Stiffness Properties of Adventitia, Media, and Full Thickness Human Atherosclerotic Carotid Arteries in the Axial and Circumferential Directions

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Arteries can be considered as layered composite material. Experimental data on the stiffness of human atherosclerotic carotid arteries and their media and adventitia layers are very limited. This study used uniaxial tests to determine the stiffness (tangent modulus) of human carotid artery sections containing American Heart Association type II and III lesions. Axial and circumferential oriented adventitia, media, and full thickness specimens were prepared from six human carotid arteries (total tissue strips: 71). Each artery yielded 12 specimens with two specimens in each of the following six categories; axial full thickness, axial adventitia (AA), axial media (AM), circumferential full thickness, circumferential adventitia (CA), and circumferential media (CM). Uniaxial testing was performed using Inspec 2200 controlled by software developed using LABVIEW. The mean stiffness of the adventitia was 3570 ± 667 and 2960 ± 331 kPa in the axial and circumferential directions, respectively, while the corresponding values for the media were 1070 ± 186 and 1800 ± 384 kPa. The adventitia was significantly stiffer than the media in both the axial ($p=0.003$) and circumferential ($p=0.010$) directions. The stiffness of the full thickness specimens was nearly identical in the axial (1540 ± 186) and circumferential (1530 ± 389 kPa) directions. The differences in axial and circumferential stiffness of media and adventitia were not statistically significant.
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Keywords: atherosclerosis, carotid artery, stiffness, tangent modulus

1 Introduction

Rupture of carotid atherosclerotic plaque can lead directly to ischemic stroke. It has been widely accepted that plaque rupture is the result of high stress concentration within the plaque structure that exceeds its material strength. With this relevance, computational models have been widely used to model stress in the vicinity of atherosclerotic lesions [1–3]. These computation models require knowledge of the material properties of plaque as well as the native vessels themselves [4].

There has been a significant effort to determine the material properties of atherosclerotic plaque [5–9]. Considerably less attention has been given to determining the material properties of the arterial wall in atherosclerosis. It is difficult to assess the risk of plaque rupture without also knowing the local material properties of the arterial wall, itself. Improved knowledge of arterial wall properties could lead to improved assessments of plaque vulnerability based on computational stress/strain predictions [2,3,9–11].

Arteries can be considered to be a layered composite of fiber oriented materials composed of three layers; intima, media, and adventitia. The mechanical properties of an intact artery result from the combined interaction of these layers with each layer displaying different material properties. In smaller arteries, the intima is very thin and comprised of the endothelial cells and the basal lamina [12]. In tension, arteries exhibit a nonlinear response which is sometimes modeled as bilinear [13]. Layer- and direction-dependent experimental data are crucial for creating realistic computational models and accurate stress–strain predictions [14].

The properties of intact carotid arteries have been studied in human, canine, porcine, and rat blood vessels. The majority of these investigators have used inflation and extension tests of cylindrical segments which result in multiaxial loading of the vessel wall. In this type of test, the stresses in the orthogonal directions are coupled through their respective Poisson's ratios which are generally unknown. To extract true material properties, such as the tangent modulus from inflation–extension tests, it is usually necessary to assume some type of constitutive model. An example of this type of approach is a paper by Learoyd and Taylor [15] who measured circumferential (tangent) modulus as a function of

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Fig. 1 Specimen preparation. Four axial strips and four circumferential rings were dissected from each artery. Two strips and two rings were further dissected to create paired adventitia and media specimens.

pressure in human carotid arteries. Inflation–extension tests of canine carotid arteries showed that at certain extension ratios, the properties were nearly isotropic [16,17]. Weizsacker et al. measured the passive mechanical properties of rat carotid arteries using inflation–extension tests and showed only a weak coupling between circumferential and longitudinal directions [18]. Uniaxial tests of porcine carotid arteries showed a relatively small difference in stiffness between the axial and transverse directions [13].

The intima in the human carotid artery is very thin, and thus, the properties of that artery are primarily determined by the properties of the media and adventitia. It has been widely accepted that atherosclerosis changes the material properties of the arterial wall. Nagaraj et al. observed the elastic modulus increases with lesion progression in porcine carotid arteries [19]. However, very little experimental data exists relating the properties of the media and adventitia of atherosclerotic blood vessels to their overall properties [20]. Teng et al. determined the ultimate strength of axial and circumferential oriented specimens of adventitia, media, and intact specimens from human carotid arteries [21]. An early study of bovine carotid arteries by von Maltzahn and Keitz determined the properties of the adventitia and media using a deductive method [22]. Extension–inflation tests were conducted using cylindrical segments of intact artery and then on the media alone after removing the adventitia. Both layers were stiffer in the longitudinal direction than in the tangential direction. Holzapfel et al. investigated layer- and direction-dependent ultimate tensile stress and stretch ratio of human atherosclerotic iliac arteries [14]. Anisotropic and highly nonlinear tissue properties were observed as well as interspecimen differences. The adventitia demonstrated the highest strength and the fibrous cap, in the circumferential direction, showed the lowest fracture stress.

This paper focuses on directly measuring and comparing the stiffness of paired samples of adventitia, media and full thickness specimens from human atherosclerotic carotid arteries. Knowledge of the stiffness of constituent layers of human atherosclerotic tissues can aid in developing an improved understanding the effects of atherosclerosis and also aid in creating more accurate computational models for predicting the effects of the disease [2].

2 Methods

The methods have been previously reported [21]. Briefly, three pairs of human carotid arteries ($n=6$) were tested (female: age 80 and males: ages 78 and 50). Carotid arteries were obtained at autopsy from the Department of Pathology, Washington University School of Medicine, and were fresh frozen in

phosphate-buffered saline. The arteries were de-identified under the auspices of a Washington University Institutional Review Board protocol. On the day of testing, each artery was thawed at room temperature and dissected into four axial strips and four circumferential rings (Fig. 1) [21]. Areas with type II and III lesions [23] were selected for testing. Only sections with fatty streak or preatheroma with extracellular lipid pools were used and sections with large lipid pool and calcification were avoided when creating the test specimens. The adventitia and media could be easily identified visually. Next, two of the axial specimens and two of the circumferential specimens were further dissected by teasing with fine tweezers to create paired media and adventitia specimens. The intima resided on the inner surface of the media specimens. Each artery yielded a total of 12 specimens with two specimens in each of the following six categories; axial intact full thickness (AI), axial adventitia (AA), axial media (AM), circumferential intact full thickness (CI), circumferential adventitia (CA), and circumferential media (CM).

The dimensions, including width and thickness, were measured using a vernier caliper (0.1 mm in precision) at three equally spaced locations along each specimen. The average value was used for further calculation of the first Piola-Kirchoff (kPa) stress. Super glue was used to attach pieces of sandpaper to the specimen. The distance between the closer edges of the two pieces of sandpaper was measured as the initial length of the tissue strip. Circumferential arterial specimens were typically 2 mm wide and 9–10 mm in length. Axial specimens were typically 2 mm wide and 15 mm long. The thicknesses of media, adventitia, and intact strips were 0.61 ± 0.21 mm, 0.60 ± 0.19 mm, and 1.24 ± 0.30 mm, respectively. The tissue specimen was mounted in a uniaxial test frame (Inspec 2200, Instron Corp., Norwood, MA) that had been modified to operate under the control of software developed using LABVIEW (National Instruments Corporation, Austin, TX). The sample was carefully positioned so that the edges of clamps and the sandpaper coincided. A load cell measured the load and clamp to clamp displacement was used to calculate the Lagrangian finite strain (Eq. (1))

$$E = \frac{1}{2}(\lambda^2 - 1) \quad (1)$$

where $\lambda = 1 + \Delta L/L_0$, ΔL is the clamp displacement, and L_0 is the initial distance between the two clamps. Based upon preliminary tests, axial specimens were preconditioned by loading twice to 10% strain, while full thickness and circumferential specimens were preconditioned by loading twice to 5% strain. After

Fig. 2 Stress–strain plots from a single artery (F, age 80): (a) axial specimens. AA: axial adventitia; AM: axial media; and AI: axial intact. (b) Circumferential specimens. CA: circumferential adventitia; CM: circumferential media; and CI: circumferential intact.

preconditioning, each specimen was loaded at 0.1 mm/s until rupture occurred. Force and displacement data were recorded at a sampling rate of 10/s. The tissue surface was maintained in a moist condition by spraying with phosphate-buffered saline.

3 Results

The stress–strain plots for each specimen generally showed a nonlinear response at lower values of strain which transitioned into a linear region. Figure 2 shows the stress–strain plots obtained from a single artery. The slope of the linear portion of each plot is a measure of the stiffness (tangent modulus) within that strain interval. The interval over which linear behavior was observed varied for each specimen but generally occurred between strains of 0.1–0.4. Loading beyond the linear region resulted in specimen failure which has been described elsewhere [21]. The stiffness data were grouped by specimen type and orientation (Fig. 3). The mean and standard error for each group were computed and a Mann–Whitney U test ($p < 0.05$, two-tailed) was used to evaluate significant differences between the means (Table 1). Underbar “_” was used to mark the last significant digit of numbers in Table 1 and throughout the paper. Our numbers had no more than three significant digits due to measurement accuracies. The adventitia was significantly stiffer than the media in both the axial and circumferential directions ($AA = 3570 \pm 667$, $AM = 1070 \pm 186$, $CA = 2960 \pm 331$, and $CM = 1800 \pm 384$; unit: kPa). The stiffness of the adventitia in the axial (AA) and circumferential (CA) directions did not differ significantly. Similarly, the stiffness of the media specimens did not differ significantly between the axial (AM) and circumferential (CM) directions. For the intact (full thickness) specimens, the axial stiffness ($AI = 1540 \pm 186$ kPa) was essentially identical to the circumferential stiffness ($CI = 1530 \pm 389$ kPa). In addition, the stiffness of the intact specimens AI and CI was similar to their respective media specimens (AM and CM).

Specimen stiffness data were successfully obtained from 71 specimens (Table 2) and showed considerable variations. It is well known that atherosclerotic plaques have complex structures and their tissue compositions are in general very inhomogeneous. Furthermore, these inhomogeneities lead directly to variations in material properties. Table 2 shows that the stiffness varies considerably from artery to artery as well as from strip to strip within the same artery. Media circumferential stiffness (CM) from artery 1 (A1) was nearly 620% higher than that from A6 (4160 kPa versus 580 kPa). That is the largest variation from all the individual artery comparisons. The largest stiffness variation within an artery

was observed from A6: AI stiffness from strip 1 was 170% higher than that from strip 2. All other comparisons can be observed from Table 2 easily.

The Mann–Whitney U test assumed data independence when doing comparisons. To account for dependence among observations, the Wilcoxon signed-rank test was performed. This test assumes that two comparing groups are paired, e.g., the data values of AA and AI on each row of Table 2 are paired (12 pairs). Thus, it partially considered the dependence among the data observations. However, it should be noted that the dependence structure was partially considered, because it does not account for dependence among the four data values of each patient. Since the

Fig. 3 Stiffness data from six human atherosclerotic carotid arteries. AA: axial adventitia; AM: axial media; CA: circumferential adventitia; CM: circumferential media; AI: axial intact; and CI: circumferential intact.

Table 1 Specimen stiffness in the linear region (mean $\pm$ standard error) of pooled data from six human carotid arteries. AA: axial adventitia; AM: axial media; CA: circumferential adventitia; CM: circumferential media; AI: axial intact; and CI: circumferential intact. The Mann–Whitney U test ($p < 0.05$, two tailed) and the Wilcoxon signed-rank test (two-tailed) were used to evaluate significance. Underbar “ $_$ ” was used to mark the last significant digit of numbers in the table.

Group	Number of specimens	Stiffness (SEM) (kPa)	Comparisons	p -values by Mann–Whitney test	p -values by Wilcoxon test (pairwise)
Axial adventitia (AA)	12	3570 (667)	AM AI CA	$P = 0.003$ $P = 0.017$ $P = 0.977$	$P = 0.002$ $P = 0.002$ $P = 0.470$
Axial media (AM)	11	1070 (186)	AA AI CM	$P = 0.003$ $P = 0.148$ $P = 0.281$	$P = 0.002$ $P = 0.042$ $P = 0.240$
Circumferential adventitia (CA)	12	2960 (331)	AA CM CI	$P = 0.977$ $P = 0.010$ $P = 0.003$	$P = 0.470$ $P = 0.016$ $P = 0.028$
Circumferential media (CM)	12	1800 (384)	AM CA CI	$P = 0.281$ $P = 0.010$ $P = 0.341$	$P = 0.240$ $P = 0.016$ $P = 0.129$
Axial intact (AI)	12	1540 (186)	AA AM CI	$P = 0.017$ $P = 0.148$ $P = 0.260$	$P = 0.002$ $P = 0.042$ $P = 0.677$
Circumferential intact (CI)	12	1530 (389)	AI CA CM	$P = 0.260$ $P = 0.003$ $P = 0.341$	$P = 0.677$ $P = 0.028$ $P = 0.129$

four data values were from the same patient, they are potentially dependent. Plaque structure is so complex and the locations of the four data points (strips) from the same patient were entirely random (we took a strip where tissue was suitable to cut), the dependence structure is totally unknown. Since data are limited, we did not want to reduce to three data values (one value for each patient). That would lose valuable information of this study. Readers could get those data from Table 2 for their own comparisons. Furthermore, Table 1 shows both methods give very consistent results, which indicates that the dependence structure likely does not play a game-changing role here. A smaller p -value from the Wilcoxon signed-rank test indicates that the two comparing groups are more significantly different under such paired comparison.

Table 2 Specimen stiffness data for the 71 specimens. A1 and A2 (F, age 80); A3 and A4 (M, age 78); and A5 and A6 (M, age 50). Abbreviations are the same as in Table 1. Underbar “ $_$ ” was used to mark the last significant digit of numbers in the table.

Artery	Strip	AA	AM	AI	CA	CM	CI
A1	1	7260	1230	2650	3680	4800	4880
	2	5000	1600	2130	2850	3510	3760
	Ave	6130	1420	2390	3270	4160	4320
A2	1	5060	N/A	1730	5400	3310	1540
	2	7650	986	1890	3770	1790	1280
	Ave	6360	986	1810	4590	2550	1410
A3	1	1690	311	1540	2040	1300	987
	2	1690	508	948	2010	984	1020
	Ave	1690	410	1240	2030	1140	1000
A4	1	4480	1750	2120	1880	1250	970
	2	3120	1750	1730	1750	1160	990
	Ave	3800	1750	1920	1820	1210	980
A5	1	1560	1700	1540	2850	1320	490
	2	2880	1340	1090	3680	1010	1050
	Ave	2220	1520	1320	3270	1170	770
A6	1	1770	370	300	3880	580	490
	2	680	230	807	1730	580	850
	Ave	1230	300	553	2810	580	670
Total	Mean	3570	1070	1540	2960	1800	1530

4 Discussion

It has been proposed that the layers of the arterial wall as well as plaque can be modeled as fiber based composite materials [20]. However, the manner in which the properties of each layer combine to create the properties of the intact artery is largely unknown. Available experimental data for the stiffness of human atherosclerotic arteries and in particular for their respective layers is very limited.

Comparing the stiffness of adventitia, media, and full thickness specimens in these arteries with American Heart Association grade II and II atherosclerotic lesions revealed statistically significant patterns (Table 1). The stiffness of the adventitia is significantly greater than the media in both the axial and the circumferential directions (Table 1). Significant differences in stiffness were not observed between the axial and circumferential directions in all three types of specimens (adventitia, media, and full thickness). The finding that the stiffness in the axial (AI) and circumferential (CI) directions were similar in full thickness specimens lends support to the current practice of using an isotropic constitutive equation, such as Mooney–Rivlin, in the computational modeling of arterial behavior [2,3,24].

Arteries under no load conditions typically contain residual stresses which can be evidenced by the existence of an opening angle when cut in the radial direction [10]. We observed a similar phenomenon [21]. Several analyses using various constitutive models have indicated that the existence of residual stresses and strains result in a more uniform circumferential stress distribution in the wall [25,26].

The stiffness data of the various specimens exhibited considerable variation both across and within donors (Fig. 3). Individual arteries did not necessarily follow the statistical pattern reported in Table 1. It should also be noted that in a specific artery the linear stiffness regions for the three types of specimens (intact, adventitia, and media) do not necessarily occur at the same strain levels. These types of variations have also been noted by others [4,27]. However, finite element models have been used to demonstrate that the calculation of stresses within the arterial wall are relatively insensitive to large variations in the material properties used in those calculations [1].

Learoyd and Taylor measured the (effective) circumferential modulus in normal carotid arteries from young and old human subjects using inflation–extension tests [15]. Their values are in

the same general range of magnitudes but cannot be directly compared to the results presented here since the inflation–extension test creates biaxial loading. Uniaxial testing of (intact) porcine carotid arteries by Silver et al. yielded similar AI and CI values for stiffness (1150 kPa and 1300 kPa, respectively) [13]. Holzapfel et al. evaluated the properties of the intima, media and adventitia in iliac arteries in both the axial and circumferential directions [4]. Their results showed anisotropy as well as a high degree of variability.

Several investigators have studied the properties of plaque. Loree et al. performed static tensile tests on plaque caps from human abdominal and thoracic aortas [6]. The mean values for the tangent moduli in the circumferential direction of cellular, calcified and hypocellular plaque were 927, 1470, and 2310 kPa, respectively, which are in the same general range of the CM media values reported in Table 1. Maher et al. also conducted circumferential uniaxial tests of plaques from human carotid artery and observed significant variability in the tangent modulus with values spanning the CM stiffness values reported here [7]. The high variability of the properties of lesions has also been observed in specimens from iliac arteries [4].

One purpose for developing experimental data concerning the arterial wall is to be able to use it in computational models of the arterial wall. Various constitutive models have been proposed for arterial wall analysis and each generally requires different types of data. These include phenomenological models based upon uniaxial and biaxial tests [28,29] as well as composite materials based models which utilize observations of tissue microstructure [13,27,30,31]. Nonetheless, all of these models incorporate some assumptions about either material behavior or structure or both. Based upon the findings in Table 1, one possible model for the human carotid artery would be a pressurized, two layer cylindrical model (media and adventitia) where each layer is isotropic and there is no slip at the interface. The solution to this problem shows that a difference in the circumferential stiffness between the two layers, such as was observed ($CA > CM$), would result in a discontinuity in circumferential stress [32].

Several factors could influence and limit the results of this study. A relatively small number of arteries were used (six vessels from three donors) as the basis for the statistical findings. Testing vessels from a larger number of donors could potentially alter the findings. An advantage of uniaxial tests is that they reveal true material properties (tangent moduli) when compared to inflation–extension tests which are a form of biaxial loading. However, the physical size the uniaxial specimens can influence the results. Uniaxial specimens should have a minimum aspect ratio (length/width) of at least five in order to avoid undue influence of end effects. Due to the relatively small diameter of the carotid artery, the aspect ratios of some circumferential specimens were 4.5. The difficulty in establishing the initial length at zero load for nonlinear soft tissues is well known and this uncertainty in initial length may have had some small effect on the calculation of strain (Eq. (1)).

Aging has been shown to result in an increased stiffening of the arterial wall in both carotid [33] and iliac arteries [34]. The age of the donors may have influenced the results presented here. The highest stiffness was recorded from specimens from the oldest donor (female, age 80). However, the stiffness from the other donors (males, ages 50 and 78) was generally similar. Altered genetics have also been shown to affect the mechanical properties of arteries [35]. Factors that may have an impact upon arterial wall mechanical properties also include hypertension, diabetes, lipidemia, and smoking [36]. It should be noted that we had only three patients and our data are not enough to support any age-related conclusions.

Dividing the atherosclerotic carotid artery into media and adventitia over-simplified the complexity of the structure of the artery. The intima resided on the inner surface of the media specimens and could not be separated as a distinct layer. The properties

of the intima have been measured in larger vessels including the abdominal aorta [27] and iliac arteries [4].

Data on the properties of atherosclerotic arteries and their constituent layers are quite limited. Detailed knowledge of the stiffness and ultimate strength of the arterial wall in atherosclerosis as well as the plaque itself are both necessary to enable an improved understanding of plaque vulnerability [37]. Teng et al. reported on the ultimate strength of the same group of specimens and found the strength of the adventitia in the axial and circumferential directions to be similar and considerably greater than the strength of the media [19]. The media was weakest in the axial direction. Subject to the limitations discussed previously, the results presented here for the common carotid artery may assist in the development of more refined mechanical models that consider the specific contributions of the adventitia and media.

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