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● *Original Contribution*

EX VIVO STUDY OF QUANTITATIVE ULTRASOUND PARAMETERS IN FATTY RABBIT LIVERS

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Abstract—Nonalcoholic fatty liver disease (NAFLD) affects more than 30% of Americans, and with increasing problems of obesity in the United States, NAFLD is poised to become an even more serious medical concern. At present, accurate classification of steatosis (fatty liver) represents a significant challenge. In this study, the use of high-frequency (8 to 25 MHz) quantitative ultrasound (QUS) imaging to quantify fatty liver was explored. QUS is an imaging technique that can be used to quantify properties of tissue giving rise to scattered ultrasound. The changes in the ultrasound properties of livers in rabbits undergoing atherogenic diets of varying durations were investigated using QUS. Rabbits were placed on a special fatty diet for 0, 3, or 6 weeks. The fattiness of the livers was quantified by estimating the total lipid content of the livers. Ultrasonic properties, such as speed of sound, attenuation, and backscatter coefficients, were estimated in *ex vivo* rabbit liver samples from animals that had been on the diet for varying periods. Two QUS parameters were estimated based on the backscatter coefficient: effective scatterer diameter (ESD) and effective acoustic concentration (EAC), using a spherical Gaussian scattering model. Two parameters were estimated based on the backscattered envelope statistics (the k parameter and the μ parameter) according to the homodyned K distribution. The speed of sound decreased from 1574 to 1565 m/s and the attenuation coefficient increased from 0.71 to 1.27 dB/cm/MHz, respectively, with increasing fat content in the liver. The ESD decreased from 31 to 17 μm and the EAC increased from 38 to 63 dB/cm^3 with increasing fat content in the liver. A significant increase in the μ parameter from 0.18 to 0.93 scatterers/mm^3 was observed with increasing fat content in the liver samples. The results of this study indicate that QUS parameters are sensitive to fat content in the liver. (E-mail: oelze@illinois.edu)

Key Words: **Quantitative ultrasound, Attenuation coefficient, Backscatter coefficient, Envelope statistics, Fatty liver.**

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is the most common cause of chronic liver disease and leads to more severe liver conditions, such as hepatocarcinomas, cirrhosis, or complete liver failure (Wieckowska and Feldstein 2008). Even though biopsy is the current gold standard for diagnosis of diffuse liver disease, this technique is not without limitations. First, liver biopsy suffers from sampling problems: liver biopsies sample as little as 1/50,000 of the total mass of the liver, often resulting in insufficient information for a definitive diagnosis (Janiec et al. 2005; Ratziu et al. 2005; Merriman et al. 2006). Second, it is an invasive method involving certain risks and added stress and expense. Finally, the histologic evaluation is subjective and dependent on the experience of the pathologist. Therefore, there is a medical need to develop noninvasive techniques that can robustly quantify diffuse liver disease such as NAFLD.

Ultrasonic imaging has been explored for many years for its ability to detect and characterize liver disease (Tchelepi et al. 2002; Mishra and Younossi 2007). Although conventional ultrasound has been successful in diagnosing some liver conditions, the use of this technique for liver disease diagnosis has several limitations. Current conventional ultrasonic techniques do not allow for quantification of the degree of fatty liver. The

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effectiveness of ultrasound for liver disease detection is reduced in patients who are morbidly obese (sensitivity is reduced to below 40%) (de Almeida et al. 2008). For conventional ultrasound to detect steatosis, the degree of fat infiltration in the liver must be above 30% (Fishbein et al. 1997; Mehta et al. 2008; Dasarathy et al. 2009). Moreover, ultrasonic imaging is highly subjective and depends on the expertise and experience of the operator (Zwiebel 1995). Researchers have investigated the liver-kidney contrast to quantify liver fat content using the ultrasound hepatic/renal ratio and the hepatic attenuation rate from ultrasound hepatic and right kidney images (Xia et al. 2011). However, this technique still needs standardization and further testing in a clinical setting.

Quantitative ultrasound (QUS) techniques have been widely used to characterize tissue microstructure and to infer the acoustical properties of the tissue. Measurements of speed of sound (Goss et al. 1978; Techavipoo et al. 2004) can provide a means of tissue characterization. The mean sound speed in soft tissue varies from approximately 1420 m/s in breast fat to 1640 m/s in muscle tissues (Goss et al. 1978; Duck 1990). Bamber and Hill (1981) reported higher mean sound speed in excised normal liver than in fatty human livers. In another *in vivo* study, researchers reported higher sound speed in normal liver than in fatty liver without fibrosis from human (Chen et al. 1987).

Spectral-based QUS parameters, such as attenuation and the backscatter coefficient (BSC) can be estimated from backscattered signals, which can be used to differentiate between normal and fat-infiltrated livers. QUS techniques have been used to quantify properties of the liver for both *in vitro* and *in vivo* studies (Bamber and Hill 1981; Fei and Shung 1985; Wear et al. 1995). Afschrift and coworkers reported increases in attenuation as the degree of steatosis exceeded 5 vol% compared to healthy liver (Afschrift et al. 1987). In a clinical trial, O’Donnell and Reilly (1985) observed higher BSCs in subjects with liver cirrhosis than in normal livers. The authors did not observe any significant differences in attenuation between normal livers and livers with cirrhosis. In an *in vivo* study, Wilson et al. (1984) reported higher attenuation in livers with cirrhosis and fatty changes compared to normal livers. Researchers have examined the use of attenuation and BSCs to monitor the stages of the liver remodeling in mice (Gaitini et al. 2004; Guimond et al. 2007). Lu and coworkers demonstrated that the BSC and attenuation in patients with diffuse liver disease were higher than in patients with healthy livers (Lu et al. 1999). Suzuki and coworkers observed that the ultrasonic attenuation depends on fatty infiltration of the liver and to a lesser extent on fibrosis (Suzuki et al. 1992).

Recent studies have examined the use of envelope statistics to characterize fibrotic liver (Tsui et al. 2009; Igarashi et al. 2010; Yamaguchi and Hachiya 2010; Ho et al. 2012). In these studies, different models for describing the envelope statistics of backscattered ultrasound (Rayleigh, Nakagami, and the K distribution) were used to characterize successfully a liver as either fibrotic or normal. In a similar study, researchers used the textural features of ultrasound B-mode images to grade hepatic steatosis in children with suspected NAFLD (Shannon et al. 2011).

Because QUS parameters have provided a unique set of descriptors to classify tissues, it is of interest to quantify how these parameters change in a liver with varying degrees of fatty infiltration. In this study, several QUS parameters, such as sound speed, attenuation, and BSC, were estimated in comparison to lipid content in fresh rabbit liver samples. Two parameters were estimated from the BSC: effective scatterer diameter (ESD) and effective acoustic concentration (EAC); and two parameters were estimated from the envelope statistics: the k parameter and the μ parameter. In subsequent sections, the experimental methods used to quantify the changes in QUS parameters versus lipid content are described. The experimental results from fresh rabbit liver samples are provided in a later section. Finally, some conclusions regarding the study are offered.

EXPERIMENTAL METHODS

Liver samples

Fresh liver samples were extracted from male New Zealand White rabbits acquired from Myrtle’s Rabbitry (Tompson’s Station, TN). The rabbits had been on a special fatty diet (King et al. 2009). The basal diet contained 10% fat, 1% cholesterol, 0.11% magnesium, 14% protein, and 54% carbohydrates (1811279, 5TZB, Purina Test Diet, Purina, Richmond, IN). A group of 5 rabbits was put on the fatty diet for 3 weeks and another group of 5 rabbits was put on the fatty diet for 6 weeks. A third group of 4 rabbits consumed a standard chow diet (2031, Harlan Teklad, Indianapolis, IN) and was used as a control for the study. Rabbits were randomly assigned to 1 of the 3 diets. Water was given ad libitum and 140 g of food was given daily to the rabbits. The feed intake was measured daily and rabbit weights were done weekly. The animals were housed individually in standard stainless steel cages at normal room temperature and light cycles. All procedures were approved by the Institutional Animal Care and Use Committee at the University of Illinois at Urbana-Champaign. Ultrasonic experiments were conducted within 15 min of the removal of the liver from the body. Bamber et al. (1977) observed insignificant changes in attenuation and significant changes in mean