Published in final edited form as:

IEEE Trans Ultrason Ferroelectr Freq Control. 2018 February ; 65(2): 188–200. doi:10.1109/TUFFC.

2017.2781654

Application of Acoustoelasticity to Evaluate Nonlinear Modulus in *ex vivo* Kidneys

Sara Aristizabal¹, Carolina Amador¹, Ivan Z. Nenadic¹, James F. Greenleaf¹, and Matthew W. Urban^{1,2}

¹Department of Physiology and Biomedical Engineering, Mayo Clinic, Rochester, MN, USA

²Department of Radiology, Mayo Clinic, Rochester, MN, USA

Abstract

Currently, dynamic elastography techniques estimate the linear elastic shear modulus of different body tissues. New methods that investigate other properties of soft tissues such as anisotropy, viscosity and shear nonlinearity would provide more information about the structure and function of the tissue and might provide a better contrast than tissue stiffness and hence provide more effective diagnostic tools for some diseases. It has previously been shown that shear wave velocity in a medium changes due to an applied stress, a phenomenon called acoustoelasticity (AE). Applying a stress to compress a medium while measuring the shear wave velocity versus strain provides data with which the third order nonlinear shear modulus, A , can be estimated. To evaluate the feasibility of estimating A , we evaluated ten *ex vivo* porcine kidneys embedded in 10% porcine gelatin to mimic the case of a transplanted kidney. Under assumptions of an elastic incompressible medium for the AE measurements, the shear modulus was quantified at each compression level and the applied strain was assessed by measuring the change in thickness of the kidney cortex. Finally, A was calculated by applying the AE theory. Our results demonstrated that it is possible to estimate a nonlinear shear modulus by monitoring the changes in strain and μ due to kidney deformation. The magnitudes of A are higher when the compression is performed progressively and when using a plate attached to the transducer. Nevertheless, the values obtained for A are similar to those previously reported in the literature for breast tissue.

Index Terms

Shear modulus; Nonlinearity; Acoustoelasticity; Kidney; Shear wave

I. Introduction

One of the most dangerous diseases that affect the health of kidneys is end-stage renal disease (ESRD), an irreversible deterioration of the renal function in which the body loses its ability to maintain the metabolic and fluid balance. The treatments available for ESRD include dialysis and kidney transplantation. For patients with ESRD, a transplant provides a better survival rate and quality of life than dialysis [1]. After the patient undergoes transplantation, biopsies are performed to monitor the health of the kidney by histological examination of the renal tissue over time. Alterations in the structure, such as fibrosis, can lead to decline in function of the kidney and ultimate rejection of the kidney transplant. The

invasive nature of renal biopsies is an impetus to investigate if other noninvasive methods such as medical imaging, or in particular elastography, could be used as indicators of the presence of renal fibrosis in the transplanted kidney.

Dynamic elastography methods rely on the propagation of shear waves for the noninvasive evaluation of the mechanical properties of soft tissues [2–5]. The basic principle of most shear wave elastography techniques relies on the use of focused ultrasound to generate acoustic radiation force to vibrate the tissue and generate shear waves. The shear wave velocity is then tracked as a function of time and distance and under the assumptions that the tissue is elastic, isotropic and homogeneous; it can then be related to the linear shear modulus of the tissue. Based on these principles acoustic radiation force-based techniques such as acoustic radiation force impulse (ARFI), supersonic shear imaging (SSI) and shear wave dispersion ultrasound vibrometry (SDUV) can accurately diagnose numerous clinical conditions by successfully discriminating healthy tissue from abnormal tissue based on the tissue stiffness which is directly related to the magnitude of the second order linear shear modulus of the tissue (μ) [2, 4, 5]. However, the diagnostic potential of these techniques is limited as the knowledge of the second order linear shear modulus might not be sufficient to reach an accurate clinical diagnosis [6–8]. To better understand pathological changes in soft tissues, advanced mechanical properties such as anisotropy, viscosity and shear nonlinearity need to be explored as they could provide more information about the structure and function of the tissue and hence become a better diagnostic tool for some diseases.

It has been demonstrated previously that soft tissues have specific nonlinear parameters that become evident when subjected to large deformations [9–12]. At low stress levels soft tissues typically exhibit linear features; on the other hand at large levels of stress tissues exhibit strain hardening, a phenomenon characterized by a nonlinear stress-strain relation where the tissue elasticity becomes strain dependent leading to an increase in the shear modulus upon applied strain [12, 13]. Krouskop, *et al.* [14] have previously shown the possibility of characterizing the nonlinear response of breast tissue by applying a mechanical force like compression loading. Plots of measured stress compared to applied strain for different types of breast tissue revealed that the elastic modulus of glandular and fibrous breast tissue remains constant over a small strain range (10%) when subjected to compression loading and thereafter it steepens with the applied strain. These results demonstrate a dependence of the elastic modulus with the pre-compression strain level. Fibrous tissue exhibited a higher elastic modulus when a higher pre-compression and therefore higher strain was applied than that observed for glandular tissue. This indicates that the elastic modulus of certain types of tissue might be indistinguishable from others at low strain levels but the modulus could be differentiated better at higher levels of strain. The fact that the nonlinear behavior exhibited by each type of tissue is unique, if measured could provide a better contrast than tissue stiffness that can help discriminate progressive pathological changes in tissues.

More recently, Sayed *et al.* introduced a new non-invasive method to characterize breast masses based on their non-linear mechanical behavior when subjected to compression using multi-compression elastography imaging. A pilot study involving ten patients showed that

the estimated nonlinear parameter, were in good agreement with the biopsy outcomes, and provided an enhanced distinction between soft tissues and stiff lesions [15].

A few studies [16–18] have previously characterized nonlinear mechanical properties of excised pig kidneys using compressive and tensile mechanical tests. In addition, Emelianov *et al.* showed the feasibility of monitoring scarring and inflammation in two *ex vivo* canine kidneys using reconstructive elasticity images by monitoring elasticity changes in response to compression before and after a crosslinking agent was injected to increase the medium stiffness [12, 19]. These studies have shown that kidneys exhibit generally nonlinear features under compression, which are commonly observed in soft tissues.

Syversveen, *et al.* evaluated the impact of transducer force on the shear wave velocity measurements obtained in transplanted kidneys by applying increasing compression weights in 31 patients and measuring the shear wave velocity after each compressive force was applied [20]. The result was an increase in shear wave velocity as stress was being applied indicating that a nonlinear phenomenon was occurring in the tissue known as Acoustoelasticity (AE) [7, 8, 20]. The acoustoelastic effect is defined as the change of wave speed when a static stress is applied to an elastic and quasi-incompressible body in the form of uniaxial compression or hydrostatic pressurization [21]. Experimentally AE consists of assessing the change of shear wave speed when the media are being subjected to compression and the third order nonlinear modulus A can then be estimated from the measured wave speed as a function of the stress being applied to the media [7, 8]. Previous studies have shown the potential of AE to evaluate the nonlinear mechanical characteristics of tissue mimicking materials and soft tissues [6–8, 22]. Bernal, *et al.* used the AE theory to study breast cancer detection using the nonlinear characteristics of the tissue. To evaluate the efficacy of the technique they evaluated the nonlinear mechanical properties of breast tissue in 8 volunteers. Manual compression was applied to the breast in six compression steps, and the shear modulus and locally applied uniaxial strain were calculated during the compression and finally a quantitative map of the nonlinear modulus was constructed using the AE theory. The results obtained by Bernal, *et al.* showed that the sensitivity of the nonlinear modulus contributed to a very good contrast of the malignant lesions to the healthy tissue. Similar findings related to image contrast have been reported by Latorre-Ossa *et al.* in agar-gelatin phantoms with soft and hard inclusions and in three beef liver samples. Guidelines for clinical use of shear wave elastography in different applications often advocate that minimal compression should be used as it is known to affect the results [20, 23–25]. We hypothesize that it is possible to use the variations of shear wave speed versus applied uniaxial stress in elastic media to estimate another parameter such as shear nonlinearity, which can further help characterize the elastic properties of the kidney.

Each tissue type has specific nonlinear elastic parameters and the progression of tissue fibrosis might be better detected when the kidney is being compressed as has been demonstrated in the case of breast and prostate cancer [14, 22]. As opposed to native kidneys which are typically deep in the body (4–5 cm), transplanted kidneys are relatively shallow because of the need to biopsy them on a regular basis [19, 20]. This presents a unique opportunity to use AE in the transplanted kidney for advanced elastographic characterization.

In this study, we wanted to assess the feasibility of estimating the third order nonlinear shear modulus in the kidney by using shear wave elastography combined with external compression [26]. A series of ten phantoms were created by embedding *ex vivo* kidneys in tissue-mimicking gelatin to create a model for a transplanted kidney. Furthermore, we conducted a parametric study to evaluate the nonlinear shear characteristics of *ex vivo* porcine kidneys under different conditions such as the presence or absence of a plate for compression, the direction of compression and the different views of the kidney and calculated the value of A using the acoustoelasticity theory. Our goal is that the aforementioned study will not only help us evaluate the feasibility of evaluating A in the kidney, but it will also help us evaluate the consistency in the estimation of A in order to guide future patient studies.

The rest of the paper is organized as follows: In Section II, the fabrication of *ex vivo* kidney phantoms, a description of the configurations evaluated as well as the experimental setup, and the analysis techniques used to evaluate the nonlinear properties of these phantoms are presented. B-mode images with SWE overlays, shear modulus variation with strain results and the summary of A and shear modulus values across the phantoms evaluated are presented in Section III. Statistical analysis of A and shear modulus results across the different configurations are evaluated in Section III. Finally, we discuss the results and conclusions in Sections IV and V, respectively.

II. Materials and methods

A. Principles of Acoustoelasticity

The principle of acoustoelasticity in soft solids is based on the effect of uniaxial compression on the speed of acoustic waves in elastic, isotropic, homogeneous and “nearly” incompressible media [7]. Experimentally, this requires estimating a nonlinear modulus, defined as A using estimates of shear modulus and strain when the media are subjected to uniaxial stress. The AE theory relates then the stress applied to the media with the shear wave speed using the following wave speed equation [7],

$$\rho V_s^2 = \mu_0 - \sigma \left(\frac{A}{12\mu_0} \right), \quad (1)$$

where, V_s represents the shear wave speed, μ_0 is the elastic shear modulus at baseline (no to minimal compression), σ is the stress applied to the media and A is the third order nonlinear modulus.

In experiments such as those performed by Latorre-Ossa, *et al.*, and Bernal, *et al.*, strain elastography was applied in progressive steps and shear wave measurements were made at each step [6, 8]. In this case the accumulated stress and strain were used. To obtain the stress at a given compression step, we need to compute the accumulated stress from all the previous compression steps. We replace the stress term σ in (1) with the following Hooke’s law definition for the cumulative stress assuming incompressibility,

$$\sigma_i = \sum_{j=1}^i \Delta\sigma_j = \sum_{j=1}^i 3\mu_j \Delta\epsilon_j, \quad (2)$$

where, μ_j is the shear modulus for compression step j and ϵ_j is the differential strain generated at compression step j then the nonlinear wave speed equation can be rewritten as

$$\rho V_{s,i}^2 = \mu_0 - \left[\sum_{j=1}^i 3\mu_j \Delta\epsilon_j \right] \left(\frac{A}{12\mu_0} \right). \quad (3)$$

In (2) we are computing the sum of the products of the Young's moduli ($3\mu_j$) and the differential strain, ϵ_j , to get the differential stress. If we further rearrange this equation and we use the relationship between the shear modulus and the shear wave velocity measured at a given compression step i ,

$$\mu_i = \rho V_{s,i}^2, \quad (4)$$

the nonlinear wave speed equation becomes,

$$\mu_i = \mu_0 - \left[\sum_{j=1}^i 3\mu_j \Delta\epsilon_j \right] \left(\frac{A}{12\mu_0} \right) \quad (5)$$

Then, by measuring the linear shear modulus at no applied stress, μ_0 , and the shear modulus μ_j and the differential strain, ϵ_j , generated due to the uniaxial stress applied at each compression step j we can estimate the nonlinear shear modulus A . The complete mathematical derivation can be found in [6–8].

B. Phantom fabrication & Experimental set up

The nonlinear shear modulus A was measured in ten *ex vivo* porcine kidneys embedded in 10% porcine 300 Bloom gelatin (Sigma-Aldrich, St. Louis, MO) (Figs. 1(a) and 1(b)). The kidneys were obtained from female pigs, with approximate weight of 30 kg. The kidneys were suspended in the middle of the gelatin block with dimensions 6.8 cm $\times$ 6.8 cm (width and height), allowing 2–3 cm between the kidney and the top and bottom surfaces of the phantom to allow room for compression. The phantoms were placed over a flat and uniform surface, and to induce a uniform uniaxial stress, a rectangular plate (11.5 cm $\times$ 4.5 cm) was attached to the General Electric (GE) 9L linear array transducer (GE Healthcare, Wauwatosa, WI) which was subsequently attached to a motorized stage (Fig. 1(c)). The linear motor was programmed to compress in steps of 2 mm from 0% compression to a total displacement of 16 mm which corresponds to 25% compression given the phantom's initial thickness. We also conducted the same test without the plate attached to the transducer. The sequence of testing can be described as follows: first, the phantoms were compressed using a plate attached to the transducer in the longitudinal view of the kidney and in two directions,

progressive direction wherein the transducer was axially displaced down onto the phantom from zero strain and then in the regressive direction wherein the compression was released by axially displacing the transducer upwards from maximal strain 2 mm at a time; the same test was then conducted in the transverse view of the kidney [26]. In the second part of the experiments, the phantoms were compressed in the absence of a plate, in both progressive and regressive directions of compression; first the compression was performed in the transverse view and then in the longitudinal view of the kidney. We performed 3–5 cycles of compression (progressive + regressive compression) for each experimental configuration [26].

C. Shear wave generation

Shear waves were generated and measured using a GE LOGIQ E9 ultrasound system (GE Healthcare, Wauwatosa, WI) equipped with a linear array transducer and running in the shear wave elastography mode [27]. Shear wave velocity maps were reconstructed using the in-phase/quadrature (IQ) data retrieved from the GE LOGIQ E9 system. The shear wave velocity was estimated from the distribution of particle motion, which was calculated by two-dimensional (2D) IQ auto-correlation method [28] after directionally filtering the left and right propagating waves [29]. A shear wave velocity map was generated at each compression step and the linear shear modulus μ_i was obtained by taking a median of the shear wave speed values within an ROI drawn in the middle portion of the kidney cortex in both the longitudinal and transverse plane as shown in Fig. 2. We obtained 8 compression steps for each AE data set; the time between compressions was approximately 5 seconds.

D. Acoustoelasticity analysis

To evaluate the nonlinear characteristics of the ten phantoms, the linear shear modulus was quantified at baseline with no compression (μ_0) and at each level of compression (μ_i) and a global measure of strain (ϵ_j) in the imaging plane was calculated by measuring the change in thickness of the kidney cortex at each compression step using the following equation,

$$\epsilon_i = \frac{\text{Change in thickness}}{\text{Original thickness}} \quad (6)$$

The nonlinear shear modulus A was then evaluated using the AE theory described in section 2.1, by fitting equation (5) to the estimations of linear shear modulus μ_j and strain ϵ_j obtained at each compression step. To fit the μ_j vs. ϵ_j data obtained experimentally to the AE theory (5), we used the MATLAB® least-squares fitting function `lsqcurvefit`. We used the least-squares fitting method (LSQ) that minimizes the sum of the squared residuals $(\mu_{\text{fit}} - \mu_i)^2$. In order to evaluate the goodness of the fit obtained using the LSQ method we used the root mean square error (RMSE) which is an error based metric and it can be defined as a measure of the difference between the values predicted by the model and the values obtained experimentally. We defined the RMSE threshold to be 1 kPa for fit acceptance based on the range of the linear shear modulus μ_i data and the presence of high error predictions due to outliers when RMSE was greater than 1 kPa. The measurements of A obtained from fits for which the RMSE was greater than the threshold were discarded, and the final values of A

were obtained from averaging the nonlinear modulus values with $\text{RMSE} < 1$ kPa obtained for each phantom from 3–5 runs of compression. Additionally, we also put a restriction on the values of A so that only negative values were retained for analysis corresponding to the available literature of measurement of A in soft tissues such as breast and liver [6] [8].

E. Statistical analysis

To compare the nonlinear and linear shear modulus values obtained across the different experimental configurations, we conducted a statistical analysis using the Wilcoxon signed-rank test, which is a non-parametric statistical test used to test the null hypothesis that the median difference between two related samples is zero [30]. We used the Wilcoxon signed-rank test to look for statistically significant differences among the values measured in the longitudinal view of the kidney with and without a plate as well as in the transverse view in both the regressive and progressive directions of compression. We considered a p-value less than 0.05 to be statistically significant.

III. Results

The B-mode images and overlaid shear wave velocity maps for one of the phantoms at three different levels of progressive and regressive compression in the longitudinal view of the kidney are illustrated in Fig. 2. Similarly, the B-mode images and overlaid shear wave velocity maps for one of the phantoms at three different levels of progressive and regressive compression in the transverse view of the kidney are illustrated in Fig. 3.

Figure 4 shows the measured linear shear modulus μ_i vs. strain ϵ_i for one of the phantoms when the compression was applied in both the progressive and regressive direction and with and without a plate attached to the transducer. The value of A was estimated by fitting the measured strain and linear shear modulus with (7) using the LSQ method. The estimates of A obtained when applying stress in the presence of a plate in the progressive and regressive direction were -12.59 and -12.25 kPa, respectively. Likewise, the estimates of A obtained when applying stress in the absence of plate in the progressive and regressive direction were -8.87 and -4.46 kPa, respectively.

The measurements of the nonlinear modulus in ten phantoms with progressive and regressive compression as well as in the presence or absence of a plate for the case where the experiment was performed in the longitudinal plane of the kidney are shown in Fig. 5. Similarly, the estimates of the nonlinear shear modulus in ten phantoms with progressive and regressive compression as well as in the presence or absence of a plate for the case where the experiment was performed in the transverse plane of the kidney are shown in Fig. 6. The error bars in Figs. 5 and 6 correspond to the standard deviation between three to five different acquisitions performed for each phantom. The range of A estimates obtained in the renal cortex for ten phantoms in the presence of a plate and in the longitudinal plane of the kidney ranged from -4.88 to -38.39 kPa when the compression was performed progressively and -3.02 to -21.49 kPa when the compression was performed regressively. In the transverse view, the nonlinear shear modulus estimates ranged from -6.01 to -35.08 kPa when the compression was performed progressively and -2.09 to -46.15 kPa when the compression was performed regressively.

In the absence of a plate the range of nonlinear shear modulus estimates in the longitudinal view were -1.53 to -24.91 kPa when the compression was performed progressively and -0.60 to -15.90 kPa when the compression was performed regressively. In the transverse view in the absence of a plate, the nonlinear modulus estimates ranged from -1.46 to -22.62 kPa when the compression was performed progressively and -1.92 to -16.94 kPa when the compression was performed regressively.

As mentioned before the strain was calculated from the B-mode images by measuring the fractional change in the thickness of the cortex when the phantoms were compressed up to 16 mm. We observed a maximum range of strain of approximately 20–35%.

The summary of the nonlinear shear modulus estimates obtained across the ten phantoms for the different parameters evaluated, in the presence or absence of a plate, both directions of compression, and two different planes of the kidney is shown in Fig. 7. The error bars correspond to the standard deviation between ten phantoms. For the estimates performed using progressive compression, the mean and standard deviations of the nonlinear shear modulus from ten phantoms in the presence of a plate in the longitudinal and transverse plane were -15.46 ± 6.42 and -17.72 ± 8.08 kPa and in the absence of a plate were -11.95 ± 7.18 and -9.24 ± 5.34 kPa, respectively. Similarly, for the estimates performed using regressive compression, the mean and standard deviations of the nonlinear shear modulus from ten phantoms in the presence of a plate in longitudinal and transverse plane were -11.97 ± 5.26 and -15.06 ± 9.50 kPa and in the absence of a plate were -6.78 ± 5.18 and -5.67 ± 3.08 kPa, respectively.

To explore the behavior of the linear shear modulus, the linear shear modulus at zero compression μ_0 was estimated across ten phantoms for the different parameters evaluated and the results are shown in Figs. 8 and 9. The error bars in Figs. 8 and 9 correspond to the standard deviation between three to five different acquisitions performed for each phantom.

The summary of the μ_0 estimates obtained in the presence or absence of a plate, both directions of compression, and two different planes of the kidney are summarized in Fig. 10. The error bars correspond to the standard deviation between ten phantoms.

For the measurements performed using progressive compression, the mean estimate of the linear shear modulus and mean standard deviations from ten phantoms in the presence of a plate in longitudinal and transverse plane were 1.95 ± 0.6 and 1.87 ± 0.6 kPa and in the absence of a plate were 1.76 ± 0.7 and 1.89 ± 0.6 kPa, respectively. Similarly, for the estimates obtained using regressive compression, the mean value of the linear shear modulus and mean standard deviations from ten phantoms in the presence of a plate in longitudinal and transverse plane were 1.85 ± 0.7 and 1.91 ± 0.6 kPa and in the absence of a plate were 1.81 ± 0.6 and 1.96 ± 0.6 kPa, respectively.

The results from the statistical analysis performed for the nonlinear modulus values using the Wilcoxon signed-rank test indicated that there was a statistically significant difference between the A estimates obtained when the experiment was performed in the presence and absence of a plate in the longitudinal and transverse view of the kidney as well as in the two directions of compression when there was a plate attached to the transducer. In the situation

where we compressed progressively with and without a plate attached to the transducer in the longitudinal and transverse view of the kidney the p -value was 0.03 and 0.003, respectively. When we compressed in the presence of a plate regressively in the longitudinal view of the kidney the p -value was equal to 0.03.

The results from the statistical analysis performed for the linear shear modulus estimates indicated that there was a statistically significant difference between the μ_0 estimates obtained when the experiment was performed in the presence and absence of a plate when doing regressive compression in the transverse view of the kidney (p -value = 0.03). The results of all statistical tests are shown in Table 1.

IV. Discussion

Phantom results showed the feasibility of estimating the nonlinear elastic properties of *ex vivo* porcine kidneys using the acoustoelasticity theory in phantoms. Bernal, *et al.*, [6] previously demonstrated the ability to measure the nonlinear shear elastic properties of liver inclusions in gelatin phantoms and breast lesions using AE. In the study presented in this manuscript, we demonstrated the feasibility of assessing the nonlinear shear modulus in kidneys by compressing *ex vivo* porcine kidneys embedded in porcine gelatin.

Figures 2 and 3 illustrate B-mode images with overlaid 2D shear wave velocity maps for one of the phantoms when compression was performed in the progressive and regressive direction and in both views of the kidney. In these figures it is possible to appreciate the variations in the color filled shear wave velocity map as compression was being applied as indicated by the color scale in the left-hand side of the figures. In the progressive direction of compression shear wave velocity increased progressively from baseline (L0) to maximum compression (L8). Similarly, the thickness of the cortex indicated by the red arrows decreased as progressive compression was being applied. In the regressive direction of compression the shear wave velocity decreased as we released the compression from L7 to back to L0; correspondingly, the thickness of the cortex increased as the compression was gradually released. The strain generally varied linearly with the compression steps used during the experiments which provided good repeatability between acquisitions.

The linear shear modulus and global strain measurements obtained at the different compression steps and the AE fits performed to obtain an estimate of the nonlinear shear modulus for one of the phantoms are shown in Fig. 4. Figs. 4(a) and (b) show the data and fits when uniaxial compression was applied in the presence and absence of a plate attached to the transducer, respectively. The values of the nonlinear shear modulus estimated from the fits were higher when we were applying stress in the progressive direction than in the regressive direction. The fact that A is higher when we compress by pushing down on phantom from zero strain than when we relax the compression from maximal strain could be a result of hysteresis, as we compress the phantom, the media will yield and when we release the compression the media may not return completely to its original configuration. Previous studies have shown that soft tissues exhibit time-dependent hysteresis in large deformations, and the stress-strain relation is nonlinear with small but significant hysteresis [31–33]. It is therefore it is advisable to precondition the tissue by applying multiple (3–10

cycles) of loading and unloading towards a steady state hysteresis loop that characterizes the most representative state of the material [34]. We found that in the first one or two cycles of compression yielded results that had higher variability in the strain compared to subsequent cycles. It is also possible to appreciate in Fig. 4 that the estimates of the nonlinear shear modulus are higher when we compress using a plate attached to the transducer than in the absence of it; this could be explained by the fact that the plate gives a more uniform stress as we axially displace the transducer; additionally as we displace the transducer attached to the compressor plate it may use the background material to apply stress to the kidney. Furthermore, the experiments performed using a plate yielded strains in the cortex of the kidney ranging from 20–25%, while the experiments performed in the absence of a plate yielded strains in the kidney cortex ranging from 35–40%.

Figs. 5 and 6 show there is some variation among the A estimates obtained for the different phantoms, which could be due to the health and age of the pigs from which we obtained the kidneys as well as the fact that the phantoms were frozen when obtained from the animal facilities and later thawed overnight before being tested. Additionally, the estimates of A are higher when we compressed using a plate attached to the transducer and when the stress was applied in the progressive direction of compression.

There were cases in which data was discarded from our analyses. For these phantoms, data was discarded because the estimated value of the nonlinear modulus was positive. Previous studies [6, 8] have shown that the nonlinear modulus has negative values in tissues such as the breast and liver. An explanation for the positive values of A we estimated may be a non-uniform stress distribution within the phantom upon compression when there was no plate attached to the transducer. Additionally, we did impose a limit on the RMSE for which a measurement was deemed successful. With these combined restrictions, we found in the longitudinal plane we had some rejections without a plate in a regressive direction (Phantom 7 ($n = 4$); Phantom 8 ($n = 1$)) and with a plate in the progressive direction (Phantom 1 ($n = 1$)). In the case where the experiment was performed in the transverse view, in the regressive direction and in the absence of a plate, we had failures in four phantoms (Phantom 5 ($n = 1$); Phantom 6 ($n = 1$), Phantom 7 ($n = 4$), Phantom 9 ($n = 4$)). In the transverse direction without a plate in the progressive direction we had to phantoms with failures (Phantom 1 ($n = 1$); Phantom 2 ($n = 1$)). In the transverse plane with a plate we had one failure in each of the progressive (Phantom 1 ($n = 1$)) and regressive direction (Phantom 5 ($n = 1$)).

The summary of the nonlinear shear modulus estimates for all the phantoms presented in Fig. 7 shows some interesting trends. First, the mean estimate of the nonlinear shear modulus in the kidneys obtained in the longitudinal and transverse view in the presence of a plate were not very different; the difference in the A estimates was only 0.54 kPa and 0.74 kPa when applying compression in the progressive and regressive direction, respectively. On the contrary, there was a larger difference in the nonlinear shear modulus estimates obtained in the two views of the kidney in the absence of a plate for both directions of compression; the difference in A estimates was 1.43 kPa and 1.66 kPa when applying progressive and regressive compression, respectively. Second, the nonlinear shear modulus estimates were higher when the experiments were performed with a plate attached to the transducer. In the longitudinal view, the estimates were approximately 30% higher when using a plate while in

the transverse plane the values were approximately 50% higher compared to the estimates obtained in the absence of a plate; such results can be the result of a different distribution of stress with respect to the dimensions of the kidney in the longitudinal and transverse views in the absence of a plate; while the stress distribution is more uniform and consistent when the study is conducted with a plate attached to the transducer.

The linear shear modulus μ_0 was also estimated at the start of each acquisition in the condition of minimal strain for each of the ten phantoms and at each compression cycle. The estimates of μ_0 for the ten phantoms obtained at all the experimental configurations are shown in Figs. 8 and 9, respectively. It is interesting to note that although the linear shear modulus values μ_i varied among the different phantoms tested due to inherent conditions of the excised kidneys mentioned before, μ_0 values remained relatively consistent among the different configurations tested, except for the case where the phantoms were evaluated under progressive compression and in the presence of a plate where the shear modulus showed a slight increase compared to the results obtained in the absence of a plate; this difference is more evident for phantoms 1 and 10 in both the transverse and longitudinal views of the kidney. The summary of all the μ_0 estimates is presented in Fig. 10 where it is possible to observe that the estimated linear shear modulus values were close among the different configurations evaluated; phantoms showed little variability even in the presence and absence of a plate.

Our results demonstrated that it is feasible to estimate a nonlinear shear modulus by monitoring the changes in strain, ϵ_i and shear modulus, μ_i , during kidney deformation. The range of values estimated for the shear modulus at zero stress and the nonlinear shear modulus, A , in the kidney are similar to the values previously reported by [6] for healthy breast tissue and by [8] in beef liver samples.

The study presented in this manuscript is a first step towards investigating the diagnostic potential of A in the kidney and can help to evaluate different factors that affect the consistency and robustness of acoustoelasticity measurements in the kidney. Nevertheless, there are several limitations in this study. First, the kidney was not perfused or pressurized during the study and therefore the effects of certain parameters such as renal blood flow and blood pressure could not be accounted for in this study. We predict that under kidney pressurization, A might vary over a larger range; this will be considered for future experiments. Second, we found that preconditioning is needed to obtain consistent repeated measurements; for this reasons 3–5 cycles of compression were performed for each experimental configuration. Another possible difficulty is the application of true uniaxial stress in our experiments; to account for this difficulty we compressed the phantoms using a rigid, motorized stage which allowed us to apply a quasi-static stress. Also, we are relying on a manual method of strain calculation which could introduce some variability especially with small strains leading to non-uniformities. Due to different levels of variation in strain, we observed that for future studies a smaller displacement increment could provide less variation. Also, automated image-based methods for computing strain may make strain measurements more robust for AE measurements.

The assessment of the nonlinear shear modulus properties of pressurized kidney phantoms will be performed in future studies in order to achieve a more precise characterization of the nonlinear modulus in the kidney. Also, a simulation model will be constructed to explore some of the differences described in this study, including the differences between using a plate and not using a plate. Additionally, we will explore the differences between the progressive and regressive results.

V. Conclusion

Ex vivo porcine kidneys embedded in porcine gelatin were evaluated with the purpose of assessing the feasibility of characterizing the nonlinear shear modulus of the kidney in a laboratory setting. These phantom results demonstrate that acoustoelasticity can be used to estimate a nonlinear shear modulus in the kidney by monitoring changes in strain and shear modulus due to deformation. The results showed that A was larger when the compression is performed in the transverse view of the kidney and in the progressive direction using a plate attached to the transducer which can help guide future experiments and improve the robustness of the method. Additional factors like perfusion or kidney pressurization can be further added to these experiments to better resemble the behavior of the *in vivo* kidney.

Acknowledgments

The authors express appreciation to the reviewers who took considerable time and effort in the review of this paper and provided a substantial improvement for the final version. The authors thank Jennifer Poston for administrative support, Randall Kinnick, Manuela Lopera and Daniel Escobar for their experimental assistance. This work was supported by grant R01 DK092255 from the National Institutes of Health. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institute of Diabetes and Digestive and Kidney Diseases or the National Institutes of Health. Mayo Clinic and some of the authors have financial interest in the shear wave elastography technology described here.

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Biographies

Sara Aristizabal Department of Physiology and Biomedical Engineering, Mayo Clinic College of Medicine, Rochester, MN, USA

Sara Aristizabal (S'14) was born in Medellin, Colombia, on January 26, 1989. She received the B.S. degree in biomedical engineering at Escuela de Ingenieria de Antioquia, Envigado, Colombia, in 2011 and the Ph.D. in biomedical engineering and physiology at the Mayo Clinic College of Medicine in Rochester, MN in 2017.

Carolina Amador Carrascal (IEEE/S'09, M'11) was born in Medellin, Colombia on April 14, 1984. She received the B.S. degree in biomedical engineering at Escuela de Ingenieria de Antioquia, Medellin, Colombia, in 2006 and the Ph.D. in biomedical engineering at the Mayo Clinic College of Medicine in Rochester, MN in 2011. She is a former research associate and assistant professor in the physiology and biomedical engineering department at the Mayo Clinic College of Medicine in Rochester, MN, USA. She is currently a senior scientist at the department of Ultrasound Imaging and Interventions with Philips Research North America in Cambridge, MA, USA. Her research interest is noninvasive evaluation of mechanical properties of soft tissue.

Ivan Nenadic (S'09 - M'11) was born in former Yugoslavia in 1983. He received B.A. with majors in physics and mathematics from Saint Olaf College, Northfield, MN, USA, in 2006, and Ph.D. in Biomedical Engineering from Mayo Clinic College of Medicine, Rochester, MN, USA, in 2011. He is currently an Assistant Professor with the Department of Physiology and Biomedical Engineering at the Mayo Clinic in Rochester, MN. His research interests are shear wave propagation in boundary sensitive geometries and clinical application of ultrasound imaging.

James F. Greenleaf (M'73-SM'84-F'88-LF'08) received the B.S. degree in electrical engineering from the University of Utah, Salt Lake City, UT, USA, in 1964, the M.S. degree in engineering science from Purdue University, Lafayette, IN, USA, in 1968, and the Ph.D. degrees in engineering science from the Mayo Graduate School of Medicine, Rochester, MN, USA, and Purdue University in 1970. He is currently a Professor of Biomedical

Engineering and an Associate Professor of Medicine with the Mayo Graduate School, and also a Consultant with the Department of Physiology and Biomedical Engineering, and Internal Medicine, Division of Cardiovascular Diseases, Mayo Clinic, Rochester. He holds 18 patents. His current interests include ultrasonic biomedical science, and he has authored over 450 articles and edited or authored five books in the field. Dr. Greenleaf is a Fellow of the American Institute of Ultrasound in Medicine, a Fellow of the American Institute for Medical and Biological Engineering, and a Fellow of the Acoustical Society of America. He was a recipient of the 1986 J. Holmes Pioneer Award, the Rayleigh Award in 2004, and the 1998 William J. Fry Memorial Lecture Award from the American Institute of Ultrasound in Medicine. He was the President of the IEEE Ultrasonics, Ferroelectrics, and Frequency Control Society (UFFC-S) in 1992 and 1993. He was the Distinguished Lecturer for the UFFC-S from 1990 to 1991. He has served on the IEEE Technical Committee for the Ultrasonics Symposium for ten years. He served on the UFFC-S Subcommittee on Ultrasonics in Medicine/IEEE Measurement Guide Editors, and on the IEEE Medical Ultrasound Committee.

Matthew W. Urban (IEEE/S'02, M '07, SM '14) was born in Sioux Falls, SD on February 25, 1980. He received the B.S. degree in electrical engineering at South Dakota State University, Brookings, SD in 2002 and the Ph.D. in biomedical engineering at the Mayo Clinic College of Medicine in Rochester, MN in 2007. He is currently an Associate Professor in the Department of Radiology, Mayo Clinic College of Medicine and Science and Associate Consultant in the Department of Radiology, Mayo Clinic Rochester. His current research interests are shear wave-based elasticity measurement and imaging applications, applications of acoustic radiation force, vibro-acoustography, and ultrasonic signal and image processing. Dr. Urban is a member of Eta Kappa Nu, Tau Beta Pi, the American Institute of Ultrasound in Medicine, IEEE, and the Acoustical Society of America.

Fig. 1.

Ex vivo kidney embedded in porcine gelatin ((a), (b)). Experimental set-up is shown in (c), the transducer array attached to a compression plate is placed in contact with the phantom.

Fig. 2.

B-mode images and overlaid shear wave velocity map at three different levels (L) of progressive (a), (b) and (c) and regressive compression (d), (e) and (f) in the longitudinal view of the kidney. All maps use the same color scale. The color variation of the velocity maps indicates the change in shear wave velocity with compression. Red arrows indicate the measured thickness of the kidney cortex at each of the compression level and red dashed boxes represent ROI where shear wave velocity measurements were obtained.

Fig. 3.

B-mode images and overlaid shear wave velocity map at three different levels (L) of progressive (a), (b) and (c) and regressive compression (d), (e) and (f) in the transverse view of the kidney. All maps use the same color scale. The color variation of the velocity maps indicates the change in shear wave velocity with compression. Red arrows indicate the measured thickness of the kidney cortex at each of the compression levels shown in the figure.

Fig. 4.

Measured linear shear modulus in kPa vs. % strain data and resulting nonlinear fits used to estimate the nonlinear shear modulus A for progressive and regressive acquisitions when compressing with (a) and without a plate (b) attached to the transducer. The % strain was calculated as the fractional change in cortex thickness from the beginning of the compression at each acquisition.

Fig. 5.

Mean estimates of the nonlinear shear modulus, A , across ten different phantoms in the longitudinal view of the kidney under four different experimental configurations, (a) progressive compression with a plate, (b) regressive compression with a plate, (c) progressive compression without a plate and (d) regressive compression without a plate. The error bars correspond to the standard deviation between three to five different acquisitions performed for each phantom.

Fig. 6.

Mean estimates of the nonlinear shear modulus, A , across ten different phantoms in the transverse view of the kidney under four different experimental configurations, (a) progressive compression with a plate, (b) regressive compression with a plate, (c) progressive compression without a plate and (d) regressive compression without a plate. The error bars correspond to the standard deviation between three to five different acquisitions performed for each phantom. Data with * indicate data missing due to not meeting the RMSE threshold (RMSE > 1000 Pa).

Fig. 7.

Summary of mean nonlinear shear modulus estimates in ten phantoms with progressive and regressive compression in the longitudinal and transverse views of the kidney with and without a plate attached to the transducer. The error bars correspond to the standard deviation from ten phantoms.

Fig. 8.

Mean estimates of the linear shear modulus, μ_0 , across ten different phantoms in the longitudinal view of the kidney under four different experimental configurations, (a) progressive compression with a plate, (b) regressive compression with a plate, (c) progressive compression without a plate and (d) regressive compression without a plate. The error bars correspond to the standard deviation between three to five different acquisitions performed for each phantom.

Fig. 9.

Mean estimates of the linear shear modulus, $\mu_0 D$, across ten different phantoms in the transverse view of the kidney under four different experimental configurations, (a) progressive compression with a plate, (b) regressive compression with a plate, (c) progressive compression without a plate and (d) regressive compression without a plate. The error bars correspond to the standard deviation between three to five different acquisitions performed for each phantom.

Fig. 10.

Summary of mean linear μ_0 estimates in ten phantoms with progressive and regressive compression in the longitudinal and transverse views of the kidney with and without a plate attached to the transducer. The error bars correspond to the standard deviation from ten phantoms.

TABLE I

Statistical analysis results over different studied configurations for measurement of A and μ_0 with color coding in Figs. 7 and 10.

Studied Configurations		p-value A	p-value μ_0
Longitudinal Progressive Plate	Longitudinal Regressive Plate	P = 0.06	P = 0.13
Transverse Progressive Plate	Transverse Regressive Plate	*P = 0.02	P = 0.27
Longitudinal Progressive No Plate	Longitudinal Regressive No Plate	P = 0.11	P = 0.37
Transverse Progressive No Plate	Transverse Regressive No Plate	P = 0.12	*P = 0.03
Longitudinal Progressive Plate	Longitudinal Progressive No Plate	*P = 0.03	P = 0.23
Transverse Progressive Plate	Transverse Progressive No Plate	*P = 0.003	P = 0.49
Longitudinal Regressive Plate	Longitudinal Regressive No Plate	P = 0.03	P = 0.84
Transverse Regressive Plate	Transverse Regressive No Plate	P = 0.06	P = 0.12

Significant differences are indicated by an *