**Viscoelasticity measured by shear wave elastography
in a rat model of nonalcoholic fatty liver disease:
comparison with dynamic mechanical analysis**

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1 Abstract

2 **Background:** Nonalcoholic fatty liver disease (NAFLD) is rapidly becoming one of
3 the most common liver diseases. Ultrasound elastography has been used for the
4 diagnosis of NAFLD. However, clinical research on steatosis by elastography
5 technology has mainly focused on steatosis with fibrosis or non-alcoholic
6 steatohepatitis (NASH), while steatosis without fibrosis has been poorly studied.
7 Moreover, the relationship between liver viscoelasticity and steatosis grade is not clear.
8 In this study, we evaluated the degree of liver steatosis in a simple steatosis rat model
9 using shear wave elastography (SWE).

10 **Results:** The viscoelasticity values of 69 rats with hepatic steatosis were measured
11 quantitatively by SWE *in vivo* and validated by a dynamic mechanical analysis (DMA)
12 test. Pathological sections were used to determine the steatosis grading for each rat. The
13 results showed that the elasticity values obtained by the two methods followed the same
14 trend, and the elasticity is significantly correlated with liver steatosis. The Pearson's
15 correlation coefficients indicate that elasticity obtained by SWE is positively linear
16 correlated with DMA ($r = 0.628$, $p = 7.85 \times 10^{-9}$). The combined Voigt elasticity
17 measurements have high validity in the prediction of steatosis (S0 vs S1-S4), with an
18 AUROC of 0.755 (95% CI = 0.6175-0.8925, $p < 0.01$) and the optimal cutoff value was
19 2.08 kPa with a sensitivity of 78% and specificity of 63%.

20 **Conclusion:** SWE might have the feasibility to be introduced as an auxiliary
21 technique for NAFLD patients in clinical settings. However, the viscosity results
22 measured by the two methods are significantly different because the two methods work

1 in different frequency bands.

2 **Keywords:** Nonalcoholic fatty liver disease, shear wave elastography, Dynamic
3 mechanical analysis, Viscoelasticity

4 **Background**

5 Nonalcoholic fatty liver disease (NAFLD) is a main cause of chronic liver disease (CLD)
6 and is rapidly becoming one of the most common liver diseases, with an approximate
7 prevalence of 20%–30% of the total population in Western countries [1, 2]. It represents
8 a spectrum of diseases ranging from simple steatosis to nonalcoholic steatohepatitis
9 (NASH), which has a close relationship with liver cirrhosis and hepatocellular
10 carcinoma [3-5]. NASH is critical in the development of liver fibrosis and liver failure
11 and has become a new challenge in the field of liver disease research.

12

13 Many different techniques have been studied in the diagnostic of NAFLD. Magnetic
14 resonance elastography (MRE) uses a modified phase-contrast method to image the
15 propagation characteristics of the shear wave in the liver. Early researches of MRE
16 suggest that it could accurately differentiate simple steatosis from NASH with or
17 without fibrosis. However, MRI is costly and time consuming for use in routine clinical
18 practice [6-8]. Recently, several ultrasound-based elastography techniques, including
19 transient elastography (TE) [9-11], acoustic radiation force impulse (ARFI) [12] and
20 shear wave elastography (SWE) [13], have been used for the noninvasive diagnosis of
21 liver disease. Compared with liver biopsy or other imaging methods, ultrasound

1 elastography has the advantages of a lower cost and a smaller time commitment. As the
2 procedure is non-invasive, it can supersede biopsy-related postoperative complications
3 and potential diagnosis error due to a small sampling range [14].

4

5 The mechanical properties of liver tissue may be helpful in the diagnosis of liver
6 diseases. Many studies had shown that liver stiffness measured by elastography
7 techniques is strongly linked to liver fibrosis [15, 16]. The World Federation for
8 Ultrasound in Medicine and Biology (WFUMB) recently produced guidelines that
9 recommended the use of elastography techniques for distinguishing advanced fibrosis
10 ($\geq F2$) from early fibrosis ($\leq F1$) [17]. However, the mechanism of hepatic steatosis
11 development is different from that of fibrosis. Some researches on steatosis by
12 elastography technology mainly focused on steatosis with fibrosis, while steatosis
13 without fibrosis has been poorly studied. The relationship between steatosis severity
14 and liver stiffness has not been determined [18-20]. Many studies have suggested that
15 liver steatosis with the accumulation of lipid deposits may cause changes in
16 viscoelasticity rather than stiffness. Zhu et al. proposed that the characterization of shear
17 wave dispersion is significantly related to various oil percentages in gelatin phantoms
18 [21]. Barry et al. showed that an increase in liver steatosis increased the viscosity in a
19 mouse liver model, *ex vivo* [22]. Deffieux et al. [23] found no obvious correlation of
20 liver viscosity and dispersion curve slope with steatosis or disease activity. However,
21 the relationship between liver viscoelasticity and steatosis grade is not clear.

22

1 Mechanical tests have been used as another method to assess tissue mechanical
2 properties [24-27]. For example, the dynamic mechanical analysis (DMA) is considered
3 a conventional “gold standard” method to assess the mechanical properties of tissues
4 and can determine the viscoelastic properties of tissues. Many studies have used DMA
5 tests to measure the mechanical properties of liver tissue for comparison of various
6 ultrasound elastography technique results [28, 29].

7

8 Our previous study showed the feasibility of assessing the viscoelasticity of liver in a
9 rat model of steatosis by the DMA test [27]. In this study, we used shear wave
10 elastography to evaluate the viscoelasticity of rat liver with steatosis. A DMA test was
11 performed by oscillatory shear test on a liver sample from the same rat model as before,
12 and then parameters, including the elasticity and the viscosity, were subjected to
13 comparative analysis with variation in the grade of liver steatosis.

14 **Results**

15 **Histologic characteristics**

16 Table 1 lists the distribution of rats with NAFLD according to histologic characteristics.
17 Liver tissue sections from mice in each group after SWE measurements were stained
18 with Oil Red O and examined under the light microscope. Fat droplets in liver tissue
19 were increased significantly as the stages of steatosis increased. Fig. 1 shows
20 representative histologic findings according to the severity of NAFLD. Fig. 1a
21 illustrates normal histologic findings without steatosis, b and c have certain

1 characteristics in steatosis, d and e show significant hepatocytes containing
 2 macrovesicular fat droplets diffused in liver sections after ORO staining, which indicate
 3 severe steatosis.

4 **Table 1 Number of rats and histopathologic results at each steatosis stage**

Days of food administration before sacrificing	Number of rats sacrificed	Steatosis stage determined by the histological assessment of the sacrificed rats				
		S0	S1	S2	S3	S4
Control group	17	17	0	0	0	0
14	12	0	7	4	7	0
28	12	0	3	5	4	0
42	14	0	1	8	5	0
60	14	0	3	2	2	7
Total Number	69	17	14	19	12	7

5
 6 **Fig. 1** Typical specimens of five rat livers at different liver steatosis stages. (a-
 7 e) S0–S4.

Use of SWE and DMA for assessing the grade of steatosis

The elasticity and viscosity values of 69 rats for S0–S4 obtained by the two methods are shown in Fig. 2. For each stage, the rats in this stage were sorted according to their mean elasticity values obtained by the DMA method. The mean elasticity and viscosity values for each stage are listed in Table 2. The mean values of elasticity obtained by SWE (from 0.86 ± 0.27 kPa to 1.05 ± 0.29 kPa) were slightly lower than those by DMA (from 1.12 ± 0.21 kPa to 1.39 ± 0.31 kPa). For viscosity, the values of DMA (from 5.40 ± 0.78 Pa*s to 6.27 ± 0.96 Pa*s) were significant greater than those of SWE (from 0.75 ± 0.10 Pa*s to 0.92 ± 0.18 Pa*s). The *p*-value for each of elasticity and viscosity, between the two types of measurements in each degree of steatosis, all less than 0.05.

Table 2 The mean viscoelasticity parameters of all rat livers measured by SWE and DMA methods (n represents the number of rats. In SWE, 10 points in each rat liver were measured in vivo, and 3 liver samples were extracted in random from each rat in DMA, values are the mean $\pm$ standard deviation. () represents the *p*-value for each of elasticity and viscosity, between the two types of measurements in each degree of steatosis. **p* < 0.05, *p* < 0.01.**

Degree of liver steatosis	ofn	SWE		DMA	
		Elasticity (kPa)	Viscosity (Pa*s)	Elasticity (kPa)	Viscosity (Pa*s)
S0	17	$0.86 \pm 0.27(*)$	$0.77 \pm 0.15(**)$	1.12 ± 0.21	5.40 ± 0.78
S1	14	$0.93 \pm 0.23(**)$	$0.76 \pm 0.20(**)$	1.25 ± 0.26	5.77 ± 0.71

S2	19	$1.05 \pm 0.29^{(**)}$	$0.79 \pm 0.14^{(**)}$	1.39 ± 0.31	6.27 ± 0.96
S3	12	$1.01 \pm 0.20^{(**)}$	$0.75 \pm 0.10^{(**)}$	1.29 ± 0.18	5.97 ± 0.68
S4	7	$0.90 \pm 0.24^{(**)}$	$0.92 \pm 0.18^{(**)}$	1.31 ± 0.14	6.20 ± 0.54

1

2

3 **Fig. 2** The mean and standard deviation of the elasticity and the viscosity in 69
4 rats for both methods. For each stage, the rats in this stage were sorted
5 according to their mean elasticity values obtained by the DMA method.

6

7 The box plots in Fig. 3 illustrate the variations of elasticity and viscosity of the two
8 methods for each stage. The results of the ANOVA test with Tukey–Kramer multiple
9 comparison tests found out the group pairs which had significant differences in
10 elasticity or viscosity.

Fig. 3 Boxplot of (a) the elasticity and (b) the viscosity obtained by the two methods at five steatosis stages. The upper and lower boundaries of the boxes represent the 25th and 75th percentiles, lines within the boxes represent medians, squares within the boxes represent means, and error bars represent ranges. *Asterisks* indicate the pairs having statistically significant differences in the Tukey–Kramer test after the ANOVA test. * $p < 0.05$, ** $p < 0.01$.

Correlation of SWE and DMA on the elasticity and the viscosity

Correlations between the SWE and DMA for the elasticity and viscosity are illustrated in Fig. 4. The Pearson's correlation coefficients indicate that elasticity obtained by SWE is positively linear correlated with DMA ($r = 0.628$, $p = 7.85 \times 10^{-9}$), while there is almost no correlation between viscosity obtained by SWE and DMA ($r = -0.01$, $p = -0.91$).

Fig. 4 Correlative analysis between the SWE and DMA about (a) the elasticity and (b) the viscosity at five steatosis stages.

Combined Voigt model analysis

However, to go further, a combined Voigt model analysis was used to fit phase velocity–frequency data, including the means and standard deviations of the phase velocities for frequencies range from 1-41 Hz (DMA), 160-380 Hz (SWE), and 1-380 Hz (DMA+SWE) at each steatosis stage, as shown in Fig. 5. The black curves a-e represent combined Voigt model analysis data at each steatosis stage, which are fitted by combined low and high frequency band (1-380 Hz) from DMA and SWE measurements. Fig .6 shows box plots of elasticity and viscosity estimates in various degrees of steatotic severity from the combined Voigt model analysis. Liver elasticity of S3 group (mean $\pm$ SD, 1.65 ± 0.23) was significantly higher than that of the S0 group (mean $\pm$ SD, 1.43 ± 0.22) ($p < 0.05$). A significant difference was found between the S2 and S0 groups in combined elasticity ($p < 0.01$), but no significant difference was observed

1 between the other groups.

2

3

4 **Fig. 5** Means and standard deviations of the shear wave phase velocity for
 5 frequencies range from 1-41 Hz (DMA), 160-380 Hz (SWE), and 1-380 Hz
 6 (DMA+SWE) at each steatosis stage.

7

8

9 **Fig. 6** Box plots of elasticity and viscosity estimates in various degrees of
 10 steatotic severity from the combined Voigt model analysis

Fig. 7 shows the combined Voigt elasticity measurements have high validity in the prediction of steatosis (S0 vs S1-S4), with an AUROC of 0.755 (95% CI = 0.6175-0.8925, $p < 0.01$) and the optimal cutoff value was 2.08 kPa with a sensitivity of 78% and specificity of 63%. Combined Voigt model analysis was also able to grading steatosis severity, $S \geq 2$ (AUROC = 0.697, 95% CI = 0.5707-0.8235, $p < 0.01$, optimal cutoff value was 2.98 kPa with a sensitivity of 51% and specificity of 83%).

Fig. 7 Receiver operating characteristic curves based on combined Voigt elasticity for differentiation of different steatosis grades by combined Voigt model analysis.

Discussion

NAFLD is known to be a complex disorder and includes simple steatosis, NASH, and fibrosis. In clinical situations, steatosis and fibrosis are usually interleaved together, and it is difficult to distinguish their separate effects on the mechanical properties of the liver tissue. Many clinical researchers of elastography technology have mainly focused

1 on steatosis with fibrosis or NASH. Grimal et al. [30] investigated the relationship
2 between liver tissue stiffness and histological inflammation score, hepatic fibrosis stage,
3 ballooning score, steatosis analyzed by SWE in rats with NASH, and found that median
4 liver stiffness values measured using SWE showed a stepwise increase with increasing
5 steatosis grade ($p = 0.03$), the results were consistent with those of [31] that
6 demonstrated that liver elasticity was effective in detecting NAFLD. However,
7 Nightingale et al. [32] performed shear wave dispersion with a linear dispersion model
8 to perform shear wave dispersion analysis in traditionally “difficult-to-image” subjects
9 in 135 NAFLD patients. Nevertheless, viscoelastic parameters were not related to the
10 steatosis stage. The presence of entirely different results in the relationship between
11 liver viscoelasticity and steatosis stage may have many causal factors, such as different
12 frequency ranges corresponding to different viscoelastic parameters, experimental
13 conditions, machines, and operators. Moreover, the role of SWE measurement *in vivo*
14 is more complicated because of the complex structure of the organism and variable
15 factors. It also becomes difficult to evaluate when a patient has more than just a single
16 disease in the clinical setting. Parker et al. [33] summarized numerous studies about
17 ultrasound shear wave dispersion in healthy and steatosis livers and concluded that
18 animal models and human populations need further investigation.

19

20 In this study, we used a rat model of liver steatosis, which avoided the confounder of
21 liver fibrosis, thus we can focus on studying the effect of steatosis on viscoelasticity.
22 Disease development in the soft tissue is always accompanied by a variety of

1 mechanical properties of the tissues. The viscoelastic properties of tissue are promising
2 for utilizing the inherent mechanical properties of tissue for grading disease. To this end,
3 we performed liver viscoelasticity measurements using SWE in rat models with various
4 degrees of steatosis severity. The DMA tests were used as a standard of reference, and
5 the values of elasticity and viscosity obtained by SWE were compared to the degree of
6 steatosis as evaluated by histological assessment. In this study, we used the Voigt model
7 to obtain the viscoelasticity parameters in a rat model of NAFLD. We found that the
8 values of elasticity obtained by SWE increased from stage S0 to S2 (Fig. 3(a)), which
9 was similar to the trend of DMA. Interestingly, the values decrease slightly from stage
10 S2 to S4, which is also similar to the DMA. Although the trend between the values of
11 elasticity obtained by SWE is consistent with DMA, the p values of elasticity obtained
12 by SWE were slightly greater than 0.05 and not statistically different among the five
13 steatosis stages. The elasticity of DMA was statistically different between stages S0 and
14 S2–S4. The values of elasticity obtained by SWE were relatively lower than those
15 obtained by DMA. However, compared with the viscosity of DMA, the results of SWE
16 were significantly lower, and there was no difference from S0 to S3. The mean value of
17 S4 was higher than in other stages (Fig. 3(b)). The trends of the five stages of viscosity
18 obtained by SWE were not consistent with those obtained by DMA [21]. Although some
19 phantom studies have shown that viscosity can provide relevant and independent
20 information about the inherent state, until now, few researchers could determine the
21 viscoelasticity parameters *in vivo* for the diagnosis of fatty liver disease.

1 Despite the results obtained by SWE based on shear wave dispersion, accurate staging
2 of the grade of liver steatosis did not prove statistically significant. However, it is
3 interesting to evaluate the values of viscoelasticity relationships between SWE and
4 DMA. In Fig. 4, the mean values of SWE were highly correlated with those obtained
5 by DMA; the correlation coefficient was 0.628, and $p = 7.85 \times 10^{-9}$, indicating significant
6 linear correlation. However, the mean values of viscosity measurements obtained by
7 SWE were relatively independent of those obtained by DMA with a correlation
8 coefficient of -0.01 . This result suggests that the elasticity may provide useful
9 information about the liver steatosis status obtained by SWE. There is little relationship
10 between SWE and DMA in terms of viscosity, suggesting that it has a weak correlation
11 with liver steatosis, which is consistent with previously reported results [23].

12

13 The viscosity results measured by the two methods are significantly different because
14 the measurements depend heavily on the frequency bands. It is focused to investigate
15 why the elasticity values measured by SWE have the same trend with the DMA but
16 without a statistical difference among the five steatosis stages. There may be several
17 reasons for this result. First, DMA has much lower frequencies than SWE, which may
18 lead to different vibrations in microstructures of liver tissue. Also, the frequency ranges
19 of the shear wave measured using SWE may not reveal the actual viscoelastic properties
20 of the tissue[34]. In DMA tests, the frequency ranged from 1 to 41 Hz, while the shear
21 wave frequency of SWE ranged from 160 to 380 Hz. Specifically, the excised liver has
22 no blood circulation in the DMA tests. Blood flow can affect the viscoelasticity

1 measurement of the liver by SWE [35]. Also, the limited size of the species utilized and
2 ultrasound imaging quality considerations can affect the results.

3

4 According to eqns (1), the elasticity parameters are dependent on the low-frequency
5 shear wave velocity. The elasticity of DMA was statistically different between stages
6 S0 and S2–S4. In this study we took a step further by using combined Voigt model
7 analysis, which combined low and high frequency band (1-380 Hz) from DMA and
8 SWE measurements, then the combined elasticity and viscosity were derived from
9 Voigt model. Fig. 5 shows that the means of the shear wave velocities at frequencies
10 from 1-380 Hz increased with increasing frequency. The results shown in the box plots
11 (Fig. 6) and the ROC curves (Fig. 7) indicate that the combined elasticity was
12 statistically significantly different between stage $S \geq 1$ and stage $S \geq 2$. The AUROC
13 values for combined elasticity for distinguishing liver steatosis stages ($S \geq 1$) were
14 approximately 0.755. These results find coincide with a previous study by Nightingale
15 et al.[32], they found that the AUROC values for distinguishing the liver steatosis stages
16 ($S \geq 2$) were 0.49 for the elasticity at 200 Hz. Although it seems reasonable that the
17 accumulation of fat may result in changes in the liver viscosity parameters, the viscosity
18 value could not be used to evaluate the level of liver steatosis. The results can
19 be attributed to multiple causes, some of them limited frequencies of the induced shear
20 wave because the viscosity becomes prominent at high frequencies. However, the shear
21 wave is difficult to detect at high frequencies because of the high level of shear wave
22 attenuation.

1 Conclusion

2 In this study, we compared the measurement of viscoelastic parameters, including
3 elasticity and viscosity, by DMA tests, SWE methods and combined Voigt model
4 analysis in a rat model of NAFLD at five steatosis stages. The results showed that the
5 elasticity values from the two methods followed the same trend, suggesting that
6 elasticity has a significant correlation with liver steatosis. To some extent, elasticity
7 values measured by SWE can reflect liver steatosis processes. SWE might have the
8 feasibility to be introduced as an auxiliary technique for NAFLD patients in clinical
9 settings.

10 Materials and Methods

11 Animal model

12 A total of 69 male Sprague-Dawley (SD) rats (weighing 200 ± 20 g, Guangdong
13 Medical Laboratory Animal Center, Foshan, China) were used in this study. They were
14 maintained under Specific Pathogen Free (SPF) conditions (relatively constant
15 temperature: 20–26°C humidity: 30–50%, 10 h: 14 h light/dark rhythm alternately) with
16 a high-calorie diet. To induce hepatic steatosis in different stages (S1–S4), a high-
17 calorie diet for different numbers of days were supplied to SD rats at a dose of 1 mL/100
18 g rat weight once a day for sixty days. The control group (S0) was fed with a regular
19 diet. Rat livers were harvested for DMA tests and histologic assessment after the rat
20 had been used for *in vivo* ultrasound measurements. The histopathologic examination

1 ultimately determined the hepatic steatosis stage. The number of animals in each group
2 was stated in Table 1. Animal care and experiments were approved by the Animal Care
3 and Use Committee of School of Medicine in Shenzhen University and Guangdong
4 Medical Laboratory Animal Center.

5 **In vivo SWE measurement**

6 In this experiment, the rat was placed in the supine position on the experimental table
7 after anesthesia at a relatively constant temperature of 24°C–26°C. The rat abdomen
8 was shaved and coated with ultrasonic gel, and then an ultrasound B-mode image was
9 used for localization of the liver and a region of interest (ROI) (size: 5mm×15mm) in
10 the median lobe of the liver, devoid of large vessels, was chosen for measurement. After
11 the ROI of the liver was selected, the operator kept the probe stable and switched to the
12 SWE mode to finish the measurement, as shown in Fig. 8. To obtain a relatively
13 accurate result, for each rat, measurements were repeated ten times in ten locations, and
14 the calculated elasticity and viscosity values were the mean of ten valid measurements.

15

16 **Fig. 8** Experimental setup for in vivo shear wave elastography (SWE)

1 measurement.

2 The SWE measurement was performed on the Verasonic vantage 256 systems
 3 (Verasonics Inc., Kirkland, WA, USA) with a linear array transducer L11-4v (Philips
 4 Healthcare, Andover, MA). The measurement procedure is similar to that used in the
 5 previous study [36]. First, a B mode image of the liver was obtained to provide imaging
 6 guidance for selecting the measurement locations, then a region of interest (ROI), 5 mm
 7 axially $\times$ 15 mm laterally, was selected for measurement. A total of 20 array elements
 8 were used in one excitation, which consisted of three successive push beams to create
 9 a Mach cone [37]. These three push beams were focused at -5 mm, 0 mm, and 5 mm
 10 deep relative to the center of the ROI with a time interval of 50 microseconds. The push
 11 beam at each depth was generated by an 80-microsecond tone burst at a center
 12 frequency of 4.4 MHz. Second, the system was switched immediately to the plane wave
 13 imaging mode after the push wave transmission. All elements were set to fire 50 detect
 14 pulses with a center frequency of 6.25 MHz and a pulse repetition frequency of 10 kHz.
 15 Next, the data post-processing steps include: (1) In-phase quadrature (IQ) data within
 16 the ROI was processed with a 2D correlation algorithm to obtain particle velocity [38];
 17 (2) 10 pixels spatial averaging was applied in the axial direction to remove spike noise
 18 after the 3×3 median filter, cubic spline interpolation with a factor of five was conducted
 19 to reconstruct the displacement profiles to increase the temporal resolution; (3) low-
 20 pass filtering with a cut off frequency of 1000 Hz was used to reduce jitter after the
 21 reverberation frames were removed, then a Fourier transform was used to estimate the
 22 phase at each frequency; (4) then a directional filter was applied to reduce the artifacts

from reflected shear waves at boundaries, a linear regression algorithm was used to calculate the shear wave phase velocities at each frequency (160–380 Hz) by estimating the average phase difference [39]; (5) finally the values of viscoelasticity were estimated by MATLAB (Mathworks, R2014a) with a nonlinear fitting method using Equation 1 based on a least-squares criterion.

Ex vivo DMA experiment

Each rat liver was harvested immediately for *ex vivo* DMA test after the *in vivo* ultrasound measurement. The livers were soaked with saline solution to prevent desiccation of liver specimens and kept in the refrigerator at a temperature of 4°C to preserve freshness.

Fig. 9 The dynamic mechanical analysis (DMA) experimental platform.

Fig. 9 shows the DMA experimental instrument (the ElectroForce3200 Series, Boss Corp., Eden Prairie, Minnesota, USA) operating at a parallel plate shear mode. Two to

1 three liver specimens were taken from each rat, and then each specimen was cut by a
 2 scalpel into the same shape (size: 15mm×5mm×4mm, thickness: 4 ± 1 mm) to match
 3 the parallel plates. During the experiment, the liver specimen was fixed to the fixture in
 4 the water tank which was filled with a saline solution at a body temperature of $38.1 \pm$
 5 0.2°C . The sandpaper was attached between the parallel plates to prevent the liver
 6 specimen from slipping. The motion controller was rotated slowly to ensure sufficient
 7 contact between the parallel plates. After the sample was placed on the parallel plates,
 8 the sample slowly received a precompression of 2–3 g to ensure sufficient contact
 9 between the plate and the sample and avoid slippage. A sinusoidal torque was
 10 engendered by the machine to make the liver specimen generate stress and strain. Then,
 11 the dynamic mechanical properties, including storage modulus G' and loss modulus G''
 12 were directly obtained from the system during the test. The phase velocities at all
 13 frequency points in the range of 1–41 Hz with an interval of 5 Hz were substituted in
 14 Equation 3 and Equation 3, and then shear elasticity μ and shear viscosity η of every
 15 sample were estimated by non-linear curve-fitting.

16 Theory

17 In this study, it is assumed that the liver consists of viscoelastic and isotropic
 18 homogenous material. The relationship between the shear wave velocity and frequency
 19 was derived from the Voigt model. The equation is as shown below [40]:

$$20 \quad c_s(\omega) = \sqrt{\frac{2(\mu^2 + \omega^2\eta^2)}{\rho(\mu + \sqrt{\mu^2 + \omega^2\eta^2})}} \quad (1)$$

21 where c_s is the shear velocity, ω the angular frequency, ρ the mass density, μ and η

1 are the shear elasticity and shear viscosity.

2

3 The principle of DMA is performed as previously described [27]. Briefly, for
 4 homogeneous biological tissue, the sinusoidal shear strain $\varepsilon(t) = \varepsilon_0 e^{j\omega t}$ induces a
 5 corresponding sinusoidal stress $\sigma(t) = \sigma_0 e^{j(\omega t + \delta)}$. The complex shear modulus G^* is
 6 defined as the ratio of stress to the strain:

$$7 \quad G^*(\omega) = \frac{\sigma(t)}{\varepsilon(t)} = \frac{\sigma_0 e^{j(\omega t + \delta)}}{\varepsilon_0 e^{j\omega t}} = \frac{\sigma_0}{\varepsilon_0} (\cos \delta + j \sin \delta) = G'(\omega) + jG''(\omega) \quad (2)$$

8 Where ω is the angular frequency, δ is a phase difference, σ_0 and ε_0 are the shear
 9 stress amplitude and strain amplitude, respectively. The real part of Equation 2, $G'(\omega)$
 10 is the storage modulus relating to the elasticity parameter, μ , while the imaginary part
 11 $G''(\omega)$ is the loss modulus relating to the viscosity η . To quantitatively analyze the
 12 viscoelastic properties of the tissues, the complex modulus is expressed by
 13 $G^* = \mu + j\omega\eta$, which is derived from the Voigt model [41]. Therefore, the storage
 14 moduli and loss moduli of the liver are expressed as follows:

$$15 \quad G' = \mu \quad (3)$$

16

$$17 \quad G'' = \omega\eta \quad (4)$$

18 **Histological assessment**

19 The excised liver tissues from middle part of the left lobe were fixed in the 10% neutral
 20 formalin liquid, and conventional methods of dehydration, paraffin-embedded sections,
 21 and Oil Red O staining was conducted by histopathology technicians in the Guangdong

1 Medical Laboratory Animal Center. Two slices, with a thickness of 7 μm , from each rat
 2 were used for the histologic examination. These pathological slices were observed
 3 under an optical microscope by pathologists who had no knowledge of the *in vivo*
 4 ultrasound measurement. Stages of steatosis were determined by quantifying
 5 percentages of hepatocellular macrovesicular steatosis: 0–4% (S0); 5–25% (S1); 26–
 6 50% (S2); 51–75% (S3); >76% (S4). Fig. 1 shows typical liver specimens for the five
 7 steatosis stages.

8 **Statistical analysis**

9 All 69 rats were divided into five groups separated by histological grade (S0–S4). For
 10 each steatosis grade, the viscoelasticity parameter values of all the rats at that grade
 11 were grouped. The mean parameter value of each rat was then used to estimate the
 12 population mean of each steatosis group. If the value exceeded more than 1.5 times the
 13 interquartile range in its group, it was eliminated as an outlier and the corresponding
 14 measurement was excluded [27]. The relationship between the SWE and DMA on the
 15 elasticity and the viscosity was analyzed using the Pearson correlation coefficient and
 16 linear regression. The accuracy of the distinction by combined dispersion curve
 17 analysis for grading steatosis severity was assessed using a nonparametric area under
 18 receiver operating characteristic curve (AUROC), and the corresponding sensitivities
 19 and specificities were calculated at optimal cut-off values of liver elasticity. All data
 20 were represented as means $\pm$ standard deviation (SD) and analyzed by using the two-
 21 tailed Student t-test, analysis of variance. The one-way analysis of variance (ANOVA)

1 with the Tukey–Kramer multiple comparison tests were used to compare the liver
 2 viscoelasticity parameters among the steatosis stages. Differences were considered
 3 significant when $*p < 0.05$, $**p < 0.01$, and $***p < 0.001$, respectively.

4 **Abbreviations**

5 NAFLD: nonalcoholic fatty liver disease; NASH: non-alcoholic steatohepatitis; SWE:
 6 shear wave elastography; DMA: dynamic mechanical analysis; MRE: magnetic
 7 resonance elastography; TE: transient elastography; ARFI: acoustic radiation force
 8 impulse; SD: Sprague-Dawley; SPF: Specific Pathogen Free; ROI: region of interest;
 9 IQ: in-phase quadrature; ANOVA: analysis of variance.

10 **Authors' contributions**

11 Zhaoke Pi and Haoming Li designed the method and drafted the original manuscript;
 12 Mengke Wang, Yanrong Guo, Xianfen Diao, and Jie Zeng participated in the
 13 experiments and analyzed the data; Siping Chen, Hui Xia, Guoqiang Liu, Xinyu Zhang
 14 and Xin Chen provided supports for the research, designed the method, and revised the
 15 manuscript. All authors read and approved the final manuscript.

16 **Availability of data and materials**

17 The data used to support the findings of this study are available from the corresponding
 18 author upon reasonable request.

19 **Ethics approval and consent to participate**

20 Animal care and experiments were approved by the Animal Care and Use Committee
 21 of School of Medicine in Shenzhen University, China (Approval No.: 20160110).

22 **Consent for publication**

1 All authors give their consent for publication.

2 **Competing interests**

3 The authors declare that they have no conflict of interest.

4 **Acknowledgments & Funding**

5 This work was supported by the National Natural Science Foundation of China (Grant
6 No. 81601503, 81871429, 91859122).

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