Ultrasound in Med. & Biol., Vol. 40, No. 4, pp. 704–713, 2014

0301-5629/$ - see front matter

ELSEVIER

http://dx.doi.org/10.1016/j.ultrasmedbio.2013.10.016

● *Original Contribution for the Festschrift in honor of Robert C. Waag*

MOUSE LIVER DISPERSION FOR THE DIAGNOSIS OF EARLY-STAGE FATTY LIVER DISEASE: A 70-SAMPLE STUDY

Christopher T. Barry,[*] Zaegyoo Hah,[†] Alexander Partin,[†] Robert A. Mooney,[‡] Kuang-Hsiang Chuang,[*] Alicia Augustine,[§] Anthony Almudevar,[||] Wenqing Cao,[‡] Deborah J. Rubens,[||,¶] and Kevin J. Parker[†]

[*] Department of Surgery, University of Rochester Medical Center, Rochester, New York, USA; [†] Department of Electrical & Computer Engineering, University of Rochester, Rochester, New York, USA; [‡] Department of Pathology and Laboratory Medicine, University of Rochester Medical Center, Rochester, New York, USA; [§] Department of Public Health Services, University of Rochester Medical Center, Rochester, New York, USA; [||] Department of Biostatistics and Computational Biology, University of Rochester Medical Center, Rochester, New York, USA; and [¶] Department of Radiology, University of Rochester Medical Center, Rochester, New York, USA

(*Received* 27 *June* 2013; *revised* 15 *October* 2013; *in final form* 21 *October* 2013)

Abstract—The accumulation of fat droplets within the liver is an important marker of liver disease. This study assesses gradations of steatosis in mouse livers using crawling waves, which are interfering patterns of shear waves introduced into the liver by external sources. The crawling waves are detected by Doppler ultrasound imaging techniques, and these are analyzed to estimate the shear wave speed as a function of frequency between 200 and 360 Hz. In a study of 70 mice with progressive increases in steatosis from 0% to >60%, increases in steatosis are found to increase the dispersion, or frequency dependence, of shear wave speed. This finding confirms an earlier, smaller study and points to the potential of a scoring system for steatosis based on shear wave dispersion. (E-mail: kevin.parker@rochester.edu)   © 2014 World Federation for Ultrasound in Medicine & Biology.

Key Words: **Fatty liver disease, Steatosis, Dispersion, Crawling waves, Shear wave elasticity imaging, Medical ultrasound imaging, Viscoelastic tissue models.**

INTRODUCTION

As a result of the unabated obesity epidemic, fatty liver disease (FLD) is the most common cause of liver dysfunction in the United States and other economically privileged countries (Angulo 2002; Dowman et al. 2010; Schreuder et al. 2008). Accumulation of fat droplets (steatosis) within the liver is most often associated with the metabolic syndrome of obesity, diabetes and dyslipidemia, but can also be caused by toxins such as alcohol and certain chemotherapeutic agents or, rarely, associated with pregnancy (Charlton 2004; Marchesini et al. 1999; Wanless and Lentz 1990). Simple hepatic steatosis is reversible, but can progress to a chronic inflammatory and fibrotic state termed NASH (non-alcoholic steatohepatitis). Cirrhosis caused by NASH is predicted to become the leading cause of end-stage liver failure and indication for liver transplant in this country within the next 8 y (Charlton 2004; Selzner and Clavien 2001).

Liver biopsy currently remains the gold standard for diagnosing and assessing FLD (Minervini et al. 2009). Because this procedure is uncomfortable and can rarely result in serious complications, the current practice is to reserve biopsy for patients in whom the suspicion for NASH is high (based on blood testing or imaging) (Strassburg and Manns 2006). Unfortunately, biochemical assessment and currently available imaging modalities are insensitive in determining the presence or degree of FLD. Furthermore, histologic assessment of liver biopsies is completely subjective (based on the individual pathologist's estimation of overall steatosis and fibrosis) and, therefore, subject to wide clinical variability.

Non-invasive techniques to assess hepatic steatosis are emerging to meet this critical need and include magnetic resonance elastography (Chen et al. 2011; Salameh et al. 2009; Schwenzer et al. 2009), magnetic resonance

Address correspondence to: Kevin J. Parker, University of Rochester, Hopeman Building 203, PO Box 270126, Rochester, NY 14627-0126, USA. E-mail: kevin.parker@rochester.edu

spectroscopy (Friedrich-Rust et al. 2010), computed tomography elastography (Castera et al. 2008), radiation force methods (Chen et al. 2013) and controlled attenuation parameter transient elastography (de Ledinghen et al. 2012; Friedrich-Rust et al. 2012; Sasso et al. 2010, 2012). We propose an ultrasound-based system that can serve as a point of care screening modality (*i.e.,* available to primary care physicians and gastroenterologists) to complement and increase efficient use of biopsy and other more costly imaging techniques.

Our previous work (Barry et al. 2012) introduced the hypothesis that increasing the amount of fat in the liver would increase the dispersion of shear wave velocity, resulting in an increase in the slope of shear speed and shear attenuation versus frequency. This is a consequence of adding a viscous element, triglycerides, to the liver medium. This previous study reported results from a preliminary study of 14 mice divided into two groups, lean (<5% steatosis) and obese (~65% steatosis). The difference in dispersion or slope of shear speed versus frequency between the two groups was found to be statistically significant ($p < 0.003$) Dispersion was low in lean livers (0.16 ± 0.03 m/s per 100 Hz) and higher in obese livers (0.23 ± 0.04 m/s per 100 Hz), as measured over a shear wave frequency band centered around 260 Hz.

In the present study, we expand the numbers of mice and attempt to titrate the response by examining subgroups with increasing steatosis. Ultimately the goal is to establish a fine gradation scoring of steatosis using shear wave dispersion, *in vivo*. The present study takes a first step toward that goal.

THEORY

Shear wave dispersion

To model shear wave propagation in sinusoidal steady state in an elastic material with losses, the general stress-strain relationship is

$$T(\omega) = \mu S(\omega) \tag{1}$$

where T and S are the shear stress and strain, respectively, ω is the frequency and μ is the shear modulus; the shear wave speed $c_s = \sqrt{\mu/\rho}$, where ρ is density. Assuming that μ can be described as $\mu(\omega) = K(\omega) + jH(\omega)$, where K is the real part and H is the imaginary part of the shear modulus, then the complex wavenumber is

$$k = \frac{\omega}{c_s} = \beta - j\alpha = \frac{\omega}{\sqrt{\frac{K(\omega)+jH(\omega)}{\rho}}} \tag{2}$$

Here, k is the wavenumber with real (β) and imaginary (α) parts (Blackstock 2000). The attenuation coefficient, α, of a propagating wave will therefore be a function of frequency depending on $K(\omega)$ and $H(\omega)$. Expanding on the real and imaginary parts of eqn (2), we have

$$\beta = \omega\sqrt{\frac{\rho}{K^2+H^2}}\left[\frac{1}{2}\left(1+\frac{1}{\sqrt{1+\frac{H^2}{K^2}}}\right)\right]^{\frac{1}{2}} \tag{3}$$

and the wave speed

$$c = \sqrt{\frac{\sqrt{K^2+H^2}}{\rho}}\left[\frac{1}{2}\left(1+\frac{1}{\sqrt{1+\frac{H^2}{K^2}}}\right)\right]^{-\frac{1}{2}} \tag{4}$$

and the absorption coefficient

$$\alpha = \omega\sqrt{\frac{\rho}{\sqrt{K^2+H^2}}}\left[\frac{1}{2}\left(1-\frac{1}{\sqrt{1+\frac{H^2}{K^2}}}\right)\right]^{\frac{1}{2}}$$

$$= \frac{\omega}{c}\sqrt{\frac{1-\frac{1}{\sqrt{1+\frac{H^2}{K^2}}}}{1+\frac{1}{\sqrt{1+\frac{H^2}{K^2}}}}}, \tag{5}$$

Note that if $H(\omega)$ is zero, then c and β are constant (over frequency), and α is zero. However, if $H(\omega)$ is non-zero, then c and α have a slope versus frequency and are termed "dispersive." From these relations, dispersion measurements are found to indicate the presence of a loss term in the material properties.

Crawling waves

The crawling wave technique, introduced by Wu et al. (2004), is an elasticity imaging method used to map elastic properties within biomaterials. It is a slowly propagating pattern of interfering shear waves, generated in the medium *via* non-invasive sources. Crawling wave (CrW) propagation can be implemented using external mechanical vibrations (Hoyt et al. 2008b; Partin et al. in press; Wu et al. 2006) as well as acoustic radiation force (Hah et al. 2012; Hazard et al. 2012). CrW data acquired by an ultrasound system can be further analyzed to estimate shear parameters (shear speed or shear modulus) within the region of interest (ROI) and, thereby, used to quantify the elasticity of the scanned medium.

In this study, mechanical vibration sources are placed at opposite sides of a phantom to induce plane shear waves into a ROI using continuous harmonic vibrations, as illustrated in Figure 1. The sources are driven by sinusoidal signals with a slight difference between the frequencies f_1 and f_2, such that $(f_2 - f_1) \ll f$. Subscripts 1 and 2 correspond to the left and right sources,