulated forward blood flow, show the effect of shear stress may be negligible compared to other influences such as arterial structure and 2-D radial-longitudinal coupling, as discussed below.

1.3.2 Ventricular-vascular coupling theory

The fascicular connection of the heart and blood vessels has been proposed to influence wall motion in early work in canines (31). More recently, cardiac motion has been suggested to influence the retrograde component of CALM (39, 42, 46). Au and colleagues in 2016 first observed cardiac motion mechanics on CALM and found a strong correlation between peak basal rotation and retrograde motion. Temporally they observed the onset of basal septal longitudinal displacement and apical rotation at initial retrograde displacement, suggesting both cardiac contraction and retrograde CALM occur at a similar time. Following waning of the forward blood velocity wave, peak instantaneous velocity is reached for both left ventricle (LV) longitudinal and rotational velocities, at which point retrograde displacement continues to its peak at end systole. This was supported by later work which manipulated LV rotation and shear rate and found a relationship between systolic retrograde motion and LV basal rotation (42). Specifically, this study altered pulse pressure (PP) and LV rotation via a serial subtraction test or a cold pressor test in an effort to increase systolic retrograde displacement (42). A case study attempted to separate out the influence of forward blood flow from cardiac motion

by looking at the impact on CALM during a PVC (49). During the PVC beat retrograde displacement was reduced along with cardiac motion parameters of apical LV rotation and LV longitudinal strain. The post-PVC beat following the compensatory electrical pause showed greater cardiac contraction and retrograde displacement. Taken together, retrograde displacement and cardiac contraction had proportional responses, supporting the ventricular vascular coupling theory. It is important to mention that this connection is not isolated because retrograde displacement reductions were also associated with decreases in PP and local blood velocity suggesting a moderating influence from distending pressure. A caveat to this study is the data was only from one participant and data were not recorded simultaneously and thus there is the potential for error in the magnitude and timing of responses. Previous work has suggested stronger links between ventricular-vascular coupling and cardiac measures such as basal and global rotation (42) which were not able to be recorded due to the spontaneity of PVCs. Due to the anatomical closeness of the aortic annulus and the base of the heart, the influence of basal rotation may be stronger, as further explored below.

Taken together, the above evidence supports a central energy theory, which considers the anatomical connection of the blood vessels to the heart which acts as a central energy source. There is a progressive attenuation of CALM amplitude moving proximal from the heart to more distal. Vessels further from the heart have a reduced displacement amplitude, with larger wall displacement amplitude seen in more central arteries with greater diameters (5, 41). CALM amplitude has also been shown to vary within the same vessel (41, 51), and between the left and right CCA (52). The anatomical difference in origin of the right common carotid artery (RCCA) from the brachiocephalic

compared to the left common carotid artery (LCCA) has been suggested as a factor in larger CALM amplitudes seen in the LCCA compared to the RCCA. Moreover, taller individuals have a smaller amplitude of displacement (39, 53). These data suggest the influence of an energy source, such as cardiac motion, whereby tissue movement will dissipate the further from the source it is (Figure 4). However, cardiac contraction also leads to forward blood flow and thus shear stress and radial distension. While the above section postulates a minimal effect of forward shear forces, there may be some influence (as noted by the calculation of 0.15 mm above (46)) which cannot be controlled in human models. *In vivo* modification of cardiac motion without forward blood flow is not possible. Thus, other hemodynamic influences are present and are temporally linked that may also influence CALM limiting the ability to determine direct coupling. Further research to observe cardiac motion without the influence of blood flow and radial distension is necessary to dissect the link to retrograde displacement.

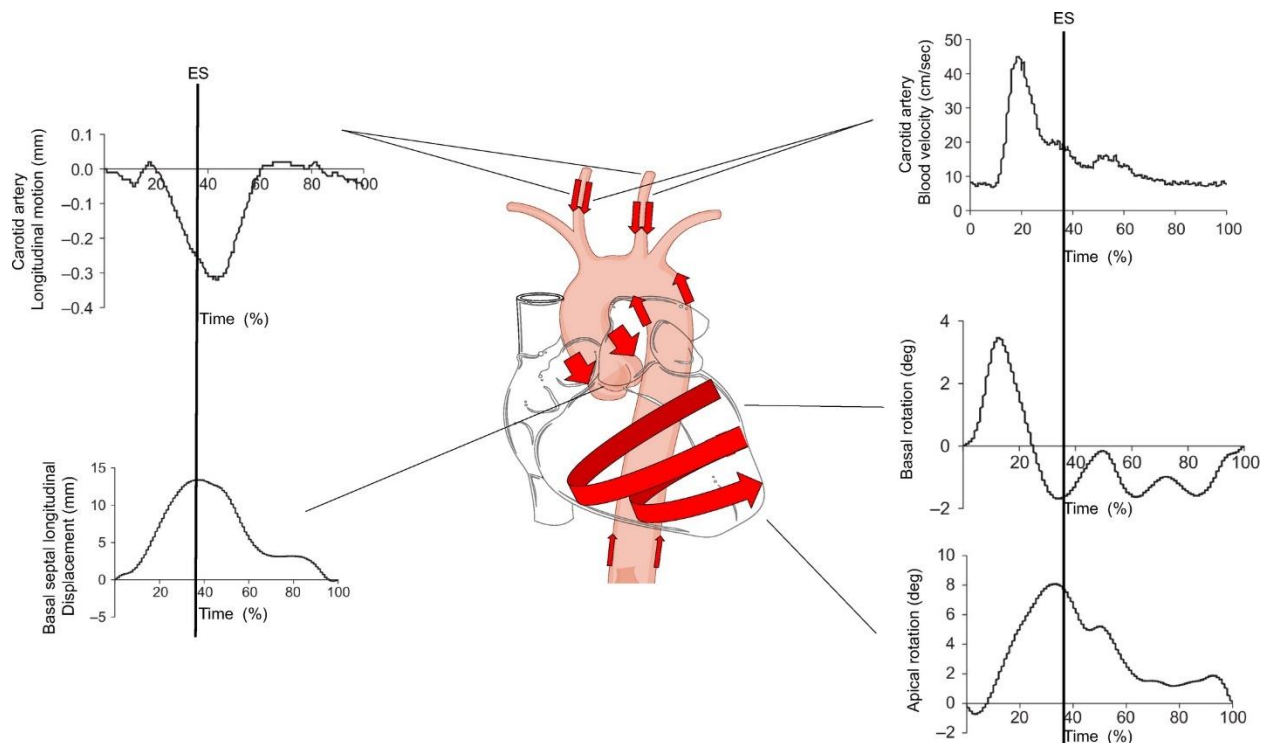


Figure 4: Image from (46). Showing the hypothesized structural ventricular vascular coupling effect of the left ventricle and proximal vasculature. The connection of the vasculature to the proximal aorta and aortic ring will lead to a pulling effect when the heart contracts, moving the vessel wall down towards the heart. More distal vessels from the left ventricle will experience less motion.

1.3.3 Structural mechanics theory

Arterial wall structure has been proposed to influence the absolute magnitude of CALM due to vessel wall elastic properties and structure orientation, and the resulting influence on wall behaviour under *in vivo* forces. As mentioned earlier the CCA wall has a helical VSMC fiber orientation which is believed to tie circumferential and longitudinal motion as blood pressure causing distension would have torsional response leading to length changes (27). In addition, elastin content decreases with age which is associated with increased arterial stiffness (23, 54–56), and advanced age is linked to smaller amplitude CALM (7, 8). CALM has been associated with driving forces from pulsatile flow rate and radial motion (44). This association with radial distension follows closely, with systolic anterograde CALM occurring during arterial dilation and systolic retrograde CALM with decreases in arterial diameter (5). Radial distension and longitudinal motion traces can be combined graphically as 2-D coupling (Figure 5C). This coupling shows a reproducible relationship, although it does not explain the retrograde phase past the end-diastolic point (46).

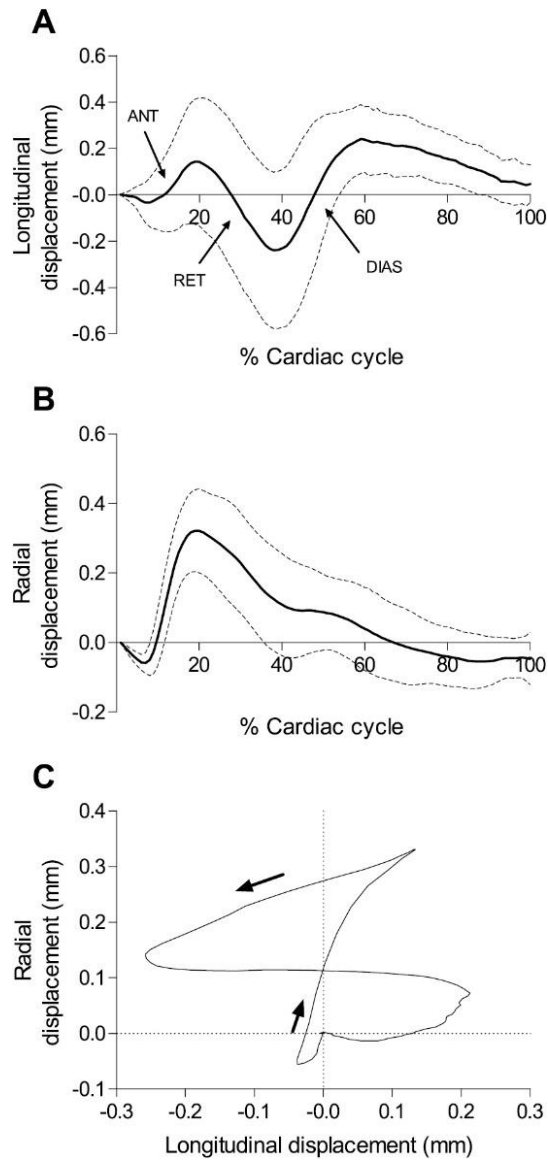


Figure 5: Image from (57). A: Longitudinal wall displacement. B: Radial wall displacement. C: 2-D coupling of radial and longitudinal displacement. All over a single cardiac cycle represented as a percentage.

Together the above evidence suggests influence from the composition of the vessel wall and radial-axial strain coupling with changing vessel diameters. Evidence for a structural link is seen in exercise training studies, where CALM following 12-week exercise protocols of either endurance, sprint interval or resistance training, showed that like wall thickness it is resistant to transient changes in lifestyle factors (43). Thus,

investigating the influence of arterial wall structure, specifically its 2-D elastic properties, can further support our understanding of CALM.

1.4 Longitudinal prestretch

While previous work focussed on the etiology of CALM, limited work has been done to correlate observable motion with the inherent tensile longitudinal prestretch of the arterial wall. Arteries are tethered by perivascular connective tissue and their side branches, providing fixation of the artery to surrounding tissues (15). This tethering holds the artery in tension, leading to it being prestretched in the axial direction *in situ* (55, 58). Longitudinal prestretch supports arterial biomechanics, improving wall load-bearing capacity by minimizing axial stress and strain variation over the cardiac cycle, decreasing circumferential stress, and allowing enhanced circumferential distensibility (58–60). Upon excision the artery will retract and the difference between the *in situ* and excised length allows for the determination of longitudinal prestretch. Changes in elastic membranes as individuals age are associated with a decline in longitudinal prestretch (Figure 6), as demonstrated by an elongation of the excised length with age (61, 62). This decline in longitudinal prestretch with age can be estimated with a power law in the abdominal aorta showing rapid decline until about age 60 at which point further decline is much smaller (55, 62). In the CCA however, the variability in the relationship between longitudinal prestretch with age is not further improved with use of power, logarithmic, or exponential functions (Figure 6) (61). As arteries age there is stiffening due to a decrease in elastin density in the arterial walls, which has been associated with the decrease observed in longitudinal prestretch (54–56, 63). PVAT has also been shown to bear some of the pressure loading by decreasing circumferential and axial stress and limiting axial

movement (21). Another study combining *in vivo* and *in vitro* biomechanical data to observe the effects of perivascular tethering, also suggested that *in vivo* arteries operate under lower wall stress (64). Axial deformation is altered depending on the degree of longitudinal prestretch when observed within the range of physiological pressures (58). Variations in the initial longitudinal prestretch for tissue testing alter behaviour such that there may be inflation in conjunction with either elongation, no length change or shortening, deemed the inversion point. These are all important considerations due to the coupling of circumferential and axial arterial behaviour and stresses. The influence longitudinal prestretch has on longitudinal length changes and stress in *ex vivo* studies provide insight in a potential relationship with CALM that has yet to be determined.

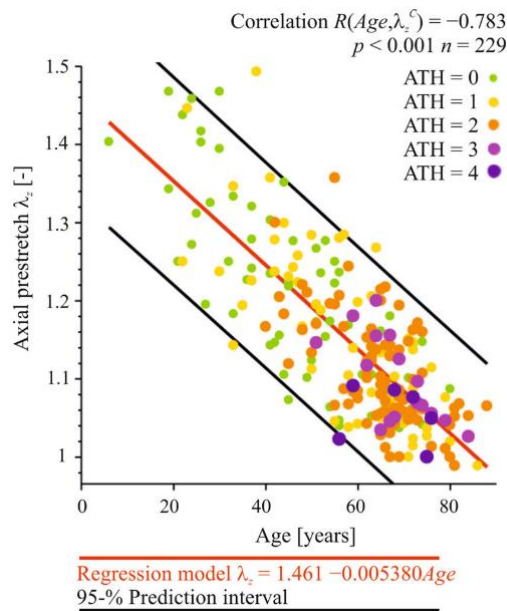


Figure 6: Image from (61). Axial prestretch predictions based on regression equations and data observed at the LCCA from 229 autopsied individuals compared to age.

1.5 Biaxial Testing

To evaluate arterial biomechanics, *ex vivo* tissue testing is necessary to disseminate the viscous and elastic wall properties to approximate *in vivo* function. Arterial tissue layers have different structures (14) and thus different mechanical properties, leading to an anisotropic wall behaviour (65, 66). Testing wall mechanics can be performed in various ways, some *in vivo* methods to estimate aortic stiffness are pulse wave velocity and arterial distensibility (56). These *in vivo* measures however are surrogates and limited to evaluation within the physiological load ranges and cannot reliably assess intrinsic material properties. In order to assess intrinsic material properties, residual stresses, or true physiologic intramural stresses *ex vivo* testing must be performed via uniaxial or biaxial testing. Uniaxial testing only observes changes in stress in one direction, although it is relatively easy to perform (67). Since arteries are anisotropic, biaxial testing is useful to observe the biaxial coupling relationship by which loading in one direction (circumferential or longitudinal) influences the behaviour of the other and vice versa (68). Uniaxial testing can be combined from longitudinal and circumferential data to observe biaxial effects, albeit without direct coupling (67). Biaxial testing can be performed in tubular or planar conditions (69). Tubular conditions maintain the tubular form and attach the ends of the artery to cannulas to observe distension with inflation in addition to longitudinal stretch, which best simulates *in vivo* conditions. Planar testing involves taking square patches of the tissue to be attached in two dimensions for testing. Planar testing is particularly useful for observing any differences between segments of the artery as well as for ease of determining arterial wall layer testing (56, 67, 70). Compared to tubular conditions, planar conditions maintain circumferential stress gradients with peak stress

(69). It is important to note that planar testing can show higher stiffness values and a deviation in pressure-inflation behaviour compared to tubular testing, although the overall conclusions are not altered as trends in the stress and dilation behaviour are maintained.

Within mechanical testing there are quasistatic and dynamic protocols that can be used to observe viscoelastic wall behaviour. Quasistatic conditions involve changing conditions slowly whereas dynamic involves fast millisecond changes (71). Under the simulated *in vivo* pulsatile conditions of dynamic testing arteries behave stiffer compared to quasistatic testing (72). Arteries respond non-linearly between stress and strain indicative of viscoelastic properties (73). The viscous and elastic properties can be disaggregated as the elastic response depends on the amount of applied force and the viscous response depends on the rate of change (72, 74). Therefore, quasistatic loading will observe static elastic properties due to the slow rate and continued force application, whereas dynamic loading will observe the viscous response due to the more rapid rates of change during testing. Viscous properties allow for time-dependent changes such as stress relaxation and creep (15, 71). For a viscoelastic material, a larger initial force is required compared to purely elastic materials to stretch (increase strain) a material rapidly to the same degree of stretch. Stress relaxation however means that over time as the viscous contribution decreases, stress will also decrease. Although on the flip side, with a sudden increase in stress, the strain is delayed, this is called creep. The relationship between viscoelastic properties of the artery and its longitudinal function have not been determined. Quasistatic testing will be performed to examine tissue elasticity and stiffness relating to longitudinal wall behaviour.

2.0 STUDY RATIONALE

Various studies have attempted to examine the determinants of CALM. However, given the complexity of isolating physiological influences *in vivo*, the determinants of beat-to-beat CALM are still not fully understood. From the limited studies that have used experimental design to provide mechanistic insight on CALM, it is clear that complete isolation of cardiac motion from the influences of blood pressure and blood flow is not possible. However, the use of human cadaveric specimens avoids this and allows for complete control over anatomy and procedures. Without the influence of shear forces on CALM from forward blood flow, the isolated impact of central force application to simulate cardiac motion can be explored. Provided this thesis refers to an isolated part of the CALM curve and is not investigating live intact systems, the term LWM will be used onwards to describe the observed retrograde displacement. Experimental conditions can be modified to produce varying force applications for comparison and ultrasound imaging has been demonstrated to be feasible in lightly embalmed cadavers (75) and thus in un-embalmed tissue the image quality is expected to be similar or better.

3.0 RESEARCH QUESTIONS AND HYPOTHESES

Research questions

- 1) Does varying incremental caudal force applications at the ascending aorta incrementally alter wall displacement of the CCA?
- 2) Does a higher degree of longitudinal prestretch correspond to greater maximal wall displacement?
- 3) Does an artery with lower stiffness have a greater maximal wall displacement?

Hypothesis

- 1) With increasing applied force, the resulting wall displacement amplitude will also increase in a linear fashion.
- 2) Arteries from donors with a greater longitudinal prestretch will have a larger maximal displacement in response to a given force application.
- 3) A less stiff artery will permit the artery to undergo a larger maximal wall displacement for a given force application.

4.0 METHODS

4.1 Ethics

This study design was reviewed and received ethics clearance through a University of Waterloo Research Ethics Committee (ORE #44176). The research methods and protocols adhere to the Biosafety level II protocols outlined by the Safety Office for working with human bodily fluids and tissues.

4.2 Study design

This study had a within-subjects experimental design. All donors underwent the dissection protocol and tissue protocol. The dissection protocol was conducted first, following coroner approval and thus this protocol typically began within a few days of death. Cadavers were obtained from donation through the Body Bequeathal program with the School of Anatomy in the Kinesiology department at the University of Waterloo. We collected a sample size of 10 donors due to resource limitations. Simulation-based power calculations (2000 simulations) were performed with a sample size of $n = 10$ using mean displacement for low (3 – 6 N, $\mu = 0.339$ mm), medium (6 – 10 N, $\mu = 0.536$ mm) and high (10+ N, $\mu = 0.868$ mm) force groups and a shared standard deviation (SD) of 0.5 mm from pilot data (Figure 7) (76). It yielded a power of 99.15% in a one-way within subjects ANOVA design and an effect size of 0.60 partial eta squared to detect a LWM difference at the common carotid artery (Figure 8). The estimated marginal means with a Bonferroni-Holm correction yielded powers of 34.05%, 70.95% and 97.05% for low, medium, and high force groups respectively (Figure 8). We collected data for 10 donors, however due to an inability to obtain displacement data from donor 10 and anterograde data with surrounding tissue from donor 9, the final sample size is $n = 8$ (5 males).

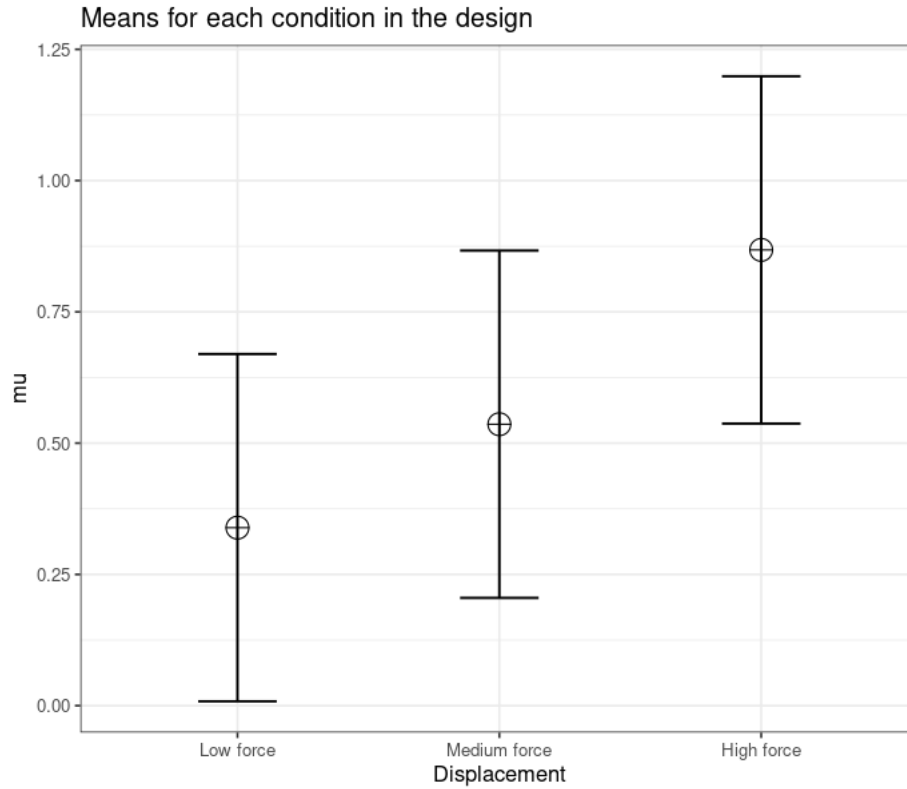


Figure 7: Change in displacement during a low (3 – 6 N), medium (6 – 10 N) and high (10+ N) force application from data of 5 donors. Error bars reflect the SD.

	power	effect_size
anova_Displacement	99.15	0.5974

	contrast	power	cohen_f
p_1	Displacement_High.force - Displacement_Low.force	97.05	1.8464
p_2	Displacement_High.force - Displacement_Medium.force	70.95	1.1485
p_3	Displacement_Low.force - Displacement_Medium.force	34.05	0.6941

Figure 8: ANOVA power (top) and estimated marginal means (bottom) of the force groups; low (3 – 6 N), medium (6 – 10 N) and high (10+ N).

Given the program is based on donation, there was no specific exclusion criteria aside from verbal screening with the family regarding communicable illnesses. Due to this, the sample age was expected within a range of 70 – 90 years of age and included individuals with various comorbidities including CVD. Donors' health information, cause of death, sex and age were all recorded. The dissection protocol started with ultrasound scanning on the RCCA for the force applications prior to scanning the LCCA for each donor. Sections of the CCA were then collected from each donor for the tissue protocol.

4.3 Dissection protocol

The dissection protocol entails the use of a full body donor. Donors were typically transported to the University within 24 – 48 hours of death, at which point donors were tagged for identification and placed in a refrigerator set at 4 °C where they were stored until the time of dissection to slow decomposition. To preserve the intactness of the cardiovascular system, our research group had access as the first protocol on the donor prior to other research teams performing their data collections. A thoracic dissection was performed removing skin, fat, and muscle tissue from the chest and removal of the chest plate to reveal the heart and great vessels. The chest plate was cut just anterior to the midaxillary line and approximately at 1/3 the length of the clavicle from the sternoclavicular joint, to provide optimal access to the internal thoracic organs and avoid damaging major blood vessels. An opening was made in the pericardium to apply an arterial clamp on the ascending aorta, within 2 cm of the exit from the heart. The clamp was then connected in series to a load cell for caudal force applications measured via commercial data acquisition software (PowerLab; ADInstruments, Castle Hill, Australia) (Figure 9). B-mode ultrasound images of the CCA were collected using a 9 – 3 MHz linear

array probe by an experienced sonographer (Vivid IQ, GE Medical Systems, Milwaukee, WI). Images were collected at a depth of 2.5 cm, with the ultrasound frame rate set to 59 fps, autogain set to 20 dB, and the persistence set to 0. Each image duration lasted approximately 6 seconds, starting just before force application and lasting for enough time to allow for the vessel wall to return to its initial position. Ultrasound images were performed starting on the RCCA for a series of caudal force applications prior to being performed on the LCCA. Various levels of force were applied ranging from 2 N to 14 N, with each pull and release remaining under 3 seconds to prevent creep. Approximately 30 images were taken on either side to account for potential wall tracking difficulties and to have multiple images for different applied forces to create a linear force-displacement model. Force applications were randomized to avoid order effects of low/medium/high forces in addition to changes with creep. Each pull was applied at a similar rate of approximately 2 – 4 N per second which was conducted by the same investigator (K.S.) for consistency.



Figure 9: Dissection protocol set-up from pilot session. Arterial clamps are applied at the ascending aorta and are held within a metal clamp attached to a load cell in series.

4.4 Experimental measures

4.4.1 Force magnitude

Force was applied caudally at the ascending aorta and was measured via a load cell connected in series to an arterial clamp. The load cell was calibrated to account for the weight of the attachment apparatus. Force readings were made in Newtons (N) using the PowerLab (ADInstruments, Castle Hill, Australia) to display in LabChart (LabChart v7.3.8, ADInstruments, PowerLab). Varying levels of force were applied between 2 N to 14 N, pulling to a maximal desired force and a return to the initial position. Maximal applied force was determined for each pull and paired to the respective ultrasound image for viewing LWM.

4.4.2 Longitudinal prestretch

A section of approximately 2 cm was marked on the left and right CCA. The average of three measurements was taken of the marked length *in situ* (l) and following excision (L) in order to calculate the degree of longitudinal prestretch (λ) (55, 62).

$$\lambda = l/L$$

Equation 1: Longitudinal prestretch.

Provided the sample population was older with multiple comorbidities, atherosclerotic plaques and/or clots were noted when seen during data collection. A previous study has shown that longitudinal prestretch is not significantly affected by atherosclerotic plaques

(77). Moreover, they found that post-mortem interval also had no significant effects on longitudinal prestretch.

4.4.3 Longitudinal wall displacement

LWM is typically measured over a cardiac cycle using B-mode ultrasound and post-scan algorithms to quantify wall movement. Given the use of cadavers, analysis in this thesis was set to the start and end of each force pull rather than a traditional cardiac cycle. Our lab uses a custom block matching algorithm (MATLAB; The MathWorks, Natick, MA) for tracking 2D arterial wall motion (10). To measure wall displacement (d), a region of interest (ROI) box is placed over the intima-media (Figure 10 red) and the adventitia (Figure 10 blue) layers. ROIs were placed on the far wall, over areas with good heterogeneity of pixel intensity to increase the success of tracking which is identified by a normalized cross correlation coefficient (NCC) of > 0.7 . For each image wall displacement is tracked in six kernels that allows for a median displacement to remove any kernels that failed to track the wall motion. Care was taken to avoid tracking near an atherosclerotic plaque as this movement may be tracked differently from the wall (78, 79).

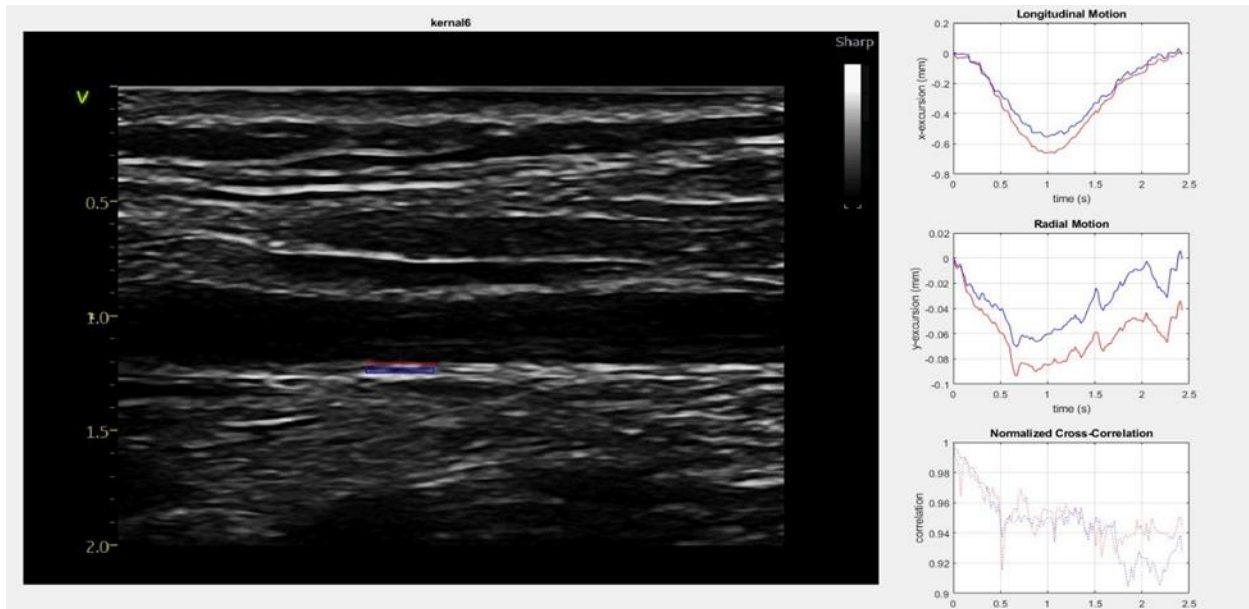


Figure 10: Example of a B-mode ultrasound wall tracking in a donor. The ROIs are placed over the intima-media complex (red) and adventitia (blue). Longitudinal motion is tracked over the duration of the pull and release as seen in the top right image.

Longitudinal wall displacement was normalized for all donors to a pull of 6 N. A linear relationship between force and displacement was assumed. An equation of the line of best fit was determined and solved for a displacement resulting from a pull of 6 N for each respective side.

4.5 Tissue protocol

Arterial samples of the left and right CCA were frozen and kept in a -80°C freezer prior to being thawed for testing. Some samples (for donors 1 – 4) were flash frozen in liquid nitrogen prior to being placed in the freezer at -80°C. Samples from donors 5 – 8 were placed in the freezer at -80°C without flash freezing. Studies comparing methods of freezing with using liquid nitrogen have noted that presence of cryopreservation agents (CPA), least changed the mechanical properties (80). However, liquid nitrogen

submersion can induce freeze fractures of the arterial sample, which were noted in our samples that were flash frozen in liquid nitrogen (81). All samples were frozen without the presence of CPA and without access to a controlled-rate freezer (CRF). While freezing without CPA can lead to bulk water loss, radial stress-strain curves showed a similar shift as those in a CRF that were thawed in a controlled bath compared to fresh tissue (82). Freezing of arteries without a medium does require a faster cooling rate which may preserve the endothelium better (83). Another study found that preservation solutions altered vessel wall permeability allowing accumulation of fluid in the internal elastic lamina (84). There are increases in length that have been seen with frozen tissue in addition to changes in mechanical properties particularly at the physiological and lower stress levels (up to 50 kPa) compared to that seen in fresh tissue (82). These changes may be due to alterations to the tissue structure from bulk water movement and ice formation (82, 85) that occur with freezing and potentially resulting changes in the alignment of fibers (82). However, given the timeline, and the inability to conduct testing on fresh tissue for every collection, the tissues were required to be frozen. Thawing slowly prior to testing is important for preserving function and was conducted the day of testing at room temperature in phosphate-buffered saline (PBS) (84).

Sample length (L_0) was determined as the average of three measures following thawing using a ruler. All arterial segments were tested twice for each protocol described. The segment was carefully cut, being mindful of orientation (proximal/distal, anterior/posterior, medial/lateral) to create two samples each 10 x 10 mm in size. Orientation was marked with a dot in the top left-hand corner on the intimal side of the sample. Wall thickness (t) was determined by taking the average of three measures for

each sample using a thickness gauge (Figure 11) (86). The dissection of the layers led to sample failure particularly of the intima-media layer due to tearing of the tissue; possibly due to the tissue being frozen rather than fresh. For this reason, all samples were tested as full thickness with the intima-media layer facing upwards for tracking. Any PVAT remaining on the artery was carefully removed prior to testing and sample measurements.

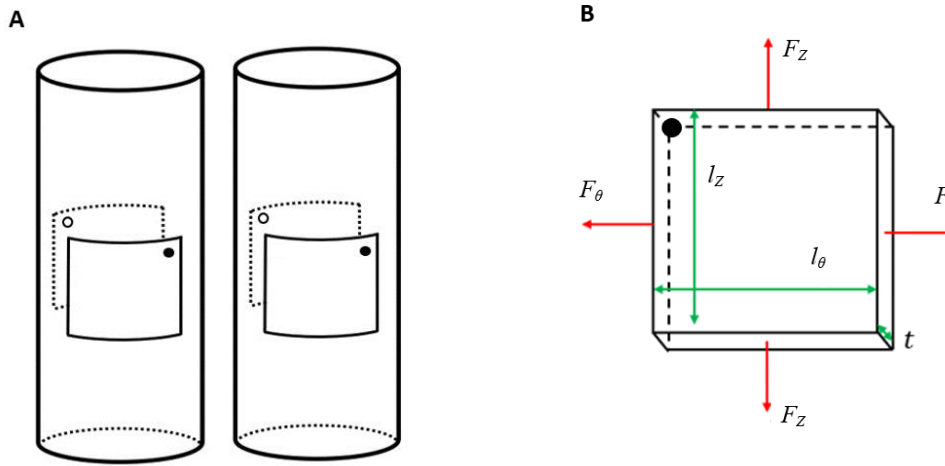


Figure 11: Measurements and directionality on the arterial segment (A) and sample (B). The solid black squares on the cylinders in A indicates samples created on the anterior surface of the arterial segment, whereas the dotted black square indicates samples created on the posterior surface of the arterial segment. The circle in image A indicates orientation, placed in the top, right-hand corner of the sample on the adventitial side (filled black circle on the anterior side, open circle on the posterior side). Image B shows a sample, the solid black circle in the top, left-hand corner indicates orientation on the intimal side for testing. Z = longitudinal direction. θ = circumferential direction. F indicates force, l indicates length, and t indicates thickness.

A BioTester 5000 test system (Cellscale Biomaterials Testing, Waterloo, Canada) was used for planar biaxial testing of the tissue samples. The load cells (allowable maximal force of 10 N) were calibrated to zero and the actuators were set to the appropriate size of the sample. Samples were placed intimal side up (87), with the orientation noted with a dot in the top left-hand corner of the sample and coated lightly

with graphite speckles for tracking. The tissue was mounted via 10 x 10 mm attachment rakes and were placed into a temperature-controlled PBS bath at 37°C for the duration of the test (Figure 12) (86).

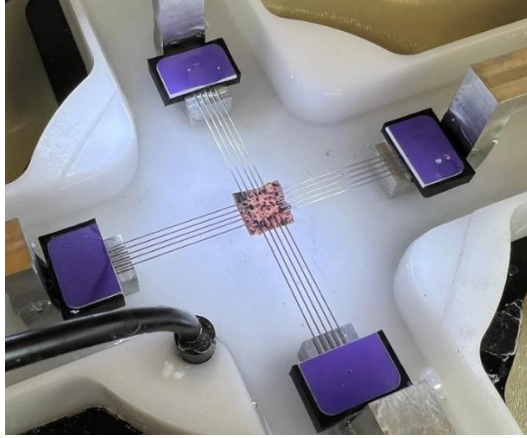


Figure 12: Set-up of a sample from a carotid artery from donor 1 using rakes. The intima-media is facing upwards and is lightly coated with CellScale graphite speckles for tracking.

A force-controlled preconditioning protocol was performed (88), with the maximal force corresponding to a calculated 1st Piola Kirchhoff stress of 150 kPa (T) (Equation 2). This protocol was repeated 6 times. The maximal stretch, corresponding to the maximal force, in the final repetition was noted for the circumferential (λ_θ) and longitudinal (λ_z) directions. The lowest stretch between the circumferential and longitudinal directions was used as the maximal stretch in the main testing protocol.

$$T = \frac{F}{A_0}$$

$$F = \frac{150.000}{A_0}$$

Equation 2: Finding maximal applied force (F) corresponding to a calculated 1st Piola Kirchhoff stress of 150 kPa (T), where A_0 is the original surface area at which this force is applied.

Quasistatic testing was performed for all samples using a stretch-controlled protocol in 7 different ratios of circumferential to longitudinal stretch: 1:1, 1:0.75, 0.75:1, 1:0.5, 0.5:1, 1:0.25 and 0.25:1 (Figure 13). The testing was performed stretching for 30 seconds followed by a 1 second hold, 30 seconds of de-stretching and a 1 second rest, prior to the next repetition. The protocol started with 10 repetitions of the 1:1 ratio for preconditioning, followed by 3 repetitions for the other ratios. The protocol ended with a 1:1 ratio (3 repetitions) to check whether the tissue was damaged during the protocol. A preload of 20 mN was set in both directions to ensure the sample was flat. Of note, following each cycle, the unloaded configuration was determined at the preload, and this point was found at different initial stretches (Figure 14). Thus, despite the same level of applied stretch, there were different resulting forces from the protocols. This was compensated by referring to the start of the 10th cycle as the “zero-stretch” and computing the starting stretch of the other protocols from the change in the sample size at the start of the cycle.

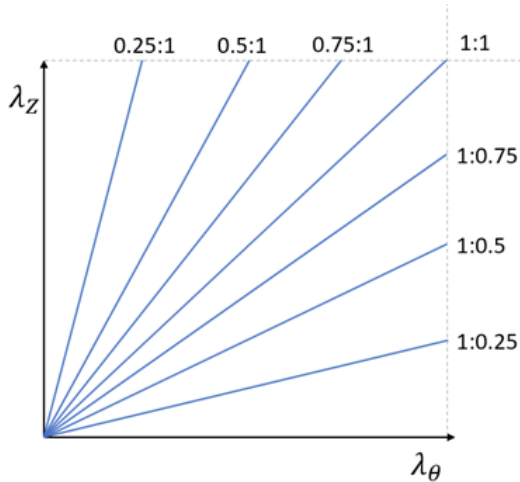


Figure 13: The various ratios to be applied to the samples for the quasistatic protocol. λ_z represents the stretch in the longitudinal direction, λ_θ represents the stretch in the circumferential direction.

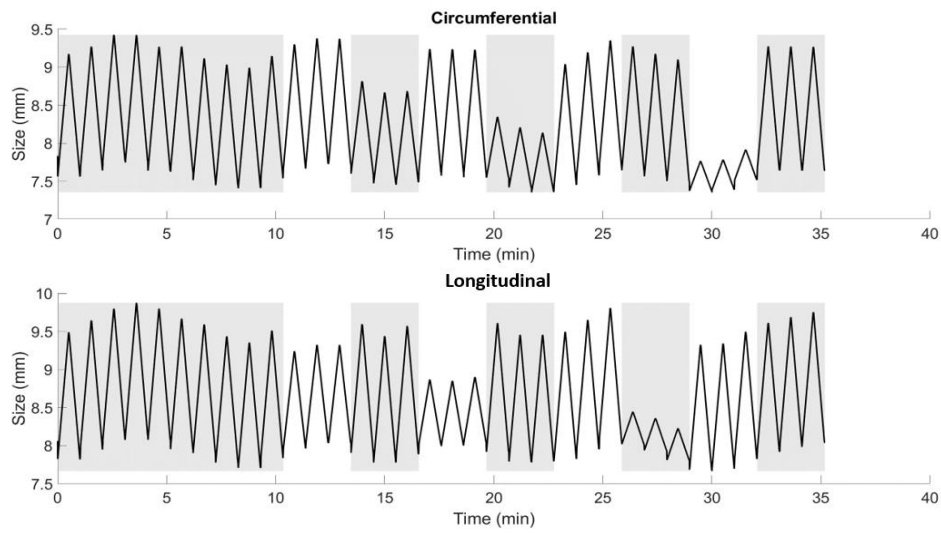
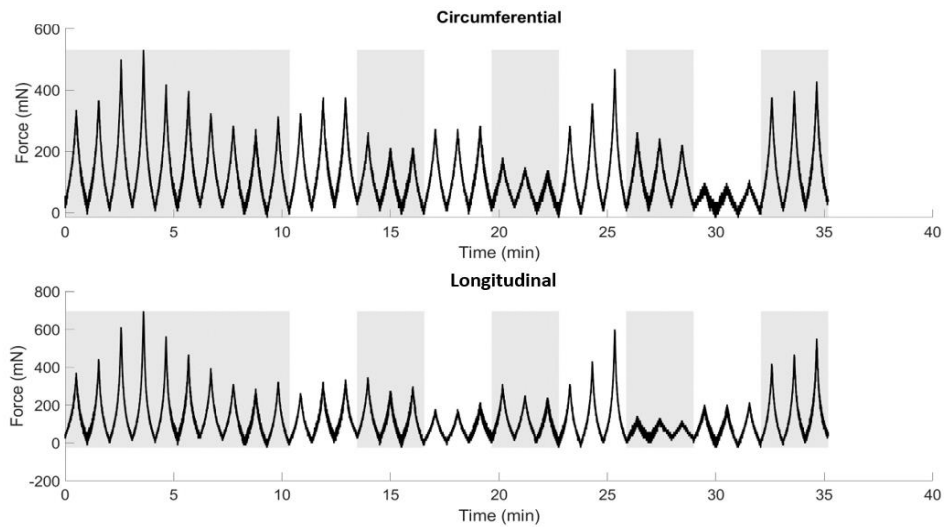
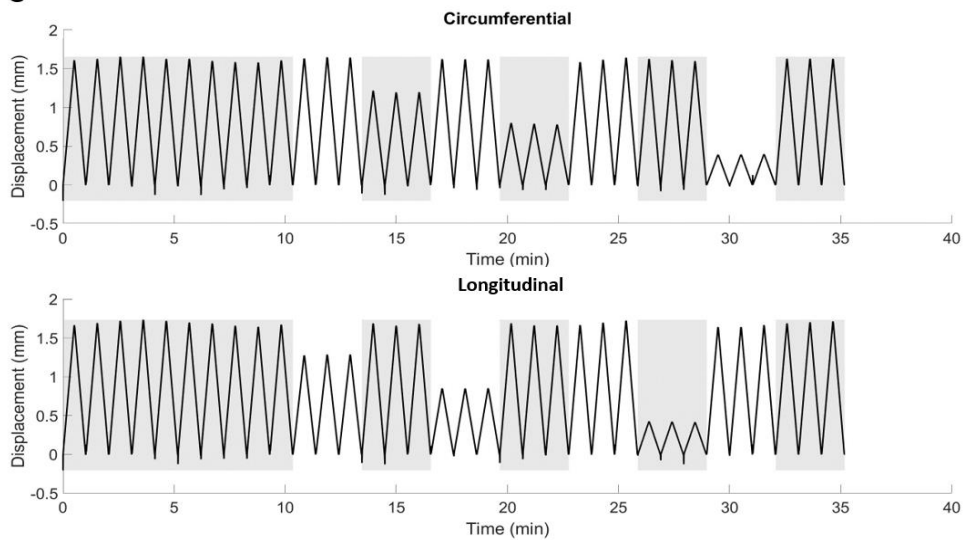
A**B****C**

Figure 14: Grey and white shading indicates different cycles. For each image A – C, the circumferential direction is on top and longitudinal direction is on the bottom. A: sample size between the rakes, variable starting size within cycles due to preload of 20 mN. B: resulting forces as measured by the load cells for the given displacement cycle from the starting size. C: displacement, consistent within a cycle as it was a displacement-controlled protocol.

Data was analyzed using the LabJoy program from CellScale (Cellscale Biomaterials Testing, Waterloo, Canada). Photographs were selected from the last cycle from each protocol. A 7 x 7 point grid was placed on the inner third of the sample for tracking. The Cauchy stress (σ) was calculated using the force and the current surface area (A) at which the force is applied (Equation 3).

$$A = A_0 / \lambda_i$$

$$\sigma = \frac{F}{A}$$

Equation 3: Calculating Cauchy stress. A represents the current surface area, A_0 represents the original surface area, and λ_i represents the strain in the specified direction.

Tracking quality was analyzed by plotting the grid points at full stretch and at the end of the cycle on top of the initial grid points. Points that did not track well were ignored for the rest of the analysis. The mean strain was computed from all remaining tracking points.

The 10th cycle in the 1:1 ratio at the start of the quasistatic protocol was used for calculating elastic modulus. Elastic modulus was determined at a constant Cauchy stress of 35 kPa. The datapoint closest to 35 ka was determined and 30 data points around this point (15 before and 15 after) were included and used to compute the slope of the curve at 35 kPa. Slope corresponds to the elastic modulus (E) as shown in Equation 4. The strain corresponding to the 35 kPa stress was also noted to compare to longitudinal wall

displacement data. Sample values within the same arterial segment were averaged for the longitudinal and circumferential directions for elastic modulus and strain at 35 kPa.

$$E = \frac{\sigma}{\epsilon} = \frac{stress}{strain}$$

Equation 4: Elastic modulus.

4.6 Statistical analysis

All statistics and plotting were performed using R and RStudio. A repeated-measures correlation (rmcorr package) was conducted on the within-subjects relationship between applied force and displacement. This accounts for non-independence among the observations and adjust for inter-individual variability via ANCOVA, generating parallel regression lines with varying intercepts to show the best linear fit for the entire donor sample (89). Simple correlations were performed for longitudinal prestretch (λ), longitudinal strain at 35 kPa, longitudinal elastic modulus (E), circumferential strain at 35 kPa, and circumferential elastic modulus (E) with wall displacement. As a secondary analysis, differences between the LCCA and RCCA were assessed with two-tailed paired t-tests for slope, displacement at 6 N, longitudinal prestretch (λ), longitudinal strain at 35 kPa, longitudinal elastic modulus (E), circumferential strain at 35 kPa, and circumferential elastic modulus (E). An *a priori* type I error rate was established at $\alpha = 0.05$, and magnitude of effect sizes are reported for each test.

5.0 RESULTS

5.1 Donor characteristics

The final sample size was $n = 8$; Table 1 displays donor characteristics. The average age of the donors was 85 ± 7 years ($n = 7$) in addition to one 19-year-old donor ($n = 1$) and included 5 males and 3 females. Donors were obtained and tested over a period of 10 months. Samples of the CCA were frozen until the day of tissue testing in June. Due to the nature of the body donation program and access to biaxial testing equipment, tissue testing was unable to be performed on fresh tissue and samples were not frozen for the same duration prior to testing.

Donor	Sex	Age (yrs)	Longitudinal prestretch LCCA (a.u.)	Longitudinal prestretch RCCA (a.u.)	Cause of death	Date of donor death	Date of testing	Preservation status	Force Measurement	Conditions/ comorbidities
1	M	19	1.24	1.16	Drug overdose	20-Dec-21	10-May-22	Fridge Dec. 20-23, frozen Dec. 23 – Feb. for first full thaw, frozen Feb. – May 9 for second full thaw	Force transducer	Healthy
2	M	94	1.13	1.21	Cardiopulmonary arrest	12-Jul-22	19-Jul-22	Fridge	Force transducer	Cardiopulmonary arrest, cardiogenic shock, hypovolemia, tachycardia, upper GI bleed, end-stage dementia
3	F	81	1.01	1.02	Not specified	09-Aug-22	12-Aug-22	Fridge	Force transducer	Congestive heart failure, calcification, and thrombosis in CCA
4	F	88	1.13	0.98	Respiratory arrest secondary to pneumosepsis	23-Aug-23	25-Aug-23	Fridge	Load cell	Respiratory arrest secondary to pneumosepsis, Dementia, and atrial fibrillation
5	M	78	1.03	1.00	COVID-19 death (diagnosis 14+ days prior to death)	13-Sep-22	15-Sep-22	Fridge	Load cell	Years living with dementia and COPD
6	F	78	0.99	0.99	Not specified	12-Nov-22	15-Nov-22	Fridge	Load cell	Liver metastases, lung metastases, and adenoid cystic carcinoma of the nasal septum
7	M	88	0.97	0.92	Sepsis	05-Feb-23	10-Feb-23	Fridge	Load cell	Sepsis, left arm cellulitis, acute chronic renal failure, atrial fibrillation, metastatic prostate cancer
8	M	78	1.05	1.03	Not specified	09-Feb-23	10-Feb-23	Fridge	Load cell	Squamous cell carcinoma of right maxillary alveolus, and had a pacemaker

Table 1: Donor characteristics including pre-stretch, date of testing and preservation method (n = 8).

5.2 Longitudinal wall displacement in response to caudal force

Data is presented for donors 1 – 8 (5 males). Longitudinal displacement data was averaged over time as a percentage of force cycle and binned by 1 N force intervals ranging from 3 – 13 N. This data is presented by side for the LCCA and RCCA (Figures 15 & 16 respectively).

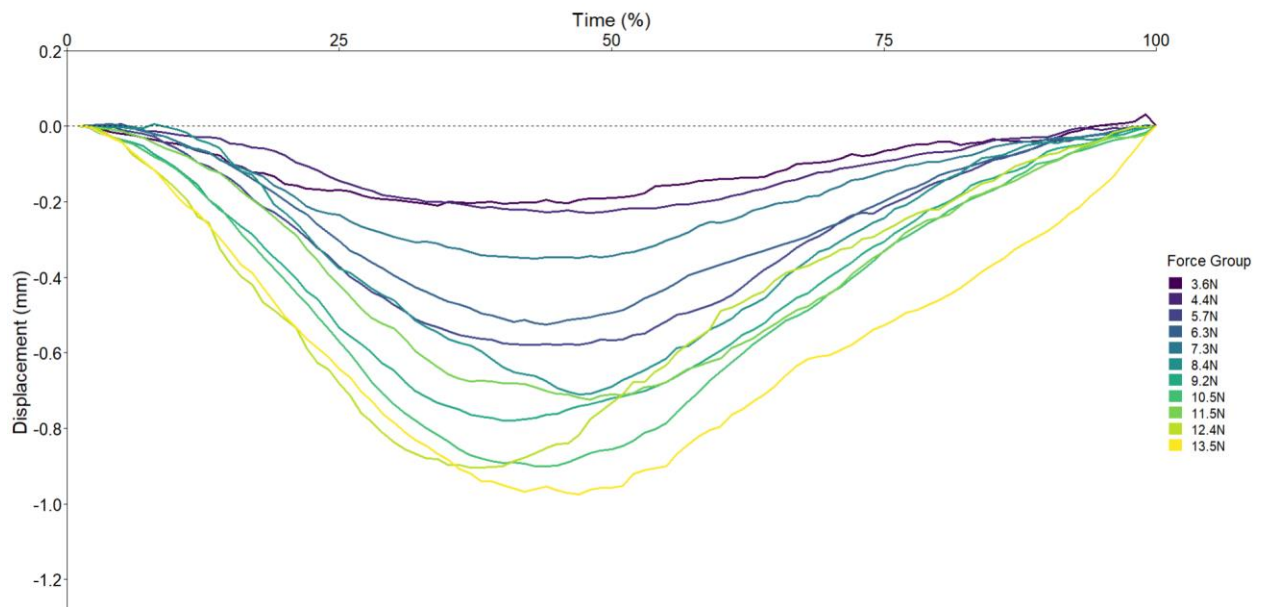


Figure 15: Average longitudinal wall displacement by force group for the LCCA ($n=8$).

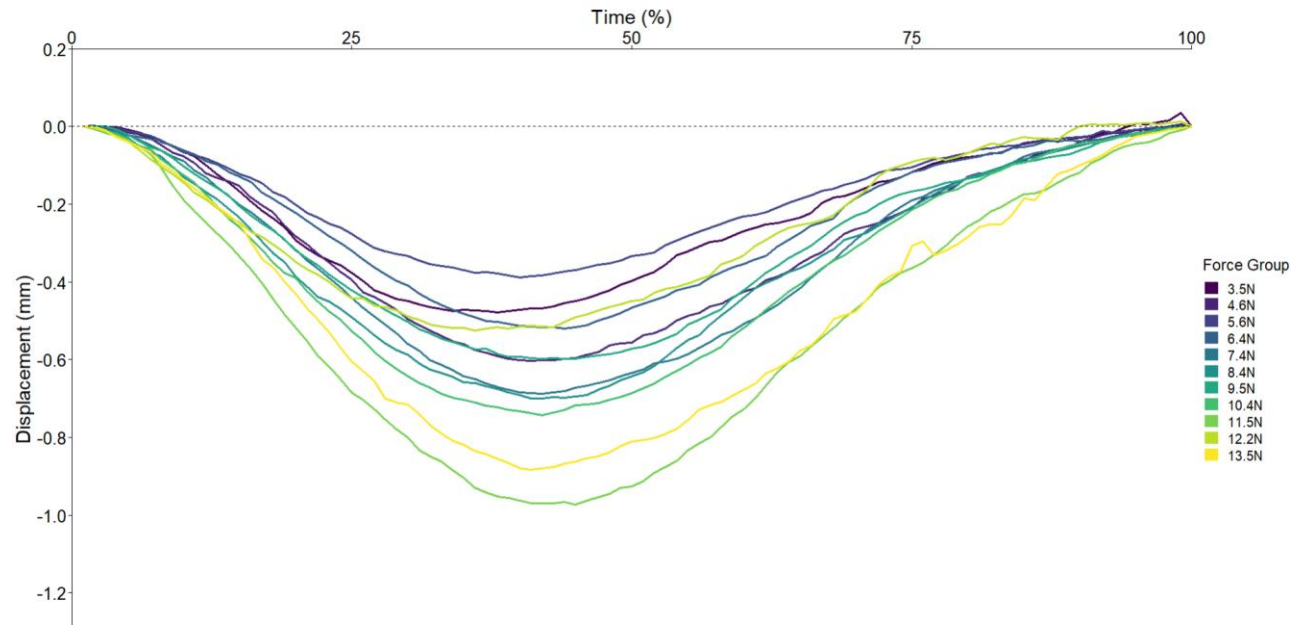


Figure 16: Average longitudinal wall displacement by force group for the RCCA (n =8).

A repeated measures correlation (Figure 17) was performed for the within-subjects relationship between applied force and longitudinal wall displacement for both sides combined, displaying a moderate negative correlation [$r = -0.68$, $p < 0.001$, CI -0.76, -0.60].

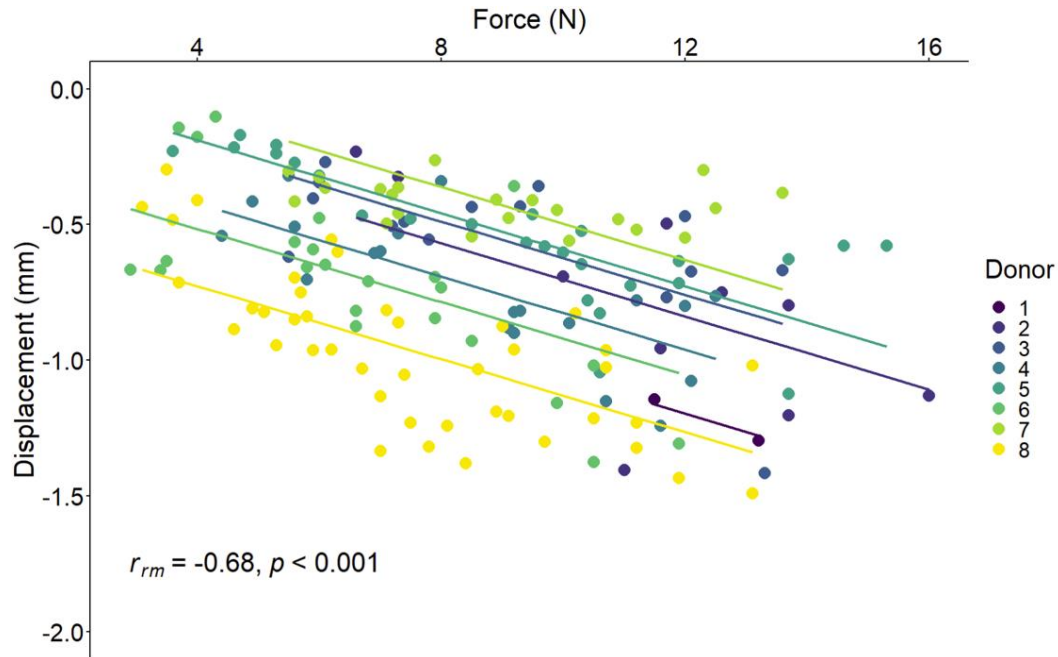


Figure 17: Repeated measure correlation ($n = 8$) on the within-subjects relationship between applied force and displacement.

A line of best fit was generated for each donor for force and displacement to predict the longitudinal wall displacement at 6 N of force (Table 2). 6 N was chosen to approximate displacement values observed *in vivo*. For the remainder of this thesis, displacement at 6 N represents the standardized force-displacement relationship for each artery segment.

Table 2: Line of best fit for each donor by side and calculated displacement at 6 N of force (n = 8).

Donor	Side	Equation of line of best fit	Displacement at 6 N (mm)
1	LCCA	$y = -0.0899x - 0.1098$	-0.65
2	LCCA	$y = -0.0795x + 0.2389$	-0.24
	RCCA	$y = -0.0283x - 0.6769$	-0.85
3	LCCA	$y = -0.117x + 0.3993$	-0.30
	RCCA	$y = -0.0091x - 0.4501$	-0.50
4	LCCA	$y = -0.1152x + 0.1964$	-0.49
	RCCA	$y = -0.0333x - 0.3663$	-0.57
5	LCCA	$y = -0.074x + 0.0843$	-0.36
	RCCA	$y = -0.0426x - 0.1003$	-0.36
6	LCCA	$y = -0.2363x + 0.807$	-0.61
	RCCA	$y = -0.0682x - 0.3087$	-0.72
7	RCCA	$y = -0.0115x - 0.3195$	-0.39
8	LCCA	$y = -0.0737x - 0.3244$	-0.77
	RCCA	$y = -0.0806x - 0.4131$	-0.90

5.3 Longitudinal prestretch

A Pearson product moment correlation was performed on the relationship between displacement at 6 N and longitudinal prestretch. There was no significant relationship between longitudinal prestretch and displacement at 6 N found for either side ($r_{LCCA} = -0.13$, $p = 0.78$; $r_{RCCA} = -0.63$, $p = 0.13$) (Figure 18).

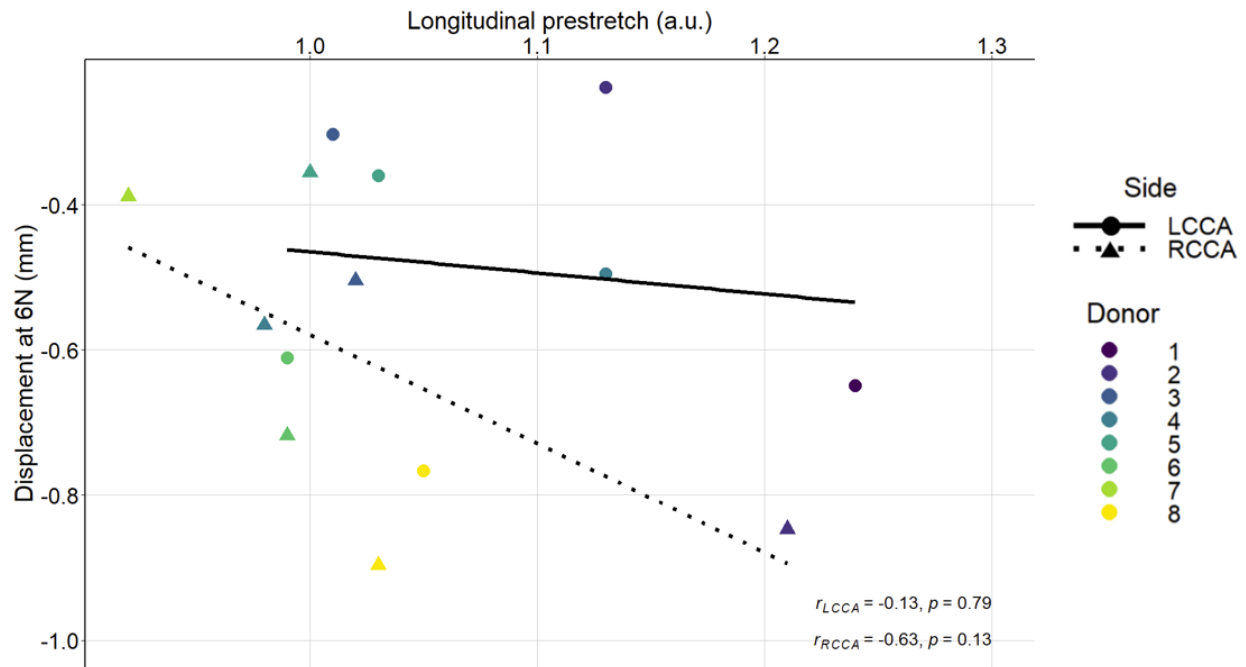


Figure 18: Correlation of displacement at 6 N compared to longitudinal prestretch for each donor. Each colour represents a different donor, with circles representing the LCCA and triangles representing the RCCA. The line of best fit for the LCCA (solid) and RCCA (dashed) is displayed.

As an external validity check, longitudinal prestretch values measured in this study for both the LCCA and RCCA were compared to values measured in fresh cadavers by Horný and colleagues in 2017 and is displayed in Figure 19. Our measurements of longitudinal prestretch for older individuals align with measurements collected by Horný et al., although our longitudinal prestretch values were lower for the 19-year-old donor.

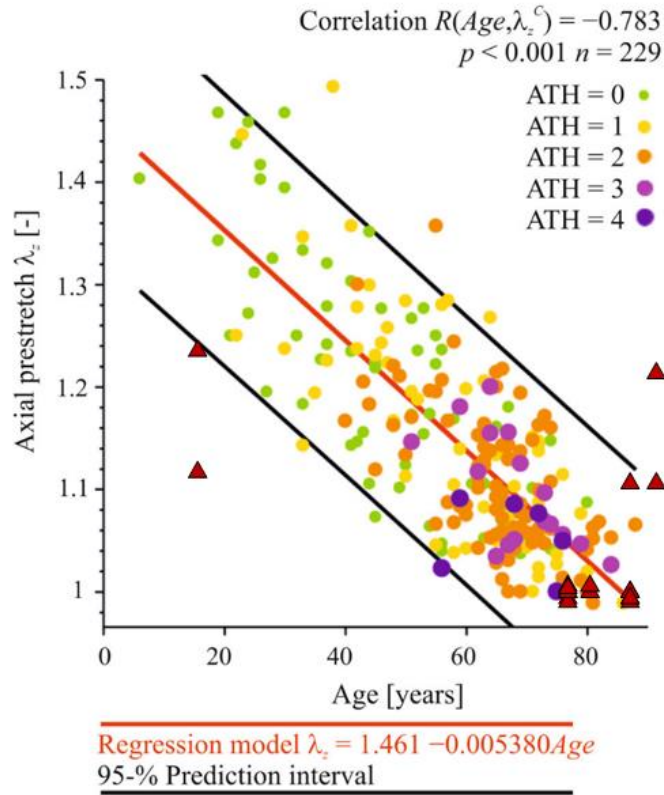


Figure 19: Image from Horný et al. 2017 (61) comparing the axial prestretch in the LCCA and age of autopsied individuals. Dot colour and size indicate atherosclerotic plaque by increasing severity from 0 – 4. The red and black outlined triangles are values from donors in this protocol ($n = 8$) of both the LCCA and RCCA.

5.4 Biaxial data correlations

Elastic moduli for the longitudinal and circumferential direction were calculated at 35 kPa Cauchy stress from stress-strain curves in order to obtain values for all donors. An example of this calculation on the stress-strain curves is shown in Figure 20. Tissue strain values at 35 kPa for the longitudinal and circumferential directions were also noted. Pearson product moment correlations were conducted on the relationships between displacement at 6 N and elastic modulus and strain at 35 kPa for the longitudinal and circumferential directions.

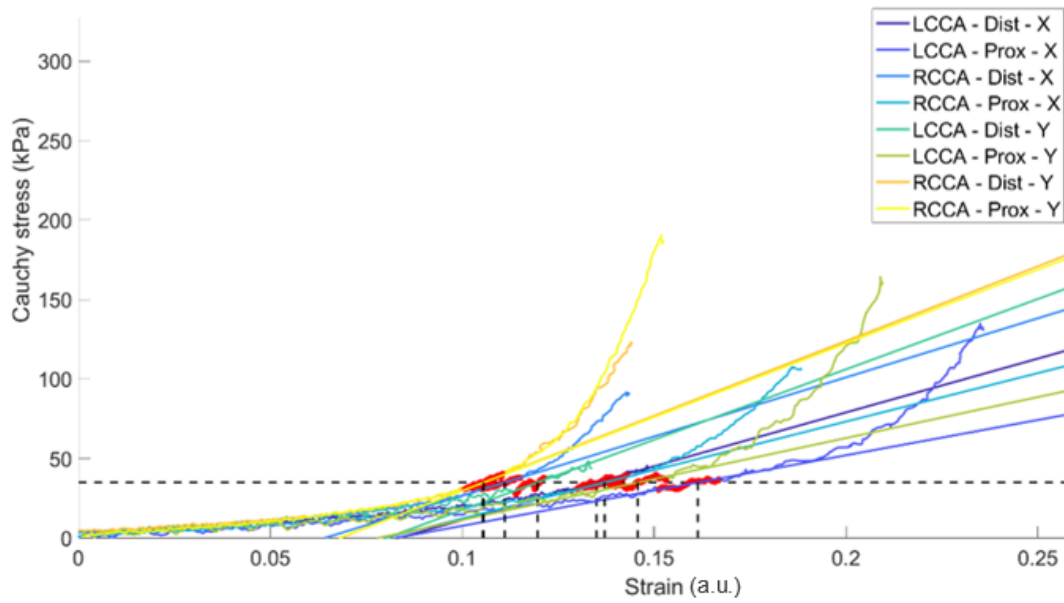


Figure 20: Biaxial data for donor 3. Two samples (distal and proximal) for both the LCCA and RCCA are displayed, showing stress-strain curves for longitudinal (Y) and circumferential (X) directions. The horizontal black dashed line indicates a Cauchy stress of 35 kPa. Red highlight indicates the collective 31 data points used for calculation of the slope. The vertical dashed lines indicate the strain of the tissue at 35 kPa. Coloured lines represent the tangential lines for each side and direction at a Cauchy stress of 35 kPa.

There was no significant relationship found between displacement at 6 N and longitudinal strain and elastic modulus at 35 kPa for either side [$r_{\text{LCCA}} = -0.72$, $p = 0.070$; $r_{\text{RCCA}} = -0.27$, $p = 0.56$] and [$r_{\text{LCCA}} = 0.50$, $p = 0.25$; $r_{\text{RCCA}} = 0.29$, $p = 0.53$] respectively (Figure 21 A & B). There was no significant relationship found between displacement at 6 N and circumferential strain [$r_{\text{LCCA}} = -0.068$, $p = 0.14$; $r_{\text{RCCA}} = 0.13$, $p = 0.79$] and elastic modulus at 35 kPa [$r_{\text{LCCA}} = 0.58$, $p = 0.23$; $r_{\text{RCCA}} = -0.31$, $p = 0.50$] for either side (Figure 21 C & D).

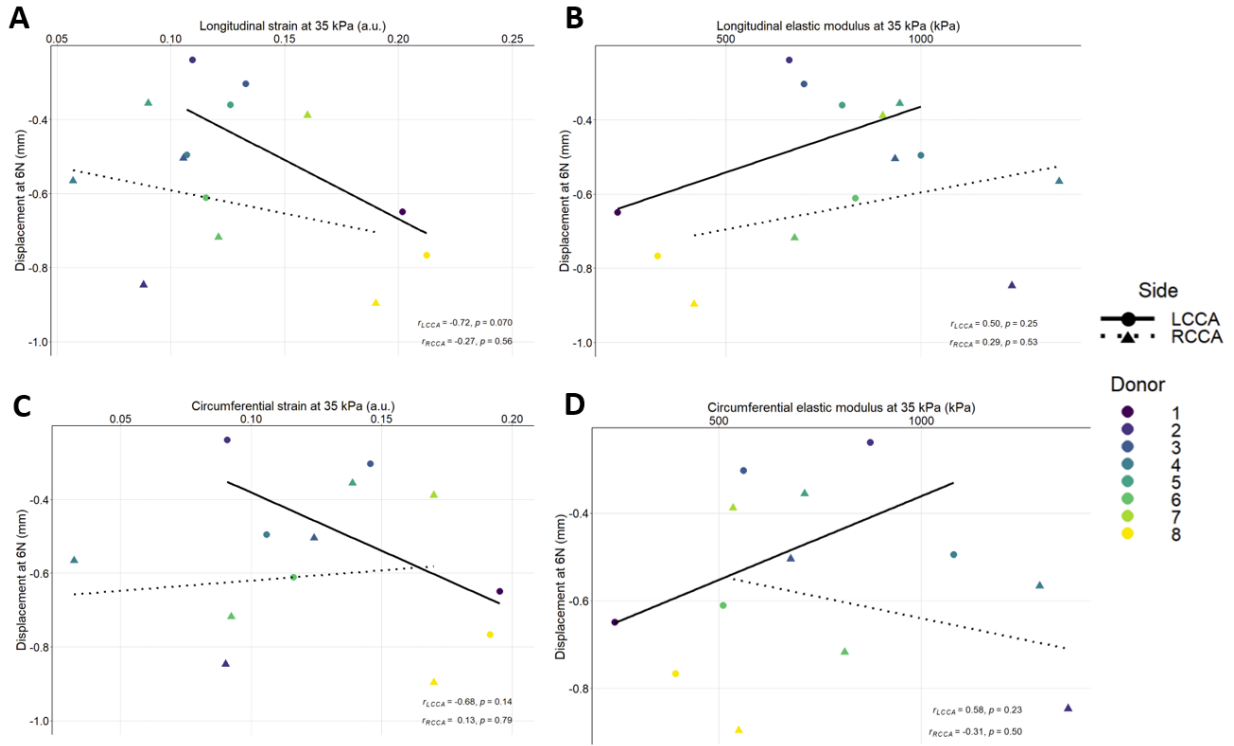


Figure 21: Correlation of displacement at 6 N compared to longitudinal strain and elastic modulus at 35 kPa (A & B respectively) and circumferential strain and elastic modulus at 35 kPa (C & D respectively) for each donor. Each colour represents a different donor, with circles representing the LCCA and triangles representing the RCCA. The line of best fit for the LCCA (solid) and RCCA (dashed) is displayed.

5.5 Differences between LCCA and RCCA

While analyzing the data it was noted that the LCCA and RCCA behaved differently. A secondary side-by-side analysis was conducted. Donors 1 and 7 had longitudinal wall displacement data for only the LCCA and RCCA respectively and are excluded in Table 3 for average values by side (n = 6).

Table 3: Average values with standard deviation compared by side (n = 6).

	LCCA	RCCA
Slope (mm/N)	-0.12 ± 0.062 †	-0.044 ± 0.026
Displacement at 6 N (mm)	-0.46 ± 0.20	-0.65 ± 0.20
Longitudinal prestretch	1.06 ± 0.060	1.038 ± 0.086
Longitudinal strain at 35 kPa	0.13 ± 0.040 †	0.11 ± 0.045
Longitudinal elastic modulus at 35 kPa (kPa)	719.65 ± 226.73	927.10 ± 345.70
Circumferential strain at 35 kPa *	0.13 ± 0.040	0.11 ± 0.048
Circumferential elastic modulus at 35 kPa * (kPa)	683.55 ± 284.38 †	900.65 ± 342.17

Values are mean ± standard deviation. * Data for n = 5, no circumferential data for LCCA from donor 5. † Significantly different ($p < 0.05$).

Two-tailed paired t-tests were performed for all variables comparing between the LCCA and RCCA. There were significant differences between the LCCA and RCCA for slope [$p = 0.0347$, Cohen's $d = 1.17$, $n = 6$], longitudinal strain at 35 kPa [$p = 0.020$, Cohen's $d = 1.37$, $n = 6$], and circumferential elastic modulus at 35 kPa [$p = 0.018$, Cohen's $d = 1.72$, $n = 5$]. There were no significant differences for displacement at 6 N [$p = 0.091$, $n = 6$], longitudinal prestretch [$p = 0.58$, $n = 6$], circumferential strain at 35 kPa [$p = 0.080$, $n = 5$], and longitudinal elastic modulus at 35 kPa [$p = 0.094$, $n = 6$]. Results of the paired t-tests are displayed in Figure 22 A – G.

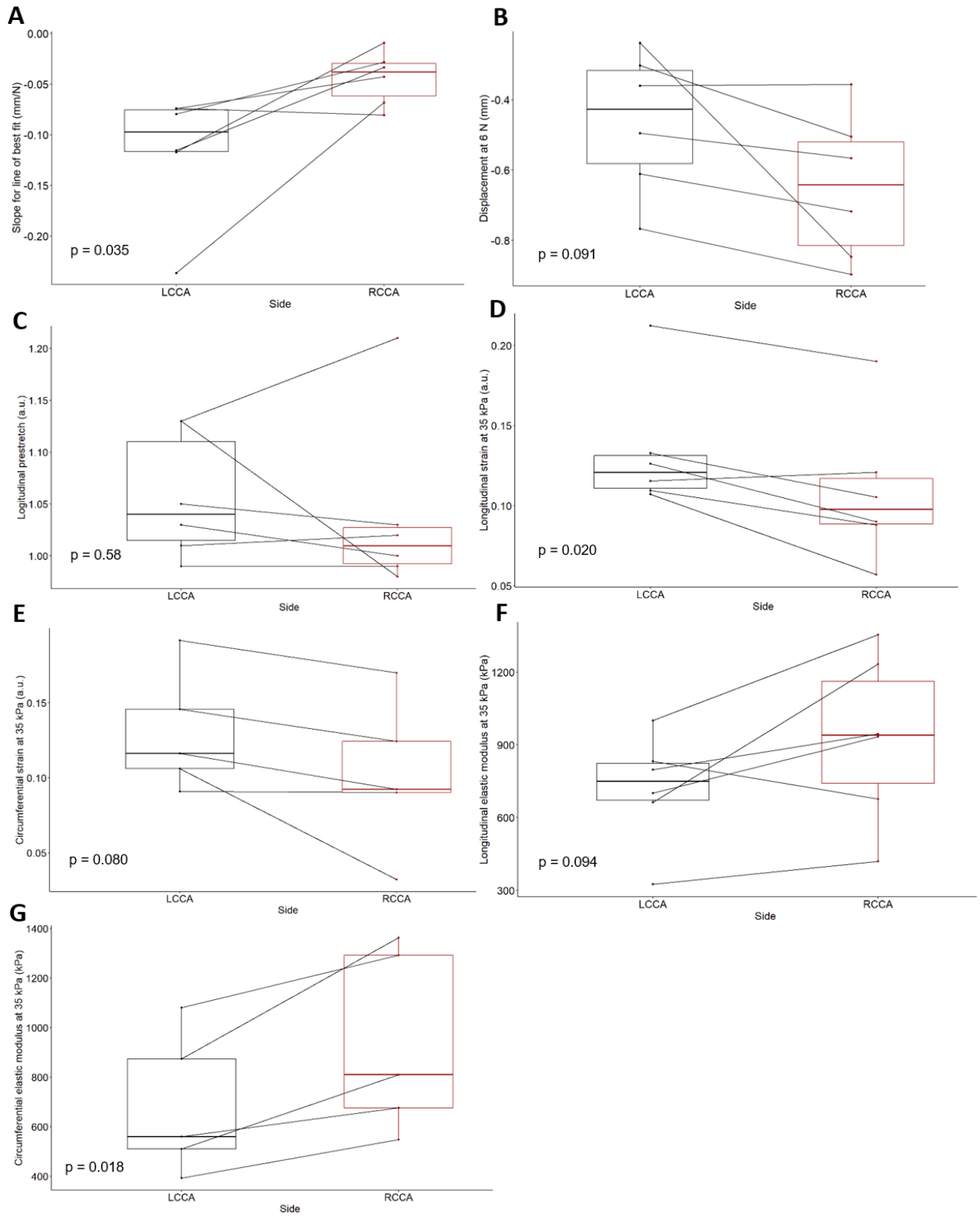


Figure 22: Paired t-test comparing the LCCA and RCCA. A: Slope for the line of best fit between the LCCA and RCCA ($p = 0.035$). B: Displacement at 6 N between the LCCA and RCCA ($p = 0.091$). C: Longitudinal prestretch between the LCCA and RCCA ($p = 0.58$). D: Longitudinal strain at 35 kPa between the LCCA and RCCA ($p = 0.020$). E: Circumferential strain at 35 kPa between the LCCA and RCCA ($p = 0.080$). F: Longitudinal elastic modulus at 35 kPa between the LCCA and RCCA ($p = 0.094$). G: Circumferential elastic modulus at 35 kPa between the LCCA and RCCA ($p = 0.018$). Significantly different ($p < 0.05$).

5.6 Visualization of the study summary

One of the overarching questions of this thesis aimed to examine if arterial stiffness modulates the force-displacement relationship. While we were underpowered to run complex analyses, it was relevant to visualize the data to observe the potential relationship. Individual lines of best fit were plotted comparing maximal wall longitudinal displacement and applied force between the different sides (Figure 23). In general, the higher the longitudinal strain, indicative of a less stiff artery, the steeper the force-displacement relationship.

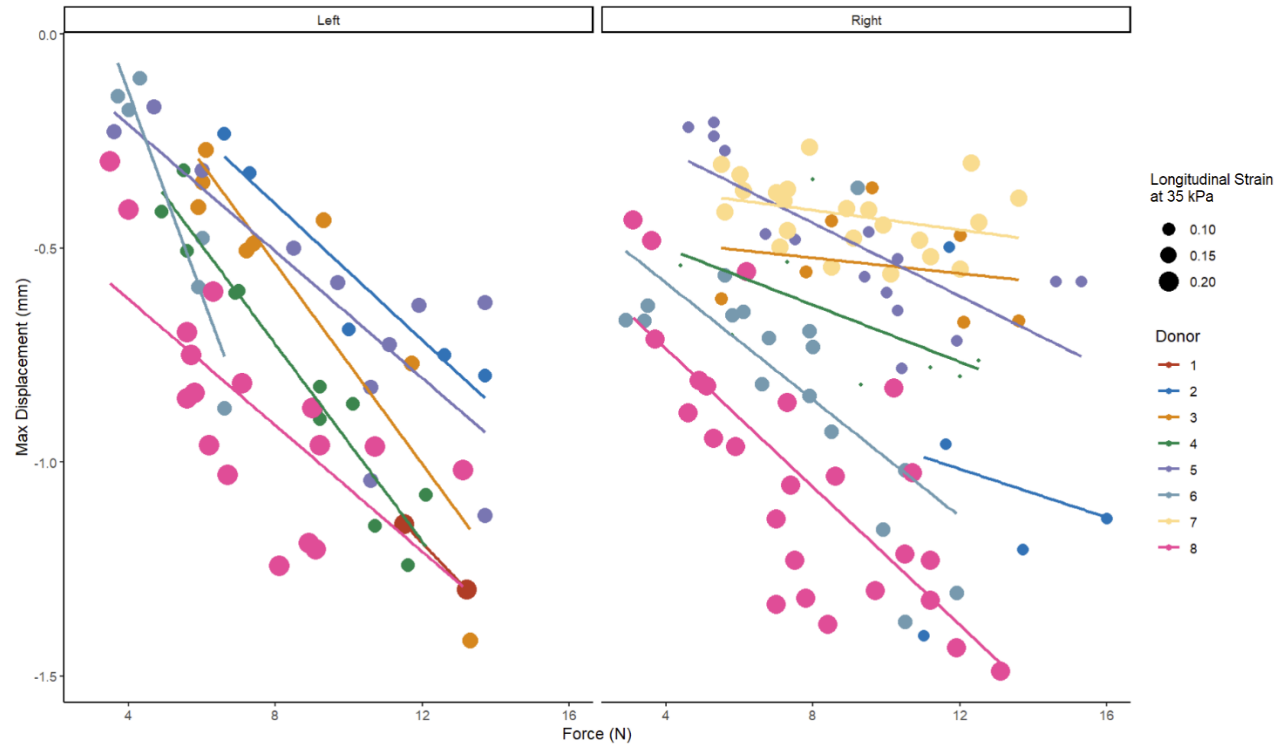


Figure 23: Individual lines of best fit comparing maximal wall longitudinal displacement in millimeters and applied force at the aorta in Newtons between the different sides (LCCA and RCCA). Each donor is represented by a different colour, including the line of best fit. Dot size represents the longitudinal strain at 35 kPa.

6.0 DISCUSSION

In this thesis we investigated the influence of simulated cardiac contraction, longitudinal prestretch and tissue biomechanics on longitudinal wall displacement in the CCA. Due to the fascicular connection of the left ventricle to the aorta via the aortic annulus, cardiac contraction has been hypothesized as a determinant for retrograde displacement in CALM (39, 42, 46, 49). Investigation into the modulation of retrograde CALM has been challenging due to the anatomical and physiological connectedness of the cardiovascular system, with hemodynamic influences and cardiac contraction occurring simultaneously. While research has demonstrated a negligible influence from wall shear stress (40, 46, 48, 49), the isolation and manipulation of cardiac contraction without forward blood flow is not possible. As the heart contracts the aortic annulus is displaced caudally which is proposed to also pull caudally on the aorta and corresponding arterial branches. This may reflect a dose-response relationship with cardiac contraction force as demonstrated by research with PVCs (49) in addition to material properties of the intermediary arterial tissue connection, such as stiffness and tissue tethering with longitudinal prestretch, which both decrease with age as does retrograde CALM magnitude (7, 8, 46, 55, 61). This study investigated longitudinal wall displacement due to simulated cardiac contraction via force application at the ascending aorta, in addition to influence from longitudinal prestretch and material stiffness. We found a moderate negative relationship between force application and retrograde wall displacement. Longitudinal prestretch and material stiffness did not appear to significantly influence longitudinal wall displacement in our small donor sample. This study suggests through simulated cardiac contraction that cardiac contraction *in vivo* is a major determinant for retrograde displacement in CALM.

6.1 Longitudinal wall displacement with force application

When applying an external force mimicking a left ventricular contraction, longitudinal wall displacement amplitude increased with increasing force application. Data were averaged within 1 N ranges and split by side as visualized in Figures 15 & 16 to demonstrate high quality wall motion tracking with a consistent pull to a maximal displacement and return to baseline. Not all donors had data within each force range, and some were represented more within a 1 N range compared to other donors. To define an individual's force-displacement relationship, displacement at 6 N was calculated as a means to compare wall displacement within a physiological range. The exertion of force at the ascending aorta is distributed across the arterial branches and displacements for a force of 6 N were similar to those seen *in vivo* [retrograde displacement: 0.42 ± 0.18] (5, 39, 42, 49, 90).

The within-subjects relationship between applied force and longitudinal wall displacement displayed a moderate negative correlation, suggesting increasing force leads to increased displacement, and is consistent between donors, albeit with individual differences in slope. The ascending aorta is connected to the heart via the aortic annulus, which moves caudally during cardiac contraction *in vivo* (5). Force applied at the ascending aorta is dispersed to the corresponding branches due to the anatomical connections. Caudal force application at the ascending aorta led to CCA wall displacement which supports the ventricular-vascular coupling theory (42, 46). Graded force applications were intended to simulate differences in the strength of cardiac contraction; this was demonstrated with varying force applications leading to corresponding changes in the wall displacement, supporting a modulatory effect on displacement amplitude from the strength of cardiac contraction. However, even for the

same force application, donors showed different amplitudes for wall displacement. The differences in slope between donors was anticipated as differences in CALM magnitude exist *in vivo* (46). While the nature of the interindividual differences has not been explored in depth, contributing factors might include differences in arterial tethering, anatomical branching, or arterial stiffness. A study found that presence of PVAT surrounding the abdominal aorta bears some portion of pressure loading (60). The PVAT tethering of the arterial branching may play a role in the individual differences by altering the distribution of force across the branching arteries. Other anatomical differences such as distance from the point of force application likely also play a role, as taller individuals have decreased CALM amplitude *in vivo* (39, 53). Although in this study we did not measure the distance from the point of force application to the imaging site, this distance (i.e., from the aortic annulus to the imaging site) may be useful for *in vivo* measurements and consistency. The influence of longitudinal prestretch and arterial stiffness is discussed in the following sections.

6.2 Longitudinal prestretch

There was no significant relationship between longitudinal prestretch and displacement at 6 N. While not significant, the directionality of the relationship between longitudinal prestretch and displacement at 6 N suggests that with an increasing longitudinal prestretch there will be a larger displacement amplitude, which is in line with our hypothesis. Longitudinal prestretch has been observed to be related to longitudinal and circumferential wall behaviour akin to material stiffness. Higher longitudinal prestretch has been shown to minimize axial stress and strain variation over the cardiac cycle and improve circumferential distensibility (58–60). An *ex vivo* study performing inflation-

extension arterial testing noted varying axial deformation and forces in response to altered initial longitudinal prestretch (58). Increasing arterial pressure at a testing longitudinal prestretch below that of the *in situ* measure leads to a decrease in force which can result in arterial buckling. Together these suggest a more longitudinally prestretched artery will have a higher load-bearing capacity. This is seen with 2-D coupling for systolic CALM (57), which is also linked to the tissue stiffness properties, which will be further discussed in the section below. Aging is related to a decrease in longitudinal prestretch (55, 61, 62) and increase in arterial stiffness (23, 54–56, 61, 62) which may further confound the ability to observe a significant difference with longitudinal prestretch and displacement at 6 N. However, we were underpowered to conduct a multivariate analysis to separate the influences of longitudinal prestretch from material stiffness properties. It is also possible that due to the lack of pressurization in the cadaveric model, we were unable to fully observe the influence of longitudinal prestretch.

There were several challenges when measuring longitudinal prestretch values. Due to the nature of the study, the dissection protocol needed to be conducted prior to longitudinal prestretch measures, which may have led to some loosening of the PVAT tethering of the tissue. Moreover, aortic samples were also collected for another project and for some donors it was excised prior to CCA longitudinal prestretch measurements. Thus, the longitudinal prestretch measures may be underestimated in some individuals. The longitudinal prestretch values measured in this study were compared to values measured in fresh cadavers by Horný and colleagues in 2017 (Figure 19). Our values were similar for older individuals. Although longitudinal prestretch was lower for the 19-year-old donor which may be attributed to multiple freeze thaw cycles and loosened

arterial tethering as the donor's arms were previously removed at the level of the axillary artery. Due to the majority of donors being of advanced age in our sample, the longitudinal prestretch values were similar and grouped closely together. This restriction of range for longitudinal prestretch values is likely further limiting the ability to identify a relationship between longitudinal prestretch and longitudinal wall displacement. Having a sample with donors of varying ages and without comorbidities was unattainable for the purposes of this study due to the nature of the body bequeathal program.

6.3 Arterial stiffness

Aging has been associated with decreases in CALM magnitude (7, 8, 46) and increases in arterial stiffness (23, 54–56). This arterial stiffening may reduce longitudinal displacement leading to the decreased CALM magnitude observed in older individuals. To investigate the effect of arterial stiffening on wall displacement we conducted biaxial testing. In order to reduce the data to a meaningful value for comparison between applied aortic force and wall displacement data, values at a Cauchy stress of 35 kPa were selected to mimic biomechanical properties seen *in vivo* (under 50 kPa) (82). Elastic modulus was used as a proxy for tissue stiffness. The tissue strain at 35 kPa was used to observe the propensity of the tissue to stretch in response to a given stress. Since arteries are anisotropic, an incremental elastic modulus, as performed in this study, is more suitable than a single point evaluation to define strain as the ratio of change in length (74). Due to different timelines for arterial tissue being frozen, biaxial testing data is not representative of fresh tissue and thus may not be representative of the tissue at the time of the dissection protocol (91). Freeze fracturing, plaque, and tissue calcification were noted during testing for some of the arterial segments. Care was taken to select samples

visually and tactilely without obvious calcified deposits. However, the degree to which plaque and in particular calcification infiltrated the tissue wall layers, such as in the media layer (92), was not investigated with histology. The presence of plaque and calcification in the arterial segments was bilateral ($n = 3$) or unilateral in the LCCA ($n = 1$).

Longitudinal stiffness

There were no significant relationships found between longitudinal elastic modulus or strain with displacement at 6 N. We predicted that arteries with a lower stiffness will have a greater propensity to stretch and lead to increased wall displacement. Provided the nature of the study, without pressurization we wanted to observe the influence of longitudinal stiffness on longitudinal displacement. Visualization of the data seen in Figure 23 compared longitudinal strain to individual lines of best fit for force-displacement data and demonstrated that generally an artery with a greater propensity to stretch will have a steeper force-displacement relationship. The direction of the relationship between longitudinal strain and displacement at 6 N indicates that a tissue with a higher strain relates to a larger displacement at 6 N. Similarly, the relationship for longitudinal elastic modulus reflected that a lower modulus related to a larger displacement at 6 N. The directionality of these relationships are in line with our hypothesis that a less stiff artery will have a greater displacement at a given force, however we were underpowered to fully examine this complex relationship.

Circumferential stiffness

Due to the anisotropic nature of arteries, consideration of stiffness in the circumferential direction is important as it may translate to stiffness in the longitudinal direction due to the

helical orientation of VSMC, collagen and elastin (19, 27). There were no significant relationships found for circumferential elastic modulus or strain with displacement at 6 N. Circumferential stiffness was evaluated to provide insight on the impact of overall arterial stiffness on longitudinal displacement. Due to the lack of pressurization for the dissection protocol it may limit the relevance of circumferential stiffness to the displacement at 6 N due to 2-D coupling behaviour. Decreases in CALM amplitude have been associated with age independent of stiffness which further points to a potential modulatory effect of material stiffness rather than a major determinant (8). Furthermore, comparison of stiffness was restricted since our sample consisted of donors of advanced age. Due to this, samples largely had an increased stiffness compared to that of donor 1 (19-year-old). Differences in stiffness related to displacement at 6 N may be more identifiable with arteries of varying degrees of stiffness associated with age.

6.4 Differences between the LCCA and RCCA

Force-displacement relationship

Measurements were conducted on both the LCCA and RCCA throughout the study. Over the course of testing and analysis, differences were noted between the sides in the force-displacement relationship. The slope of the relationship was steeper for the LCCA indicating that for a given force, there is greater wall displacement in the LCCA compared to the RCCA, as seen in the individual lines of best fit in Figure 23. This difference in slope may be due in part to the anatomical differences for origin off the aortic arch. The LCCA originates directly off the aortic arch, compared to the RCCA, which originates from a bifurcation off the brachiocephalic trunk from the aortic arch. The direct branching of the LCCA from the aortic arch may lead to a higher proportional force distributed to the LCCA

from the ascending aorta compared to that exerted on the RCCA. *In vivo* measures noted greater CALM displacement in the LCCA compared to the RCCA [0.055 ± 0.157 mm in adults >50 yrs.] (52). This displacement difference measured *in vivo* is smaller than the shared standard deviation predicted for our study of 0.5 mm for our power simulation. Furthermore, displacement at 6 N was not significantly different between sides and was greater for the RCCA which may be due to fewer measurements for the RCCA for forces at or below 6 N due to poor image quality. As a methodological note, force-displacement imaging was conducted on the RCCA prior to the LCCA for all donors. There is the potential that throughout the duration of varying force applications for the RCCA images, tissue tethering the arterial branches were loosened at the high force levels (non-physiological >10 N), which may also have led to the increased displacement seen for the LCCA compared to the RCCA for higher force applications. We were underpowered to examine side differences further as we only had complete data for 6 donors, particularly at low forces between 3 – 6 N.

Biaxial testing data

In this study the longitudinal strain was significantly lower at 35 kPa in the RCCA compared to the LCCA. The circumferential elastic modulus at 35 kPa was significantly higher for the RCCA compared to the LCCA. Together these data indicate that the RCCA has an increased stiffness compared to the LCCA. A study investigating the biaxial mechanical properties of human CCA in fresh tissue found some donors showed considerable differences between the circumferential stiffness between the LCCA and RCCA (66). Although, biaxial tubular testing for pulsatile conditions performed in murine carotid arteries found no differences between the LCCA and RCCA (72). Overall

differences between sides have not been examined in depth, in part due to the majority of biaxial testing studies grouping data together based on the artery (i.e., CCA, femoral, etc.) likely due to the smaller sample sizes. Calcification of plaques has been shown to be more prominent in the RCCA compared to the LCCA (93). Calcified deposits interact with the surrounding tissue leading to global stiffening of the tissue (94). Computational modelling for calcification presence and distribution within an artery noted increased peak circumferential stress (95) and variation of the elastic modulus between the calcified region and surrounding tissue (96). There is a possibility of tissue differences due to non-visible calcification within the arterial segments of the RCCA compared to the LCCA that led to the increased stiffness observed in the RCCA, particularly given the advanced age of the donors. Furthermore, blood flow differences can lead to remodeling of the artery as seen in blood flow restriction of murine carotid arteries (97). Hemodynamic differences due to the anatomical variation in the origin between the LCCA and RCCA (93) therefore may influence remodeling and thus material differences. Considering the small sample size in this study, more work needs to be conducted to inform the potential differences between the RCCA and LCCA material stiffness. Due to the direct branching of the LCCA from the aorta it may experience more proportional force exerted from cardiac contraction. Thus, we suggest CALM measurements *in vivo* be taken on the LCCA to be more reflective of the influence from cardiac contraction.

7.0 LIMITATIONS

Due to the nature of the body bequeathal program, selection based on inclusion/exclusion criteria is limited. Exclusion criteria was based on donor tissue preservation. To avoid disruption to the arterial tree tethering, the protocol was conducted on donors that had the head, neck, torso, and upper arms intact with the exception of data from donor 1, who was frozen for an extended period of time prior to the study start. The majority of donors were of advanced age and had various cardiovascular diseases, including atrial fibrillation, lung carcinoma, congestive heart failure, chronic obstructive pulmonary disease (COPD), renal failure, thrombosis and calcification of regions of the aorta and common carotid arteries. Atrial fibrillation is an irregular heart rhythm that can lead to blood clots in the heart, increasing the risk for stroke and heart failure. Lung cancer increases the risk of blood clots increasing the risk of pulmonary embolism. Congestive heart failure results from a weakened heart, leading to blood backing up and causing fluid buildup in extremities and/or other organs. COPD is a chronic inflammatory lung condition that causes obstructed airflow from the lungs and can cause heart failure in the right ventricle. Renal failure places more stress on the heart and leads to changes in blood pressure that can lead to increased arterial stiffness. For this reason, data from these donors may not reflect healthy arteries that transfer energy along the ventricular-vascular continuum. Generalizable vascular health is also complicated by the advanced age of donors, which implies arterial stiffening. Longitudinal prestretch values were also similar across donors leading to a restriction of range which may have led to a decrease in statistical power.

Due to the methodological choices to isolate the ventricular-vascular coupling we removed the chest plate during dissection. This 'open chest' model removed some tissue tethering between the ribs and the heart and lungs. Force application was applied via a clamp at the ascending aorta which compressed the aorta rather than maintaining the tubular form at the site of force application. The arteries were not pressurized which further prevented our ability to investigate 2-D coupling further with circumferential distension and longitudinal wall displacement. The lungs were also not pressurized as breathing (and in turn thoracic pressure changes) has been demonstrated to shift CALM tracings (51). Due to the 'open chest' model the lungs were not restricted in the thorax and thus were not compressing the heart which would have limited the ability to investigate changes in CCA wall displacement in response to altered lung volumes if performed.

Biaxial testing was unable to be performed on fresh tissue and was conducted on arterial tissue that had been frozen and kept at -80°C for varying periods of time for each donor, as the biaxial testing protocols were all conducted within a 1-month period. Since freezing can alter water content due to ice formation within the arterial tissue, stiffness indexes may be impacted due to potentially more rapid increases in tissue stress in frozen compared to fresh tissue particularly at lower stress regions (82). Although other studies have noted minimal to no significant differences for arteries preserved at -80°C as performed in this study (98–100), there may be changes related to differences in the length of freezing prior to testing between donors.

Stiffness values measured at 35 kPa are from the 1:1 protocol which can vary between specimens due to anisotropy and nonlinearity. We did not perform histology or

multiphoton analysis to determine microstructure and thus were unable to determine the stress/strains corresponding to the start or end of the transition zone to compare initial slope with elastin and final slope for collagen engagement. We selected the stiffness values at 35 kPa to be consistent across all donors, however due to inter-individual variability the point corresponding to *in vivo* stresses and strains will vary between donors which may limit the ability to identify a significant relationship between this specific stress point and displacement at 6 N.

8.0 CONCLUSION

Increasing caudal aortic force application as a surrogate for cardiac contraction induced a resulting increase in longitudinal wall displacement of the CCA supporting the ventricular-vascular coupling theory. There were no significant relationships between longitudinal prestretch and biomechanical tissue properties on longitudinal wall displacement. This supports cardiac contraction involvement in CALM; however, our data also indicate that CALM may not truly represent a measure of vascular stiffness. Our work suggests that CALM measurements should be performed with cardiac imaging to supplement interpretations to potentially normalize carotid motion to cardiac displacement. There may be a modulatory effect of longitudinal prestretch and material stiffness, however, the small sample size and advanced age of donors may limit the ability to identify significant differences. The LCCA displayed a greater change in displacement for a given force application compared to the RCCA due to the direct branching from the aorta and thus a greater proportional force distributed. Future work should consider the anatomical differences between the LCCA and the RCCA and the impact this may have on CALM motion *in vivo*. CALM imaging should be conducted with cardiac imaging, particularly when comparing clinical populations to healthy controls to observe changes in wall displacement due to cardiac contraction.

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