Published in final edited form as:

IEEE Trans Med Imaging. 2016 January ; 35(1): 13–28. doi:10.1109/TMI.2015.2453194.

Piecewise Pulse Wave Imaging (pPWI) for detection and monitoring of focal vascular disease in murine aortas and carotids *in vivo*

Iason Zacharias Apostolakis,

Department of Biomedical Engineering Columbia University, New York, NY 10027 USA.

Sacha D. Nandlall, and

Department of Biomedical Engineering Columbia University, New York, NY 10027 USA.

Elisa E. Konofagou [Member, IEEE]

Departments of Biomedical Engineering and Radiology, Columbia University, New York, NY 10027 USA (ek2191@columbia.edu)

Abstract

Atherosclerosis and Abdominal Aortic Aneurysms (AAAs) are two of the most common vascular diseases. Current clinical criteria in monitoring and risk assessment of these diseases exhibit profound weaknesses. Since both diseases are associated with mechanical changes in the arterial wall, Pulse Wave Imaging (PWI), a technique developed by our group to assess and quantify the mechanical properties of the aortic wall *in vivo*, may provide valuable diagnostic information. However, using PWI to acquire a single regional Pulse Wave Velocity (PWV) value of the entire imaged section of the diseased artery would not provide sufficiently localized information on the pathology. Hence, the implementation of piecewise Pulse Wave Imaging (pPWI) aims at addressing the issue of focal vascular disease. The feasibility of this novel technique is tested on murine normal, aneurysmal and atherosclerotic arteries in order to assess its effectiveness in monitoring each disease. Sub-regional PWVs were then estimated within 2-4 mm segments along the length of the arterial wall and estimates of the local stiffness of each segment referred to as local PWI Modulus (E_{PWV}) values were calculated using the Moens-Korteweg equation.

Stiffness maps were constructed for each case and provided localized spatial information on sub-regional PWV and E_{PWV} measurements. Overall stiffness was found to increase in the atherosclerotic cases. The mean sub-regional PWV was found to be 2.57 ± 0.18 m/s for the normal aortas ($n = 7$) with a corresponding mean E_{PWV} of 43.82 ± 5.86 kPa. A significant increase ($p = 0.001$) in the group means of the sub-regional PWVs was found between the normal aortas and the aortas of mice on high-fat diet for 20 (3.30 ± 0.36 m/s) and 30 weeks (3.56 ± 0.29 m/s). The mean of the sub-regional PWVs (1.57 ± 0.78 m/s) and E_{PWV} values (19.23 ± 15.47 kPa) decreased significantly in the aneurysmal aortas ($p = 0.05$). Furthermore, the mean coefficient of determination (r^2) of the normal aortas was significantly higher ($p = 0.05$) than those of the aneurysmal and atherosclerotic cases. These results demonstrated that pPWI may be able to provide useful biomarkers for monitoring focal vascular diseases.

Keywords

Pulse wave velocity; Pulse Wave Imaging; localized stiffness mapping; atherosclerosis; abdominal aortic aneurysm (AAA); speckle tracking; ultrasound

1. Introduction

Several pathological conditions, such as myocardial infarction, stroke and lower limb ischemia have been shown to be atherosclerosis-related [1], [2] and are some of the major causes of overall morbidity and mortality in Western populations [3], [4]. Atherosclerotic plaques are characterized by a focal accumulation of lipids, complex carbohydrates, blood cells, fibrous tissues, and calcified deposits [5]. Currently, despite major advances in the diagnosis of atherosclerosis, predicting atherosclerotic plaque rupture remains imprecise [6]. Thus, the biggest challenge for screening and diagnostic methods is to identify patients at high risk who have lesions that are vulnerable to thrombosis, before the event occurs. In the current clinical practice, the risk assessment of plaque rupture is based on the degree of endoluminal stenosis [7], [8]. However, in the case of vulnerable plaques, luminal area is often not significantly reduced [9]. Additionally, imaging methods that rely on surveying changes in luminal diameter, area and flow resistance to identify the severity of luminal obstruction by stenosis, such as quantitative coronary angiography, are limited by arterial remodeling [10], [11]. Over the past few years, research has primarily focused on improving the quantification of the arterial wall thickness and the characterization of plaque morphology and composition using magnetic resonance- and ultrasound-based imaging techniques [12], [13], [14], [15], [16].

The current understanding regarding the mechanisms that underlie plaque formation and development indicates that monitoring of the mechanical properties of the atherosclerotic plaques and the surrounding wall tissue may provide information crucial to patient care. More specifically, the development of these lesions causes the composition of the arterial wall to change compared to its normal state, thereby making the wall tissue less homogeneous [17] and altering its mechanical properties [18], [19]. Moreover, the development of elastographic [20], [21] and radiation force-based methods [22], [23] has also made it possible to obtain localized measurements of arterial and plaque material properties. However, elastographic methods usually require pressure values in order to provide stiffness values. These pressure values can be obtained either with a catheter or by using a pressure cuff; the former method is invasive, while the second can induce errors because of the considerable differences between peripheral pressure values from site to site and also compared to the central blood pressure [18]. On the other hand, ARFI methods involve manually pushing tissue using the acoustic radiation force from a focused transducer, and also suffer from additive noise due to the physiological motion of tissue and the small magnitude of the induced displacements.

The abdominal aortic aneurysm (AAA) is the most common form of aneurysm. It is typically defined as a permanent and irreversible dilation of the abdominal section of the aorta by at least 50% over its normal diameter. AAA is a frequently lethal disease of the

older population, with more than 10,000 deaths in the United States annually [24]. It affects more men than women and the overall mortality following rupture of an aortic aneurysm exceeds 75%, including emergency surgical intervention [25]. In humans, most AAAs occur in the infrarenal abdominal aorta [26]. The typical diagnostic techniques for detecting AAA are abdominal ultrasound and computed tomography (CT) [27], [28] and the highest risk of rupture is identified when the transverse diameter of the aneurysm reaches or exceeds 5-5.5 cm [29], [30]. However, the size of the aneurysm as a risk assessment criterion is not reliable and does not have a physically sound theoretical basis [28]. Consequently, the rupture risk cannot be properly predicted given the current diagnostic techniques available in the clinic.

AAAs are known to affect the mechanical properties of tissues [31] and, since the aneurysm ruptures when the acting wall stresses exceed the tensile strength of the degenerated AAA wall [32], the study of the local mechanical properties of the AAA wall has come in the foreground in order to assess the AAA rupture risk. Noninvasive methods to assess the stiffness of the AAA wall tissue have been investigated by several groups including our own. Both ultrasound [33], [34], [31] and CT [35] methods have been used for this purpose.

As mentioned previously, the localized assessment of stiffness in different regions of major blood vessels remains of paramount importance in monitoring focal vascular diseases such as atherosclerosis [19], [36], [37] and AAAs [28], [38].

The Pulse Wave Velocity (PWV) is typically used to estimate the stiffness of arteries [18]. Pulse waves are pressure pulses generated by the heart during the contraction-relaxation cycle that travel through the vessel wall as pressure waves [17]. In a straight elastic tube containing a nonviscous liquid, the disturbances in flow velocity, pressure, tube diameter, and wall displacement, propagate as waves along the tube at a certain velocity [39]. The Young's modulus of the tube is related to the wave velocity through the corrected Moens-Korteweg equation as follows [18]:

$$PWV = \sqrt{\frac{Eh}{2R\rho(1-v^2)}} \quad (1)$$

where E is the Young's modulus of the elastic tube wall, h is the wall thickness, ρ is the density of the wall, R is the inner radius of the tube and v is the Poisson's ratio (the latter appears in the equation to compensate for the finite thickness of the wall).

The PWV is defined as ratio of the distance between two measurement sites to the time shift of the waveforms at these two sites. Despite this simple definition, some problems in the techniques currently used to measure it remain, thus limiting the general applicability of the PWV measurement.

The foot-to-foot method that is typically used to calculate the PWV suffers from errors of distance measurements and time-delay measurements [40], [41]. Thus, given that it is difficult to measure small time-shifts, because the wave travels very fast (on the order of several meters per second), the foot-to-foot method focuses on obtaining a PWV estimate over a relatively long distance (typically between the common carotid and femoral arteries).

Therefore, this method indicates general arterial stiffness and is incapable of providing localized measurements of focal mechanical change.

Several medical imaging-based methods have been previously developed for noninvasive measurements of regional PWV, mainly using magnetic resonance imaging [42], [43], [44] and ultrasound [45], [46], [47], [48], [49]. However, all of the aforementioned techniques lack either the temporal resolution, spatial resolution, or both to capture the propagation of the pulse wave along of a few millimeters of the arterial wall. Pulse Wave Imaging (PWI) is a noninvasive technique developed by our group for tracking the propagation of pulse waves along the wall of the aorta at high spatial and temporal resolutions [31], [50], [51]. This technique has been previously validated *in silico*, *in vitro* and *ex vivo* [52], [53], [54], and also *in vivo* for both mice and humans [31], [50], [55], [56], [57], [58]. One advantage of PWI over other techniques is that the pulse wave propagation can be visualized and thus linear regression of the spatio-temporal variation of the displacement waveform of the arterial wall yields a single PWV value along with the corresponding coefficient of determination r^2 , which has been shown to be indicative of the quality of the measurement [55].

However, improvements can be made to several aspects of this technique. First, as stated previously, only a single PWV value is estimated for the entire segment imaged and consequently localized stiffness measurements within the imaged arterial segment are not available. Furthermore, it assumes the imaged arterial wall to be a homogeneous medium. This is a precarious assumption, especially given the fact that in diseased arteries it does not hold. In pathological vessels, the arterial wall is less homogeneous and exhibits a certain degree of tortuosity in the imaged segments, which diverges from the straight elastic tube model assumed in the Moens-Korteweg equation. To address these issues, the present work aims at enhancing the performance of PWI by introducing piecewise Pulse Wave Imaging (pPWI) to map the localized stiffness of the arterial wall *in vivo* and utilizing this technique to detect and monitor the development and progression of localized vascular diseases such as atherosclerosis and AAA.

II. Materials and methods

Animal Study

All procedures performed in this study were approved by the Institutional Animal Care and Use Committee (IACUC) of Columbia University (protocol AC-AAAG4951). For the purpose of the atherosclerosis study, six-month old ApoE knockout mice (ApoE^{-/-}, $n = 7$) were obtained from the Jackson Laboratories (Bar Harbor, ME, USA) and were fed an atherogenic Western-type diet (20% protein, 50% carbohydrate, 21% fat, 0.21% cholesterol; Research Diets, New Brunswick, NJ, USA). ApoE^{-/-} mice are known to spontaneously develop atherosclerosis throughout the arterial tree, a process which is accelerated by maintaining them on a diet rich in cholesterol [59]. One of the acquired mice died after 17 weeks of high-fat diet from myocardial infarction during anesthesia, while another two of them were euthanized due to poor health and deep skin ulcerations after 25 weeks on high-fat diet. The remaining mice ($n = 4$) were euthanized after approximately 35 weeks of high-fat diet. The abdominal aortas of each mouse were scanned once every three weeks in order

to monitor the progress of the disease. After 5 weeks of high-fat diet the same routine was applied to the carotid arteries of the same mouse cohort. For the purpose of this study we focused on scanning the right carotid arteries of the mice. Additionally, the right carotid artery of a different ApoE knockout mouse on regular chow diet (not included in the mouse cohort) was scanned as a control.

In the case of the aneurysm development study, six ($n = 6$) male ApoE/TIMP-1 knockout mice were infused with angiotensin II (A9525, Sigma-Aldrich, St. Louis, MO, USA) for 30 days via subcutaneously implanted osmotic pumps (Alzet Model 2004, Durect, Cupertino, CA, USA). The mice were given a dose of 2.2 mg/kg/day (milligrams of angiotensin II per kilogram of body mass per day) and were monitored 2-3 times every week over the course of 30 days. The timelines of both animal studies used in the present work are shown in Fig. 1.

It should also be noted that after the formation of an AAA in this mouse model, atherosclerosis is known to develop in the proximity of the aneurysm [60]. However, for the purposes of this study, aneurysmal aortas were kept in a separate analysis category from atherosclerotic ones.

B. Data Acquisition

A diagram of the methodology that was used to acquire and process the aortic data is depicted in Fig. 2. First, the RF frames were acquired with a previously developed high framerate data acquisition system [51]. A 30-MHz ultrasound probe (RMV-707B, VisualSonics, Toronto, ON, Canada) was placed on the mouse abdomen to obtain a long-axis view of the abdominal aorta in the suprarenal region. Thus, major reflection sites such as the aortic bifurcation were avoided. The field of view was $12 \times 12 \text{ mm}^2$, and the axial and lateral image resolutions of this probe were 55 and 115 μm , respectively. Additionally, a 40-MHz ultrasound probe (RMV-704, VisualSonics) was placed on the mouse neck to obtain a long-axis view of the right common carotid artery along with its respective bifurcation. The field of view was equal to $10 \times 12 \text{ mm}^2$, and the axial and lateral image resolutions of this probe (40 MHz) were equal to 40 and 80 μm respectively.

In the EKV (ECG-based Kilohertz Visualization) mode provided by the imaging system (Vevo 770, VisualSonics), the single-element transducer operated on a line-by-line-basis using ECG gating. The transducer transmitted and received at a pulse-repetition frequency (PRF) of 8 kHz. The data acquisition was triggered by a synchronization signal on the RF signals, which indicated the time of pulse transmission from the transducer. The ECG was acquired using the electrode leads on the heating mouse platform. A two-channel 14-bit waveform digitizer (CompuScope 14200, Gage Applied Technologies, Lachine, QC, Canada) was used to acquire the RF signals and the associated ECG simultaneously at a sampling rate of 200MHz. Each acquisition lasted approximately 7 minutes. After data acquisition, the received RF lines were synchronized based on the R-wave peak (a reliable peak of the ECG during the cardiac cycle) of the simultaneously recorded ECG signals to reconstruct the RF image sequence for a complete cardiac cycle at an effective frame rate of 8kHz [61], [62], [31], [51].

Motion Estimation

The axial wall velocity of the aortic and carotid wall velocity were estimated off-line from the same RF data acquired, using a time-efficient, 1-D normalized cross-correlation technique described elsewhere [63]. The window size was approximately 10λ in each case and the window overlap was 90%. In order to ensure the quality of the results of the motion estimation algorithm, the estimated displacements were considered to be of acceptable quality if their associated correlation coefficients were at least 0.85. The wall velocity estimates were smoothed by a Savitzky-Golay filter followed by a 3×3 median filter to remove any peak hopping errors. The rigid motion induced by respiration was also removed by subtracting the velocity of the surrounding tissue from the velocity of the wall. This was done on a line-by-line basis by calculating the mean velocity of the surrounding tissue for each line and then subtracting it from the axial wall velocities calculated within the corresponding line. The wall velocities were color-coded and superimposed onto the grayscale B-mode images. The sequence of PWI wall velocity images were generated over the entire cardiac cycle and formed a cine-loop. Only the wave on the anterior wall was shown, in part due to better visualization reasons and in part due to the fact that the posterior wall exhibits little motion *in vivo* because of its close proximity to the spine. The contours of the arterial walls were delineated in the first frame of each acquisition sequence and then were updated for each subsequent frame based on the incremental axial displacements using automated contour tracking described elsewhere [64]. In that study, the effectiveness of the technique was validated within the framework of myocardial contour tracking of murine left ventricles *in vivo*. The forward pulse wave was clearly visualized, which propagated from the proximal (heart in both cases) to the distal (kidneys in the case of the aorta and head in the case of the carotid) positions of the vessel.

D. Wall Segmentation

For the purpose of acquiring the extent of the arterial wall, a semi-automated segmentation algorithm was developed. First, each image was subjected to smoothing Gaussian and homomorphic filtering in order to reduce the noise in the image and also impose uniform illumination conditions. Next, each image was thresholded given a user-defined threshold, after which a region around either the anterior or the posterior wall was selected. The wall was segmented using a Sobel-based edge detection method. However, since the resulting segmentation provides a surplus of information for the purposes of this method, a final step of fitting a smoothing spline through the resulting segmentation was implemented. As a result, a smooth line that accurately delineates the arterial wall was obtained.

E. Spatio-Temporal Imaging of Pulse Wave Propagation

The PWI axial wall velocities of the arterial wall segment at a specific beam position were plotted as a function of the time elapsed after the R-wave. Thus, the PWI axial wall velocity variation over distance and time of the pulse-wave propagation was depicted in a 2-D image, indicating the spatiotemporal variation of the pulse-wave propagation. The distance was calculated by estimating the length of the aortic wall, as delineated by the segmentation algorithm, since the width of the image does not always coincide with the length of the imaged arterial wall. This is because the arterial wall does not usually extend straight across

the image, as it presents curvature and local angles with the ultrasound beam. However, it should be noted that these angles between the arterial wall and the ultrasound beam do not affect the PWV measurements since it is possible to track the pulse wave arrival time at each point of the arterial wall as long as the tissue motion has a measureable axial component.

On the 2-D spatio-temporal images, the 50% upstroke point of the PWI axial wall velocity versus time was selected as the tracking feature in order to estimate the PWV. The 50% upstroke was defined as the time-point, at which the axial wall velocity was closest in value to the average between the foot and the peak of the axial wall velocities at each beam location. Thus, each beam position is associated with one 50% upstroke marker. The choice of this tracking feature is in part justified because the early wavefront is less affected by the generated reflected waves [18] and also because previous studies have shown that it exhibits the greatest separation in normal subjects [57] as well as that it is the most robust compared to alternatives (foot, 25% upstroke, 75% upstroke etc.) [65]. In order to maintain consistency, the 50% upstroke points were used for tracking the pulse wave in all cases. Finally, no normalization or scaling of the temporal PWI axial wall velocities waveforms was implemented, in order to retain all essential local stiffness information.

Linear regression of the relationship between the 50% upstroke arrival time and the distance along the anterior wall yielded the slope as the regional PWV value for the whole imaged segment and the corresponding coefficient of determination r^2 as an approximate measure of the pulse wave propagation uniformity.

F. Piecewise PWV and Localized PWI Modulus Mapping

In order to acquire localized PWV measurements and the corresponding stiffness maps, the 50% upstroke points were divided in 50% overlapping kernels of 65 points (4 mm) in the case of the aortic wall and 40 points (2.4 mm) in the case of the carotid wall. Linear regressions were performed for each sub-region, thereby providing a localized PWV value along with the corresponding coefficient of determination r^2 , indicative of the quality of the linear fit [55]. The size of the kernels was chosen accordingly, to balance the tradeoff between the resolution of the resulting stiffness maps (smaller kernels) and quality of the linear regression (larger kernels). Most importantly, the resulting resolution of the stiffness maps needed to be comparable to the size of the focal wall disease. The 4 mm kernel size was selected because it is at least 4 times the diameter of the mouse aorta even in aneurysmal cases, thus approximating the Moens-Korteweg condition of having a vessel whose length is significantly greater than its diameter.

For the purpose of deriving stiffness estimates for each sub-region, the Moens-Korteweg equation was utilized. The diameter of each sub-region of the vessel was estimated using the segmentation curves along the anterior and posterior walls. More specifically, the vertical separation of the segmented walls was estimated for each line lying within that particular sub-region, after which the diameter was calculated by compensating for the angle of that particular sub-region and then averaging the results. The arterial thickness of the aortic wall was measured for each sub-region using a standard B-mode method of taking the average of three measurements of the anterior wall within each segment. Given the high axial and lateral resolutions of the 30 MHz probe used in this study, this allows for accurate

quantification of the aortic wall thickness in mice as seen in previous studies [50], [66]. A similar approach was employed for the mouse carotids by taking advantage of the higher axial and lateral resolutions of the 40 MHz probe. More specifically, its theoretical resolution of 40 μm in the axial direction and 80 μm in the lateral direction has been shown to be capable for accurate arterial wall thickness measurements in all of the major peripheral vascular systems in mice [67]. Additionally, micro-ultrasound wall thickness measurements in atherosclerotic mouse carotids have been shown to correlate well with measurements performed in histology [68]. In order to ensure uniformity and comparability of the arterial wall thickness and vessel diameter measurements, both of them were measured using B-mode data recorded at the R-peak of the QRS complex of the ECG signal in each mouse, which is the most easily identifiable and detectable feature of the ECG signal.

Finally, in both aortas and carotid arteries the density of the arterial wall was assumed to be equal to 1060 kg/m^3 and Poisson's ratio was set to 0.5 [18]. Consequently, using the aforementioned parameters along with the calculated PWV, a stiffness estimate – PWI modulus (E_{PWI}) was obtained for each sub-region using the previously mentioned Moens-Korteweg equation.

III. Results

A. Sequences of PWI Images

A sequence of PWI images at intervals of 1.25ms is shown in Fig. 3 for the baseline case of a normal aorta (no high-fat diet or angiotensin II pump implantation). As mentioned previously, only the velocities on the anterior wall of the aorta and the surrounding tissue are shown. The propagation of the wave is denoted by solid blue arrows. Uniform wave propagation along with similar peak PWI velocities across the tissue can be observed.

Fig. 4 shows the propagation of the pulse wave in the aorta of the same mouse after it has been on high-fat diet for 30 weeks and has also developed a case of stenosis on the distal end of the aorta as indicated by the solid red arrow. These two images also show that the magnitude of the wall velocities has decreased and also that the propagation of the wave is nonuniform.

Similar sequences were produced in the case of aneurysmal aortas such as the case in Fig. 5. As shown by the red arrow, the magnitudes of the anterior wall velocities induced by the pulse wave are lower in the aneurysmal sac compared to those developed in the proximal segments of the vessel. It can also be observed that the peak magnitudes of the PWI wall velocities attenuate as the pulse wave approaches the aneurysmal region of the aorta.

In Fig. 6, the propagation of the pulse wave is shown in a normal murine carotid artery. To maintain consistency with the aortic cases, only the velocities on the anterior wall are shown. The propagation of the wave is characterized by uniformity as it linearly propagates from the proximal to the distal end of the field of view. The case of an atherosclerotic carotid artery is shown in Fig. 7. The location of the atherosclerotic plaque is indicated by the solid red arrow. As in Figs. 4 and 5, the magnitude of the wall velocities diminishes as the pulse wave propagates through the diseased tissue.

B. Spatio-Temporal Imaging of Pulse Wave Propagation and Piecewise Linear Regression

Fig. 8 depicts the spatio-temporal plot of the anterior aortic wall velocity for three cases of (a) a normal mouse aorta, (b) an atherosclerotic mouse aorta and (c) an aneurysmal mouse aorta. On each image, the 50% upstroke points are shown along with the piecewise PWV measurements and the corresponding r^2 coefficients for each sub-region. On top of each image the regional PWV value of the entire imaged segment is shown. Information on the PWV of each segment is given along with its quality measure, namely the square of the coefficient of determination (r^2). Until now, PWI provided us with only the regional PWV and corresponding r^2 for the whole imaged segment, which is provided in each case on top of each subplot. Hence, the piecewise PWV method provides additional information on the propagation of the pulse wave in localized diseased tissue. Fig. 8 also shows that in the case of the normal aorta, the PWV values are uniform and close in magnitude accompanied by high r^2 values.

In the case of the atherosclerotic aorta, the uniformity of the pulse wave propagation is reduced and, correspondingly, lower r^2 values are obtained, particularly in the segments close to the atherosclerotic region. Furthermore, a small decrease in the peak PWI wall velocities can be observed between the normal and the atherosclerotic case, as well as an overall increase of the estimated sub-regional PWVs.

In the case of the aneurysmal aorta, the uniformity of the wave propagation is further compromised, resulting in a nonuniform spatio-temporal plot. A more prominent decrease of the r^2 values can be observed in the region of the aneurysm compared to the normal aorta and there is also a drop in the peak PWI wall velocities inside the aneurysmal sac compared to those in both the surrounding wall tissue and the normal aorta.

Analogous spatio-temporal plots were acquired for the carotid artery cases. More specifically, in Fig. 9 the spatio-temporal plots for the case of (a) a normal carotid artery and (b) an atherosclerotic carotid artery are provided. Relatively high degree of linearity of the 50% markers and uniform magnitude of peak PWI wall velocities are indicative of the normal carotid artery. Additionally, in the same case, a backward-propagating reflected wave can be seen, which appears in the spatio-temporal plot as a characteristic V-shape pattern that has also been reported in clinical studies of PWI in human carotid arteries [49], [56]. On the other hand, there is greater nonlinearity in the atherosclerotic case.

Additionally, as previously noted for the PWI images, the magnitudes of peak PWI wall velocities are reduced in the plaque region of the spatio-temporal plot.

C. PWV and Stiffness Maps

Using the segmentations of the arterial walls, the PWV and E_{PWI} values were overlaid onto the B-modes of each case, to produce maps of PWV and relative stiffness. Fig. 10(a) depicts such maps for a normal mouse aorta; it can be seen that both the PWVs and the E_{PWI} maintain consistent magnitudes while remaining uniform across the length of the aorta. In Fig. 10(b), similar maps are shown for the same mouse after 20 weeks of high-fat diet. A stiffness increase is evident at the proximal end of the aorta, as indicated by both the higher PWVs and E_{PWI} values. Finally, in Fig. 10(c), the results for the same mouse after 30 weeks

of high-fat diet are shown. In addition to generally higher stiffness estimates, the uniformity of the PWV and the modulus values is virtually nonexistent. Similar results were found for the case of murine aneurysm development. Fig. 11(a) shows the results from a baseline PWI acquisition (i.e. the mouse aorta before the angiotensin II pump implantation). In Fig. 11(b), the results are depicted for the same mouse aorta six days after the angiotensin II pump implantation and a sharp increase in average stiffness of the aortic sub-regions can be observed. Finally, in Fig. 11(c), the fully developed aneurysm is shown 16 days after pump implantation along with the corresponding estimated E_{PWI} . The stiffness of the aortic wall at the site of the aneurysm sac has decreased, while a region proximal to the heart of slightly higher stiffness remains.

Finally, PWV and stiffness maps were also produced for mouse carotids. In Fig. 12(b), both are provided for the baseline PWI acquisition. Both the PWV and E_{PWI} values are similar to those obtained in the previous baseline cases of aorta, with the exception of a slight increase in stiffness close to the bifurcation. Fig 12(d), on the other hand, provides these maps in the case of an atherosclerotic carotid artery. The focal point of the disease is indicated with a red arrow. In that particular sub-region, a higher PWV is observed; however, due to the size of the plaque, wall thickness has greatly increased and vessel diameter has decreased, resulting in lower E_{PWI} values in the vicinity of the plaque. This finding may also reflect increased lipid content in this particular advanced atherosclerotic lesion.

D. Statistical Analysis

Statistical analysis was performed on seven ($n = 7$) normal aortas, six ($n = 6$) aneurysmal aortas, six aortas ($n = 6$) after 20 weeks of high-fat diet and four ($n = 4$) aortas after 30 weeks of high-fat diet. An independent t-test was used to verify the statistical significance of the difference between the normal and diseased aortas, with p-values of less than 0.05 considered indicative of statistical significance. In the statistical results, only PWVs and E_{PWI} that correspond to an r^2 coefficient of at least 0.5 were taken into account, to avoid including unreliable values in the analysis. However, all of the r^2 values (including those less than 0.5) were used to estimate the mean sub-regional r^2 values for each mouse population. Figs. 13(a) and (b) show the mean aortic PWVs and E_{PWI} , respectively, from normal and aneurysmal mice and also from mice on high-fat diet for 20 and 30 weeks averaged over the whole mouse population of each cohort. Fig. 13(c) shows the mean r^2 coefficient over the aortic wall averaged over the mouse population of each case. Fig. 13(d) shows the standard deviation of the PWVs within the whole imaged segment of the aortic wall averaged over the mouse population of each cohort. Figs. 13(c) and 13(d) change in a complementary fashion, both provide a measure of the homogeneity of the aortic wall, since a higher standard deviation of the sub-regional PWVs corresponds to greater variation of the wall stiffness between sub-regions. Similarly, lower mean sub-regional r^2 coefficients imply that there is more local variation and inhomogeneity in the mechanical properties of the arterial wall. Fig. 13(e) shows the mean arterial wall thickness of the anterior aortic wall in each case as measured with pPWI. Finally, Fig. 13(f) shows the mean peak PWI wall velocities over the anterior wall in each case. It must be noted that in the case of Figs. 13(a), (b), (c) & (f), in aneurysmal aortas the measurements were limited to the aneurysmal tissue,

while in the case of Fig. 13(d) peri-aneurysmal tissue was included in order to generate sufficient segments to calculate the standard deviation of the sub-regional PWVs.

Finally, in Table I, the mean piecewise PWVs and E_{PWV} values are provided along with the mean r^2 coefficients for the case of the mouse carotid arteries after 5, 20 and 30 weeks of high fat diet. Although there is an increase in the mean of both the mean sub-regional PWV and E_{PWV} , the only significant changes are the decrease of the mean r^2 coefficient between the cases of carotid arteries after 5 and 20 weeks of high fat diet, as well as the increase of the wall thickness between the cases of carotid arteries after 5 and 30 weeks of high-fat diet ($p < 0.05$).

IV. Discussion

In this study, the PWI method was extended and enhanced in order to track the sub-regional PWVs, over the length of a few millimeters of the arterial wall. Additionally, the feasibility of this new method was demonstrated in normal, aneurysmal and atherosclerotic murine aortas, as well as in normal and atherosclerotic murine carotids *in vivo*. The piecewise measurements allow for more detailed inspection of the mechanical properties of the arterial wall, since higher resolution is achieved in both the PWV and E_{PWV} maps. Stiffness maps of higher resolution are of particular importance in pathological arteries (i.e., calcifications, plaques and aneurysms), since the arterial wall loses its homogeneity and becomes nonuniform. Consequently, different sub-regions of the same imaged segment exhibit different levels of stiffness, the quantification and comparison of which may provide critical patient care information.

Additionally, this is the first PWI study that utilizes a semiautomated segmentation algorithm, in order to more precisely trace the extent of the arterial wall. Its use enhances the proposed method since it allows for more precise and user-independent PWV measurements both regionally and sub-regionally. Further work is needed to fully automate and further establish this part of the post-processing algorithm.

A. Sequences of PWI Images

This novel technique was applied to both atherosclerotic and aneurysmal aortas as well as atherosclerotic carotids in an effort to assess its performance in the monitoring of the progression of each disease. The effectiveness of the developed technique needs to be further tested in predicting and assessing the early onset of cardiovascular diseases, which may be more associated with changes in the composition of the arterial wall rather than geometrical changes in the morphology of the vessel. Such changes can be seen in the data presented herein, both in the propagation pattern and the velocity of the pulse wave. As shown in the PWI propagation images, the propagation pattern becomes more irregular and nonuniform in the aneurysmal and atherosclerotic cases of mouse aortas and carotids indicating underlying changes in the mechanical properties of the arterial wall. As seen in Figs. 4 and 7, which respectively show an atherosclerotic aorta and carotid the magnitude of the peak PWI wall velocities are significantly lower than those of their respective baseline cases. This indicates an increase in the stiffness of the vasculature of atherosclerotic ApoE^{-/-} mice, which is consistent with the results presented in previous studies [69], [70].

Additionally, in the case of the aneurysmal abdominal aorta shown in Fig. 5, the aneurysmal sac exhibits the lowest PWI axial wall velocity which has also been reported in prior PWI studies [31]. These results are also confirmed by the statistical analysis depicted in Fig. 13(f), where the mean peak PWI axial wall velocities are shown to be significantly lower in the atherosclerotic (A30, $p = 0.05$) and aneurysmal cases ($p = 0.01$) than the normal ones. A possible explanation for the significant decrease of aneurysmal wall motion could be due to the reduced intra-aneurysmal pressure. The etiology behind this drop may be more complex than the aneurysm's larger diameter alone. For example, it has been shown that the thrombus reduces the effect of the pressure load on the AAA wall [71], [72], also leading to decreased aneurysm expansion [73].

B. Spatio-Temporal Imaging of Pulse Wave Propagation

Piecewise PWI increases the amount of information that traditional regional PWI provides by obtaining highly localized measurements. The spatio-temporal maps of Figs. 8 and 9 demonstrate that sub-regional PWV measurements provide additional insights into the wall mechanical properties. Specifically, while in previous studies [31], [50] it was reported that aneurysmal and calcified aortas can be differentiated from normal aortas with the use of the r^2 coefficient of determination, in Fig. 8, the r^2 coefficients in all three cases (normal, atherosclerotic, aneurysmal) have similar high values thus impeding their characterization using regional PWV. However, the sub-regional PWV measurements are affected not only in their magnitude but also in their quality (i.e. sub-regional r^2 coefficients) in all three cases shown in Fig. 8. In the atherosclerotic aorta, the PWV is shown to increase overall and a decrease of the r^2 coefficient is observed close to the atherosclerotic region, while in the aneurysmal aorta there is a significant decrease in the average of the r^2 coefficients, corroborating the nonuniform propagation of the pulse wave. In the aneurysmal case, significant degradation of the r^2 values occurs around and inside the aneurysm sac. This can be attributed to the nonlinear transmission of the pulse wave caused by the gradient in both geometry and composition of the aortic wall effected by the aneurysmal sac. Furthermore, in several aneurysmal cases there are some reflections, caused by aortic wall inhomogeneities, which may lead to narrow regions of negative slope of the 50% upstroke markers, as seen in Fig. 8(c). However, given that their size is small we can expect that the measured PWV in each kernel, which represents the resolution of our method, reflects the stiffness of the tissue within it [74]. The associated r^2 is a measure of inhomogeneity of the arterial wall in each kernel and thus can be used to identify regions with extensive presence of reflections.

Finally, it has to be noted that in all three cases the sub-regional PWV measurements are in good agreement with the values reported in literature for normal [31], [44], [48], [50], [69], aneurysmal [31] and atherosclerotic [44], [69] mouse aortas.

Similarly, Fig. 9 shows that in the normal case, the propagation of the wave is uniform and is similar to the spatio-temporal plots of normal carotid arteries presented in clinical studies [56] in that it contains a reflection wave and a dicrotic notch. This reflection wave does not significantly affect the tracking of the pulse wave since the tracked features (50% upstroke markers) are detected on the early part of the forward wave. In contrast, in the case of atherosclerotic carotid, the propagation of the wave exhibits a marked nonlinearity along

with a drop of the magnitude of the wall velocities in the atherosclerotic region. As can be seen in Fig. 9, in both cases the quality of the linear fit drops close to the distal end of the carotid, with the decrease being more prominent in the atherosclerotic case. One possible cause of this is the intravascular turbulence near the bifurcation of the carotid, which may create nonlinearities in the propagation of the pulse wave. Another reason for the decrease in linearity could be that because of the narrow diameter of the carotid, any curvature of the vessel can induce significant out of plane motion, which may affect PWV calculation and cannot be detected by our technique as currently implemented. Nevertheless, the values of the sub-regional PWVs estimated for the healthy mouse are comparable with the ones provided in this older PWV study in carotid arteries in mice *in vivo* [75]. Similarly, the mean sub-regional PWV values estimated for the case of mice on high-fat diet shown in Table I are found to be in the range of the measurements for healthy carotid arteries [75]. However, further investigation of atherosclerotic mouse carotids is needed to form further conclusions on the effectiveness of the present technique.

C. PWV and Stiffness Maps

A notable finding implied by Figs. 10(a), (b) and (c) is that as the duration of high-fat diet increases, and as atherosclerosis progresses further, the average sub-regional PWV increases. This is corroborated by the bar plot in Fig. 13, which demonstrates that between normal and 20- and 30-week high-fat-diet aortas, there is a significant increase in the mean sub-regional PWV, with an accompanying significant increase in the mean sub-regional E_{PWV} values in the case of mice on 20-weeks of high-fat diet. This result is in good agreement with several other studies, which have shown that both PWV and vessel stiffness increase with atherosclerosis and also that the elastic response of the vessel decreases [44], [69], [70]. While these increases in the PWV as atherosclerosis progresses may seem counterintuitive because of the increased fat deposition in the arterial wall, additional longitudinal studies in monkeys [76], [77] and humans [19], [36] have all similarly shown increased PWV measurements in atherosclerotic arteries. Farrar et al. [77] attributes this increase in the PWV mainly to increases in the total material in the wall (i.e. increases in arterial elastin and collagen content) caused by atherosclerosis. Moreover, according to Fig. 13, the r^2 coefficient is shown to decrease significantly ($p < 0.05$) between the normal and the 20- and 30-week high-fat-diet aortas indicating that the aortic wall is less homogeneous. Additionally, the thickness measurements used in the Moens-Korteweg equation are shown to increase significantly in the cases of mice on 20 (0.16 ± 0.01 mm) and 30 (0.19 ± 0.01 mm) weeks of high-fat diet compared to the normal aortas (0.13 ± 0.004 mm), a finding that has been noted in other studies [78], [79]. It should be noted that in the case of ApoE^{-/-} mice fed a high-fat diet, increases in both PWV and aortic wall thickness have also been observed in a recent study by Chatterjee et al. [80] after 20 weeks and 36 weeks of high-fat diet.

In the case of the stiffness maps of the carotid artery (Fig. 12), in the normal carotid artery, an increase in relative stiffness is observed close to the bifurcation, which is mainly due to the narrowing of the common carotid into one of its two branches. In the case of the atherosclerotic carotid artery, there is an increase in the PWV at this site. This trend, although not statistically significant, is also indicated by the results in Table I. More specifically, an increase in the mean of sub-regional PWVs is observed between the mice

after 5 and either 20 or 30 weeks of high-fat diet. Moreover, r^2 coefficients derived for carotid arteries were found to decrease significantly ($p < 0.05$) after 20 weeks of high-fat diet, which indicates that r^2 could be used as potential biomarker of atherosclerosis in the carotid artery. A significant increase was also found between the thicknesses of the carotid arteries after 5 and 30 weeks of high-fat diet ($p < 0.05$). Plaque thickness of the atherosclerotic mice after 30 weeks of high-fat diet was found to be close to those of other ultrasound biomicroscopy studies [67], [68]. In general, it has been shown that ultrasound biomicroscopy slightly overestimates wall thickness compared to histological measurements. One suggested reason for this is the fact that tissue tends to shrink during histological preparation.

An anomaly observed in the atherosclerotic mouse data was the fact that, according to Table I, a decrease was observed in the piecewise PWVs and E_{PWV} of the carotid arteries of the mice on 20 and 30 weeks of high-fat diet. Additionally, according to Fig. 13, a non-significant decrease was also observed in the mean piecewise E_{PWV} between the mice on 20 and 30 weeks of high-fat diet. One factor that may have contributed to the aforementioned drops in stiffness could be the small number of animals in the population of the 30-week-high-fat-fed mice. Furthermore, similar decreases in the PWV and arterial Young's modulus have been observed in an extensive study by Farrar et al. in which the aortic PWV of a large population of monkeys were fed an atherogenic diet over an extensive period of time [76]. Farrar et al. hypothesized that arteries respond to the hypercholesterolemia caused by the high-fat diet by remodeling and more specifically by proliferating smooth muscle cells and loosening connective tissue. This in combination with the increased lipid accumulation would result to a more compliant vessel overall [76]. This same explanation may also apply to the results in mice presented here. Additionally, given that E_{PWV} values are calculated through the Moens-Korteweg equation, the decrease in their mean from 20 to 30 weeks of high fat diet in both the carotid arteries and the aortas is a result of a combination of the wall thickening due to the advancement of the atherosclerotic lesion which results to greater wall thickness coupled with a lower value of arterial diameter. These changes in the vessel, coupled with the fact that advanced, lipid-laden lesions appear in ApoE^{-/-} mice after 20 and up to 40 weeks [59], could be another reason for this decrease in stiffness. It should be noted that lipid-rich murine atherosclerotic plaques have been shown to yield lower stiffness values by means of *in situ* mechanical testing [81].

In the case of AAA development, Figs. 11(a), (b) and (c) imply an interesting pattern in the formation of the aneurysm. Sub-regional PWV and E_{PWV} values increase sharply after a week of angiotensin II administration, thus indicating an increase in the vessel's stiffness. However, at that stage there are no signs of aneurysm formation. According to existing literature [82], within 1-10 days after implantation of the osmotic pump that infuses mice with a dose of angiotensin II, elastin fragmentation is promoted. Hence, one explanation for these observations is that the degradation of elastin transfers the mechanical load to the collagen fibers present in large arterial vessels, thus increasing the overall stiffness of the vessel [83]. These findings have also been verified in an *ex vivo* setting [84], where angiotensin II-infused murine arteries were subjected to mechanical testing. In the same study higher PWV was measured *in vivo* in the angiotensin infused murine aortas via

Doppler velocimetry. However, in Fig. 11(c), after formation of the aneurysm, it can be seen that both the sub-regional PWVs and E_{PWI} have regressed to lower magnitudes. This is also confirmed by Fig. 13, where there is a significant decrease ($p < 0.05$) in both the mean sub-regional PWVs and E_{PWI} values of the aneurysmal cases compared to normal aortas. Furthermore, normal and aneurysmal cases as seen in Fig. 13 can also be distinguished based on the quality of the linear fit, as quantified by the r^2 coefficient, since the value of this parameter is significantly lower ($p < 0.001$) in AAAs than in normal aortas. Similar findings were seen in previous work by Luo et al. [31]. Finally, it has to be noted that according to Fig. 13, a significant increase ($p < 0.001$) was found between the arterial wall thickness of aneurysmal (0.20 ± 0.03 mm) and normal aortas (0.13 ± 0.004 mm). Similar increases in the wall thickness have also been observed in this MRI study on murine elastase-induced AAAs [85] and also comparable ultrasonic measurements have been made using a Vevo 660 (VisualSonics, Toronto, ON, Canada) [86].

D. PWI Modulus Values

The derived PWI modulus values were also compared against stiffness values from other studies involving noninvasive techniques or mechanical testing. This was done to provide preliminary validation for the computed values. The E_{PWI} of the ApoE^{-/-} mouse abdominal aortas were estimated at 43.82 ± 5.86 kPa in normal aortas, while in 20- and 30-week high-diet-fed aortas the mean E_{PWI} were 68.09 ± 20.66 kPa and 57.72 ± 13.12 kPa respectively. According to Fig. 13(b), E_{PWI} changes significantly between normal aortas and aortas of mice on 20 weeks of high-fat diet, while the increase between normal and mice on 30 weeks of high-fat diet was non-significant (albeit only narrowly so with $p = 0.057$). However, the inclusion of this biomarker appears to supplement the differentiating strength of the proposed method and also reflects the atherosclerosis-induced stiffening of the arteries. In the case of healthy mouse aortas, the calculated E_{PWI} values were lower compared to the Young's modulus values measured *in vivo* in this previous PWI study by Fujikura et al. [50], since in that study the simplified version of the Moens-Korteweg equation was employed, ignoring the contribution of Poisson's ratio. Furthermore, a number of *ex vivo* studies have attempted to estimate the stiffness of murine vasculature using atomic force microscopy (AFM) and mechanical testing. Notably, Tracqui et al. [81] used AFM to probe the stiffness of the elastic lamina and individual components of plaque structure. The mean Young's modulus for the elastic lamina space of the aortic wall was estimated to be 58.3 ± 6.3 kPa while individual components of plaques (lipid rich areas, fibrotic areas) exhibited a wider range of stiffness values that included both lower (5.5 kPa) and higher (59.4 kPa) stiffness values. Additionally, in another study by Santelices et al. [87], an experimental system was designed for measuring the stiffness of the murine aorta by simulating the *in vivo* pulsatile pressurization loop environment and using the pressure-diameter response. The reported moduli in 8-week old wild-type mice were in the range of 80 kPa. In similar studies, the stiffness of the proximal to the heart murine abdominal aorta has been measured to be in the range of 50 kPa [88], while the circumferential elastic modulus of elastin in rat carotid arteries has been measured at 48.42 kPa [89]. In all of the aforementioned studies, there is variability in the arterial stiffness measurements due to the different assumptions in each methodology. More specifically, it must be noted that those studies were performed *in vitro*, which differs from the *in vivo* environment that the present study was performed in. Thus,

some of the factors that influence material stiffness such as axial prestretch and perivascular support may have not been simulated realistically in the *in vitro* setting. However, despite those limitations, the E_{PWI} values estimated here are in the range of the experimental measurements presented in the aforementioned studies.

E. Limitations and Future Work

One of the limitations of the current study is caused by the sensitivity of the PWV estimation to the SNR of the image sequence. This factor is even more important in the case of piecewise PWV estimation, since the linear fitting is done over smaller segments of 2-4 mm and hence the presence of image artifacts or noise could influence the resulting PWV and r^2 measurements more than at larger scales. Consequently, the quality of the image is of critical importance in our method and for this reason several precautions (i.e. use of degassed gel, careful probe placement, complete removal of abdominal and throat hair, compensation for rigid motion) were taken to ensure that the quality of the acquisitions was as high as possible. Additionally, acquisitions that contained artifacts or low SNR regions were discarded. Another possible limitation of the proposed technique may arise in the case of mice that suffer from arrhythmias, as in that case an irregular heartbeat may compromise the results of the stroboscopic ECG gating technique. However, with the further development of ultrafast imaging [90] high frequency plane wave acquisitions may be utilized to achieve high frame-rates without relying on the acquired ECG.

The choice of using r^2 as a measure of nonuniformity in the current study may seem surprising; however, it is justified in part because of previous PWI studies showing its effectiveness in differentiating normal from calcified mouse aortas [50], normal from aneurysmal mouse aortas [31] as well as normal from aneurysmal human aortas [57]. Furthermore, the r^2 has been shown to be capable of staging the development of AAA in mouse aortas [58]. Finally, a previous simulation study [91] has linked r^2 with the degree of tissue homogeneity. In general, nonuniform wave propagation has been associated with reduced r^2 values and has been attributed to the underlying arterial wall inhomogeneity [31], [57]. The r^2 coefficient may present a limitation, which is the fact that by only looking at its values, no inference can be made on the nature of the inhomogeneity. This is another case where the E_{PWI} maps provide useful information. More specifically, the E_{PWI} maps match the piecewise PWVs and their associated r^2 values with specific regions on the B-mode image. Thus, the arterial wall inhomogeneity can be easily identified on the B-mode, through changes in the arterial diameter or wall thickness. Fig. 14 shows an example of combining the B-mode and B-mode derived morphological values with PWI-derived results.

The semi-automated segmentation algorithm implemented for the purpose of this study has two aims: first, to assist a trained observer into performing the segmentation; and second, to reduce the inter-user variability of the wall segmentation. Previous PWI studies [31], [55], [57] have utilized manual segmentation of the arterial wall which while inducing a certain degree of inter-user variability, has been tested and found not to greatly affect the pulse wave velocity measurements. Additionally, testing of the method against manual tracing of the wall was not found to affect PWV measurements. However, the fact that there is still manual initialization in this algorithm remains as one of its limitations. Ongoing work will

aim at implementing a fully automatic method to obtain the wall segmentation according to a single set of rules.

An additional factor that could have potentially affected the PWV measurements performed in this study is the bigger length of the pulse wave's wavelength compared to the size of the investigated inhomogeneities. However, it should be noted that sub-regional PWVs are estimated by tracking the wavefront velocity of the pulse wave which has been shown to be a good measure of the characteristic velocity of all of the pulse wave's components and has also been shown to be less affected by reflections [18]. Thus, by focusing on the wavefront, the influence of the long wavelength on the pPWI measurements is reduced. Furthermore, a long wavelength is required in our study, since it is a necessary condition for the validity of the Moens-Korteweg equation [92].

Additionally, while the development of standing waves within arterial wall inclusions has been previously reported in a simulation study [54], no standing waves were observed in any of the *in vivo* results presented in this study or in previous *in vivo* studies [31], [57], [58]. This may be attributed to the simplified assumptions used *in silico* [54] (e.g., use of a linear elastic model with no branching, submerged in water with a Heaviside step function as the inlet velocity function).

In the case of aneurysmal aortas absence of axisymmetric properties may result in less homogeneous propagation of the pulse wave within the circumference of the vessel. However, within the shorter sub-regions (2-4 mm) used in this study to calculate the pulse wave velocity more homogeneous propagation can be assumed, which again demonstrates the importance of using pPWI to assess such focal vascular pathologies. However, in order to fully address this issue, there is ongoing work by our group that aims at the implementation of 3-D ultrafast ultrasonic imaging to track the pulse wave propagation within the circumference of the artery.

Another limitation, inherent to the application of the Moens-Korteweg equation to major arterial vessels *in vivo*, is the fact that the surrounding tissue elasticity is not taken into account. Nevertheless, it has to be noted that this particular limitation applies to all major longitudinal clinical studies employing tonometry or arterial wall tracking at two distal sites to infer PWV and report on arterial wall stiffness.

Because of the aforementioned limitations in Moens-Korteweg equation, the derived metric is dependent on but not necessary equal to the stiffness. Thus, the name "PWI modulus" was used to indicate its nature as a relative (rather than an absolute) measure of stiffness. Its significance was demonstrated in the current study as a new parameter that takes into account and combines three major parameters that have been separately shown to change in the presence of focal vascular disease (PWV [93], [94], arterial wall thickness [95], [96] and vessel diameter [97], [98]) to successfully differentiate between normal and pathological arterial tissue.

Finally, it has to be noted that no pressure measurements were made in mice for the purpose of this study. This may be considered a limitation, since part of the increased PWV in atherosclerotic mice could be due to arterial hypertension. However, PWV has been shown

to be able to detect changes in arterial stiffness, caused by factors such as atherosclerosis and increased arterial wall thickness, after adjusting for pressure [19] or within groups of similar pressures (only hypertensive patients seen in [94]). Consequently, given that the inspection of the mouse arteries from the B-mode images has confirmed the presence of both atherosclerosis as well as increases in arterial wall thickness, it seems to be perfectly logical to assume that, in the current study, PWV tracks the change in arterial wall mechanical properties. However, in order to decouple the effects of arterial stiffness from those of the pressure on the PWV, a correction for the pressure needs to be implemented, but goes beyond the scope of this paper. For this purpose, the effects of pressure on PWV will be investigated in future studies which will include phantom and *in vivo* experiments consisting of localized pressure and PWV measurements. Moreover, intra-aneurysmal pressure measurements in mice could provide more information on the effects of thrombus on the reduced PWV wall velocities at the aneurysmal aortic wall.

In the current study, the kernel size for piecewise linear fitting was chosen after experimenting with different kernel sizes, keeping in mind that the length of each sub-region needed to be comparable with the focal expression of each disease. Moreover, 50% overlapping of the sub-regions was employed to further increase resolution. However, even more localized inhomogeneities can also be detected by using the r^2 quality indicator. Thus, a potential usage of pPWI would be to detect local inhomogeneities inconspicuous on the B-mode image by using the r^2 coefficient as mentioned previously and subsequently adjusting and focusing the pulse wave measurements in the pathological sub-region or performing further analysis at that particular region. The feasibility of using localized PWV measurements to detect local inhomogeneities in the arterial wall has already been shown by our group via FSI simulations [91].

Future work aims to optimize the resolution of this method (i.e. optimal kernel size and overlap) to increase the accuracy of the PWV measurements. There is also ongoing work to validate the E_{PWI} values derived using the Moens-Korteweg equation via both AFM and micro-indentation measurements with encouraging preliminary results [99]. Finally, there will be further development of adaptive multi-resolution stiffness maps, in which PWVs are estimated with a maximization criterion based on sub-regional r^2 coefficients and where dynamic programming is used to estimate the optimal kernel size.

V. Conclusion

Estimation of the mechanical properties in the vascular wall may lead to timely and critical diagnosis, and is essential for monitoring localized cardiovascular diseases. The novel technique of piecewise Pulse Wave Imaging presented herein provides detailed maps of the PWV along the vascular wall based on pulse wave propagation. Stiffness maps of normal and pathological aortas as well as carotids were generated and utilized to assess initial feasibility of the new methodology in the detection of the early onset of atherosclerotic and aneurysmal disease. The mean sub-regional PWV in $n = 7$ normal mouse aortas was found to be 2.57 ± 0.18 m/s while in the cases of mice on 20- and 30-week periods of high-fat diet, the PWV was found to increase significantly ($p = 0.001$) to 3.30 ± 0.36 m/s and 3.56 ± 0.29 m/s, respectively, in line with previously reported findings. Furthermore, the r^2 coefficient of

determination was found to be significantly higher in normal aortas than atherosclerotic aortas (20 and 30 weeks of high-fat diet, $p = 0.05$) and aneurysmal ones ($p = 0.001$). Finally, the mean E_{PWI} was found to be 43.82 ± 5.86 kPa in normal aortas while in atherosclerotic aortas it increased significantly ($p = 0.05$) to 68.09 ± 20.66 kPa after 20-weeks of high-fat diet. A significant difference ($p = 0.05$) was also found between the sub-regional r^2 coefficient values of the mouse right carotid arteries after 5 and 20 weeks of high-fat-diet. PWI-based stiffness mapping was thus shown to be capable of differentiating fully developed atherosclerosis and aneurysms from normal walls in major arteries, as well as monitoring the early stages of the aforementioned diseases.

Acknowledgment

The authors would like to thank Dr. Shunichi Homma for the use of the Visualsonics Vevo 770 ultrasound system. The authors would also like to thank Fusako Sera, M.D., Ronny Li, M.S. and Gary Hou, Ph.D.

This work was supported in part by from the National Institutes of Health (NIH R01-HL098830) and from the General Michael Arnaoutis Foundation.

REFERENCES

1. Libby P, Ridker PM, Maseri A. Clinical cardiology: new frontiers. *Circ.* 2002; 105:1135–1143.
2. Naghavi M, Libby P, Falk E, Casscells SW, Litovsky S, Rumberger J, Badimon JJ, Stefanidis C, Moreno P, Pasterkamp G, Fayad Z, Stone PH, Waxman S, Raggi P, Madjid M, Zarrabi A, Burke A, Yuan C, Fitzgerald PJ, et al. From vulnerable plaque to vulnerable patient: a call for new definitions and risk assessment strategies. *Circ.* 2003; (108):1664–1672.
3. Roger VL, Go AS, Lloyd-Jones DM, Benjamin EJ, Berry JD, Borden WB, Bravata DM, Dai S, Ford ES, Fox CS, Fullerton HJ, Gillespie C, Hailpern SM, Heit JA, Ho PM, Howard VJ, Kissela BM, Kittner SJ, Lackland DT, et al. Heart disease and stroke statistics - 2012 update : a report from the american heart association. *Circ.* 2012; 125:e2–e220.
4. Go AS, Mozaffarian D, Roger VL, Benjamin EJ, Berry JD, Borden WB, Bravata DM, Dai S, Ford ES, Fox CS, Franco S, Fullerton HJ, Gillespie C, Hailpern SM, Heit JA, Howard VJ, Huffman MD, Kissela BM, Kittner SJ, Lackland DT, et al. Heart disease and stroke statistics - 2013 update: a report from the american heart association. *Circ.* 2013; 127:e6–e245.
5. Maurice RL, Fromageau J, Brusseau E, Finet G, Rioufol G, Cloutier G. On the potential of the Lagrangian estimator for endovascular ultrasound elastography: in vivo human coronary artery study. *Ultrasound Med. Biol.* 2007; 33(8):1199–1205. [PubMed: 17466446]
6. Ohayon J, Finet G, Le Floch S, Cloutier G, Gharib AM, Heroux J, Pettigrew RI. Biomechanics of coronary plaque: site, stability and in vivo elasticity modeling. *Ann. Biomed. Eng.* 2013 in press.
7. Saam T, Hatsukami TS, Takaya N, Chu B, Underhill H, Kerwin WS, Cai J, Ferguson MS, Yuan C. The vulnerable, or high-risk, atherosclerotic plaque: noninvasive mr imaging for characterization and assessment. *Radiology.* 2007; 244(1):64–77. [PubMed: 17581895]
8. Huang X, Yang C, Canton G, Ferguson M, Yuan C, Tang D. Quantifying effect of intraplaque hemorrhage on critical plaque wall stress in human atherosclerotic plaques using three-dimensional fluid-structure interaction models. *J. Biomech. Eng.* 2012; 134:12004–1-12004-9.
9. Hansen, HHG.; de Korte, CL. *Ultrasound and Carotid Bifurcation Atherosclerosis*. Springer; London: 2012. Noninvasive carotid elastography; p. 341-353.
10. Glagov S, Weisenberg E, Zarins CK, Stankunavicius R, Kolettis GJ. Compensatory enlargement of human atherosclerotic coronary arteries. *N. Engl. J. Med.* 1987; 316(22):1371–1375. [PubMed: 3574413]
11. Taylor A, Shaw LJ, Fayad Z, O'Leary D, Brown BG, Nissen S, Rader D, Raggi P. Tracking atherosclerosis regression: a clinical tool in preventive cardiology. *Atherosclerosis.* 2005; 180:1–10. [PubMed: 15823269]

12. Saam T, Ferguson MS, Yamykh MS, Yarnykh VL, Takaya N, Xu D, Polissar NL, Hatsukami TS, Yuan C. Quantitative evaluation of carotid plaque composition by in vivo mri. *Arterioscler. Thromb. Vasc. Biol.* 2005; 25(1):234–239. [PubMed: 15528475]
13. Staub D, Schinkel AFL, Coll B, Coli S, van der Steen AFW, Reed JD, Krueger C, Thomenius KE, Adam D, Sijbrands EJ, ten Cate FJ, Feinstein SB. Contrast-enhanced ultrasound imaging of the vasa vasorum. *JACC Cardiovasc Imaging.* 2010; 3(7):761–771. [PubMed: 20633855]
14. Wohlin M, Sundstrom J, Andren B, Larsson A, Lind L. An echoluent carotid artery intima-media complex is new and independent predictor of mortality in an elderly male cohort. *Atherosclerosis.* 2009; 205(2):486–491. [PubMed: 19243779]
15. Velgimigli M, Rodriguez-Granillo GA, Garcia-Garcia HM, Malagutti P, de Jaegere P, de Feyter P, Serruys PW. Distance from the ostium as an independent determinant of coronary plaque composition in vivo: an intravascular ultrasound study based radiofrequency data analysis in humans. *Eur. Heart J.* 2006; 27(6):655–663. [PubMed: 16415094]
16. Naim C, Douziech M, Therasse E, Robillard P, Giroux MF, Cloutier G, Soulez G. Vulnerable atherosclerotic carotid plaque evaluation by ultrasound, computed tomography and magnetic resonance imaging: an overview. *Can. Assoc. Radiol. J.* 2013 in press.
17. Mercuri, M.; McPherson, DD.; Bassiouny, H.; Glagov, S. *Non-Invasive imaging of atherosclerosis.* Springer; 1997.
18. Nichols, WW.; O'Rourke, MF.; McDonald, DA. *McDonald's Blood Flow in Arteries: Theoretical, Experimental and Clinical Principles.* Hodder Arnold; New York: 2005.
19. van Popele NM, Grobbee DE, Bots ML, Asmar R, Topouchian J, Reneman RS, Hoeks AP, Deidre AM, van der Kuip DAM, Hofman A, Witteman JCM. Association between arterial stiffness and atherosclerosis: the rotterdam study. *Stroke.* 2001; 32(2):454–460. [PubMed: 11157182]
20. deKorte CL, Pasterkamp G, van der Steen AFW, Woutman HA, Born N. Characterization of plaque components with intravascular ultrasound elastography in human femoral and coronary arteries In vitro. *Circ.* 2000; 102:617–623.
21. Schmitt C, Soulez G, Maurice RL, Giroux MF, Cloutier G. Noninvasive vascular elastography: toward a complementary characterization tool of atherosclerosis in carotid arteries. *Ultrasound in Med. & Biol.* 2007; 33(12):1841–1858. [PubMed: 17698283]
22. Zhang XM, Greenleaf JF. Noninvasive generation and measurement of propagating waves in arterial walls. *J. Acoust. Soc. Am. Suppl.* 2006; 119(2):1238–1243.
23. Dumont D, Behler RH, Nichols TC, Merricks EP, Galippi CM. Arfi imaging for noninvasive material characterization of atherosclerosis. *Ultrasound Med. Biol.* 2006; 32(11):1703–1711. [PubMed: 17112956]
24. Hoyert DL, Xu J. Deaths: preliminary data for 2011. *Natl. Vital Stat. Rep.* 2012; 61(6):1–52. [PubMed: 24984457]
25. Wassef M. Views in research on abdominal aortic aneurysms. *Ann. N. Y. Acad. Sci.* 2006; 1085:xv–xvii.
26. Berguer R, Bull JL, Khanafer K. Refinements in mathematical models to predict neurysm growth and rupture. *Ann. N. Y. Acad. Sci.* 2006; 1085:92–109. [PubMed: 17182926]
27. Sakalihasan N, Limet R, Defawe D. Abdominal aortic aneurysm. *Lancet.* 2005; 365:1577–1589. [PubMed: 15866312]
28. Vorp DA. Biomechanics of abdominal aortic aneurysm. *J. Biomech.* 2007; 40:1887–1902. [PubMed: 17254589]
29. Lederle FA, Johnson GR, Wilson SE, Ballard DJ, Jordan WD, Blebea J, Littooy FN, Freischlag JA, Bandyk DD, Rapp JH, Salam AA. Rupture rate of large abdominal aortic aneurysms in patients refusing or unfit for elective repair. *JAMA.* 2002; 287:2968–2972. [PubMed: 12052126]
30. Brewster DC, Cronenwett JL, Hallett JWJ, Johnston KW, Krupski WC, Matsumura JS. Guidelines for the treatment of abdominal aortic aneurysms. Report of a subcommittee of the joint council of the american association for vascular surgery and society for vascular surgery. *J. Vasc. Surg.* 2003; 37(5):1106–1117. [PubMed: 12756363]
31. Luo J, Fujikura K, Tyrie LS, Tilson MD III. Pulse wave imaging of normal and aneurysmal abdominal aortas in vivo. *IEEE Trans. on Med. Imag.* 2009; 28(4):477–486.

32. Vande Geest JP, Di Martino ES, Bohra A, Makaroun MS, Vorp DA. A biomechanics-based rupture potential index for abdominal aortic aneurysm risk assessment. *Ann. N. Y. Acad. Sci.* 2006; 1085:11–21. [PubMed: 17182918]
33. Long A, Rouet L, Bissery A, Rossignol P, Mouradian D, Sapoval M. Compliance of abdominal aortic aneurysms evaluated by tissue Doppler imaging: correlation with aneurysm size. *J. Vasc. Surg.* 2005; 42:18–26. [PubMed: 16012447]
34. Hansen HHG, Lopata RGP, Holewijn S, Truijers M, de Korte CL. Non-invasive vascular ultrasound strain imaging: different arteries different approaches. 4th European Conference of the International Federation for Medical and Biological Engineering. 2008; 22:298–302.
35. Truijers M, Pol JA, SchultzeKool LJ, van Sterkenburg SM, Fillinger MF, Blankensteijn JD. Wall stress analysis in small asymptomatic, symptomatic and ruptured abdominal aneurysms from 2-d ultrasound. *Eur. J. Vasc. Endovasc.* 2007; 33:401–407.
36. Cecelja M, Chowienszyk P. Role of arterial stiffness in cardiovascular disease. *JRSM Cardiovasc Dis.* 2012; 1(4):1–11.
37. Ohayon J, Finet G, Gharib AM, Herzka DA, Tracqui P, Heroux J, Rioufol G, Kotys MS, Elagha A, Pettigrew RI. Necrotic core thickness and positive arterial remodeling index: emergent biomechanical factors for the risk of plaque rupture. *Am J Physiol Heart Circ Physiol.* 2008; 295:H717–H727. [PubMed: 18586893]
38. Sonesson B, Hansen F, Lanne T. Abdominal aortic aneurysm: a general defect in the vasculature with focal manifestations in the abdominal aorta? *J Vasc Surg.* 1997; 26(2):247–254. [PubMed: 9279311]
39. Fung, YC. *Biomechanics: Circulation*. 2nd ed.. Springer-Verlag; New York: 1997.
40. Xu J. Do we need a better approach for measuring pulse-wave velocity? *Ultrasound in Med. & Biol.* 2003; 29(9):1373. [PubMed: 14553816]
41. Karamanoglu M. Errors in estimating propagation distances in pulse wave velocity. *Hypertens.* 2003; 41(6):E8.
42. Bolster BD, Atalar E, Hardy CJ, McVeigh ER. Accuracy of arterial pulse-wave velocity measurement using MR. *J. Magn. Reson. Imaging.* 1998; 45(6):878–888. [PubMed: 9702890]
43. Yu HY, Peng JL, Wang JL, Wen CY, Tseng WYI. Quantification of the pulse wave velocity of the descending aorta using axial velocity profiles from phase-contrast magnetic resonance imaging. *Magn. Reson. Med.* 2006; 28(6):876–883. [PubMed: 16947380]
44. Herold V, Parczyk M, Moerchel P, Ziener CH, Klug G, Bauer WR, Rommel E, Jakob P. In vivo measurement of local aortic pulse-wave velocity in mice with mr microscopy at 17.6 tesla. *Magn. Res. Med.* 2009; 61:1293–1299.
45. Meinders JM, Brands PJ, Willigers JM, Kornet L, Hoeks AP. Assessment of the spatial homogeneity of artery dimension parameters with high frame rate 2-D B-mode. *Ultrasound Med Biol.* 2001; 27(6):785–794. [PubMed: 11516538]
46. Brands PJ, Willigers JM, Ledoux LA, Reneman RS, Hoeks AP. A noninvasive method to estimate pulse wave velocity in arteries locally by means of ultrasound. *Ultrasound Med Biol.* 1998; 24(9):1325–1335. [PubMed: 10385955]
47. Meinders JM, Kornet L, Brands PJ, Hoeks APG. Assessment of local pulse wave velocity in arteries using 2d distension waveforms. *Ultrason. Imaging.* 2001; 23(4):199–215. [PubMed: 12051275]
48. Hartley CJ, Taffet GE, Michael LH, Pham TT, Entman M. Noninvasive determination of pulse-wave velocity in mice. *Am J. Physiol. Heart Circ. Physiol.* 1997; 273(1):H494–H500.
49. Couade M, Pernot M, Messas E, Emmerich J, Hagege A, Fink M, Tanter M. Ultrafast imaging of the arterial pulse-wave. *IRBM.* 2011; 32(2):106–108.
50. Fujikura K, Luo J, Gamarnik V, Pernot M, Fukumoto R, Tilson MD III, Konofagou EE. A novel noninvasive technique for pulse-wave imaging and characterization of clinically-significant vascular mechanical properties in vivo. *Ultrasonic Imag.* 2007; 29:137–154.
51. Pernot M, Fujikura K, Fung-Kee-Fung SD, Konofagou EE. Ecg-gated, mechanical and electromechanical wave imaging of cardiovascular tissues in vivo. *Ultrasound in Med. & Biol.* 2007; 33(7)

52. Shahmirzadi D, Li RX, Konofagou EE. Pulse-wave propagation in straight geometry vessels for stiffness estimation: theory, simulations, phantoms and in vitro findings. *J. Biomech. Eng.* 2012; 134(11):1145021–1145026.
53. Shahmirzadi D, Nrayanan P, Li RX, Qaqish WW, Konofagou E. Mapping the longitudinal wall stiffness heterogeneities within intact canine aortas using pulse wave imaging ex vivo. *J. Biomech.* 2013; 46(11):1866–1874. [PubMed: 23764176]
54. Shahmirzadi D, Konofagou E. Detection of aortic wall inclusions using regional pulse wave propagation and velocity in silico. *Artery Res.* 2012; 6(3):114–123.
55. Vappou J, Luo J, Konofagou EE. Pulse wave imaging for noninvasive and quantitative measurement of arterial stiffness in vivo. *Am. J. Hypertens.* 2010; 23:393–398. [PubMed: 20094036]
56. Luo J, Li RX, Konofagou EE. Pulse wave imaging of the human carotid artery: an in vivo feasibility study. *IEEE Trans. Ultrason. Ferroelectr. Freq. Control.* 2012; 59(1):174–181. [PubMed: 22293749]
57. Li RX, Luo J, Balam SK, Chaudry FA, Shahmirzadi D, Konofagou E. Pulse wave imaging (pwi) in normal, hypertensive and aneurysmal human aortas in vivo: a feasibility study. *Phys. Med. Biol.* 2013; 58:4549–4562. [PubMed: 23770991]
58. Nandlall SD, Goldklang MP, Kalashian A, Dangra NA, d'Armiento JM, Konofagou EE. Monitoring and staging abdominal aortic aneurysm disease with pulse wave imaging. *Ultrasound Med Biol.* 2014; 40(10):2404–2414. [PubMed: 25130446]
59. Nakashima Y, Plump AS, Raines EW, Breslow JL, Ross R. Apoe-deficient mice develop lesions of all phases of atherosclerosis throughout the arterial tree. *Arterioscler. Thromb. Vasc. Biol.* 1994; 14:133–140.
60. Fillinger M. The long-term relationship of wall stress to the natural history of abdominal aortic aneurysms (finite element analysis and other methods). *Ann. N. Y. Acad. Sci.* 2006; 1085:22–28. [PubMed: 17182919]
61. Pernot M, Konofagou E. Electromechanical imaging of the myocardium at normal and pathological states. *Ultrasonics Symposium, 2005 IEEE.* 2005
62. Cherin E, Williams R, Needles A, Liu G, White C, Brown AS, Zhou Y-Q, Foster FS. Ultrahigh frame rate retrospective ultrasound microimaging and blood flow visualization in mice in vivo. *Ultrasound in Med & Biol.* 2006; 32(5):683–691. [PubMed: 16677928]
63. Luo J, Konofagou EE. A fast normalized cross-correlation calculation method for motion estimation. *IEEE Trans. Ultrason. Ferroelectr. Freq. Control.* 2010; 57:1347–1357. [PubMed: 20529710]
64. Luo J, Konofagou EE. High-frame rate, full-view myocardial elastography with automated contour tracking in murine left ventricles in vivo. *IEEE Trans. Ultrason. Ferroelectr. Freq. Control.* 2008; 55(1):240–248. [PubMed: 18334330]
65. Li RX, Qaqish WW, Shahmirzadi D, Konofagou E. Performance assessment and optimization of Pulse Wave Imaging (PWI) in ex vivo canine aortas and in vivo normal human arteries. *Conf Proc IEEE Eng Med Biol Soc.* 2012
66. Martin-McNulty B, Vincelette J, Vergona R, Sullivan ME, Wang Y-X. Noninvasive measurement of abdominal aneurysms in intact mice by a high-frequency ultrasound imaging system. *Ultrasound in Med. & Biol.* 2005; 31(6):745–749. [PubMed: 15936490]
67. Gan L-M, Gronros J, Hagg U, Wikstrom J, Theodoropoulos C, Friberg P, Fritsche-Danielson R. Non-invasive real-time imaging of atherosclerosis in mice using ultrasound biomicroscopy. *Atherosclerosis.* 2007; 190:313–320. [PubMed: 16677654]
68. Ni M, Zhang M, Ding S-F, Chen W-Q, Zhang Y. Micro-ultrasound imaging assessment of carotid plaque characteristics in apolipoprotein-E knockout mice. *Atherosclerosis.* 2008; 197(1):64–71. [PubMed: 17870080]
69. Wang Y-X, Halks-Miller M, Vergona R, Sullivan ME, Fitch R, Mallari C, Martin-McNulty B, De Cunha V, Freay A, Rubanyi GM, Kauser K. Increased aortic stiffness assessed by pulse wave velocity in apolipoprotein E-deficient mice. *Am. J. Physiol. Heart Circ. Physiol.* 2000; 278:H428–H434. [PubMed: 10666072]

70. Agianniotis A, Stergiopoulos N. Wall properties of the Apolipoprotein E-deficient mouse aorta. *Atherosclerosis*. 2012; 223:314–320. [PubMed: 22770991]
71. Mower WR, Quinones WJ, Gambhir SS. Effect of intraluminal thrombus on abdominal aortic aneurysm wall stress. *J Vasc Surg*. 1997; 26(4):602–608. [PubMed: 9357460]
72. Vorp DA, Mandarino WA, Webster MW, Gorcsan J3. Potential influence of intraluminal thrombus on abdominal aortic aneurysm as assessed by a new non-invasive method. *Cardiovasc Surg*. 1996; 4(6):732–739. [PubMed: 9013001]
73. Thubrikar MJ, Robicsek F, Labrosse M, Chervenkov V, Fowler BL. Effect of thrombus on abdominal aortic aneurysm wall dilation and stress. *J Cardiovasc Surg*. 2003; 44(1):67–77. [PubMed: 12627076]
74. Fouque, JP.; Garnier, J.; Papanicolaou, G.; Solna, K. Wave propagation and time reversal in randomly layered media. Springer; 2007.
75. Williams R, Needles A, Cherin E, Zhou Y-Q, Henkelman RM, Adamson SL, Foster FS. Noninvasive ultrasonic measurement of regional and local pulse-wave velocity in mice. *Ultrasound in Med. & Biol*. 2007; 33(9):1368–1375. [PubMed: 17561330]
76. Farrar DJ, Bond MG, Riley WA, Sawyer JK. Anatomic correlates of aortic pulse wave velocity and carotid artery elasticity during atherosclerosis progression and regression in monkeys. *Circulation*. 1991; 83:1754–1763. [PubMed: 2022028]
77. Farrar DJ, Bond MG, Sawyer JK, Green HD. Pulse wave velocity and morphological changes associated with early atherosclerosis progression in the aortas of cynomolgus monkeys. *Cardiovasc Res*. 1984; 18:107–118. [PubMed: 6697337]
78. Li R-J, Yang Y, Wang YH, Song L, Wang Z, Zhang YZ, Qin YW, Li ZA, Zhang XS. Micro-ultrasound imaging of atherosclerotic progression and correlation with inflammatory markers in apolipoprotein-E knockout mice. *Tex Heart Inst J*. 2011; 38(4):364–370. [PubMed: 21841862]
79. Itskovich VV, Choudhury RP, Aguinaldo JGS, Fallon JT, Omerhodzie S, Fisher EA, Fayad ZA. Characterization of aortic root atherosclerosis in apoe knockout mice: high-resolution in vivo and ex vivo mrm with histological correlation. *Magn Reson Med*. 2003; 49:381–385. [PubMed: 12541260]
80. Chatterjee S, Bedja D, Mishra S, Amuzie C, Avolio A, Kass DA, Berkowitz D, Renahan M. Inhibition of glycosphingolipid synthesis ameliorates atherosclerosis and arterial stiffness in apolipoprotein e^{-/-} mice and rabbits fed a high fat and cholesterol diet. *Circulation*. 2014; 126:2403–2413. [PubMed: 24710030]
81. Tracqui P, Broisat A, Toczek J, Mesnier N, Ohayon J, Riou L. Mapping elasticity moduli of atherosclerotic plaque in situ via atomic force microscopy. *Journal of Structural Biology*. 2011; 174(1):115–123. [PubMed: 21296163]
82. Daugherty A, Manning MW, Cassis LA. Angiotensin II promotes atherosclerotic lesions and aneurysms in apolipoprotein E-deficient mice. *J. Clin. Invest*. 2000; 105(11):1605–1612. [PubMed: 10841519]
83. Wagenseil J, Mecham RP. Elastin in large artery stiffness and hypertension. *J. Cardiovasc. Transl. Res*. 2012; 5(3):264–273. [PubMed: 22290157]
84. Tham DM, Martin-McNulty B, Wang YX, Cunha VD, Wilson DW, Athanassios CN, Powers AF, Sullivan ME, Rutledge JC. Angiotensin II injures the arterial wall causing increased aortic stiffening in apolipoprotein E-deficient mice. *Am. J. Physiol. Regul. Integr. Comp. Physiol*. 2002; 283:R1442–R1449. [PubMed: 12388474]
85. Bartoli MA, Kober F, Cozzone P, Thompson RW, Alessi MC, Bernard M. In vivo assessment of murine elastase-induced abdominal aortic aneurysm with high resolution magnetic resonance imaging. *Eur J Vasc Endovasc Surg*. 2012; 44(5):475–481. [PubMed: 22939881]
86. Martin-McNulty B, Vincelette J, Vergona R, Sullivan ME, Yi-Xin W. Noninvasive measurement of abdominal aortic aneurysms in intact mice by a high-frequency ultrasound imaging system. *Ultrasound Med Biol*. 2005; 31(6):745–749. [PubMed: 15936490]
87. Santelices LC, Calano SJ, Erhart JC, Prantil RL, Haney JL, Vorp DA, Ahearn JM. Experimental system for ex vivo measurement of murine aortic stiffness. *Physiol. Meas*. 2007; 28:N39–N49. [PubMed: 17664666]

88. Guo X, Kassab GS. Variation of mechanical properties along the length of the aorta in C57bl/6 mice. *Am. J. Physiol. Heartt Circ. Physiol.* 2003; 285:H2614–H2622.
89. Zullinger MA, Fridez P, Hayashi K, Stergiopulos N. A strain energy function for arteries accounting for wall composition and structure. *J. Biomech.* 2004; 37:989–1000. [PubMed: 15165869]
90. Tanter M, Fink M. Ultrafast imaging in biomedical ultrasound. *IEEE Trans Ultrason Ferroelectr Freq Control.* 2014; 61(1):102–119. [PubMed: 24402899]
91. Shahmirzadi D, Konofagou EE. Quantification if arterial wall inhomogeneity size, distribution and modulus contrast using FSI numerical pulse wave propagation. *Artery Research.* 2014; 8:57–65. [PubMed: 24795784]
92. Fung, YC. *Biomechanics: mechanical properties of living tissues.* Springer-Verlag; New York: 1993.
93. Farrar DJ, Green HD, Bond MG, Wagner WD, Gobbeé RA. Aortic pulse wave velocity, elasticity, and composition in a nonhuman primate model of atherosclerosis. *Circ Res.* 1978; 43:52–62. [PubMed: 95906]
94. Blacher J, Asmar R, Djane S, London GM, Safar ME. Aortic pulse wave velocity as a marker of cardiovascular risk in hypertensive patients. *Hypertens.* 1999; 33:1111–1117.
95. Thubrikar MJ, Labrosse M, Robicsek F, Al-Soudi J, Fowler B. Mechanical properties of abdominal aortic aneurysm wall. *J Med Eng Technol.* 2001; 25(4):133–142. [PubMed: 11601439]
96. Bots ML, Hoes AW, Koudstaal PJ, Hofman A, Grobbee DE. Common carotid intima-media thickness and risk of stroke and myocardial infarction. *Circulation.* 1997; 96:1432–1437. [PubMed: 9315528]
97. O'Gara PT. Cardiology patient page: aortic aneurysm. *Circulation.* 2003; 107:e43–e45. [PubMed: 12591767]
98. Schillinger M, Exner M, Mlekusch W, Sabeti S, Amighi J, Nikowitsch W, Timmel E, Kicking B, Minar C, Pones M, Lalouschek W, Rumpold H, Maurer G, Wagner O, Minar E. Inflammation and carotid artery - risk for atherosclerosis study (icaras). *Circulation.* 2005; 111:2203–2209. [PubMed: 15851593]
99. Nandlall, SD.; Shahmirzadi, D.; Apostolakis, IZ.; Finan, JD.; Delpratt, N.; Morisson, B., III; Konofagou, EE. Mapping the local stiffness of murine aortas using pulse wave imaging and validation with atomic force microscopy. 9th International Conference in Ultrasonic Biomedical Microscanning; Eddleston, Scotland, United Kingdom. 2014;

Figure 1.
Timeline of the mouse experiments designed in the current study. (A20: mice on 20 weeks of high-fat diet, A30: mice on 30 weeks of high-fat diet)

Figure 2.

Illustration of the post-processing methodology used in the current study

Figure 3.

Sequence of PWI images every 1.25 ms (i.e. 10 frames) in a normal aorta. The axial wall velocities are color-coded and overlaid onto the B-mode image. Positive velocities (in red) represent upward motion while negative velocities (in blue) represent downward motion. The solid blue arrows indicate the propagation of the pulse wave from the proximal to the distal site ((a)-(d)).

Figure 4.

Sequence of PWI images every 1.25 ms in an aorta of a mouse that has been under high-fat diet for 30 weeks. The solid blue arrows indicate the propagation of the pulse wave, while the solid red arrow indicates the stenotic region.

Figure 5.

Sequence of PWI images every 1.25 ms in a mouse receiving angiotensin II. The solid blue arrows indicate the propagation of the pulse wave, while the solid red arrow indicates the aneurysmal region.

Figure 6.

Sequence of PWI images every 1.25 ms in a carotid artery of a mouse that before the initiation of the high-fat diet. The solid blue arrows indicate the propagation of the pulse wave.

Figure 7.

The sequence of PWI images every 1.25 ms in a carotid artery of a mouse that has been under high-fat diet for 30 weeks. The solid blue arrows indicate the propagation of the pulse wave, while the solid red arrow indicates the stenotic region.

Figure 8.

Spatio-temporal imaging of pulse-wave propagation in murine aortas along with the piecewise linear regression of the 50% upstroke markers overlaid on each spatio-temporal plot. The sub-regional PWV and r^2 coefficient values are shown next to each segment, while the regional PWV and r^2 coefficient values for the whole imaged aortic region are shown on top of each spatio-temporal plot. The spatio-temporal plots above correspond to (a) a normal mouse aorta, (b) a stenotic mouse aorta after 30 weeks of high-fat diet and (c) an aneurysmal mouse aorta. In the latter two plots, the focal region of the disease is also indicated.

Figure 9.

Spatio-temporal imaging of pulse-wave propagation in murine carotid arteries along with the piecewise linear regression of the 50% upstroke markers overlaid on each spatio-temporal plot. The sub-regional PWV and r^2 coefficient values are shown next to each segment, while the regional PWV and r^2 coefficient values for the whole imaged aortic region are shown on top of each spatio-temporal plot. The spatio-temporal plots above correspond to (a) a normal mouse carotid artery and (b) an atherosclerotic mouse carotid artery. In (a) a characteristic V-shape is delineated using dashed lines to indicate forward propagation of the forward pulse (left dashed line of the V) as well as the backward propagation of a reflected wave (right dashed line of the V).

Figure 10.

Localized PWV and stiffness maps for the case of a mouse aorta (a) before the start of the high-fat diet, (b) after 20 weeks of high-fat diet and (c) after 30 weeks of high-fat diet. In the latter case stenosis has developed on the distal end of the aorta as indicated by the solid red arrow. Both the sub-regional PWVs and E_{PWI} have been color-coded and overlaid onto the B-mode images.

Figure 11.

Localized PWV and stiffness maps for a mouse aorta (a) before the implantation of the angiotensin II pumps, (b) 6 days after pump implantation and (c) 16 days after pump implantation. In the latter case an aneurysm has developed on the distal end of the aorta as indicated by the solid red arrow. Both the sub-regional PWV and E_{PWV} have been color-coded and overlaid onto the B-mode images.

Figure 12.

B-mode images along with localized PWV and stiffness maps for the case of a normal mouse carotid artery (a, b) and an atherosclerotic carotid artery (c, d). In the atherosclerotic carotid, stenosis has developed on the distal end of the artery as indicated by the solid red arrow (c, d). Both the sub-regional PWV and E_{PWV} values have been color-coded and overlaid onto the B-mode images (b, d).

Figure 13.

Statistical results on seven normal ($n = 7$, Normal) aortas along with six aortas after 20 weeks of high-fat ($n = 6$, A20), four aortas ($n = 4$, A30) after 30 weeks of high-fat diet and six aneurysmal aortas ($n = 6$, AAA). (a) Mean sub-regional PWVs over the aortic wall averaged over the corresponding mouse population. (b) Mean sub-regional E_{PWV} values over the aortic wall averaged over the corresponding mouse population in each case. (c) Mean sub-regional r^2 coefficient over the aortic wall averaged over the corresponding mouse population in each case. (d) Standard deviation of the sub-regional PWVs over the whole imaged aortic wall averaged over the corresponding mouse population in each case. (e) Mean arterial wall thickness averaged over the corresponding mouse population in each case. (f) Mean peak PWV wall velocities over the whole imaged aortic wall averaged over the corresponding mouse population in each case. Error bars denote standard deviation and significances are marked on the plot.

Figure 14.

Examples of using B-mode, B-mode-derived values and PWI-derived data to infer the nature of nonlinear propagation in (a) a normal mouse aorta, (b) an atherosclerotic mouse aorta and (c) an aneurysmal mouse aorta. In each case PWV and E_{PWI} maps are followed by plots of local diameter, arterial wall thickness measurements and local r^2 values.

Table I

Mean piecewise PWVs, E_{PWV} and piecewise r^2 coefficients for the mouse carotid arteries after 5, 20 and 30 weeks of high fat diet

High fat diet duration	Mean piecewise PWVs (m/s)	Mean piecewise E_{PWV} (kPa)	Mean piecewise r^2 coefficient	Mean arterial wall thickness (μm)
5 weeks (n = 7)	2.03 ± 0.20	19.50 ± 5.15	0.87 ± 0.04	85 ± 9
20 weeks (A20, n = 6)	2.12 ± 0.07	21.17 ± 4.036	0.62 ± 0.25^I	99 ± 81
30 weeks (A30, n = 4)	2.08 ± 0.22	15.24 ± 4.87	0.80 ± 0.12	449 ± 69^I

^I Significant (p 0.05) versus the 5-week case