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● *Original Contribution*

A NEW METHOD FOR MEASURING THE SPEED OF SOUND IN RAT LIVER *EX VIVO* USING AN ULTRASOUND SYSTEM: CORRELATION OF SOUND SPEED WITH FAT DEPOSITION

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Abstract—The speed of sound correlates well with the fat content of the liver. Therefore, non-invasive quantification of sound speed in the liver might be of diagnostic value. Here we describe a new non-invasive method that would be clinically applicable for measurement of sound speed in the liver. Sprague–Dawley rats were divided into two groups: a control group and a fatty liver group prepared by keeping the rats on a choline-deficient diet for 6 wk. The livers were subjected to pathologic and biochemical analysis; the speed of sound through the liver tissue was measured using our proposed method and a pulser–receiver as standard. Our results indicated that use of the proposed method makes it feasible to diagnose fatty liver with good accuracy on the basis of sound speed. This approach would have considerable potential for non-invasive diagnosis of fatty liver and would be a valuable adjunct to conventional liver diagnostic procedures. (E-mail: h-kumagai@jichi.ac.jp) **© 2014 World Federation for Ultrasound in Medicine & Biology.**

Key Words: **Fatty liver, Non-invasive diagnosis, Pulser–receiver, Rat, Speed measurement, Speed of sound, Steatosis, Tissue characterization, Ultrasonics, Ultrasound.**

INTRODUCTION

Some progressive liver diseases are associated with various complications such as cirrhosis, portal hypertension and hepatocarcinoma. It is thus important to evaluate the condition of the liver to make treatment decisions or prognoses. For this purpose, biopsy is the current diagnostic "gold standard," but has certain disadvantages such as a risk of serious complications, sampling error and variability in histologic evaluation among pathologists (Bedossa et al. 2003; Brunt and Tiniakos 2010; Mehta et al. 2009). Therefore, there is a need to develop non-invasive, repeatable and accurate techniques for quantitative evaluation of liver tissue characteristics (Adams and Feldstein 2011; Ghoshal et al. 2012). Although evaluation of liver disease using ultrasonic imaging is non-invasive and effective, it is highly subjective and depends on the expertise and experience of the operator (Schwenzer et al. 2009; Zwiebel 1995). A different technique for quantitative characterization of liver tissue is needed. There have been several attempts at non-invasive external measurement of acoustic or physical properties, such as the speed of sound (Bamber et al. 1987), frequency-dependent attenuation (Fujii et al. 2002; Itoh et al. 1988) and elasticity (Abenavoli and Beaugrand 2012; Myers et al. 2012). Diagnostic techniques that evaluate frequency attenuation or elasticity have been developed in the clinical setting, but evaluation based on sound speed has not yet been applied clinically. As reported in a number of previous publications, the speed of sound is well correlated with the fat content of the liver (Bamber et al. 1981; Chen et al. 1987). Therefore, non-invasive quantification of sound speed in the liver might be of diagnostic value. The conventional reflection-mode ultrasound system provides reflective intensity information coordinated with ultrasonic propagation time in a single sound path. The reason for the difficulty in measuring sound speed in a clinical situation is that it is impossible to calculate sound speed or depth independently on the basis of the

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ultrasonic propagation time in a single sound path. Here we describe a new non-invasive method that would be clinically applicable for measurement of sound speed in the liver. We focused on the fact that a reflection ultrasound system obtains echo information from multiple oscillators in simultaneous reflections. We considered that it would be possible to calculate different multiple ultrasonic propagation on the basis of this information and to calculate the sound speed and depth separately. Our ultimate goal was to develop a non-invasive diagnostic technique capable of tissue characterization by measuring the speed of sound in the liver. Sound speed through liver tissues was measured using not only our proposed method, but also the pulser–receiver method, which yields true values for sound speed. The livers we studied were then subjected to pathologic and biochemical analyses to yield "gold standard" data for comparison.

METHODS

Animals

Animal experiments were carried out in a humane manner after receiving approval from the Institutional Animal Experiment Committee of Jichi Medical University, and in accordance with the Institutional Guidelines for Proper Conduct of Animal Experiments and Related Activities in Academic Research Institutions under the jurisdiction of the Ministry of Education, Culture, Sports, Science and Technology.

Sprague–Dawley rats aged 5 wk were obtained from Charles River Laboratories (Yokohama, Japan). Eight rats were fed a choline-deficient diet (F2CDD, Oriental Yeast, Tokyo, Japan) for 6 wk, and eight controls were fed standard chow (CE-2, Clea Japan, Tokyo, Japan). The choline-deficient diet model of fatty liver disease exploits the fact that choline is essential for correct assembly and export of very-low-density lipoprotein, the main vehicle for export of fat from the liver to peripheral adipose tissue. Being unable to correctly produce and export very-low-density lipoprotein, the choline-deficient animals accumulate fat in the liver (Koteish and Diehl 2001).

The animals were housed in standard stainless-steel cages at normal humidity and room temperature with a diurnal 12-h light cycle, and provided with water *ad libitum*. At 1, 2, 4 and 6 wk after the start of diet feeding, rats were sacrificed by intraperitoneal infusion of thiopental and their livers were removed.

Ultrasonic methods

The largest lateral left lobe was excised (5–10 mm thick) for measurement of sound speed by our new method. In parallel, the second largest lobe was excised for measurement of sound speed with a pulser–receiver, which provides the actual speed of the ultrasound. Both samples were completely submerged in 0.9% degassed saline solution at 36.3 ± 0.2°C with a water bath temperature controller, and sound speed was measured after 10 min to allow temperature equalization between the samples and the saline solution. Ultrasonic measurements were conducted once with each method within 20 min after removal of the liver from the body to prevent possible errors caused by changes in liver properties (Hachiya et al. 1993). However, for each measurement, we obtained multiple data. Moreover, we had preliminarily confirmed that the difference in sound speed between the two lobes of a rat liver was within 10 m/s, which indicated the same resolution value as the local sound speed.

New method for determination of sound speed

We obtained ultrasonic information using our ultrasound system after covering the removed liver sample with a polymeric hydrogel-based tissue phantom (OST, Kashiwa, Japan) (20 mm thick, sound speed 1580 m/s at 36°C) to simulate subcutaneous fat. Sound speed was measured using a 7.0-MHz linear array-type scanner (Panasonic, Osaka, Japan) equipped with our prototype ultrasound system (Fig. 1). Raw data were saved by the system (sampling rate = 40 MHz), and measurements were conducted in multifocus mode in 4-mm steps between 11 and 60 mm (total of 13 focuses). Sound speed was calculated with our C++ program.

The ultrasound system creates individual point signal images by adjusting the time delay of the signals received by the probe oscillator. It is necessary to set the sound speed level of the tested material to adjust the time delay (sound speed setting). In a conventional ultrasound system, the sound speed setting is fixed at one

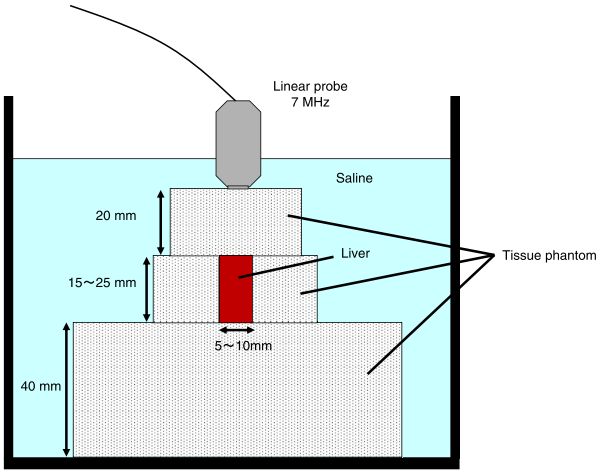

Fig. 1. Ultrasound system of new method for determination of sound speed. The removed liver sample is covered with a polymeric hydrogel-based tissue phantom to simulate subcutaneous fat. Sound speed was measured using a 7.0-MHz linear array-type scanner equipped with our prototype ultrasound system.