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Arterial stiffness identification of the human carotid artery using the stress-strain relationship *in vivo*

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Abstract

Arterial stiffness is well accepted as a reliable indicator of arterial disease. Increase in carotid arterial stiffness has been associated with carotid arterial disease, e.g., atherosclerotic plaque, thrombosis, stenosis, etc. Several methods for carotid arterial stiffness assessments have been proposed. In this study, *in-vivo* noninvasive assessment using applanation tonometry and an ultrasound-based motion estimation technique was applied in seven healthy volunteers (age 28 ± 3.6 years old) to determine pressure and wall displacement in the left common carotid artery (CCA), respectively. The carotid pressure was obtained using a calibration method by assuming that the mean and diastolic blood pressures remained constant throughout the arterial tree. The regional carotid arterial wall displacement was estimated using a 1D cross-correlation technique on the ultrasound radio frequency (RF) signals acquired at a frame rate of 505–1010 Hz. Young's moduli were estimated under two different assumptions: (i) a linear elastic two-parallel spring model and (ii) a two-dimensional, nonlinear, hyperelastic model. The circumferential stress (σ_θ) and strain (ϵ_θ) relationship was then established in humans *in vivo*. A slope change in the circumferential stress-strain curve was observed and defined as a transition point. The Young's moduli of the elastic lamellae (E_1), elastin-collagen fibers (E_2) and collagen fibers (E_3) and the incremental Young's moduli before ($E_{0 \leq \epsilon_\theta < \epsilon_\theta^T}$) and after the transition point ($E_{\epsilon_\theta^T \leq \epsilon_\theta}$) were determined from the first and second approach, respectively, to describe the contribution of the complex mechanical interaction of the different arterial wall constituents. The average E_1 , E_2 and E_3 from seven healthy volunteers were found to be equal to 0.15 ± 0.04 , 0.89 ± 0.27 and 0.75 ± 0.29 MPa, respectively. The average $E_{0 \leq \epsilon_\theta < \epsilon_\theta^T}^{Int}$ and $E_{\epsilon_\theta^T \leq \epsilon_\theta}^{Int}$ of the intact wall (both the tunica adventitia and tunica media layers) were found to be equal to 0.16 ± 0.04 MPa and 0.90 ± 0.25 MPa, respectively. The average $E_{0 \leq \epsilon_\theta < \epsilon_\theta^T}^{Ad}$ and $E_{\epsilon_\theta^T \leq \epsilon_\theta}^{Ad}$ of the tunica adventitia were found to be

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equal to 0.18 ± 0.05 MPa and 0.84 ± 0.22 MPa, respectively. The average $E_{\epsilon_{\theta}^T \leq \epsilon_{\theta}}^{Me}$ and $E_{\epsilon_{\theta}^T \leq \epsilon_{\theta}}^{Me}$ of the tunica media were found to be equal to 0.19 ± 0.05 MPa and 0.90 ± 0.25 MPa, respectively. The stiffness of the carotid artery increased with strain during the systolic phase of cardiac cycle. In conclusion, the feasibility of measuring the regional stress-strain relationship and stiffness of the normal human carotid artery noninvasively was demonstrated in human *in vivo*.

Keywords

Arterial stiffness; Carotid artery; Collagen; Elastin; Motion estimation; Tonometry; Ultrasound

1. Introduction

Arterial stiffness has been shown to be an excellent indicator of cardiovascular morbidity and mortality in a large percentage of the population [1], patients with hypertension [2, 3], atherosclerosis [4] and myocardial infarction [5]. Several carotid stiffness indices have been proposed based on the pressure-diameter relationship of arterial distension from the end-diastolic to the end-systolic phase, e.g., arterial distensibility, arterial compliance, Peterson's elastic modulus (E_p) and stiffness index (β) [6–17]. They, however, represent a global stiffness measurement of the entire arterial wall based on a single measurement site. The Young's modulus can depict more subtle changes in the relative proportions of the constituents of the arterial wall [18]. The *in-vivo* noninvasive Young's modulus estimated from the regional stress-strain relationship includes more comprehensive information on the arterial wall properties regarding the effects of the different constituents [19]. Only a few studies have reported on the *in-vivo* Young's modulus measurement of the carotid artery based on the pressure-strain relationship [18, 20–22] or from the slope of the stress-strain relationship [23] at end-diastole and end-systole. Previous studies, however, have not investigated on the complex mechanical interaction of the arterial wall constituents in humans *in vivo*.

It is hypothesized that the slope change of the stress-strain relationship is related to the change in material properties resulting from the elastin and collagen contribution in the aortic wall [6, 24]. The elastic lamellae of the arterial wall are primarily dilated at low pressure levels. As the lumen pressure increases, the collagen fibers start elongating [25, 26] and reach high tensile lengths. The tunica adventitia stiffness thereby increases with pressure in order to prevent the artery from overstretching or rupture [27].

Higher vascular stiffness is typically found in older subjects because the elastic lamellae decrease in number with age while the connective tissue and collagen fibers increase [28]. The elastic modulus and spatial arrangement of the arterial wall constituents depend on the level of circumferential stress. The incremental Young's modulus of the carotid artery, as a function of blood pressure and circumferential stress, was shown to be higher with age and hypertension [3]. The change in the carotid artery stiffness with age has been shown similar to that of the abdominal aorta [7]. The stiffness of the carotid artery has been shown to be higher than in the abdominal aorta in young subjects (< 20 years old) [12, 29] and in children [30]. The difference in stiffness between the carotid artery and the abdominal aorta

is due to their difference in elastin and collagen proportionality [10]. The linear relationship between the carotid stiffness and aortic pulse wave velocity (APWV) was proposed as a rough estimate of the cardiovascular risk factor [10, 31]. In the aforementioned studies, however, insufficient information on the contribution of the arterial wall constituents was shown, which may be useful for the early detection of cardiovascular disease.

Two well-known mechanical models, i.e., the Kelvin-Voigt and generalized Maxwell models, have been applied to describe the dilation mechanism of the arterial wall during the systolic phase of the cardiac cycle. Dobrin et al. [32] identified the series elastic component (SEC) representing the elastin, collagen and smooth muscle cell behavior, in the intact canine carotid artery via the Kelvin-Voigt and generalized Maxwell models. They found that the generalized Maxwell model represented the carotid arterial wall more accurately than the Kelvin-Voigt model. The smooth muscle cells which are overlaid and arranged in the circumferential direction mainly exhibit the viscosity behavior. Therefore, it regulates the caliber of the blood vessels and results in a time delay between the pressure and the dilation waveforms [6]. In order to describe the constituents' mechanism of the abdominal aortic wall of mice *in vivo*, Danpinid et al. [19] modified a generalized Maxwell model (i.e., a two-parallel spring model) by compensating for the time delay between the pressure and the dilation waveforms and thus ignoring the viscous properties. However, this model, viscosity was not expected to affect the stress-strain relationship, only the time delay between the pressure and the dilation waveforms, which was removed for the purposes of this study. Due to the nonlinear and anisotropic characteristics of the artery, other approaches based on the use of strain-energy functions have been proposed. *In-vivo* hyperelastic constitutive equations (stress and strain relations) have been proposed [33, 34] to identify material properties and to calculate wall stress in human arteries based on *in-vivo* clinical images.

To obtain the stress-strain relationship, the carotid pressure and wall displacement need to be simultaneously measured. Applanation tonometry can provide noninvasive assessment of the wall pressure. It provides a continuous measurement of the blood pressure waveform [35] and has been shown to accurately provide peripheral arterial pressure waveforms, e.g., in the femoral and radial arteries. In this paper, measurements in the left CCA were performed since it is more accessible for imaging while at the same time more closely correlated with the central aorta conditions [18]. The calibration method for the carotid pressure waveform assumed that the mean and diastolic blood pressures remained constant throughout the arterial tree [36], i.e., were equivalent to those of the radial artery [37].

Ultrasound imaging is a well-known noninvasive method for direct visualization of the vessels. The arterial wall motion can be estimated using motion estimation, or speckle tracking, techniques on the RF signals. These techniques have been mainly used on the brachial, femoral and carotid arteries and the abdominal aorta [38]. In this study, a 1D cross-correlation technique was applied noninvasively to estimate and image the local carotid arterial wall displacement in humans *in vivo* [39].

In this paper, we noninvasively applied the applanation tonometry methodology and ultrasound-based estimation motion technique [39] to obtain local, *in-vivo* pressure measurements and wall displacements, respectively, in the left CCA of healthy humans. The

in-vivo regional stress-strain relationship was proposed to characterize the complex mechanical interaction of the arterial wall constituents and determine the respective Young's moduli from (i) a linear elastic two-parallel spring model and (ii) a two-dimensional, nonlinear, hyperelastic model.

The experimental procedure, pressure and diameter relation, and stress and strain calculation derived from (1) the *in vivo* measurements (2) the two-parallel spring model and (3) the two-dimensional hyperelastic model are first provided in the Methods section. The Young's moduli of the elastin and collagen fiber in both the tunica media and tunica adventitia in the left CCA of healthy humans are presented in the Results section, followed by discussion and conclusions on the methodology and results presented.

2. Methods

2.1 Experimental procedure

The study was performed on seven healthy male volunteers of ages varying between 22 and 32 years (28 ± 3.6 years old, average $\pm$ std). The brachial blood pressure of the subjects was first measured using a sphygmomanometer to allow for calibration of the radial pressure waveform via a SphygmoCor system (AtCor Medical, Sydney, NSW, Australia). The applanation tonometer (Millar SPT-301 probe; Millar Instruments, Houston, TX) was then placed on the subject's wrist against the radial artery where the strongest pulse signal was manually detected. The diastolic and systolic blood pressures of the radial artery were assumed equal to those of the brachial artery [37]. The mean blood pressure was estimated using numeric integration of the radial pressure waveform [36]. In order to obtain the carotid pressure waveform, the subjects were placed in the supine position. The carotid pressure waveform was obtained with the sensor placed perpendicularly on the left CCA where the strongest pulse signal was detected. The carotid blood pressure was calibrated by assuming that the mean and diastolic blood pressures were equivalent to those of the radial artery [36, 37].

In order to acquire RF signals, the subjects were placed in a sitting position. The ultrasound transducer was placed on the left CCA using coupling gel. High temporal resolution RF frames of the left CCA were obtained with a 10-MHz linear array transducer using a clinical ultrasound system (Sonix TOUCH; Ultrasonix Medical, Burnaby, British Columbia, Canada). The radial plane of the carotid artery was closely aligned with the axial direction of the ultrasound beams in the longitudinal view. The local incremental wall displacement along the carotid arterial wall was determined using a 1D cross-correlation technique [39] between consecutive RF frames acquired at a sampling frequency of 20 MHz, depth of 30 mm, width of 38 mm, and a line density depending on the subject from 16 to 32 beams per full sector. Since the frame rate is associated with the beam density, the frame rate was varied between 505 and 1010 Hz depending on the depth scanned in each subject that had to be sufficient to determine the diameter of the lumen in each subject. At least three measurements were performed and averaged in each subject case.

2.2 Pressure and diameter relation

During the systolic phase of the cardiac cycle, the carotid wall was assumed to exhibit a purely passive elastic behavior. The viscosity effects were thus ignored. Uniform pressure acting on the inner arterial wall was assumed. The external pressure was assumed to be zero.

The carotid pulse pressure acquired from applanation tonometry was calibrated to obtain absolute values. The carotid blood pressure was estimated over the entire cardiac cycle (Fig. 1A) according to the aforementioned assumptions of calibration.

The carotid artery region most perpendicular to the ultrasound beam was selected in order to obtain the most accurate displacement estimation [19]. Selected points on the envelope-detected B-mode image (Fig. 1B) were defined as ‘near wall’ (wall nearest to the ultrasound probe (top)) and ‘far wall’ (wall furthest from the ultrasound probe) position and they were mapped onto the B-mode image. The radial near and far wall incremental displacements along the carotid arterial wall were determined using a 1D cross-correlation technique [39] and were averaged across the wall thickness. The cumulative displacement was calculated using Eq. (1) (see below). The red and blue dots represent near and far wall cumulative displacements towards and away from the ultrasound transducer (top), respectively (Fig. 1C). The diameter of the carotid artery (Fig. 1D) was, then, calculated from the difference between the near and the far wall cumulative displacements added on the reference diameter measured on the first frame as follows.

$$u_{cum}(i) = u_{cum}(i-1) + u_{inc}(i), \quad (1)$$

$$d(i) = d_{ref} + [u_{cum,near}(i) - u_{cum,far}(i)], \quad (2)$$

where $u_{cum}(i)$ is the cumulative displacement, $u_{inc}(i)$ is the incremental displacement, $u_{cum,near}(i)$ and $u_{cum,far}(i)$ are the near and far wall cumulative displacement, respectively, $d(i)$ is the diameter, d_{ref} is the reference mean diameter, and i is the frame index.

However, a delay between the pressure and the dilation waveforms during the systolic phase was noted due to the viscoelastic behavior of the carotid wall. According to the aforementioned assumptions regarding the arterial properties, the minima and maxima of the carotid pressure and diameter waveforms were aligned and matched to remove the viscosity effect [19], i.e., only the dilation of the carotid artery was considered with negligible vascular vasodilation effects.

2.3 Stress and strain calculation

The carotid arterial wall is well-defined into three layers which are the tunica intima, tunica media and tunica adventitia respectively arranged from the innermost to outermost wall layer, as illustrated in Fig. 2. The mechanical behavior is different for each wall layer depending on the constituents. The functional components of the tunica media are elastin lamellae, collagen fibers and smooth muscle cells while that of the tunica adventitia are collagen fibers and some elastin merged with the surrounding connective tissue. Regarding its actions, the tunica media and tunica adventitia predominantly adjust the mechanical

behavior at the lower and higher pressure levels respectively [25]. The tunica media and tunica adventitia, therefore, were in the focus in this study in order to determine the stress-strain relationship. The two-layer wall, i.e., both the tunica media and tunica adventitia, was defined as the ‘intact wall’.

2.3.1 Stress and strain relation calculated directly from experimental data—

Laplace’s law was applied to calculate the stress in the circumferential direction, the most dominant of the arterial wall deformation. Infinitesimal strain theory was assumed. The circumferential stress-strain relationship, therefore, was established along the carotid artery via Eqs. (4) and (5) to characterize the complex mechanical interaction of arterial wall constituents. The mean circumferential stress, $\sigma_\theta(t)$, was calculated as

$$\sigma_\theta(t) = \frac{P_i(t)d_i(t)}{2h}, \quad (3)$$

or in terms of $d(t)$ as follows:

$$\sigma_\theta(t) = \frac{P_i(t)d(t)}{2h} - \frac{P_i(t)}{2}, \quad (4)$$

where h denotes the carotid arterial wall thickness (intact wall thickness; the addition of the tunica adventitia and tunica media thickness), $P_i(t)$ and $d_i(t)$ denote the inner pressure and the diameter of the carotid arterial wall, respectively. The carotid arterial wall thickness of 0.48 ± 0.047 and 0.61 ± 0.018 mm (mean $\pm$ std) was applied for ages varying between 22 to 29 and 30 to 32 years, respectively [40]. In this study, the wall thickness was evaluated on the B-mode image though manual tracing by a trained expert.

Using the Cauchy strain definition, the mean circumferential strain, $\varepsilon_\theta(t)$, was equal to the ratio of the diameter change to the reference mean diameter, $d_{\min}$, defined as the minimum diameter over a cardiac cycle, i.e.,

$$\varepsilon_\theta(t) = \frac{d(t) - d_{\min}}{d_{\min}}. \quad (5)$$

Fig. 3 shows the locations of the five selected points along the carotid arterial wall corresponding to five circumferential stress-strain relationships as shown in Fig. 4A. In this study, 5 to 9 points arranged over the longitudinal locations of the carotid wall were selected for each subject in order to calculate the mean and standard deviation. As mentioned above, different numbers (5 to 9 points) were selected depending on the number of points on the carotid artery wall in each subject that were most perpendicular to the ultrasound beam in order to obtain the most accurate displacement estimation.

2.3.2 The two-parallel spring model—The arterial model was assumed to be an axisymmetric, single-walled layer cylindrical tube with isotropic, linearly elastic, homogeneous, incompressible and non-viscous properties. As shown in Fig. 4A, the stress-strain relationship of the carotid artery in humans was observed a clear inflection point similar to the abdominal aorta in mice [19]. The Young’s moduli of the elastic lamellae and

elastin-collagen fibers were defined as E_1 and E_2 , respectively, from a bilinear fit of the non-linear stress-strain curve (Fig. 4B). At the change of the slope, defined as the transition point, it was hypothesized that the collagen fibers start engaging and the Young's modulus of the vessel wall depends on the elastin-collagen fibers modulus instead. E_1 and E_2 , therefore, were assessed using two linear regression fits separated by the transition point (Fig. 4B). In this study, the transition point was defined as the maximum of the second derivative of the ratio of the circumferential stress to the circumferential strain (Fig. 4C). The difference between E_1 and E_2 was defined as the modulus of the collagen fibers (E_3). The relationship between E_1 , E_2 and E_3 is thus as follows [19]:

$$E_1 = \frac{\sigma_\theta - \sigma_0}{\varepsilon_\theta}; 0 \leq \varepsilon_\theta < \varepsilon_\theta^T \quad (6)$$

$$E_1 = E_2; \varepsilon_\theta = \varepsilon_\theta^T \quad (7)$$

and

$$E_2 = \frac{\sigma_\theta - E_1 \varepsilon_\theta^T}{\varepsilon_\theta - \varepsilon_\theta^T} = E_1 + E_3; \varepsilon_\theta^T \leq \varepsilon_\theta \quad (8)$$

where σ_0 is the stress at diastolic reference state (the stress at the minimum diameter over a cardiac cycle), ε_θ^T is the strain at the transition point, i.e., where the collagen fibers start

engaging. The strain ratio of the actively engaged collagen fibers, $\frac{\varepsilon_\theta^T}{\varepsilon_\theta}$, was unity at $\varepsilon_\theta = \varepsilon_\theta^T$, when the collagen fibers started engaging and underwent tension.

2.3.3 The two-dimensional hyperelastic model—Using the noninvasively acquired pressure-diameter data *in vivo*, the approach was proposed by Schulze-Bauer et al. based on the two-dimensional Fung's type model [33]. The arterial wall was assumed to be infinitesimally thin with respect to the arterial diameter. Radial stress and residual stresses, therefore, were ignored. The arterial model was assumed to be an axisymmetric, double-walled layer cylindrical tube with plane strain, anisotropic, nonlinearly elastic, homogeneous (in each layer), incompressible and non-viscous properties. The collagen fiber orientation was assumed to align only in the circumferential direction (no dispersion). The hyperelastic constitutive equation in the circumferential and axial directions associated with the strain-energy function (W) and principal stretches (λ) was proposed [27] as follows, respectively:

$$\sigma_{\theta\theta}^{\text{mod}} = \lambda_\theta \frac{\partial W}{\partial \lambda_\theta} \quad (9)$$

and

$$\sigma_{zz}^{\text{mod}} = \lambda_z \frac{\partial W}{\partial \lambda_z}, \quad (10)$$

where $\sigma_{\theta\theta}^{\text{mod}}$ and σ_{zz}^{mod} are the circumferential and axial stresses predicted by the model, and λ_θ and λ_z are the circumferential and axial stretches defined as $\lambda_\theta = D_i(A + d_i^2\pi)/d_i(A + D_i^2\pi)$ and $\lambda_z = z/Z = 1$, respectively. $A = d_i h \pi$ is the cross-sectional wall area. D_i and d_i respectively denote unloaded and loaded inner diameters, and z and Z are the actual and unloaded lengths of the arterial segment, respectively.

The two-dimensional strain-energy function was equal to

$$W = \frac{C}{2}(e^Q - 1) \quad (11)$$

with

$$Q = c_{\theta\theta}(\varepsilon_{\theta\theta}^G)^2 + 2c_{\theta z}\varepsilon_{\theta\theta}^G\varepsilon_{zz}^G + c_{zz}(\varepsilon_{zz}^G)^2, \quad (12)$$

where C , $c_{\theta\theta}$, $c_{\theta z}$, and c_{zz} are the constitutive parameters. $\varepsilon_{\theta\theta}^G$ and ε_{zz}^G are the circumferential and axial Green-Lagrange strains defined as $\varepsilon_{\theta\theta}^G = \frac{1}{2}(\lambda_\theta^2 - 1)$ and $\varepsilon_{zz}^G = \frac{1}{2}(\lambda_z^2 - 1)$, respectively.

Four material parameters of the model, i.e., D_i , C , $c_{\theta\theta}$, and $c_{\theta z}$, were optimized using a nonlinear least-squares method by minimizing the sum of differences between the circumferential and axial stresses calculated experimentally and from the model defined as

$$\chi^2 = \sum_{j=1}^n [(\sigma_{\theta\theta}^{\text{mod}} - \sigma_{\theta\theta})_j^2 + (\sigma_{zz}^{\text{mod}} - \sigma_{zz})_j^2], \quad (13)$$

where j was the j^{th} of n data points. $\sigma_{\theta\theta}$ and σ_{zz} are the circumferential and axial stresses calculated directly from the experimental data using the equilibrium equation of the force acting on the arterial wall with the inflation as follows:

$$\sigma_{\theta\theta} = \frac{P_i d_i^2}{d_o^2 - d_i^2} \left(1 + \frac{d_o^2}{d^2} \right), \quad (14)$$

$$\sigma_{zz} = \frac{\pi d_i^2 P_i + 4F}{4\pi h(d_i + h)}. \quad (15)$$

where d_o denotes the outer diameter. F is the external axial force determined explicitly by a known constant ratio of the axial to the circumferential stress of the intact wall (both the tunica adventitia and tunica media layers), tunica adventitia and tunica media defined as $\kappa^k = \sigma_z^k / \sigma_\theta^k$ which index k represents the intact wall (κ^{Int}), tunica adventitia (κ^{Ad}) and tunica media (κ^{Me}).

In order to investigate the contribution of the elastin lamellae and the collagen fibers in the intact wall, tunica adventitia and tunica media, κ was adopted from existent experimental data of an *in vitro* human layer-dissected CCA at physiologic loading ($P_i = 13.3$ kPa) [41].

In this study, the axial stretches of the intact wall, tunica adventitia and tunica media *in vivo* were assumed to be unity (0% axial stretch) as the arterial wall was subjected to arterial pressure. The κ^{Int} , κ^{Ad} and κ^{Me} at 0% axial stretch was assumed to be equal to 0.361, 0.195 and 0.482, respectively [41]. The d of the tunica adventitia and tunica media defined as $d^{Ad} = d + h - h^{Ad}$ and $d^{Me} = d - h + h^{Me}$, respectively. h^{Ad} and h^{Me} denote the tunica adventitia and tunica media thicknesses. The h^{Ad} and h^{Me} were respectively assumed to be equal to 40% and 60% of the carotid arterial wall thickness (h) [41]. Eqs. (9) – (15) were applied to determine the material parameters and stresses of the intact wall, tunica adventitia and tunica media of the carotid arterial wall, which underwent strain before ($0 \leq \varepsilon_\theta < \varepsilon_\theta^T$) and after the transition point ($\varepsilon_\theta^T \leq \varepsilon_\theta$). A generalized reduced gradient algorithm was used to determine the best-fit parameters including convexity [27]. The incremental Young's moduli before and after the transition point of the intact wall, tunica adventitia and tunica media were determined using Eq. (17), i.e., differentiated the strain energy function twice with respect to the Green-Lagrange strain, i.e.,

$$\sigma_{\theta\theta} = \lambda_\theta \frac{\partial W}{\partial \lambda_\theta} = \frac{\partial W}{\partial \varepsilon_{\theta\theta}} = E \varepsilon_{\theta\theta}, \quad (16)$$

thus

$$E = \frac{\partial^2 W}{\partial \varepsilon_{\theta\theta} \partial \varepsilon_{\theta\theta}}. \quad (17)$$

Since E changed with deformation, the determined E was separately averaged for the stress-strain curves before and after the transition point, which was respectively defined as

$E_{0 \leq \varepsilon_\theta < \varepsilon_\theta^T}$ and $E_{\varepsilon_\theta^T \leq \varepsilon_\theta}$, e.g., $E_{0 \leq \varepsilon_\theta < \varepsilon_\theta^T}^{Int}$ and $E_{\varepsilon_\theta^T \leq \varepsilon_\theta}^{Int}$ for the intact wall, $E_{0 \leq \varepsilon_\theta < \varepsilon_\theta^T}^{Ad}$ and $E_{\varepsilon_\theta^T \leq \varepsilon_\theta}^{Ad}$ for the tunica adventitia, and $E_{0 \leq \varepsilon_\theta < \varepsilon_\theta^T}^{Me}$ and $E_{\varepsilon_\theta^T \leq \varepsilon_\theta}^{Me}$ for the tunica media.

3. Results

Fig. 4A shows an example of the circumferential stress-strain relationship along five longitudinal locations, indicated in different colors on the envelope-detected B-mode images of the human carotid artery as shown in Fig. 3. Fig. 4B shows the mean circumferential stress-strain relationship of Fig. 4A. The stress-strain relationship shows nonlinearity and a transition point.

E_1 , E_2 and E_3 were calculated from the two-parallel spring model using Eqs. (6) – (8). The Pearson product moment correlation coefficients (r) of the two linear fits were calculated. Good correlation between the two linear stress-strain relationships was found with $r^2 = 0.967$ and 0.996 before and after the transition point, respectively. The three Young's moduli in seven subjects, averaged across all longitudinal locations of the carotid artery, are shown in Table 1. Fig. 5 shows the averaged Young's modulus of E_1 , E_2 and E_3 over all subjects. The E_1 , E_2 and E_3 were found to be equal to 0.15 ± 0.04 , 0.89 ± 0.27 and 0.75 ± 0.29 MPa, respectively.

The stress-strain relationship of the intact wall (Fig. 6A), tunica adventitia and tunica media at constant 0% axial stretch before and after the transition point were estimated using the two-dimensional hyperelastic model with optimized material parameters (Fig. 6C and 6D). Good correlation was found with $r^2 = 0.961$, 0.965 and 0.964 in the intact wall, tunica adventitia and tunica media before the transition point, and $r^2 = 0.993$, 0.991 and 0.991 in those after the transition point. Using Eq. (17), the $E_{0 \leq \varepsilon_\theta < \varepsilon_\theta^T}$ and $E_{\varepsilon_\theta^T \leq \varepsilon_\theta}$ were determined and average from seven subjects for the intact wall, tunica adventitia and tunica media, as shown in Table 2A and 2B. The average $E_{0 \leq \varepsilon_\theta < \varepsilon_\theta^T}^{Int}$ and $E_{\varepsilon_\theta^T \leq \varepsilon_\theta}^{Int}$ of the intact wall were respectively found to be equal to 0.16 ± 0.04 MPa and 0.90 ± 0.25 MPa. The average $E_{0 \leq \varepsilon_\theta < \varepsilon_\theta^T}^{Ad}$ and $E_{\varepsilon_\theta^T \leq \varepsilon_\theta}^{Ad}$ of the tunica adventitia were respectively equal to 0.18 ± 0.05 MPa and 0.84 ± 0.22 MPa. The average $E_{0 \leq \varepsilon_\theta < \varepsilon_\theta^T}^{Me}$ and $E_{\varepsilon_\theta^T \leq \varepsilon_\theta}^{Me}$ of the tunica media were respectively equal to 0.19 ± 0.05 MPa and 0.90 ± 0.25 MPa. The resulting $E_{0 \leq \varepsilon_\theta < \varepsilon_\theta^T}$ and $E_{\varepsilon_\theta^T \leq \varepsilon_\theta}$ were related to the elastin lamellae and collagen fibers contribution, respectively, based on the two-parallel spring model [19] as depicted in Fig. 6B.

4. Discussion

In this paper, the *in-vivo* stress-strain relationship was determined in order to characterize the complex mechanical interaction of constituents of the carotid arterial wall. To establish the stress-strain relationship *in vivo*, the arterial pressure and wall displacement measurements are respectively acquired and estimated *in vivo*. In addition, this study was performed in healthy humans; therefore noninvasive assessment was a requirement. The noninvasive (indirect) pressure assessments avoided the effects of local flow turbulence and pressure encountered in invasive (direct) procedures through catheterization in the small arterial lumen. The invasive pressure assessments can affect the elasticity measurements of the arterial wall [4]. The applanation tonometry showed the high accuracy when peripheral arterial pressure waveforms from superficial vessels were acquired [42]. However, it can be affected by movements from either the subject or user during the measurement. This noninvasive assessment, therefore, required the steady acquisition by the trained experimentalist and short duration of each measurement to acquire an accurate pressure waveform. The carotid pressure waveform was calibrated using the assumptions of constant mean and diastolic blood pressures throughout the arterial tree. The mean and diastolic blood pressures of the carotid artery were equal to those of the radial and brachial arteries. To obtain the brachial pressure, the auscultation method [12, 43, 44] was performed by a physician. This method is generally used by physicians to acquire systolic and diastolic brachial blood pressure by monitoring a pressure gauge or mercury sphygmomanometer together with a pressure cuff and stethoscope. They tend to provide lower and higher values than the true intra-arterial pressure for systolic and diastolic blood pressures, respectively [45]. Underestimation of the pulse pressure, therefore, may occur.

For the purposes of this study, the stress calculation using Laplace's law, however, did not use the pulse pressure. The stress is dependent on the pressure and the increasing transmural

pressure produced the arterial wall dilation during the systolic phase over a cardiac cycle. The transmural pressure is equal to the difference between the existing intravascular and extravascular pressures whereas the latter pressure cannot be measured *in vivo*. The surrounding tissue provides constraints on the vessels and may also affect the passive mechanics of the arterial wall. A previous study [46] has reported the surrounding tissue effect as a reduction of the arterial wall stress and strain in the circumferential direction by 70 and 20%, respectively. A limitation of this study is thus the extravascular pressure and surrounding tissue which have been ignored in the models.

The strain was calculated in the circumferential direction based on the diameter waveform obtained using a 1D cross-correlation technique [39]. Due to the most dominant deformation in the circumferential direction, only the circumferential strain was considered in the two-parallel spring model and assumed to be isotropic. The approach based on the two-dimensional hyperelastic model, however, can characterize nonlinear anisotropic material responses. Knowledge on the external axial forces was required and was adopted from pre-existent data since they could not be measured *in vivo*. The arterial wall viscous parameters were ignored because the higher nonlinear deformation under physiologic loading was not clearly visualized *in vivo*. As indicated in the introduction and methods sections, the minima and maxima of the carotid pressure and diameter waveforms were aligned and matched to eliminate the viscosity effects. Only the dilation of the carotid artery was considered. The carotid artery was assumed to react passively with negligible vascular vasodilation effects from the smooth muscle cells. The mechanical properties of the carotid artery were mainly influenced by the contribution of the elastin and collagen fibers, which reacted as the predominant elastic response [6]. Therefore, the models used provided the contribution by the elastin and collagen constituents in both the tunica adventitia and tunica media using the stress-strain relationship and our ultrasound-based method. The models, however, do not account for the angular dispersion or 3D geometry of the collagen fibers [47], i.e., the collagen fibers in the tunica media are mainly oriented in the circumferential direction while that in the tunica adventitia are more dispersed. This negligence would affect the estimated stress-strain relationship and also the Young's modulus, especially in the tunica adventitia but not the tunica media. The strain estimated from the models, furthermore, may be overestimated due to two limitations. First, as previously indicated, the extravascular pressure and surrounding tissue were ignored. Second, the carotid arterial wall was assumed to dilate symmetrically in the radial direction.

Two separate models [19, 33] were employed in this study to estimate the Young's moduli of the carotid arteries. The first model was used for the Young's moduli of the elastin lamellae (E_1), elastin-collagen fibers (E_2) and collagen fibers (E_3). Since the passive behavior of the carotid artery was only considered here, its dilation was assumed to occur without vascular vasodilation. The carotid artery was mainly constituted of elastin and collagen fibers which provided predominantly elastic response [6]. A modified generalized Maxwell model, i.e., a two-parallel spring model [19] was thus applied. Furthermore, the stress-strain relationship of the carotid artery established noninvasively using our ultrasound-based method characterizes the nonlinear elastic behavior with a clear inflection point defined as the transition strain. Due to the clear inflection point, the stress-strain

relationship can be separated into two linear relationships, i.e., the two-parallel spring models before and after the transition point. The transition point split the stress-strain relation into two curves and was defined as the maximum of the second derivative of the circumferential stress and strain ratio. The maximum of the second derivative of the circumferential stress and strain ratio (Fig. 4C) shows the maximum change of stress as strain increases to approximately 23%. The second was used for the incremental Young's moduli of the intact wall, tunica adventitia and tunica media to characterize the complex mechanical interaction between the arterial wall constituents.

Regardless of the aforementioned limitations, these moduli qualitatively represent the stiffness of the carotid arterial wall constituents. E_2 was the highest modulus (0.89 ± 0.27 MPa). It was approximately one-fold and six-fold higher than E_3 (0.75 ± 0.29 MPa) and E_1 (0.15 ± 0.04 MPa), respectively. The collagen fibers were thus found to be significantly stiffer than the elastic lamellae ($P < 0.05$) in agreement with previous reports [25, 26].

Regarding the quantities of these Young's moduli, the relation of E_1 and E_2 to $E_{0 \leq \epsilon_\theta < \epsilon_\theta^T}$ and $E_{\epsilon_\theta^T \leq \epsilon_\theta}$ was noticed. E_1 and E_2 were in agreement with $E_{0 \leq \epsilon_\theta < \epsilon_\theta^T}^{Int}$ and $E_{\epsilon_\theta^T \leq \epsilon_\theta}^{Int}$ of the intact wall ($E_{0 \leq \epsilon_\theta < \epsilon_\theta^T}^{Int} = 0.16 \pm 0.04$ MPa and $E_{\epsilon_\theta^T \leq \epsilon_\theta}^{Int} = 0.90 \pm 0.25$ MPa), respectively. It was thus hypothesized that at small strains (prior to the transition point) the arterial wall contribution was dominated by the elastin lamellae. At transition point, collagen fibers were assumed to start engaging and undergo tension in a sharp change of the Young's modulus. Beyond the transition point, the arterial stiffness thus increased as the strain increased due to the dominant collagen fiber contribution, i.e., the collagen fibers reached their straightened lengths and protected the vessel from overstretching or rupture. The tunica media was not significantly stiffer than the tunica adventitia ($P > 0.05$) both before

($E_{0 \leq \epsilon_\theta < \epsilon_\theta^T}^{Me} = 0.19 \pm 0.05$ MPa and $E_{0 \leq \epsilon_\theta < \epsilon_\theta^T}^{Ad} = 0.18 \pm 0.05$ MPa) and after transition point ($E_{\epsilon_\theta^T \leq \epsilon_\theta}^{Me} = 0.90 \pm 0.25$ MPa and $E_{\epsilon_\theta^T \leq \epsilon_\theta}^{Ad} = 0.84 \pm 0.22$ MPa).

Previous studies in healthy subjects have reported that the stiffness of the carotid artery was higher than that of the abdominal aorta [5, 7, 8, 12]. The difference in stiffness between the carotid and the abdominal aorta is mainly due to the difference in elastin and collagen constituency [10]. Because of this constituency difference, the ratio of $E_1 : E_2 : E_3$ was found to be 1 : 6 : 5 and 1 : 3 : 2 in the human carotid artery (this study) and murine abdominal aorta (previous study) [19], respectively. Previous studies in the ascending and descending porcine aorta [26] have reported that the Young's moduli, determined from uniaxial testing, of the tunica media were approximately four-fold higher than that of the

tunica adventitia. In this study, the $E_{0 \leq \epsilon_\theta < \epsilon_\theta^T}^{Me}$ and $E_{\epsilon_\theta^T \leq \epsilon_\theta}^{Me}$ of the tunica media was found to be not significantly higher than those of the tunica adventitia ($P > 0.05$) since the tunica media deformation underwent small strain with *in-vivo* physiologic pressure and the angular dispersion of collagen fibers was ignored in the model. As a result of the pressure and the angular dispersion, the $E_{0 \leq \epsilon_\theta < \epsilon_\theta^T}^{Me}$ and $E_{\epsilon_\theta^T \leq \epsilon_\theta}^{Me}$ of the tunica media increase with deformation when it is subjected to higher pressure, i.e., in case of pathologic condition or under *in-vitro*

testing moreover the $E_{0 \leq \varepsilon_\theta < \varepsilon_\theta^T}^{Ad}$ and $E_{\varepsilon_\theta^T \leq \varepsilon_\theta}^{Ad}$ of the tunica adventitia also decrease when the angular dispersion of collagen fibers are considered. In arterial histology, the tunica adventitia has more dispersion in the collagen fibers than the tunica media (almost oriented in the circumferential direction or without dispersion) and this structure arrangement provides the lower strength in the tunica adventitia. Furthermore, the material response in the tunica adventitia, i.e., pressure-radius relationship, has been reported to be highly sensitive to the angular dispersion of the collagen fibers [47].

5. Conclusion

The *in-vivo* regional stress-strain relationship in the healthy human carotid arterial wall was established noninvasively. A transition point of the stress-strain relationship was detected representing the change in the contribution of the elastin and collagen fiber during the systolic phase of the cardiac cycle. The carotid arterial wall constituents were characterized by two separate models that yielded the Young's moduli of the elastin lamellae (E_1), elastin-collagen fibers (E_2) and collagen fibers (E_3) or the incremental Young's moduli of the intact wall ($E_{0 \leq \varepsilon_\theta < \varepsilon_\theta^T}^{Int}$ and $E_{\varepsilon_\theta^T \leq \varepsilon_\theta}^{Int}$) which is composed of the incremental Young's moduli of the tunica adventitia ($E_{0 \leq \varepsilon_\theta < \varepsilon_\theta^T}^{Ad}$ and $E_{\varepsilon_\theta^T \leq \varepsilon_\theta}^{Ad}$) and tunica media ($E_{0 \leq \varepsilon_\theta < \varepsilon_\theta^T}^{Me}$ and $E_{\varepsilon_\theta^T \leq \varepsilon_\theta}^{Me}$). The tunica media was not significantly stiffer than the tunica adventitia ($P > 0.05$), while the collagen fibers were found to be five times higher in stiffness than the elastin lamellae.

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Highlights

- > The *in-vivo* regional stress-strain relationship in the healthy human carotid wall was established noninvasively.
- > The carotid wall constituents were characterized by two separate models.
- > The stiffness of the carotid artery increased with strain during the systolic phase of cardiac cycle.
- > The tunica media was not significantly stiffer ($P > 0.05$) than the tunica adventitia.
- > The collagen fibers were found to be five times higher in stiffness than the elastin lamellae.

A

B

c

D

Figure 1.

(A) The carotid pressure variation of the human carotid artery over 1 cardiac cycle. (B) Envelope-detected B-mode image. (C) The cumulative displacements of the carotid arterial wall. The red and blue curves indicate the cumulative displacement at the near and far wall, respectively. The black curve indicate the difference of the near and far wall cumulative displacement. (D) The diameter variation with time.

Figure 2.

Diagram of the elastic carotid arterial wall is well-defined into three layers which are tunica intima, tunica media and tunica adventitia respectively arranged from the innermost to outermost wall layer (Adapted from Humphrey, 2002).

Figure 3.

Envelope-detected B-mode image with 5-color mark indicating the longitudinal locations of the determined stress-strain relationship of the human carotid artery

A

B

c

Figure 4.

(A) The circumferential stress-strain relationship of the human carotid artery with 5 longitudinal locations. (B) The mean stress-strain relationship corresponding to Fig. 4A. The stress-strain relationship was separated into 2 linear relations by the transition point. (C) The 1st and the 2nd derivative of the circumferential stress and strain ratio over systolic phase of the cardiac cycle.

The transition point is defined as the maximum of the 2nd derivative of the circumferential stress and strain ratio.

Figure 5.
The averaged Young's moduli of E_1 , E_2 , and E_3 (mean $\pm$ std) over 7 subjects.

A

B

c

D

Figure 6.

(A) The circumferential stress-strain relationship of the human carotid artery averaged along longitudinal locations. The solid red line indicates the model at 0% axial stretch of the intact wall. (B) The modified two-parallel spring model depicted $E_{0 \leq \epsilon_{\theta} < \epsilon_{\theta}^T}$ and $E_{\epsilon_{\theta}^T \leq \epsilon_{\theta}}$ of the tunica adventitia and tunica media. (C) and (D) The relations of the tunica adventitia and tunica media before and after the transition point were fitted using the two-dimensional hyperelastic model, respectively. The solid blue and magenta lines indicate the model at 0% axial stretch of the tunica adventitia and tunica media, respectively.

Table 1

E_1 , E_2 , and E_3 of 7 subjects (age 28 ± 3.6 years old).

Subject	E_1 (MPa)	E_2 (MPa)	E_3 (MPa)
1	0.13	0.70	0.57
2	0.13	1.06	0.93
3	0.18	0.60	0.42
4	0.12	1.40	1.28
5	0.10	0.94	0.84
6	0.15	0.77	0.62
7	0.22	0.78	0.56
Average	0.15 ± 0.04	0.89 ± 0.27	0.75 ± 0.29

Table 2

$E_{0 \leq \varepsilon_\theta < \varepsilon_0^T}$ and $E_{\varepsilon_\theta^T \leq \varepsilon_\theta}$ (mean $\pm$ std) of 7 subjects (age 28 ± 3.6 years old) determined at 0% axial stretch of (A) the intact wall (B) the tunica adventitia and tunica media.

A				
Subject	$E_{0 \leq \varepsilon_\theta < \varepsilon_\theta^T}^{Int}$ (MPa)	$E_{\varepsilon_\theta^T \leq \varepsilon_\theta}^{Int}$ (MPa)		
1	0.17 $\pm$ 0.00	0.70 $\pm$ 0.09		
2	0.15 $\pm$ 0.00	1.07 $\pm$ 0.05		
3	0.22 $\pm$ 0.01	0.63 $\pm$ 0.03		
4	0.13 $\pm$ 0.01	1.37 $\pm$ 0.24		
5	0.11 $\pm$ 0.00	0.92 $\pm$ 0.09		
6	0.17 $\pm$ 0.00	0.80 $\pm$ 0.06		
7	0.19 $\pm$ 0.00	0.80 $\pm$ 0.03		
Average	0.16 $\pm$ 0.04	0.90 $\pm$ 0.25		

B				
Subject	$E_{0 \leq \varepsilon_\theta < \varepsilon_\theta^T}^{Ad}$ (MPa)	$E_{0 \leq \varepsilon_\theta < \varepsilon_\theta^T}^{Me}$ (MPa)	$E_{\varepsilon_\theta^T \leq \varepsilon_\theta}^{Ad}$ (MPa)	$E_{\varepsilon_\theta^T \leq \varepsilon_\theta}^{Me}$ (MPa)
1	0.17 $\pm$ 0.00	0.23 $\pm$ 0.01	0.66 $\pm$ 0.09	0.70 $\pm$ 0.10
2	0.14 $\pm$ 0.00	0.16 $\pm$ 0.00	0.90 $\pm$ 0.04	1.06 $\pm$ 0.04
3	0.26 $\pm$ 0.02	0.27 $\pm$ 0.02	0.63 $\pm$ 0.04	0.64 $\pm$ 0.03
4	0.15 $\pm$ 0.01	0.16 $\pm$ 0.01	1.28 $\pm$ 0.24	1.38 $\pm$ 0.26
5	0.12 $\pm$ 0.00	0.12 $\pm$ 0.00	0.80 $\pm$ 0.08	0.92 $\pm$ 0.09
6	0.19 $\pm$ 0.00	0.19 $\pm$ 0.00	0.80 $\pm$ 0.07	0.80 $\pm$ 0.05
7	0.20 $\pm$ 0.00	0.20 $\pm$ 0.00	0.80 $\pm$ 0.05	0.83 $\pm$ 0.03
Average	0.18 $\pm$ 0.05	0.19 $\pm$ 0.05	0.84 $\pm$ 0.22	0.90 $\pm$ 0.25