

Viscoelastic properties of liver measured by oscillatory rheometry and multifrequency magnetic resonance elastography

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Abstract. The mechanical properties of liver can sensitively indicate the progression of hepatic fibrosis. Mechanical tissue characterization involves the analysis of the complex shear modulus measured either by oscillatory rheometry or by *in vivo* elastography. In this study, bovine liver specimens were investigated by oscillatory rheometry and multifrequency magnetic resonance elastography (MRE) in a common frequency range between 25.0 and 62.5 Hz. The results were compared with *in vivo* MRE of human liver. Storage and loss moduli were quantified, and the data were also analyzed employing a springpot model, yielding a stiffness-related parameter of 2.96 ± 0.53 kPa in bovine liver by rheometry and of 2.20 ± 0.45 kPa in human liver by *in vivo* MRE. Furthermore, MRE of excised bovine liver showed that stiffness tended to increase with decreasing sample temperature. In conclusion, mechanical tissue characterization by multifrequency MRE agrees well with oscillatory rheometry, which validates MRE as a method for investigating the rheology of liver tissue.

Keywords: Magnetic resonance elastography, rheometry, liver viscoelasticity, springpot

1. Introduction

One of the most traditional diagnostic methods in medicine, manual palpation, is based on the correlation between pathological conditions of soft tissue and changes in its mechanical properties. In the past, a mechanical characterization of biological tissue was limited to excised samples. For instance, compression tests were performed to determine the grade of hepatic fibrosis from liver stiffness [19]. Dynamic magnetic resonance elastography (MRE) [8,12] and shear-wave based ultrasound elastography (USE) [11,18] are noninvasive techniques capable of measuring tissue mechanical properties inside the human body. Both modalities have been used for noninvasive staging of hepatic fibrosis [2,3,13, 20]. In conventional MRE, mechanical vibrations of a single frequency, the so-called drive frequency, are introduced into the target tissue, which is positioned inside the magnetic resonance (MR) imager.

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Motion-sensitive image acquisition techniques can measure minimal phase variations of the MR signal induced by shear vibrations. The resulting wave images are analyzed by solving the inverse problem of elastography [9,10]. Multifrequency MRE relies on broadband motion encoding, which allows the acquisition of multiple harmonic vibrations in one experiment [1,6]. In this experiment the dispersion of the complex shear modulus, $G^*(\omega)$, with ω being the angular drive frequency, is accessible by a single time-resolved MRE scan. Hence, material parameters can be calculated by fitting the analytical $G^*(\omega)$ function of any rheological model to the data. Recently, it has been shown that the springpot model, also referred to as the “single fractional element” in the literature [15], is very well suited for analyzing multifrequency MRE of *in vivo* liver and brain [5,17]. The springpot represents a linear interpolation of spring and dashpot, yielding two independent material parameters: a stiffness-related parameter μ with the dimension kPa and the dimensionless α , which is a parameter that characterizes the alignment of mechanical structure-building elements in the tissue. Although *in vivo* elastography, in particular of the liver, is extensively investigated in patients, there has been no systematic study determining whether the derived material parameters can be reproduced by more established test methods. A material model fitting to elastographic data of liver would improve the significance of *in vivo* studies and would enable the comparison of data acquired by different modalities at different drive frequencies.

Therefore, this study aims to determine the complex shear modulus of liver by rotational rheometry and multifrequency MRE in the common frequency range applicable by MRE on humans. The experiments consist of three parts: (i) rotational rheometry of bovine liver at 1°C, (ii) MRE of bovine liver at different temperatures and (iii) *in vivo* human liver MRE.

2. Materials and methods

2.1. Multifrequency MRE

Seventeen volunteers (10 female; mean age: 33.6 ± 6.4 years) were repeatedly examined by multifrequency MRE of the liver. This study was approved by the local ethics committee and written informed consent was obtained from all subjects. Two to three examinations were performed per subject. The vibration generator used is described in [4]. Details of the image acquisition sequence and data processing are given in [6]. $G^*(\omega)$ was calculated at four drive frequencies (25.0, 37.5, 50.0 and 62.5 Hz), which were synchronously applied in a superimposed waveform.

The same protocol was applied to a bovine liver specimen stuffed into a cubic vessel ($11 \times 12 \times 14$ cm) and one examination was performed each at 4°C, 12°C and 22°C. In these experiments, a square vibration plate on top of the sample was used for introducing planar waves into the liver.

2.2. Oscillatory shear rheometry

Cylindrical samples of fresh bovine liver with 50 mm diameter and a slice thickness of 0.9–2.4 mm were tested by an oscillatory shear device (MCR 301, Anton Paar, Austria). The sample temperature was adjusted to 1°C, which is further discussed below. The linear regime of viscoelasticity was estimated by four amplitude sweeps at frequencies of 5, 20, 40 and 60 Hz using a single tissue specimen. The strain amplitude γ_0 was varied between 0.1% and 2.0%. The limit γ_{\max} of the linear viscoelastic regime was set to 5% deviation of both real (G') and imaginary part (G'') of G^* from the plateau value ($\gamma_0 \rightarrow 0.1$) in all four amplitude sweeps. Then, G^* was measured within 2.5 and 62.5 Hz frequency range by increments of 2.5 Hz. γ_0 was set below the previously determined linear strain limit given in the results section. The experiments were repeated on six different tissue samples for estimating the variability of the method.

2.3. Calculation of viscoelastic parameters

The viscoelastic parameters were calculated according to the springpot model [15]. The complex shear modulus $G_S^*(\omega)$ was fitted to $G^*(\omega)$ for obtaining the viscoelastic parameters μ and α (see the Appendix). In all experiments, μ and α were determined by a least-square-fit routine [6]. Mean values and standard deviations (SD) of G' , G'' , μ and α were derived from repeated experiments of oscillatory rheometry and *in vivo* MRE.

3. Results

In four amplitude sweeps, the deviation of the loss and the storage modulus from their plateau values was lower than 5% for all strain amplitudes $\gamma_0 < 0.43\%$. Tests at 5 Hz and at 60 Hz frequency are plotted in Fig. 1. Thus $\gamma_0 = 0.3\%$ was set in the rheometer frequency sweeps to ensure the determination of $G^*(\omega)$ in the linear viscoelastic regime.

The complex modulus data and fitted results are displayed in Fig. 2a for the storage modulus G' and in Fig. 2b for the loss modulus G'' . In addition, the complex moduli at MRE frequencies are given in Table 1. For all data of drive frequencies > 25 Hz, G^* appears to be slightly lower in rheometry

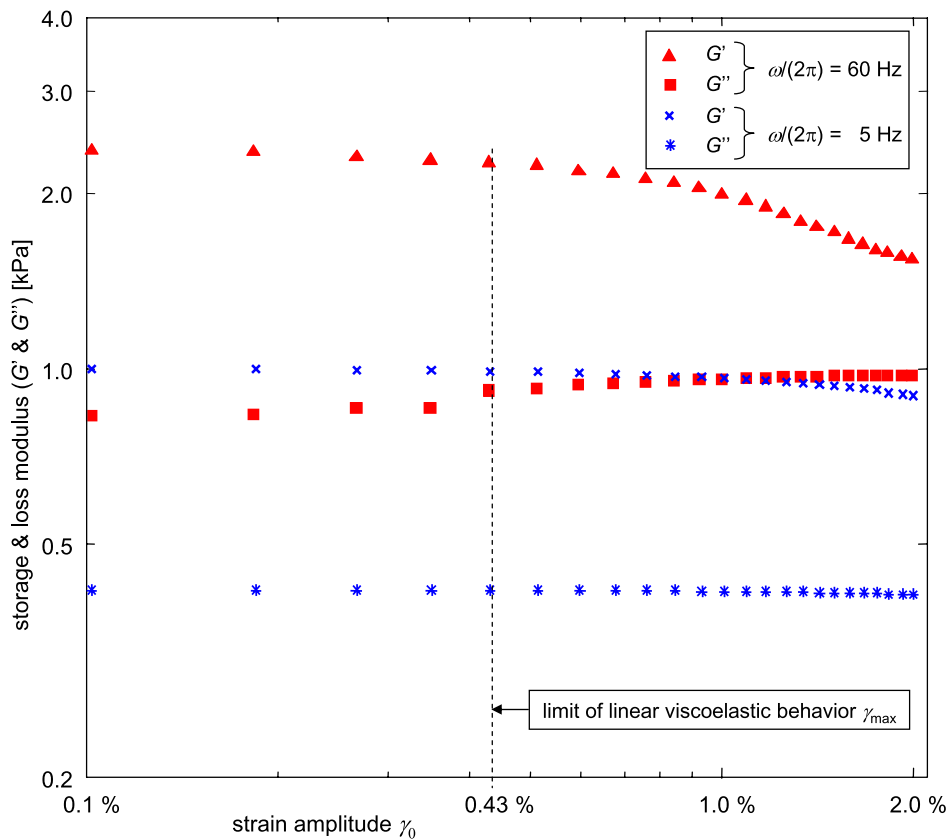


Fig. 1. Amplitude sweeps of bovine liver using an oscillatory shear rheometer at 5 and 60 Hz frequency. The dashed line marks the linear viscoelastic limit γ_{\max} .

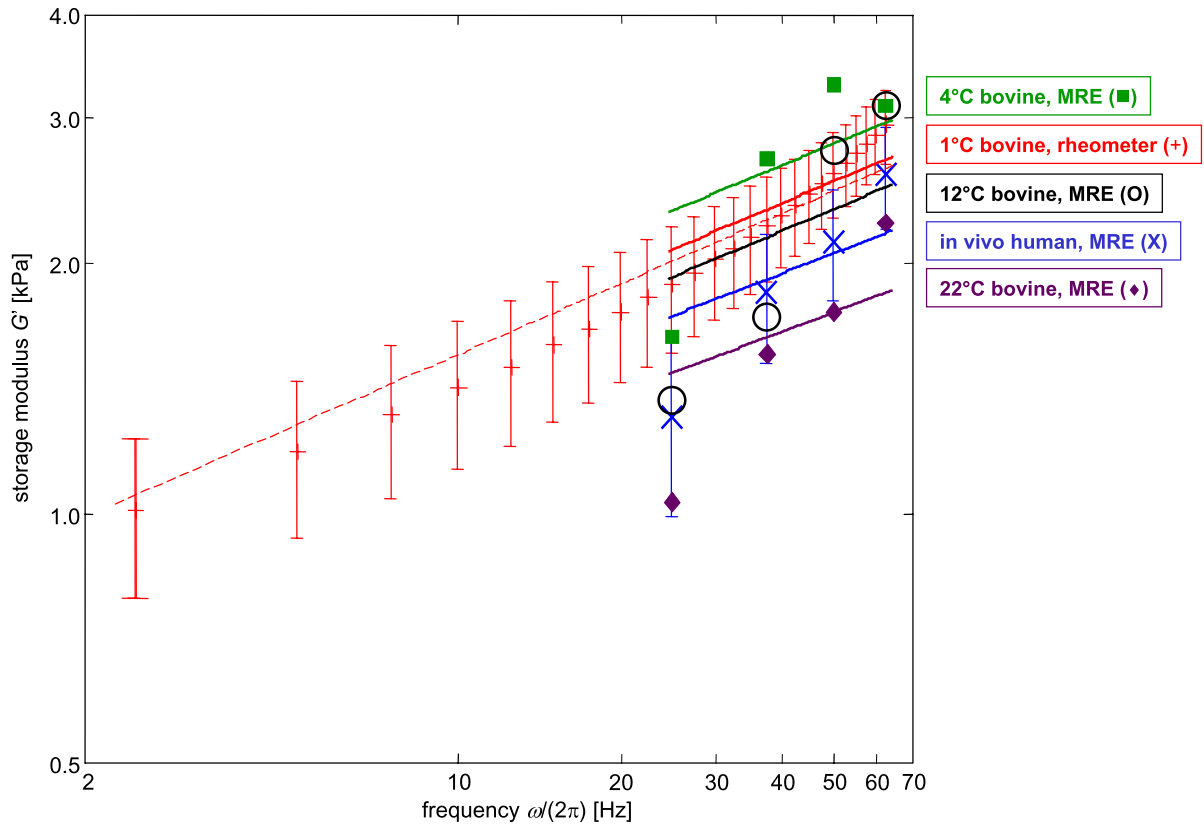


Fig. 2a. Storage modulus $G'(\omega)$: The experimental data and the fitted functions according to the springpot model are represented by symbols and lines, respectively. The data obtained by the rheometer tests and in the *in vivo* examinations represent mean values as described in the caption of Table 2. The error bars correspond to the standard deviation (SD). Please note that, for assessing the quality of the fit, it is important to consider both G' and G'' data (shown in Fig. 2b) as well as their ratios. Rheometer data were separately fitted once within the entire displayed frequency range and secondly within the dynamic range of multifrequency MRE.

than observed by MRE on 4°C bovine tissue samples. MRE further reveals that G^* decreases with increasing temperature resulting in lower values at 22°C than measured by rheometry at 1°C. The fitted parameters μ and α at different temperatures are listed in Table 2. While the slopes of all fitted curves (determined by α) in Fig. 2a and b are similar, the positions of the curves vary considerably due to significant differences in μ . Figure 3a shows that the variation of α is small, whereas a decline of μ with temperature T is perceptible in Fig. 3b. A rate $\Delta\mu/\Delta T = -67 \text{ Pa}/^\circ\text{C}$ ($R^2 = 0.85$) was observed for *ex vivo* bovine liver in the temperature range from 1°C to 22°C. Similar to μ , this rate depends on the assumption made for η (see the Appendix).

4. Discussion

The present study focused on the viscoelastic properties of *in vivo* liver and excised tissue samples. For that reason, the tissue samples were tested without any chemical treatment to ensure the comparability of rheometry and MRE. As a consequence, shear rheometry had to be performed at the lowest possible

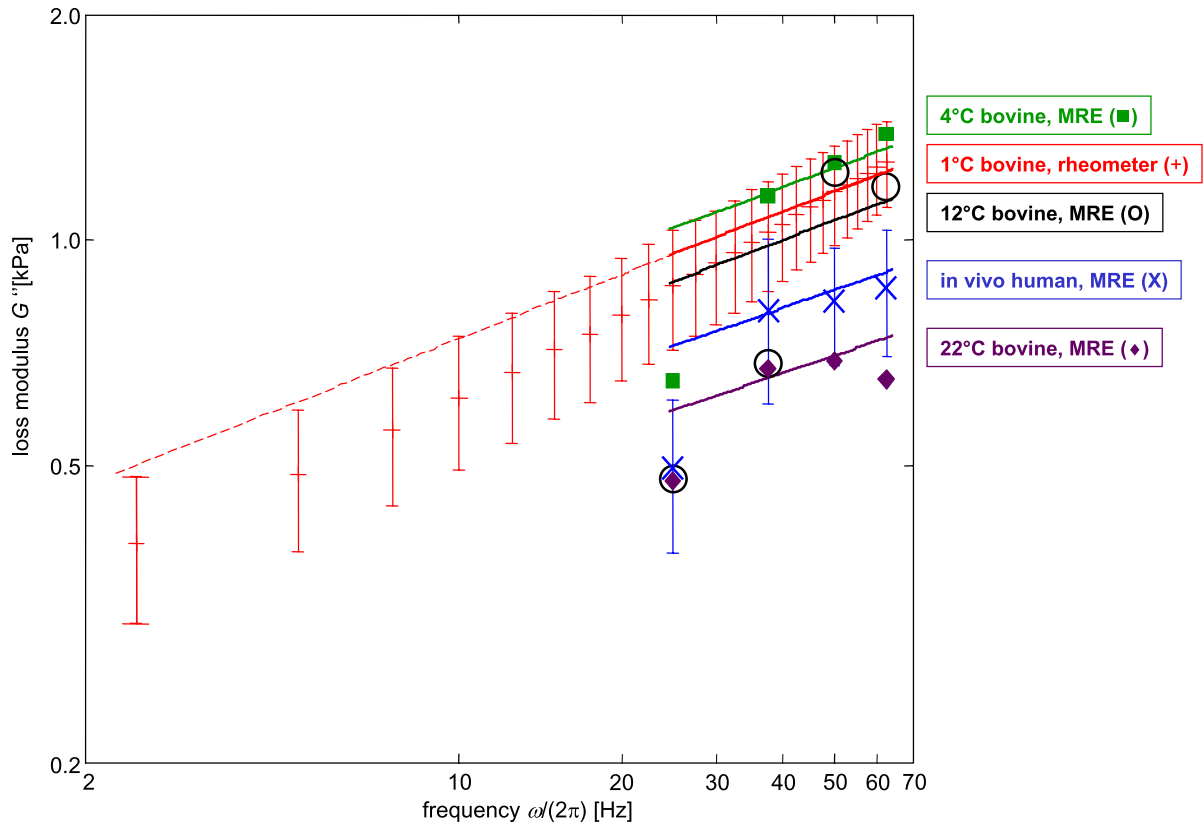


Fig. 2b. Loss modulus $G''(\omega)$ equivalent to Fig. 2a (please see the caption of Fig. 2a for details).

Table 1
Storage modulus G' and loss modulus G'' at mechanical excitation frequencies of MRE

Frequency (Hz)	Storage modulus G' (kPa)				Loss modulus G'' (kPa)			
	25.0	37.5	50.0	62.5	25.0	37.5	50.0	62.5
1°C – rheometry – bovine	1.89 (0.33)	2.22 (0.32)	2.57 (0.30)	2.94 (0.30)	0.87 (0.16)	1.02 (0.17)	1.16 (0.18)	1.27 (0.17)
4°C – MRE – bovine	1.63	2.68	3.28	3.10	0.65	1.14	1.27	1.39
12°C – MRE – bovine	1.37	1.72	2.74	3.10	0.48	0.69	1.23	1.18
22°C – MRE – bovine	1.03	1.56	1.75	2.24	0.48	0.67	0.69	0.65
36.5°C – MRE – <i>in vivo</i> human	1.30 (0.31)	1.84 (0.33)	2.13 (0.32)	2.56 (0.37)	0.50 (0.11)	0.80 (0.20)	0.83 (0.15)	0.86 (0.16)

Note: Data of rheometry and *in vivo* MRE are given as mean values of repeated studies with their standard deviations in brackets.

temperature (here 1°C) to avoid clotting. Other authors performed rheometry studies of *ex vivo* liver at 37°C to simulate body temperature [7]. However, in this approach, the tissue samples had to be prepared using a sodium chloride solution and Vaseline to avoid swelling and clotting. The sample preparation and the different test temperatures may be the reason for the lower limit of the linear viscoelastic regime $\gamma_{\max} = 0.2\%$ given in [7]. In contrast, we found $\gamma_{\max} = 0.43\%$ at 1°C in our study.

The springpot model represents a power law of G^* . This implies a frequency-independent ratio of G''

Table 2
The viscoelastic parameters α and μ according to the springpot model with temperature T

Method	Oscillatory rheometry		Multifrequency MRE			
	<i>Ex vivo</i> bovine		<i>Ex vivo</i> bovine			<i>In vivo</i> human
Liver tissue						
T ($^{\circ}\text{C}$)	1	1	4	12	22	36.5
α	0.282 ± 0.012	0.275 ± 0.017	0.267	0.272	0.242	0.251 ± 0.011
μ (kPa)	2.88 ± 0.54	2.96 ± 0.53	3.38	2.65	1.76	2.20 ± 0.45
Fit range (Hz)	2.5–62.5	25.0–62.5			25.0–62.5	

Note: Rheometry and *in vivo* MRE parameters represent mean values (\pm standard deviation) of repeated measurements on 6 samples and 17 subjects, respectively.

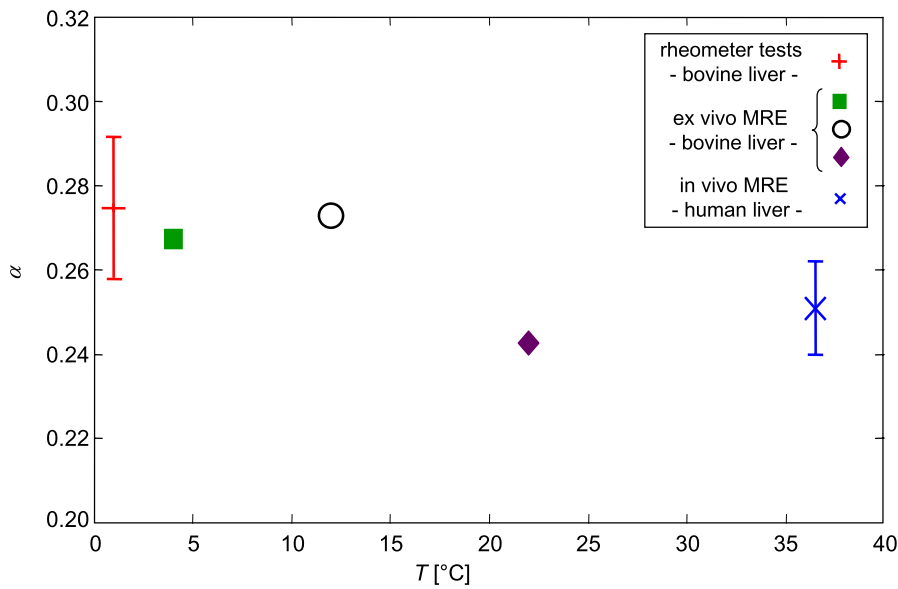


Fig. 3a. Viscoelastic parameter α according to the springpot model over test temperature T fitted in the MRE frequency range (25.0–62.5 Hz) as given in Table 2.

and G' given as a function of the power α (see Eq. (A-2)). Therefore, the lines in Fig. 2a and b represent a combined fit to both G' and G'' of only two parameters, which is not the same as two individual linear fitted curves to G' and G'' comprising four independent variables. A better fit of model and experiment may be achievable using higher order rheological models. However, this is bought by having a larger number of free variables which decreases the inter-study reproducibility [6]. To our knowledge, the springpot model represents the best characterization of the mechanical behavior of liver among two-parameter models of linear viscoelasticity [5].

The given fitted curves are characterized by almost parallel slopes, indicating a similar α , although the experimental conditions varied with regard to temperature, boundary conditions and mechanical stimulation. Differences between *ex vivo* specimens and *in vivo* liver such as intercellular pressure and blood perfusion also appeared to have no significant influence on α . Our results suggest that the slope of G^* and the ratio G''/G' depend on the inherent constitution of the liver rather than temperature and vital functions. On the other hand, a linear temperature dependence of μ was observed within the bovine liver. However, the tolerance limit of MRE, which is on the order of 10–15% [10], precludes a definitive

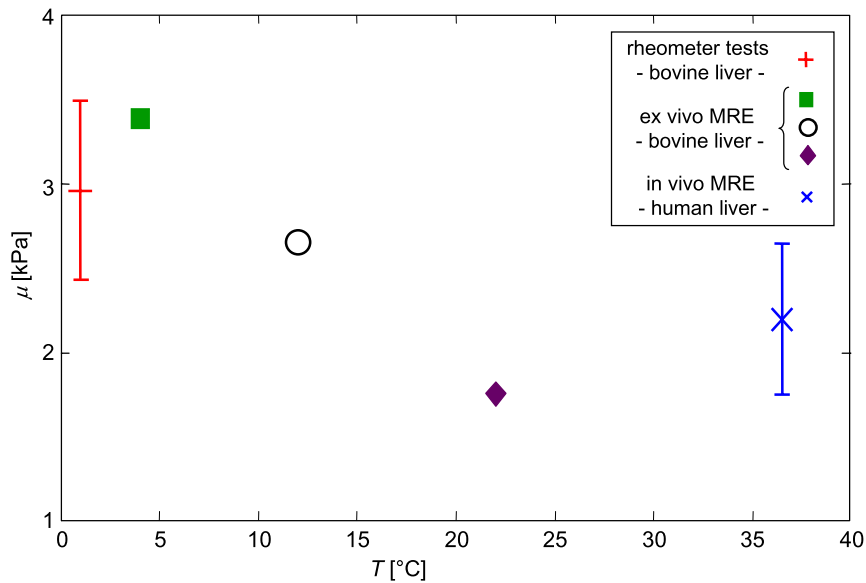


Fig. 3b. Viscoelastic parameter μ according to the springpot model over test temperature T as given in Table 2 and equivalent to Fig. 3a.

conclusion about the elasticity–temperature function of liver. Still, μ seems to correlate with T , while α shows no dependence. This is consistent with the interpretation of the springpot model as a hierarchically aligned structure of springs and dashpots, where μ and α are related to the spring constants and the number of connections, respectively [14]. In this interpretation a decrease in temperature does not impact the geometry of the structure but increases the value of the spring constants.

The variability of *in vivo* MRE observed in this study is partially due to individual variations of the viscoelastic properties of liver. For example at 37.5 Hz, G' was 1.47 ± 0.15 kPa in a 42-year-old woman and 2.64 ± 0.42 kPa in a 35-year-old woman. Such a significant difference ($P < 0.05$) among healthy subjects confirms the inter-individual variation of liver MRE reported in [5].

Comparing MRE with rheometry requires careful consideration of mechanical boundary conditions [16]. For example, slipping of the sample may cause underestimation of G^* by oscillatory rheometry [7,16]. This may also be the reason why our rheometer data obtained at 1°C are slightly lower than MRE-derived values at 4°C.

In summary, a similar frequency behavior of the complex shear modulus of liver tissue was found by multifrequency MRE and oscillatory shear rheometry. Using the springpot model, a temperature dependence of μ was found, while α showed no correlation. The viscoelastic parameters determined by multifrequency MRE are in very good agreement with the data acquired through oscillatory rheometry. This study validates multifrequency MRE as a tool for investigating the rheology of liver and further motivates *in vivo* applications of the technique to humans.

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Appendix

In this study, the viscoelastic parameters were calculated according to the springpot model [15]. Its complex modulus is represented by

$$G_S^*(\omega) = k^{1-\alpha}(i\omega)^\alpha = k^{1-\alpha}\omega^\alpha \left(\cos \frac{\alpha\pi}{2} + i \sin \frac{\alpha\pi}{2} \right), \quad (\text{A-1})$$

with the two independent parameters k and α , $0 < \alpha < 1$. α is related to the angle δ of the loss tangent:

$$\tan \frac{\alpha\pi}{2} = \tan \delta = \frac{G''}{G'}. \quad (\text{A-2})$$

The springpot model can be interpreted as an interpolation between a pure elastic solid with a spring-constant μ ($\alpha \rightarrow 0$) and a viscous fluid with a dashpot-parameter η ($\alpha \rightarrow 1$), as becomes clear by inserting

$$k^{1-\alpha} = \mu^{1-\alpha} \cdot \eta^\alpha \quad (\text{A-3})$$

into Eq. (A-1). In this study k is represented by μ and η in order to avoid dependence of the units of the fitted parameter k , i.e. $\text{Pa} \cdot (\text{Pa s})^{\alpha/(1-\alpha)}$, on the second free variable α . Since μ and η are linearly-dependent, any assumption for the value of either μ or η can be made. We assumed $\eta = 7.3 \text{ Pa s}$, which is the dashpot-parameter in human liver according to the standard linear solid model given in Asbach et al. [1]. This assumption of η does not impact the significance of the data. The assumed η -value solely scales k to μ via Eq. (A-3), while the α -parameter remains unchanged. Of note, μ is independent of frequency and thus represents a material-inherent property different from the frequency-dependent shear modulus commonly used in elastography.

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