Obesity and Liver Disease: Evaluation of Fatty Infiltration of the Liver Using Ultrasonic Attenuation

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Summary We developed an in vivo ultrasonic attenuation measurement system with which we attempted to evaluate the degree of fatty infiltration in the liver. In an animal study, fatty liver was induced in rabbits, and ultrasonic radiofrequency waveforms from the liver were obtained using a 10 MHz A mode transducer. Frequency-dependent attenuation of the ultrasound, which was correlated with total lipid content, was calculated using a spectral difference method. In a human study, ultrasonic waveforms were obtained using a 3.5 MHz transducer. Frequency-dependent attenuation also showed a significant correlation with the grading of fatty infiltration of the liver. These results suggested that fatty infiltration of the liver could be evaluated quantitatively and noninvasively using frequency-dependent attenuation of the ultrasound.

Key Words human, fatty liver, rabbit, experimental fatty liver model, attenuation of ultrasound

Abnormal liver tests are often found in obese patients, and fatty infiltration of the liver is the most common histopathological change in these patients. Some fatty livers are reported to progress to liver fibrosis, even to liver cirrhosis (1). These findings suggest that accurate evaluation and treatment of fatty infiltration of the liver is very important in preventing the progression of chronic liver disease at an early stage. By using commercially available B-scan equipment, fatty infiltration of the liver can be evaluated by characteristic echo patterns, for example, “bright liver”. However, these echo patterns are not specific for fatty liver (2–3) and the ultrasonic findings depend on the subjective judgement of the sonographer. Furthermore, sonographic grading of the disease is not precise enough to predict the severity of the pathological changes (4).

We developed an ultrasonic attenuation measurement system, with which we attempted to quantitatively evaluate fatty infiltration of the liver. In the present study, we first used an experimental rabbit fatty liver model. Ultrasonic data were obtained in vivo directly from the liver, and attenuation of ultrasound was compared with the fat content measured by a quantitative method. Secondly, we performed a human study. Ultrasonic waveforms were obtained noninvasively, using a 3.5 MHz linear transducer, and ultrasonic attenuation was compared with the grading of fatty infiltration of the liver.
Fig. 1. Block diagram of system for the measurement of the ultrasonic attenuation coefficient of rabbit liver.

MATERIALS AND METHODS

A. Animal Study

Materials. Twelve male albino rabbits weighing 2.0–2.8 kg were used. Six of these rabbits received intravenous administrations of 10% Intrafat (Daigo Nutritive Chemicals, Osaka, Japan) at 80 ml/kg body weight daily for 3–9 weeks (Intrafat is a fat emulsion for intravenous infusion, containing soy been oil 10.0 g., phospholipid 1.2 g, and glycerol 2.5 g per 100 ml). The other six rabbits were not treated.

Instrumentation. The equipment consisted of a 10 MHz A-mode transducer (7mm in diameter)(Shimadzu Corp., Kyoto, Japan), a pulse generator (Shimadzu Corp.), an A/D converter (R/390AD; SONY-TEKTRONIX, Tokyo, Japan), a personal computer (PC-9801 VM2; NEC, Tokyo, Japan), and an oscilloscope (T912; TEKTRONIX, Beaverton, OR, USA). A block diagram of the system is shown in Fig.1.

Data acquisition. Rabbits were anesthetized with pentobarbital and the liver was exposed through a midline incision of the abdomen. Then, while the transducer was held in contact with the liver, ultrasonic radiofrequency (RF) signals from the liver were digitized and transferred to the personal computer memory. One hundred waveforms were obtained and stored on a floppy disk. Off-line analysis, using the personal computer, was applied to the waveforms.

Acoustic attenuation measurement. Frequency-dependent attenuation (FDA) was calculated using the spectral difference method (5). FDA measurement is shown schematically in Fig.2. Non-overlapping data segments were determined in the waveforms; the length of the data segments corresponded to 3 mm in the liver. Both the near data and far data segments were fast Fourrier transformed to yield the power spectra. By averaging one hundred log-power spectra in each data segment, we were able to obtain the corresponding average log-power spectra. The attenuation coefficients were determined from the difference between the two average log-power spectrum values. The FDA is the slope of the attenuation coefficient versus frequency curve. This slope was
Fig. 2. Schema for ultrasonic attenuation measurement. Digitized ultrasonic signals from the liver are shown in the upper panel. The signals inside the ROI (region of interest) are Hamming windowed, fast Fourrier transformed, and averaged in each ROI to yield the corresponding averaged power spectra (middle panel). By subtracting two averaged power spectra and dividing by twice the distance between the ROI, we obtained the spectral difference (lower panel). The estimate of the slope of the least square line fit to this spectral difference represents the frequency-dependent attenuation coefficient value, A.

determined by the least square fit over the frequency range of 6.3–11.7 MHz.

Evaluation of fatty infiltration of the liver. After ultrasonic data acquisition, the rabbit was sacrificed by the injection of an overdose of pentobarbital. The liver was resected immediately and divided into pieces; one piece was homogenized for measurement of total lipid content. Total liver lipid was extracted following the method of Folch (6), and the total lipid content per g wet weight of the liver was determined. Another piece of the liver was fixed in formalin and histologically examined for fatty
infiltration, using hemotoxylin-eosin and Sudan III staining.

**B. Human study**

**Subjects.** The subjects of the study were 17 inpatients at Osaka University hospital. They all showed abnormal liver tests and underwent liver biopsy. Their liver conditions were: nonspecific changes (NS, n=5), fatty liver (FL, n=5), chronic hepatitis (CH, n=5), and liver cirrhosis (LC, n=5). Written informed consent was obtained from each patient before data were collected.

**Instrumentation.** A modified B-mode ultrasonic scanner with a 3.5 MHz linear transducer was used (SDU-500; Shimadzu Corp., Kyoto, Japan); this system can provide RF waveforms before nonlinear processing. A block diagram of the system is shown in Fig.3, left.

**Data acquisition and analysis.** Data were acquired after routine abdominal ultrasound examination on the day before the liver biopsy. RF waveforms of the scan line, denoted by a dotted line on the B-mode image (Fig.3, right), were provided and stored on a floppy disk of the personal computer. One hundred waveforms were obtained for each patient and the FDA of the ultrasound was calculated. Here, the frequency range for least square fit to determine FDA was 1.0–4.7 MHz.

**Evaluation of fatty infiltration of the liver.** Liver specimens from the patients were classified in 4 groups based upon the microscopic gradings for fatty infiltration: 0= no or
rare fat droplets, 1=10%–30% of cells affected, 2=30%–60% affected, 3=60%–100% affected.

C. Statistical methods. Values were expressed as mean ± SD. Student’s unpaired t test was used to compare total lipid content and FDA in the fatty liver model with these values in the normal controls. Simple linear regression was performed to assess the relationship between FDA and the total lipid content of the rabbit liver. Spearman’s rank correlation was used to assess the relationship between FDA and the grading of fatty infiltration of human liver.

Fig. 4. Microphotograph of hematoxylin-eosin stained liver specimen obtained from a rabbit which received intravenously administered fat emulsion. Fatty infiltration is seen in the liver, but no apparent fibrosis is observed.

Fig. 5. Relationship between frequency dependent attenuation and total lipid content in rabbit liver. There is a good correlation between the two ($r=0.92$, $p<0.001$).

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RESULTS

The liver of rabbits intravenously administered with Intrafat showed diffuse fatty infiltration, with some predisposition to the pericentral area (Fig. 4). If we applied the grading of steatosis, fatty infiltration of grade 1–2 was observed in the treated group. However, no apparent fibrosis was observed in the livers, either in the fatty liver model or in the normal controls. Total liver lipid content was significantly increased \( (p<0.01) \) in the fatty liver model \( (95.7 \pm 19.2 \text{ mg/g liver} \) compared with the normal controls \( (45.0 \pm 7.3 \text{ mg/g liver} \). FDA was significantly increased \( (p<0.01) \) in the fatty liver model \( (0.69 \pm 0.11 \text{ dB/MHz/cm} \) compared with the normal controls \( (0.37 \pm 0.06 \text{ dB/MHz/cm} \).

Figure 5 shows the relationship between total lipid content and FDA in the rabbit liver. There was a good correlation between total lipid content and FDA \( (r=0.92, p<0.001) \).

In the human study, FDA was increased with fatty infiltration of the liver. There was a significant correlation between FDA and the microscopic grading of fatty infiltration in the liver \( (r_s=0.836, p<0.01, \text{Fig. 6}) \). Fatty livers associated with chronic hepatitis or liver cirrhosis showed almost the same FDA values as fatty livers of the same grade without chronic hepatitis or cirrhosis.

![Graph showing correlation between total lipid content and FDA in the rabbit liver.](image)

**Fig. 6** Relationship between frequency dependent attenuation and microscopic degree of fatty infiltration in human liver. There is a significant correlation \( (r=0.836, p<0.01) \).
DISCUSSION

In this study, we produced an experimental rabbit fatty liver model. We were able to produce mild to moderate fatty liver, and the distribution of fat droplets was mainly centrilobular. The distribution of fat in the liver of obesity patients is centrilobular in milder cases, and diffuse in severe cases (7), so our experimental fatty liver model seems to be an appropriate model of human fatty liver associated with obesity. The FDA of the fatty liver model was correlated with the total liver lipid content. Attenuation of ultrasound is the sum of the energy loss due to reflection and scattering, as well as to absorption. In addition to the fact that pure fat itself absorbs ultrasound, fat droplets in the liver cause scattering. Thus, elevated attenuation of ultrasound in fatty liver may be due to the increased absorption and scattering of ultrasound.

In the human study, FDA was correlated with the grading of fatty infiltration. Although the number of cases was small, we found that pathological conditions of the liver, other than fatty infiltration, seemed to have little effect on the attenuation coefficient. These results suggested that fatty infiltration of the liver could be evaluated quantitatively and noninvasively by measuring the FDA of the ultrasound. Further investigation is needed to clarify the usefulness of the FDA of ultrasound in the assessment of fatty infiltration of the liver.

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