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Dynamic mechanical analysis to assess viscoelasticity of liver tissue in a rat model of nonalcoholic fatty liver disease

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ABSTRACT

Nonalcoholic fatty liver disease (NAFLD) is the most common liver disorder in both developed and developing countries. A noninvasive method of detecting early stage NAFLD and distinguishing non-alcoholic steatohepatitis (NASH) from simple steatosis (SS) would be useful. The over-accumulation of fat in hepatocytes alters the physical microstructure and chemical contents of the liver tissue. This study included dynamic mechanical analysis (DMA) testing on liver samples from a rat model of NAFLD to determine whether the tissue shows any significant changes in viscoelasticity due to the histological changes. Liver steatosis was induced in 57 rats by gavage feeding of a high fat emulsion; 12 rats received a standard diet only and served as controls. Each rat provided 2 or 3 samples for DMA tests. The shear modulus and loss modulus were measured at 9 frequency points evenly-spaced in the range from 1 Hz to 41 Hz. The phase velocity of shear wave was calculated from the measured modulus. Multivariate T^2 test was used to assess the significance of intra-group difference. The results showed significant changes ($p < 0.05$) in storage modulus in livers with moderate to severe (S2 to S4) steatosis in comparison with livers without steatosis (S0), while the loss modulus demonstrated significant changes earlier in stage S1, indicating that fat accumulation affects the mechanical properties of liver, particularly viscosity. However, no significant differences were observed between the steatosis grades. These results also suggest that mild inflammation may affect the mechanical properties, which requires further verification. These findings provide new information about the mechanical properties of livers with NAFLD in low frequency range and suggest that it is possible to distinguish normal livers from livers with NAFLD.

1. Introduction

Nonalcoholic fatty liver disease (NAFLD), also called hepatic steatosis, is one of the most common causes of chronic liver disease (CLD) and has become a severe threat to human health in many parts of the world [1]. The prevalence of NAFLD is estimated to be 20%–30% in the general population of western countries [2], and it has also increased rapidly in China due to changes in lifestyle over the past 30 years. A recent study indicated that the prevalence of NAFLD was 19.3% in non-obese subjects and 60.5% in obese subjects in the Chinese population [3]. NAFLD is characterized by over-accumulation of fat in the liver, including simple steatosis (SS) and non-alcoholic steatohepatitis (NASH). Although the prognosis of SS is optimistic, NASH can progress to fibrosis and lead to end-stage liver disease. About 20% of patients with NASH finally develop liver cirrhosis [4]. A liver biopsy remains the gold standard method to grade the severity of the disease and thus estimate prognosis. As an invasive method, it is unsuitable for screening large numbers of subjects because of its risks and poor patient acceptance [5]. Furthermore, the heterogeneity of hepatic disease also poses a challenge to liver biopsy because of the small size of the sample [6]. A noninvasive method is desirable to detect early stage NAFLD and to differentiate NASH from SS. To date, various imaging methods have been utilized to evaluate patients with NAFLD, including ultrasound (US), computed tomography (CT) [7], magnetic resonance imaging (MRI) [8], magnetic resonance elastography (MRE) [9], and magnetic resonance spectroscopy (MRS) [8]. Among these methods, US is the only one that is suitable for screening large numbers of subjects; however, conventional US is limited by low accuracy in detecting mild steatosis [10,11], operator

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dependency [12], and its qualitative nature [13]. Therefore, less invasive, highly accurate, and affordable screening tools are required.

The main histological characteristic of NAFLD is the overaccumulation of fat in the form of a single large intracytoplasmic fat droplet in hepatocytes or smaller well-defined droplets displacing the nucleus to the cell periphery [14]. It is hypothesized that the changes in physical microstructure and chemical contents of liver tissue alters the density and mechanical properties of the tissue, leading to the changes in some of mechanical parameters. Therefore, some studies have investigated the mechanical property changes of liver during the development of NAFLD, and most of these studies used elastography technique. However, these studies provided conflicting results. Salameh et al. evaluated the mechanical properties of rat livers in a model of NASH using in vivo 7.0T MR elastography and found that liver elasticity was significantly elevated in animals with NASH whereas it was not significantly elevated in rats with SS [15,16]. Later, the same group measured liver stiffness in 58 NAFLD patients using MRE and found that liver stiffness had high accuracy for discriminating patients with NASH, even before the onset of the fibrosis, from those with SS [17]. Guzmán-Aroca et al. fed chickens a hyperlipidemic diet and found that the mean shear wave group velocity (gSWV) measured in vivo by acoustic radiation force impulse (ARFI) techniques in chicken livers increased with the severity of liver steatosis [18]. Their study in patients with morbid obesity suggested that the gSWV was higher in NASH patients than that in SS patients [19]. Barry et al. found that an increase in liver steatosis clearly increased the viscosity and resulted in an increased slope of the shear wave dispersion curve [20]. Later, they measured the shear wave phase velocity (pSWV) in frequencies ranging from 200 to 360 Hz using crawling wave techniques in liver samples from 70 mice with progressive increases in steatosis from 0% to 60%, and found that the increase in steatosis increased the dispersion [21]. However, Nightingale et al. measured the mechanically related parameters, such as shear wave group velocity, phase velocity at 200 Hz, and dispersion slope, in livers of 135 NAFLD patients and found no correlation between steatosis stage and any of these parameters [22].

The classical dynamical mechanical analysis (DMA) test is a conventional “gold standard” technique to determine the mechanical properties of a specimen. DMA tests have been used to investigate the mechanical properties of biological tissues and there are several reports of its use in liver tissue [23-25]. However, these studies investigated either normal livers or livers with fibrosis, and few studies have focused on livers with NAFLD. The objective of the current work is to determine whether there is any discernible change in biomechanical properties of liver tissue in different stages of steatosis at low-frequency ranges in a rat model of NAFLD using the DMA test. With an independent measurement of a completely different principle, our goal is that these results will extend our knowledge of viscoelastic properties of NAFLD livers below 50 Hz and help researchers to develop specific ultrasound-based measurement techniques.

2. Materials and methods

2.1. Principle of DMA

DMA is usually performed for 2 purposes: first, to measure the stiffness and damping of a material under certain conditions (temperature, frequency, stress, strain levels, humidity, etc.); and second, to monitor the changes of stiffness and damping with a change in temperature, frequency, or time. For linear-viscoelastic material, the applied sinusoidal stress induces a corresponding sinusoidal strain for which the amplitude and phase shift can be determined. The complex shear modulus G* is the ratio of stress to the strain in shear oscillation mode and is expressed as follows:

$$G^*(\omega) = \frac{\sigma(t)}{\varepsilon(t)} = \frac{\sigma_0 e^{j(\omega t+\delta)}}{\varepsilon_0 e^{j\omega t}} = \frac{\sigma_0}{\varepsilon_0}(\cos\delta + j\sin\delta) = G'(\omega) + jG''(\omega), \quad (1)$$

$$G'(\omega) = |G^*(\omega)|\cos\delta, \quad (2)$$

$$G''(\omega) = |G^*(\omega)|\sin\delta, \quad (3)$$

where ω, σ_0, ε_0 and δ are angular frequency, shear stress amplitude, shear strain amplitude and phase shift, respectively. The real part of the complex modulus, $G'(\omega)$ is called the storage modulus, reflecting the stiffness of a specimen, while the imaginary part, $G''(\omega)$, is called the loss modulus, reflecting its viscosity.

For a homogeneous tissue, the mechanical properties can also be described by the shear wave propagation velocity, which depends on the frequency of the shear wave and the viscoelastic properties of the tissue. The relationship between the phase velocity and the complex modulus is expressed as Eq. (4), which is independent of the specific rheological model:

$$V(\omega) = \sqrt{\frac{2\left(G'^2 + G''^2\right)}{\rho G'\left(1 + \left(\frac{G''}{G'}\right)^2\right)}} = \sqrt{\frac{2\left(G'^2 + G''^2\right)}{\rho\left(G' + \sqrt{G'^2 + G''^2}\right)}}, \quad (4)$$

where ρ is the density of tissue and assumed as 1 kg/m^3 in this study.

To characterize the viscoelastic properties of the tissues quantitatively, we first need to describe the tissue using a simple rheological model, and then derive the relationship showing that the speed of shear wave is dependent on the rheological parameters of frequency and density. Various rheological models have been investigated to describe the tissue, including the Maxwell, Zener, and Voigt models and the Voigt model is one of the most commonly used, especially in the studies for assessing liver viscoelasticity [26]. The Voigt model consists of a spring and a dashpot in parallel, describing the elasticity and viscosity respectively. By introducing the Voigt model, pSWV can be expressed by Eq. (5) [27]:

$$V(\omega) = \sqrt{\frac{2\left(\mu^2 + \omega^2\eta^2\right)}{\rho\left(\mu + \sqrt{\mu^2 + \omega^2\eta^2}\right)}}. \quad (5)$$

Here, μ is the shear elasticity, and η is the shear viscosity of the tissue. Therefore, the elasticity and viscosity properties can be estimated from the SWV dispersion curve according to Eq. (5).

2.2. Animal model

NAFLD was induced in male Sprague–Dawley (SD) rats by means of a high fat diet at Guangdong Medical Laboratory Animal Center, Guangdong, China. In total, 69 rats weighing 180–200 g were used in this study. They were housed in specific pathogen free rooms with a 10 h:14 h light/dark cycle at constant room temperature (20–26 °C) and humidity (40–70%). Rats were randomly divided into 2 groups. The control group (F0), including 12 rats, were fed with normal water and food. The remaining 57 rats were included in the treatment group to induce NAFLD; in addition to normal water and food, this group was also fed with a high fat emulsion by gavage at a dose of 1 mL/100 g body weight once a day. The high fat emulsion was composed of 20% lard, 10% cholesterol, 2% sodium cholate, 0.5% propylthiouracil, 30% fructose and 37.5% pure water. Furthermore, 55% olive oil was injected subcutaneously twice a week at a dose of 0.6 mL/100 g rat weight initially and 0.3 mL/100 g rat weight thereafter. Every 2 weeks after

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