ELSEVIER

Ultrasound in Med. & Biol., Vol. 23, No. 1, pp. 1–8, 1997

0301-5629/97 $17.00 + .00

PII S0301-5629(96)00181-0

●*Original Contribution*

ULTRASOUND ATTENUATION AND BACKSCATTER IN THE LIVER DURING PREDNISONE ADMINISTRATION

Z. F. LU,* J. A. ZAGZEBSKI,* R. T. O'BRIEN† and H. STEINBERG‡
*Department of Medical Physics, The University of Wisconsin Medical School, Madison, WI, USA;
and Departments of †Surgical Sciences and ‡Pathobiology,
University of Wisconsin School of Veterinary Medicine, Madison, WI, USA

(*Received* 12 *March* 1996; *in final form* 18 *March* 1996)

Abstract—Ultrasound attenuation and backscatter changes resulting from glucocorticoid administration were investigated in a dog model. Ten beagle dogs were randomized into two groups: five were given 2 mg/kg/day IM injections of prednisone to induce steroid hepatopathy and five served as controls. Histology showed vacuolization in most hepatocytes of treated animals on the third day of treatment, and larger, midzonally distributed vacuoles from day 7 on. An increase in both ultrasonic attenuation and backscatter was observed in treated dogs during *in vivo* measurements. Pooled data from the two groups suggest that attenuation elevations precede backscatter changes. Attenuation was significantly higher in the treated animals than in the controls by day 7. Both attenuation and backscatter were significantly higher in livers of treated than untreated dogs when measured by direct application of the transducer on the liver following euthanasia. We conclude that attenuation and backscatter coefficients can detect early changes in the liver associated with steroid hepatopathy. This may be a useful model to investigate detection of diffuse liver disease with ultrasound tissue characterization. Copyright © 1997 World Federation for Ultrasound in Medicine & Biology.

Key Words: **Ultrasound attenuation, Ultrasound backscattering, Liver, Quantitative ultrasound imaging, Prednisone, Steroid Hepatopathy, Ultrasound, Veterinary ultrasound.**

INTRODUCTION

Estimations of acoustic attenuation and backscatter are used routinely for diagnosing and assessing diffuse disease conditions in organs (Garra 1993). Currently, these estimates are done qualitatively during clinical ultrasound studies by noting the overall image brightness of an organ, the ability to penetrate and visualize deep margins of the organ and/or the system receiver gain settings required to produce acceptable ultrasound images. *In vivo* quantitation of acoustic parameters, such as attenuation (Garra et al. 1987; Hartman et al. 1993; He 1989; Ophir et al. 1985; Wilson et al. 1984; Zagzebski et al. 1993), backscatter (Garra et al. 1987; Zagzebski et al. 1993) and scatterer diameter (Garra et al. 1987), is possible. However, this is seldom done during routine patient scans because specialized equipment and computer software are required for reliable measurements. Also, equivocal results, mainly for attenuation (Garra et al. 1987; Lin et al. 1988; Taylor et al. 1986), may have led to skepticism over the diagnostic role of such measurements.

Qualitative assessments of echogenicity and backscatter also are common in veterinary ultrasound. Relative B-mode image brightness and visualization depth into the tissue are used for diagnosing and monitoring diffuse disease conditions in the liver of dogs (Nyland and Park 1983), cats (Yeager and Mohammed 1992) and cattle (Acorda et al. 1994). Assessments of muscles and ligaments in horses are also made on the basis of echogenicity (Nicoll et al. 1993).

This report focuses on ultrasound attenuation and backscatter changes in the liver accompanying experimentally induced steroid hepatopathy. Steroid hepatopathy is a commonly diagnosed sequelum to naturally occurring hyperadrenocorticism (Cushing's disease) and administration of corticosteroids to dogs (Rogers and Ruebner 1974). Studies have demonstrated hepatocellular vacuolation with glycogen granule deposition as early as 2 days after administration of corticosteroids at low immunosuppressive doses (Dillon et al. 1980; Fittschen and Bellamy 1984). Over time, these histological changes result in a diffuse, subjec-

Address correspondence to: Dr. J. A. Zagzebski, Department of Medical Physics, University of Wisconsin-Madison Medical School, 1530 Medical Sciences Center, 1300 University Avenue, Madison, WI 53706-1532, USA.

tively hyperechoic image pattern during ultrasound examinations of the liver (Schelling 1991). This opens the possibility that ultrasound might be sensitive to detect liver vacuolar degeneration brought on by Cushing's disease or drug therapy. No research, however, has quantified the acoustic changes in the liver leading to these subjectively detected changes on ultrasound images, nor have studies determined the time course of these variations.

The purpose of this study was to measure ultrasound attenuation and backscatter coefficients in the liver of dogs during prednisone administration. The results could provide a basis to make assessments of the ability of quantitative ultrasound methods to detect acoustic changes accompanying subtle liver vacuolar degeneration. The study also addresses the feasibility of experimental steroid hepatopathy in dogs as a model for the quantification of diffuse liver disease in both human and veterinary patients.

MATERIALS AND METHODS

The study was done in conjunction with an investigation of ultrasound B-mode image changes during steroid hepatopathy (O'Brien et al. 1996) and was done under an approved protocol from the University of Wisconsin Research Animal Resources Center.

Initially, six healthy beagle dogs each weighing approximately 20 kg were randomized into two groups of three treated and three controls. Treated animals were given daily IM injections of prednisone at a dose of 2 mg/kg/day. Ultrasound echo signals were recorded on days 0, 3, 7, 10, 14 and 18 of the experiment, and weekly thereafter until day 31. During a subsequent stage of the study, four additional animals, two treated and two controls, were followed, but only until day 14.

The animals were fasted for at least 12 h before each experimental session. Ultrasound echo data were recorded from a left paraxyphoid and a right intercostal window to acquire signals from different regions of the liver. Prior to ultrasound scanning, dogs were given an IM sedative (acepromazine, 0.2 mg/kg) and the skin hair was clipped. Coupling gel and alcohol provided acoustic coupling.

A Siemens Sonoline SL-1 sector scanner equipped with a 7.5-MHz mechanical transducer was used to acquire echo data. The frequency chosen was that used in conventional veterinary examinations of animals of this size. The scanner was modified by the manufacturer to output linearly amplified, radio frequency (RF) echo signals. RF signals from a region of interest, delineated using B-mode images from the scanner, were digitized at 50 MHz by a transient recorder (LeCroy TR8828C) and transferred to a MicroVax (Digital Equipment Corporation) computer using a GPIB interface (National Instruments).

All data in this study were acquired without the experimenter being aware of which animals were treated. The area selected for RF data acquisition typically was 4–5 cm in depth and consisted of 60–80 consecutive acoustic lines, free of echoes from major vessels. RF data from five image planes were acquired from each region of the liver. After echo data were acquired from the liver, RF echo signals were also recorded from a tissue-mimicking phantom, which served as an acoustic reference. The same transducer, instrument gain and output settings were used for the reference phantom data as for the echo data from the liver. The attenuation and backscatter coefficients of the reference phantom were known, so ratios of the processed echo signals from the liver and from the phantom could be used to estimate the acoustic parameters in the liver (Yao et al. 1990).

After ultrasound data were acquired and while dogs were sedated, two percutaneous biopsy samples were obtained from the liver. These were taken using a 14-ga needle and automatic biopsy instrument. One sample was fixed in 10% buffered formalin; half of the other sample was placed in 100% ethanol, and the other half was frozen unfixed at −70°C. Formalin- and alcohol-fixed sections were stained with both hematoxylin and eosin (H&E) with and without diastase and evaluated for histopathological changes. Alcohol-fixed sections were stained with periodic acid Schiff (PAS) to determine amounts of glycogen. Frozen sections were stained with oil red-O and evaluated for lipid content.

Biopsy samples were evaluated semiquantitatively on a "0–3" scale for evidence of histopathological change and the presence of glycogen and lipid content increase. Presence of vacuoles was graded by the number of hepatocytes with intracellular vacuoles per hepatic lobule. A score of "0" was given to samples with < 10% of hepatocytes vacuolated and "3" represented 70%–100% vacuolization. Semiquantitation of hepatic glycogen or lipid content was based on the number of glycogen granules or lipid per hepatic lobule in PAS-stained sections. A score of "1" represented a 10%–50% increase in the number, and "3" (marked increase) represented a 100% increase in the number (Dillon et al. 1980).

Dogs were humanely euthanized at the conclusion of the experiment: on day 31 for five of the first six animals and day 14 for the second group. The liver was surgically exposed, and *in situ* RF echo data were recorded from both right and left sides of the liver with the 7.5-MHz mechanical transducer coupled directly on the organ. This allowed attenuation and backscatter estimates without having to correct for losses through

body wall layers. One dog in the first group was euthanized on day 18 because of complications following biopsy.

Echo signal analysis

Similar to the data acquisition, data analysis was done without knowing which RF files were from treated or control dogs. The signal analysis methods have been previously described (Yao et al. 1990; Zagzebski et al. 1993), and so will be outlined only briefly here. Signals along each beam line within a uniform, operator-selected region of interest in the liver were subjected to quadrature detection off-line. Computer programs branched each time-dependent waveform into two channels, multiplying signals in one channel by $\sin\omega_o t$, and in the other by $\cos\omega_o t$, where ω_o is the analysis frequency and t the echo arrival time. Signals were then convolved with a 4-μs Blackman–Harris window, which served as a low-pass filter, the window duration establishing the analyzed signal bandwidth (Zagzebski et al. 1993). The filtered echo signals in each quadrature channel were then squared and the two waveforms were added, producing a single time-dependent waveform. Averaging these time-dependent signals over all beam lines in the selected region resulted in "filtered, signal intensity vs. depth" data for the liver. With the 7.5-MHz transducer, this process was done for six frequency components, *viz*, 5, 5.5, 6, 6.5, 7 and 7.5 MHz.

The same analysis was applied to the signals from the reference phantom. Depth-dependent ratios of filtered echo signal intensities from the liver to intensities from the reference phantom then were obtained. Our analysis shows that this ratio is independent of the ultrasound instrument and transducer beam, depending only on the acoustic properties of the sample and reference (Yao et al. 1990).

For a uniform region in the sample, the logarithm of the intensity ratio plotted vs. depth forms a straight line. The slope of this line is proportional to the difference between the attenuation coefficient of the liver and that of the reference phantom. Because the latter is known, the attenuation in the sample can be deduced. Quantitative "backscatter estimator" images of the liver are then produced by compensating the intensity ratio for the attenuation along the acoustic path in the liver and multiplying by the backscatter coefficient of the reference phantom. The backscatter coefficient of the liver at the analysis frequency is obtained by averaging backscatter estimators over at least a 4-cm × 4-cm region.

For the *in vivo* measurements, attenuation losses through the body wall must also be compensated for to estimate the backscatter coefficient in the liver. In this study, we computed an effective attenuation coefficient for the body wall by comparing closely the echo signals acquired *in vivo* on the last measurement day for one animal with signals acquired *in situ* just minutes later. For each scanning plane, signals from a 2-cm × 0.3-cm region of interest in the liver, centered at a depth, z, of 2.5 cm, were isolated and the Fourier transform computed. This was done for all five data acquisition planes, and the results were averaged, yielding the function $I(\omega_o, z)$ where ω_o is the analysis frequency. The same approach was applied to the RF echo signals from the liver *in situ*, yielding $I'(\omega_o, z)$. An effective attenuation coefficient for the body wall, $\alpha_{eff}(\omega_o)$, was then computed using:

$$\alpha_{eff}(\omega_o) = \frac{1}{4z_{wall}} \ln\left(\frac{I'(\omega_o,z)}{I(\omega_o,z)}\right) + \alpha_{liver}(\omega_o), \quad (1)$$

where z_{wall} is the body wall thickness and α_{liver} the attenuation coefficient of the liver, measured using the method described in the previous paragraph. The attenuation coefficient is expressed in dB/cm by multiplying the value in eqn (1) by 8.686.

The effective attenuation coefficient slope of the body wall tissue (including muscle, skin and fat) measured in this study was 1.4 dB/cm-MHz for the right window and 1.2 dB/cm-MHz for the left window. The body wall attenuation correction for each dog was done by measuring the body wall thickness estimated from B-mode images and multiplying by the effective attenuation coefficient for each frequency component of the received echo signal.

RESULTS

Figure 1 shows biopsy samples from a treated dog with H&E stain on days 0, 3 and 14. Histological changes in these treated dogs were already evident on day 3 (Fig. 1b), when samples from these animals scored "3" for vacuolization. Small vacuoles were predominantly PAS and oil red-O negative, indicative of water accumulation (hydropic degeneration). During the course of the treatments, there was a change in the distribution of vacuoles, from diffuse to midzonal hepatocytes, and in the score, from 3 to 2; vacuoles also increased in size (arrows, Fig. 1c). Increased amounts of oil red-O positive lipid outside of the vacuoles were seen in day 14 experimental animals. An increase of glycogen granule deposition reported by other groups using histochemical analysis (Dillon et al. 1980; Rogers and Ruebner 1974) was not observed in this study.

In situ results for attenuation and backscatter coefficients averaged over all animals are plotted in Figs. 2 and 3 as a function of ultrasonic frequency. These

(a)

(b)

(c)

Fig. 1. H&E stained liver histology sections. (a) Day 0, hepatocytes with uniform nonvacuolated diffuse eosinophilic cytoplasm. 125× magnification. (b) Day 3, 70%–100% of hepatocytes (score of 3) spanning hepatic lobule with vacuoles of variable sizes. 200× magnification. (c) Day 14, 11%–69% of hepatocytes (score of 2) vacuolated, with increases in vacuole size segregated to the midzonal region (arrows) of hepatic lobule. 200× magnification.

data were obtained at the termination of the experiment, which was day 31 for three controls and two treated animals, day 18 for one treated dog (because of a complication following biopsy) and day 14 for the last four animals. The error bars represent ± 1 SD among results for all animals in a group. Both the mean attenuation and mean backscatter increased significantly in the treated group. At 6 MHz, the mean attenuation coefficient divided by frequency for controls is about 0.74 dB/cm-MHz. The mean for treated animals is 0.87 dB/cm-MHz at this frequency. No differences were found in the results from the two sides (right vs. left) of the liver.

At 6 MHz, the backscatter coefficient of normal dog liver is approximately 37×10^{-4} $cm^{-1}sr^{-1}$. The backscatter coefficient increased to approximately three times this value in dogs in the steroid hepatopathy group. Interestingly, the mean backscatter coefficient frequency dependence does not appear to exhibit any variations between the normal liver and the liver in the treated group for the analysis frequency range.

To study the time course of attenuation and backscatter changes in *in vivo* scanning, the backscatter coefficient and the slope of the attenuation coefficient vs. frequency were determined for each measurement session. For each dog, an average attenuation coefficient slope (dB/cm/MHz) and an average backscatter coefficient were computed from the 5.5-, 6- and 6.5-MHz frequency components. Scatter plots presenting *in vivo* results of data acquired from individual dogs as a function of time are presented in Figs. 4 and 5. Although there is considerable overlap among data for the two groups, there is a clear trend towards increasing

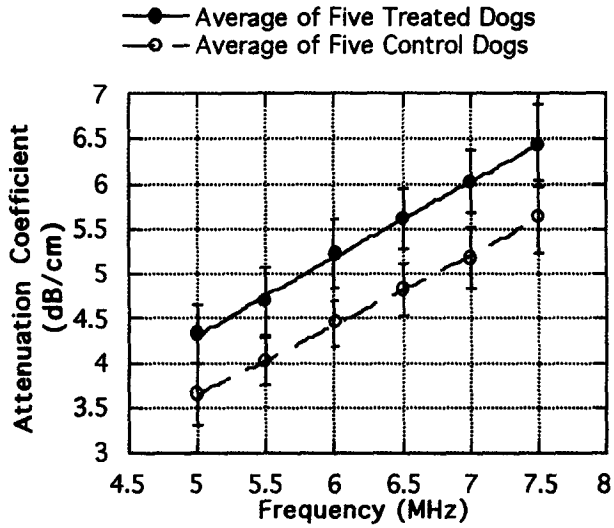

Fig. 2. Attenuation coefficient vs. frequency measured *in situ*, with the transducer placed directly on the liver surface at the conclusion of treatment. Attenuation in livers of treated animals (top curve) is greater than attenuation for controls. Error bars represent ± 1 SD of results among animals of a group.

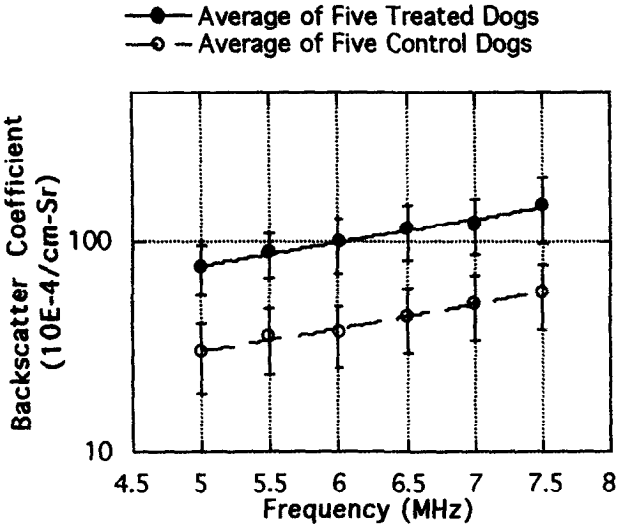

Fig. 3. Backscatter coefficient vs. frequency measured *in situ*. The average backscatter coefficient in livers of control animals (bottom curve) is significantly lower than the backscatter coefficient in livers of treated animals (top curve). Error bars represent ± 1 SD of results among animals of a group.

attenuation and backscatter with treatment time in the treated animals.

The trend is more easily discerned in pooled results for each group. Figure 6 shows the average attenuation coefficient slope as a function of treatment time for both controls (squares) and treated (circles) animals. Error bars represent ± 1 SD of results for each day. As early as day 3, the pooled results indicate an increase in the attenuation coefficients of treated dogs compared to controls. Control dogs maintained similar levels of attenuation and backscatter as measured on day 0. A two-tailed *t*-test was applied, assuming the measured results were samples from normal distributions. The *t*-test indicates a statistically significant difference ($p \leq 0.05$) between attenuation coefficient slopes of the two groups starting at day 7. On day 10, the p value was ≤ 0.02, while on day 14 it was ≤ 0.003.

Average backscatter coefficients measured from the treated group are compared with the results measured from the control group in Fig. 7. Starting at approximately day 7, the backscatter coefficient of the treated dogs was noticeably higher than that of controls. Liver backscatter coefficient from treated dogs continued to increase with treatment time, while the backscatter coefficient of the controls did not change significantly during the study. A two-tailed *t*-test demonstrated significant differences ($p \leq 0.01$) in backscatter coefficients of the two groups on day 14.

DISCUSSION AND CONCLUSIONS

Both the ultrasound attenuation and backscatter coefficients increased with time in livers of dogs administered therapeutic doses of prednisone. The increased backscatter results were anticipated, based on subjective veterinary findings (Schelling 1991). Clinical ultrasound images from normal dogs exhibit a lower echogenicity in the liver than in the spleen; experimental steroid hepatopathy reverses this pattern, producing a higher liver echogenicity than that of the spleen.

The dose of prednisone administered is commonly used in veterinary clinical medicine for skin, joint and neurological diseases. For this dose, by day 14 the average backscatter coefficient at 6 MHz in treated dogs was three times the average value observed in normal dog livers. The backscatter coefficient increased continuously during treatments, even in animals for which the study was extended to 31 days. There was no noticeable variation in backscatter with frequency between the treated and untreated groups, but this may be a result of using only a 2.5-MHz measurement bandwidth. A useful follow-up study might be to investigate scatter over a broader frequency range.

Although we anticipated an elevated backscatter in livers of dogs treated with prednisone, a more immediate finding in this study was an increase in ultrasound attenuation very early during treatments. Differences between the attenuation of liver in normals and that of treated animals, measured through the intact body wall, were not statistically significant until day 7; however, the trends in the data strongly suggest that attenuation values in treated animals were higher than normals as early as day 3. By the end of the treatment period,

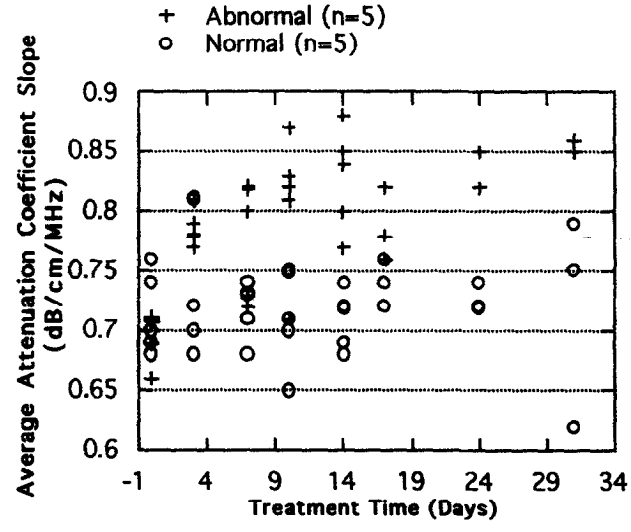

Fig. 4. Slope of the attenuation coefficient vs. frequency, averaged for 5.5-, 6- and 6.5-MHz frequency components, from livers of individual dogs, vs. time following initiation of treatment. Treated animals (+) exhibit increasing attenuation with time, whereas controls do not.

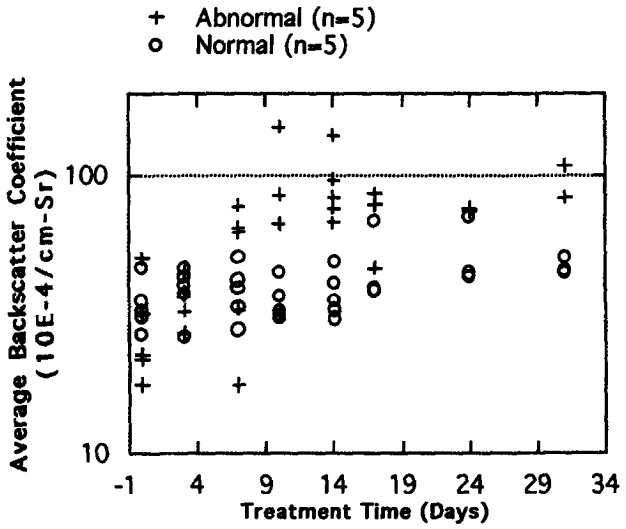

Fig. 5. Backscatter coefficients vs. time from liver of treated dogs (+) and controls (O). Treated animals (+) exhibit an increasing backscatter coefficient with time, but there is considerable overlap in the data.

pooled attenuation values had risen from an initial level of 0.74 dB/cm-MHz to 0.87 dB/cm-MHz. Duration of the changes after cessation of corticosteroid administration and rate of return to normal have not been investigated. (Badylak and Van Vleet 1981).

These attenuation increases cause difficulty imaging the liver on B-scans. Subjective interpretation of ultrasound images obtained at the same time as RF data acquisition was unable to discriminate between the livers of experimental and control dogs from days 0–7 (O'Brien et al. 1996). By day 10 of the experiment, however, the radiologist (RTO) was certain which animals were treated on the basis of image changes as well as on the basis of liver size and subjective spleen–liver comparisons. The attenuation and backscatter measurement results provide a quantitative assessment of these changes and appear to allow earlier detection than subjective analysis of images.

The data in Figs. 6 and 7 suggest that attenuation increases occurred earlier than backscatter elevations. Similar tendencies were reported by quantifying the video image data produced during this same study (O'Brien et al. 1996). This finding might be explainable from the histology results. By day 3, all treated dogs had significant vacuolization of hepatic cells. At this time, it was possible to measure an increase in attenuation but not in backscatter. A redistribution of vacuoles to the midzone and to larger sizes was noticed from day 7 on, and inspection of the mean backscatter levels strongly suggests that elevated scatter also becomes evident at this time. We hypothesize that elevated attenuation is associated with the mere presence of vacuolar material, whereas elevated backscattering is affected not only by the amount of vacuolar material but also by the size and spatial distribution of vacuoles. The progression from diffuse vacuoles to vacuoles that were larger and predominantly midzonal seems to be associated with elevations in backscatter. Analogous observations have been made for attenuation and backscatter in graphite powder-in-gel tissue phantoms. Attenuation for this phantom material is proportional to the mass of graphite, but backscatter levels are sensitive to the size distribution of graphite grains (Wu et al. 1992).

Steroid hepatopathy in this experimental model appeared to be characterized by vacuolar degeneration, imbition of water and, possibly, derangement of fat and glycogen metabolism. The major component of the vacuole content was predominantly PAS and oil red-O negative material, consistent with water. Evidence of smaller amounts of lipid and glycogen in vacuoles was also seen.

Our values for attenuation in untreated dog livers (0.74 dB/cm-MHz) are higher than that of normal human liver (0.5 dB/cm-MHz) (Zagzebski et al. 1993). We are not certain if this is a result of different frequencies used in human studies or histological differences. Overlapping the typical frequency range of both the animal and human measurements would be worthwhile in future studies.

Parker et al. (1988) and Tuthill et al. (1989) investigated acoustic changes in liver with glycogen content and for various drug treatments. Both studies reported elevated attenuation, but with no significant

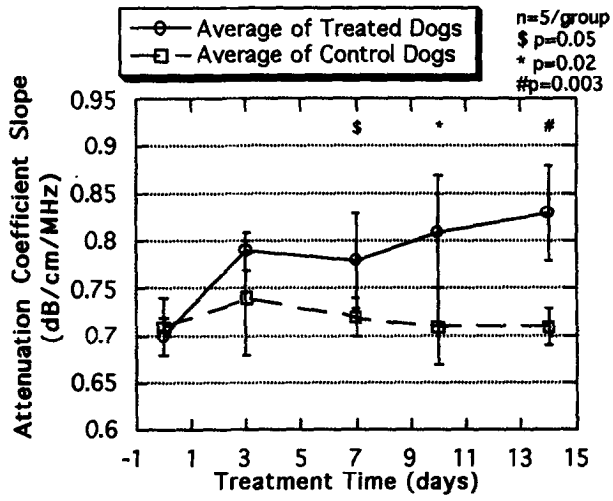

Fig. 6. Pooled results of attenuation coefficient slopes vs. time in livers of treated and control animals. Error bars represent ± 1 SD of results among animals of a group. Elevated attenuation seems to occur as early as day 3 after initiation of treatments. The data exhibit a statistically significant elevation in attenuation in the treated group by day 7 ($p < 0.05$).

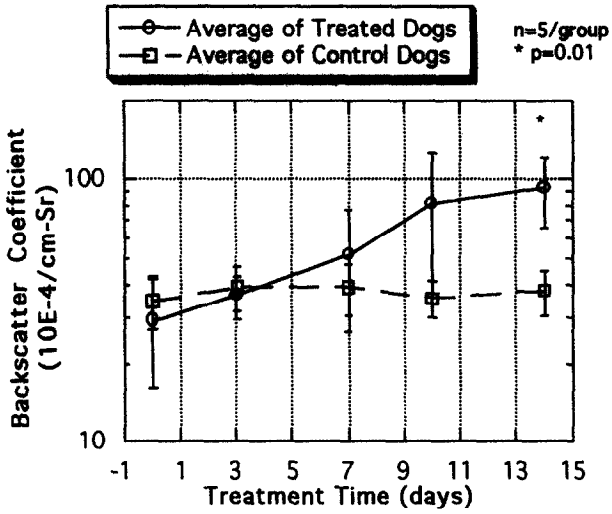

Fig. 7. Pooled results of *in vivo* backscatter coefficient measurements vs. time from the liver. Error bars represent ± 1 SD of results among animals of a group. Elevated backscatter is seen in the treated group, but not until at least day 7 of the study. On day 14, the backscatter coefficient elevation in the treated group was statistically significant ($p < 0.001$).

variations in ultrasonic backscatter. In contrast, our study demonstrates marked variations in both ultrasound backscatter and attenuation in the livers of dogs with steroid hepatopathy. Differences between our results and those of Parker et al. (1988) and Tuthill et al. (1989) conceivably reflect actual differences in liver tissue for the different experimental conditions; they also may be due to the latter experimenters' use of B-mode image echogenicity to judge backscatter, whereas we measured backscatter directly.

We believe that accurately quantitating backscatter and attenuation can be a useful supplement to conventional methods of judging echogenicity and attenuation in ultrasonography. Spleen–liver echogenicity comparisons are done routinely in veterinary ultrasound to monitor the liver during drug therapy. During prednisone administration, the increases in both attenuation and backscatter in livers of treated animals could have an offsetting effect on the echo level from a given depth. This could mask acoustic changes judged only on the basis of echogenicity. In ultrasound examinations of humans, quantification of backscatter and attenuation could provide more objective assessments than judgements of echogenicity and attenuation based on comparisons of echo signals from organs or evaluations of the ability of sound waves to penetrate to the extreme margins of an organ.

The dog steroid hepatopathy model appears to be useful for investigating sensitivity and accuracy of ultrasound methods for detecting diffuse liver disease in humans. The *in vivo* measurements exhibit greater variations than the *in situ* measurements, rendering this a challenging environment to test a measurement method applied to humans. Variations are caused mainly by the uncertainties from the acoustic scanning windows and the involuntary motion of the dogs. In addition, the fact that dogs were fasted for at least 12 h prior to the study resulted in a distended gall bladder. This reduced the region in the liver from which data that was subjectively judged to be acceptable on the basis of no vessels or large interfaces could be obtained. This is a more severe problem in the dog model than is generally observed for human liver scans. Nevertheless, we believe that this model can serve as a useful test bed for evaluating *in vivo* quantitative ultrasound techniques.

Acknowledgements—We are grateful to Dr. Ernie Madsen for providing the reference phantom and to Thadeus Wilson and Drs. Deborah Darien, Lisa Forrest and Mauria O'Brien who assisted with the experiments. This work was supported in part by Grant RO1 CA 39224 from the National Institutes of Health, The UW School of Veterinary Medicine Companion Animal Research Fund and the Wisconsin Clinical Cancer Center.

REFERENCES

Acorda JA, Yamada H, Ghamsari SM. Evaluation of fatty infiltration of the liver in dairy cattle through digital analysis of hepatic ultrasonograms. Vet Radiol Ultrasound 1994;35:120–123.

Badylak SF, VanVleet JF. Sequential morphologic and clinicopathologic alterations in dogs with experimentally induced glucocorticoid hepatopathy. Am J Vet Res 1981;43:1310–1318.

Dillon AR, Spano JS, Powers RD. Predinosolone induced hematologic biochemical and histologic changes in the dog. J Am Anim Hosp Assoc 1980;16:831–837.

Fittschen C, Bellamy JEC. Predinisone-induced morphologic and chemical changes in the liver of dogs. Vet Pathol 1984;21:399–406.

Garra BS. In vivo liver and splenic tissue characterization by scattering. In: Shung GA, Thieme GA, eds. Ultrasonic scattering in biological tissues. Boca Ratan, FL: CRC Press, 1993:347–391.

Garra BS, Insana MF, Shawker TH, Russell MA. Quantitative estimation of liver attenuation and echogenicity: Normal state versus diffuse liver disease. Radiology 1987;162:61–67.

Hartman PC, Oosterveld BJ, Thijssen JM, Rosenbusch GJ, van den Berg J. Detection and differentiation of diffuse liver disease by quantitative echography: A retrospective assessment. Invest Radiol 1993;28:1–5.

He P. Acoustic attenuation estimation for soft tissue from ultrasound echo envelope peaks. IEEE Trans Ultrasound Ferroelec Freq Cont 1989;36:197–203.

Lin T, Ophir J, Potter G. Correlation of ultrasonic attenuation with pathologic fat and fibrosis in liver disease. Ultrasound Med Biol 1988;14:729–734.

Nicoll RG, Wood AK, Martin IC. Ultrasonographic observations of the flexor tendons and ligaments of the metacarpal region of horses. Am J Vet Res 1993;54:502–506.

Nyland TG, Park RD. Hepatic ultrasonography in the dog. Vet Radiol 1983;24:74–84.

O'Brien RT, Zagzebski JA, Lu Z, Steinberg HA. Measurement of acoustic backscatter and attenuation in the liver of dogs with experimentally-induced steroid hepatopathy. Am J Vet Res 1996;57:1690–1694.

Ophir J, McWirt RE, Maklad NF, Jaeger PM. A narrowband pulse-echo technique for in vivo ultrasonic attenuation estimation. IEEE Trans Biomed Eng 1985;32:205–212.

Parker KJ, Asztely MS, Lerner RM, Schenk EA, Waag RC. In-vivo measurements of ultrasound attenuation in normal or diseased liver. Ultrasound Med Biol 1988;14:127–136.

Rogers WA, Ruebner BH. A retrospective study of probable glucocorticoid-induced hepatopathy in dogs. J Am Vet Med Assoc 1974;170:603–606.

Schelling CG. Ultrasonography of the adrenal glands. In: Kaplan PM, ed. Problems in veterinary medicine: Ultrasound. Philadelphia, PA: JB Lippincott, 1991:604–617.

Taylor KJ, Riely CA, Hammers L, et al. Quantitative ultrasound attenuation in normal liver and in patients with diffuse liver disease: Importance of fat. Radiology 1986;160:65–71.

Tuthill TA, Baggs RB, Parker KJ. Liver glycogen and water storage: Effect on ultrasound attenuation. Ultrasound Med Biol 1989; 15:621–627.

Wilson LS, Robinson DE, Doust BD. Frequency domain processing for ultrasonic attenuation measurement in liver. Ultrason Imaging 1984;6:278–292.

Wu EX, Goodsitt MM, Madsen EL. Microscopic mechanism of attenuation of compressional ultrasonic waves in tissue-mimicking phantom materials. Ultrason Imaging 1992;14:121–133.

Yao L, Zagzebski J, Madsen E. Backscatter coefficient measurements using a reference phantom to extract depth-dependent instrumentation factors. Ultrason Imaging 1990;12:58–70.

Yeager AE, Mohammed H. Accuracy of ultrasonography in the detection of severe hepatic lipidosis in cats. Am J Vet Res 1992;53:597–599.

Zagzebski J, Lu Z, Yao L. Quantitative ultrasound imaging: In vivo results in normal liver. Ultrason Imaging 1993;15:335–351.