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Quantification of Regional Aortic Stiffness Using MR Elastography: A Phantom and Ex-vivo Porcine Aorta Study

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Abstract

MR Elastography (MRE) is a noninvasive technique for measuring tissue stiffness that has been used to assess the average stiffness of the abdominal aorta. The utility of aortic MRE would be improved if it could provide information about local variations in aortic stiffness. We hypothesize that regional variations in aortic stiffness can also be measured with MRE and the purpose of this work was to demonstrate that MRE can measure regional stiffness variations in a vascular phantom and in ex vivo porcine aortas.

A vascular phantom was fabricated, containing two silicone tubes embedded in gel. A segment of one of the tubes was modified to increase its stiffness. MRE was performed on the phantom with a continuous flow of water through the tubes. The stiffness distribution along the modified tube was measured and compared to the reference tube. MRE was also performed in porcine aortas embedded in gel with segments treated with saline or formalin for 4 days. The stiffness difference between saline- and formalin-treated aortic segments was measured by MRE and mechanical tests.

A positive correlation was found between the regional stiffnesses measured by MRE and mechanical tests. The results indicate that MRE can be used to evaluate the local stiffness distribution in silicone tubes and ex vivo porcine aortas. It may therefore be possible to apply MRE to measure regional stiffness variations of the aorta in vivo.

Keywords

MR elastography; Aorta; Stiffness

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1. Introduction

Increased aortic stiffness has been proven to have a significant association to the onset and progression of cardiovascular diseases, such as hypertension [1], systolic hypertension, atherosclerosis [2–4], and aortic aneurysm [5, 6]. Aortic stiffness is also a very important clinical indicator to predict future cardiovascular events and death, especially among hypertensive patients [7, 8]. Thus, aortic stiffness assessment by using pulse wave velocity (PWV) has become a routine procedure in clinical practice.

Recent studies have demonstrated regional variation of stiffness can provide more information for clinical diagnosis. From animal study, local pulse wave velocity (PWV) can provide early information about the local progression of atherosclerosis [3]. Higher regional aortic stiffness, especially at aortic root and ascending aorta, is associated with higher rates of surgical aortic replacement and aortic root dilation in children and young adults with connective tissue disorders [9]. Regional PWV was found correlated with different cardiovascular risk factors [10], different incidence and prognosis of various cardiovascular diseases in hypertensive patients [11].

PWV is one of the methods to evaluate global aortic stiffness [12]. However, because of the inaccurate estimation of aortic length from body surface anatomical landmarks, the accuracy of PWV assessment is controversial [13]. Regional aortic stiffness may better reflect the mechanical property distribution of the aorta. The local change in aortic diameter or area due to pressure along the aorta can be used to evaluate regional aortic stiffness, but the central pressure required for this analysis must typically be measured invasively by catheterization[14]. With the development of MR and ultrasound derived PWV, regional arterial stiffness of different aortic segments has shown the potential capability to evaluate the cardiovascular health and risk of patients [15–17].

MRE has been used in a few studies to assess aortic stiffness [18–20]. However, it has not been tested if MRE is capable of measuring regional aortic stiffness. In the present study, our aim was to investigate the feasibility of measuring regional differences in aortic stiffness using a silicone tube model of the aorta and ex vivo porcine aortas.

2. Materials and Methods

2.1 Phantom Study

A phantom study was first performed to assess wave propagation along an aorta with different elastic properties in two segments. Half of a 30-cm long silicone tube was wrapped with waterproof tape (Kendall™, Covidien) to simulate an aortic segment with higher wall stiffness. A tissue-simulating 10% B-gel (Bovine Skin Powder, Sigma-Aldrich, St. Louis, MO) was made in a rectangular container to mimic the abdominal tissue that mechanical vibrations normally pass through during in vivo MRE. The tube was embedded at the bottom of the gel phantom and another silicone tube of the same type but without tape was embedded as well to serve as a control. Tap water was circulated through the embedded tubes during the scans.

Figure 1 shows the driver and phantom setup. A 10-cm-long plastic stick was attached to a plastic drum-like passive driver (6-cm diameter), which was connected to MRE active driver [19, 21]. The driver stick was positioned on one side of the phantom where the tap water flew out of the two tubes; the stick was also perpendicular to the two tubes so that it tapped the phantom right above the two tubes simultaneously. Tap water was continuously flowing in the two tubes to maintain stable pressure in case of collapse of the lumen. The exact flow rate was not recorded during the experiments, but it was set closed to the blood flow velocity during diastolic phase in vivo.

The phantom with the attached passive driver was placed in a single-channel transmit-receive (T/R) head coil. Imaging was performed on a 1.5-T whole-body MR system (Signa HDxt, GE Medical Systems, Waukesha, WI). For localization, coronal images were first acquired using a T2-weighted single-shot fast spin echo (SSFSE) sequence. A 2D, non-gated, flow-compensated, gradient-echo MRE sequence was used to image the wave propagation in the phantom [22].

The data acquisition parameters for MRE were as follows: single 6-mm-thick coronal slice, repetition time/echo time (TR/TE) = 50.1/26.6 ms, 256×96 acquisition matrix with a 0.75 FOV in the phase-encoding direction (interpolated to a 256×256 matrix), flip angle = 30° , FOV = 40 cm, and bandwidth = 31.25 kHz. The imaging plane was oriented along the longitudinal axis of both tubes. One 16.7-ms, 1.6-G/cm, first gradient moment nulled motion-encoding gradient was applied in the slice direction (dominating motion direction in the tubes). Ten phase offset images were acquired equally spaced over one cycle of the motion.

The MRE wave images were analyzed using MRE-Lab (Mayo Clinic, Rochester, MN). The coronal images were masked to the region of the tubes for MRE data analysis, including the wall and lumen. A one-dimensional directional filter was applied to the wave data to isolate wave propagation in one direction along the tubes and to remove the reflected wave information (Figure 2). The directional filter incorporated a 4th-order Butterworth bandpass filter with cutoff values of 1 – 40 waves/FOV to remove longitudinal waves and high-frequency noise [23]. The directionally filtered wave images were then processed using the local frequency estimation (LFE) and phase gradient (PG) inversion algorithms to obtain effective shear stiffness maps of the tube, μ_{MRE} [19, 20].

2.2 Ex Vivo Porcine Aorta

4 ex vivo porcine aortas were obtained from seven-month-old male domestic healthy pigs (100–130 kg) within 15 minutes of slaughter from a local commercial slaughterhouse. The aortas were immersed in 0.9% saline solution (0.9% sodium chloride USP pH 5.0(4.5 to 7.0) mEq/L solidum 154 chloride 154 osmolarity 308 mOsmol/L, Baxter Healthcare, IL, USA) and preserved at room temperature prior to examination. All the side branches were tied off. Figure 3 shows a picture of one of the excised porcine aortas.

The porcine aortas were cut to about 28 cm in length and were embedded in 10% B-gel as was done for the phantom study described above. 3 aortas were embedded in a single gel, and the other aorta used for control was embedded in another gel phantom. Glass rod was

placed in each aortic lumen before being embedded in the hot gel to prevent the collapse due to the weight; when the B-gel cooled down and solidified, the glass rod was removed from the aortic lumen for MRE experiments. MRE was performed on each porcine aorta with the same driver arrangement and MRE protocol as the phantom study. Same to the phantom study, tap water was circulated through the aortas during the scans. The total time from death of the animal to completion of all examinations on the aortas was approximately 12 hours.

After MRE scan, all the aortas were removed from the gel, and the 3 aortas were wrapped with 2 pieces of gauze, one piece covering each half of the length of the aorta. One piece of gauze was soaked with saline and the other was soaked with 10% neutral buffered formalin (NBF) (Fisher Scientific Company L.L.C., Kalamazoo, MI). The fourth aorta sample was wrapped with only saline gauze for standard reference. Aortic samples were reexamined using the same driver arrangement and MRE protocol two times at 2, 4 days. The aortas were embedded in gel 6 hours before each reexamination with gauze removed. During the course of the study, saline and NBF was added to the gauze every 3 hours to prevent evaporation.

All the images were analyzed with the same procedure that was used for the phantom study. The effective shear stiffness maps were used to report μ_{MRE} for all of the aorta samples.

Within 6 hours after the last MRE scan, mechanical testing was performed on each aorta sample. We used disk geometries with 15-cm diameter and 2-mm thickness for all of our aorta samples that underwent RheoSpectris test (RheoSpectrisTM C500+, Rheolution Inc., Canada). Each sample was fixed horizontal on the circular cavity without visible curvature. (Figure 4) Optical beam was then focused at the center of the circular upper surface of the disk sample for mechanical testing at 60Hz. 3 positions in the 3 treated aortas, including the saline segment, formalin segment and junctional part were chosen for mechanical testing, as well as two locations in the reference aorta.

2.3 Statistical analysis

Continuous data were expressed as mean $\pm$ standard deviation. The changes in segmental aortic μ_{MRE} among different days were compared using Friedman's test. Correlation between days of preservation and μ_{MRE} of the formalin-fixed aorta samples, as well as the stiffnesses measured with MRE and mechanical testing was calculated with Spearman's rank correlation coefficient. A p value less than 0.05 was considered to indicate a statistically significant difference. A statistical software package (SPSS 19.0, SPSS Inc., USA) was used for statistical analysis.

3. Result

3.1 Phantom Study

The mechanical waves were easily transmitted to and well visualized within the lumen of the silicone tubes embedded in the B-gel phantom (Figure 2); Figure 2A shows the original wave propagating in the tubes, while Figure 2B and 2C show directional filter processed forward and backward wave propagation, respectively. The longest wavelength appeared at

the taped part of the silicone tube. The wavelength of the untaped part was similar to that in the reference standard tube (Figure 5).

Figure 6 shows that the MRE-derived stiffness in the taped part of the tube was 110.9 kPa using the PG algorithm, and 102.0 kPa using LFE by averaging within a rectangular ROI. The stiffness in the untaped portion was similar to the stiffness in the reference tube (PG: 57.9 kPa vs. 48.2 kPa, LFE: 57.2 kPa vs. 45.1 kPa, respectively).

As shown in Figure 2A, in the area where PG inversion had “high-stiffness artifact”, but not LFE, the wave amplitude was very low due to attenuation. Therefore, we excluded this area in calculating aorta stiffness because neither PG nor LFE was able to do the inversion accurately in this area with such a low wave amplitude.

3.2 Ex Vivo Porcine Aorta

Wave propagation could be well visualized in all of the aortas and wavelength and stiffness differences were observed between the different segments of the aortas treated with saline and formalin (Figure 7).

As shown in Table 1, there was no significant difference in stiffness μ_{MRE} between the two halves of the fresh aortas before treatment. After treatment with saline and formalin, there was a significant increase of μ_{MRE} in the formalin-treated segments, but no significant increase in the saline-treated segments.

Linear correlation was found between μ_{MRE} and the days of treatment for the formalin-treated aortic segments ($\rho_{PG}=0.900$, $P=0.001$; $\rho_{LFE}=0.843$, $P=0.004$) (Figure 8).

Using mechanical testing, the Young's modulus (another measure of stiffness) of the aorta samples increased from day 0 (the reference aorta) to day 4 of formalin treatment (234.6 ± 81.0 kPa to 442.8 ± 75.7 kPa). Young's modulus of junctional parts was 346.7 ± 119.6 kPa. Linear correlations were observed between the MRE-measured stiffness μ_{MRE} and the Young's modulus from the mechanical tests in 11 regions of the 4 aortas ($\rho_{PG}=0.733$, $P=0.010$; $\rho_{LFE}=0.609$, $P=0.047$) (Figure 9).

4. Discussion

In this phantom study, we were able to observe a difference in the stiffness of taped and untaped regions of a silicone tube by using MRE. Furthermore, we were also able to differentiate the stiffness of saline- and formalin-treated regions of ex vivo porcine aortas using the same technique. There was also a moderate correlation between the stiffness measurements using MRE and mechanical testing in the treated and untreated aortas.

The results indicate that MRE can be used to measure regional stiffness changes of the aorta. The shear stiffness of the fresh ex vivo porcine aortas calculated using the LFE inversion algorithm in this study was 8.4 ± 1.4 kPa, which was comparable with the results in the literature for in vivo human aortic stiffness measurements using MRE with 60-Hz vibrations by Damughatla et al. (4.0 ± 0.9 kPa for young people, 7.4 ± 2.1 kPa for the old) [20]. And using PG inversion algorithm, also similar to Kolipaka et al study (3.7 ± 0.8 kPa for

normotensive people, 9.3 ± 1.9 kPa for the hypertensives), shear stiffness of fresh ex vivo porcine aortas was 6.6 ± 2.5 kPa. Fixatives can alter the cells or tissues on a molecular level to increase their mechanical strength or stability, and can help to preserve the shape and structure of the sample [24]. Because of the volatilization of formalin, the stiffness of the portions of the saline-treated segments adjacent to the formalin-treated segments was affected by the formalin as indicated by the mechanical testing result. Therefore, there was a gradual stiffness variation between the saline- and formalin-treated areas of the aortas. This reduced the delineation between the two regions in the MRE stiffness images. Furthermore, the MRE inversion algorithms themselves suffer from boundary effects which blur the boundaries between regions with different stiffnesses as the inversions assume wave propagation in homogeneous materials.

By using Eq. (1), assuming aorta (soft tissue) to be incompressible (Poisson's ratio (ν) = 0.5), μ_{MRE} can be converted to Young's modulus. Young's modulus should be 3 times larger than μ_{MRE} with the same frequency. But in this study, the MRE results are about a factor of 5–6 lower than the static shear modulus (Young's modulus/3). The reason may be due to geometric change, which includes (1) imperfect horizontal fixation on the circular cavity without curvature; (2) inconsistent compression at the sample edges; (3) uneven thickness of the vascular wall (not equal to 2mm exactly) during mechanical test. Additionally, the room temperature was different between the MRI scanner room and the RheoSpectris lab

$$\mu_{MRE} = \frac{\text{Young's modulus}}{2(1+\nu)} \quad (1)$$

Reflected waves can significantly impact the accuracy of MRE stiffness measurements. Because of the constrained wave propagation along the tube and aortas in this study, we used 1D directional filters to remove the reflected waves. After directional filtering, there was still some reflected wave information at distal ends of the tube and aortas. In the PG stiffness map, hot spot artifacts were observed in these locations. The LFE inversion algorithm did not create these same hot spots.

MRE has been demonstrated as a possible technique to assess the stiffness of the aorta in vivo [18–20]. However, the relatively long wavelength observed in the aorta in those studies has limited the spatial resolution of aortic MRE. There are several reasons for the relatively long wavelength in the aorta. First, the abdominal aorta is located in the retroperitoneal area of the body, so there is significant attenuation of the externally applied vibrations required for MRE as the waves propagate to the aorta. Thus, low-frequency external vibrations of 60 Hz [18–20] were used to generate shear waves in those studies. Second, aortic MRE can be interpreted as being affected by waveguide effects because the wavelength is long compared to the width the aorta [18–20, 24, 25]. The waveguide effect can increase the apparent shear wave speed and wavelength. Third, the stiffness of the aorta itself affects the wavelength, and similar with myocardium [26], this stiffness may even change during the cardiac cycle due to pressure change within blood pulsation through the aorta.

In this study, we artificially divided the aorta samples into 2 segments with lengths of 15 cm each and the difference in the stiffness between the two segments could be seen using MRE.

It is still unclear if the stiffness changes could be assessed in shorter aortic segments. Improvements in the spatial resolution for MRE of the aorta may be required to investigate stiffness variations of smaller segments and will be the subject of future investigations.

There were several limitations to the current study. The current MRE technique did not directly assess the absolute aortic wall stiffness, but rather used shear wave motion along the aorta that was conducted into the intraluminal fluid as an effective measure of stiffness. Taking into account the geometry and boundary effects of the aorta may be required to get the true stiffness. A 2D single-slice acquisition and analysis was performed, whereas a 3D vector MRE technique may provide additional information to improve the stiffness measurements. A constant flow of water through the aortic lumen was used and pressure changes during the cardiac cycle were not considered. In phantom study, there was no control tube for the tape-wrapped segment of the silicone tube, and we don't have independent mechanical test results for silicone tube stiffness. Finally, these experiments were performed with the vessels embedded in gel to approximate the impact the body has on waves as they propagate toward the vessels, including scattering, attenuation, and dispersion effects. However, the in vivo environment is much more complicated than our model, including heterogeneous tissue properties and numerous tissue boundaries, and this may affect how well waves in the aorta can be detected.

5. Conclusion

In conclusion, this study demonstrated the feasibility of using MRE to assess altered regional stiffness in a vascular phantom of a silicone tube by comparison with a reference homogeneous tube. The results also showed that MRE can depict a significant difference in the stiffness of saline- and formalin-treated aortic tissue segments. MRE stiffness values correlated with tissue stiffness measurements made with mechanical tensile testing. These results provide motivation for further developing and assessing MRE as a tool for potentially assessing regional aortic stiffness in vivo.

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Figure 1.
Schematic of the driver and phantom setup

Figure 2.

Phantom MRE Wave Images

A) One offset of the acquired wave data in the phantom. B) and C) The forward and reflected waves isolated using a 1D directional filter.

Figure 3.
Excised porcine aorta

Figure 4.
Aortic sample fixed horizontal on the holder with circular cavity

Figure 5.

Wave Profiles in the Phantom

A) Profile in the taped tube: The wavelength in the half of the tube with tape around it was longer than in the untaped half and the reference tube. B) Profile in the reference tube: The wavelength is uniform and is similar to the untaped region of the taped tube.

Figure 6.

Stiffness Maps of the Phantom

The shear stiffness of the taped half of the tube was higher than the untaped half and the reference tube in both the PG and LFE stiffness maps. A high-stiffness artifact created by wave reflections at the distal part of the reference tube in the PG stiffness map was avoided in the analysis.

Figure 7.

Wave profiles and stiffness maps of 2 aorta samples after 4 days of treatment.

(A,B) Wave profile and shear stiffness maps obtained from an aorta sample after 4 days of treatment – the top half with saline and the bottom half with formalin. Differences in the wavelength and shear stiffness in the two regions can be observed. The shear stiffness in the formalin-treated half (PG: 17.9 kPa, LFE: 19.4 kPa) was higher than the saline-treated half (PG: 3.9 kPa, LFE: 4.9 kPa). (C,D) Reference aorta sample only treated with saline for 4 days. The wavelength and shear stiffness distributions are more homogenous in the reference sample (PG: 9.2 kPa, LFE: 13.3 kPa).

Figure 8.

Relationship between μ_{MRE} and the number of days of formalin treatment of the aorta samples.

From MRE results, stiffness of formalin-treated aortic segments increased with the days of treatment, and linear correlation was found ($r_{PG}=0.917$, $P=0.001$; $r_{LFE}=0.887$, $P=0.001$).

Figure 9.

Relationship between the MRE-measured shear stiffness and the Young's modulus from mechanical testing of the aorta specimens.

Compared with mechanical testing, linear correlation was found between the MRE-measured stiffness μ_{MRE} and the Young's modulus from the mechanical tests ($r_{PG}=0.751$, $P=0.08$; $r_{LFE}=0.638$, $P=0.035$).

Table 1

MRE-measured stiffness μ_{MRE} of the aorta samples during treatment.

Treated ^a	Inversion Algorithm	Fresh	2 days	4 days	X ²	P
Saline	μ_{MREPG} (kPa)	5.6±2.5	7.6±2.6	7.1±1.4	4.667	0.097
	μ_{MRELFE} (kPa)	8.0±1.2	10.1±2.1	11.0±1.7	4.667	0.097
Formalin	μ_{MREPG} (kPa)	8.6±2.5	16.4±2.4	19.3±0.5	6.000	0.05
	μ_{MRELFE} (kPa)	8.8±1.8	16.7±3.3	19.3±1.2	6.000	0.05

^aContinuous data are presented as mean ± standard deviation.