Measurement of acoustic backscatter and attenuation in the liver of dogs with experimentally induced steroid hepatopathy

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Objective—To determine the usefulness of a new method of measuring acoustic backscatter and attenuation in the liver of dogs with experimental steroid-induced hepatopathy.

Animals—10 clinically normal dogs.

Procedure—Steroid hepatopathy was induced by daily injections of prednisone (2 mg/kg of body weight, IM). Dogs were evaluated histologically and were sonographically imaged on days 0, 3, 7, 10, and 14. Acoustic backscatter and attenuation were measured from in vivo images of dogs, using a video signal mod and, compared with results obtained from analysis of the unprocessed radio frequency signal.

Results—Histologic evaluation revealed midzonal, predominantly water-filled vacuoles in hepatocytes by day 7, which persisted for the remainder of the study and significantly \((P = 0.0001)\) increased liver weight on day 14. Attenuation and backscatter increased during the experimental period. Mean effective attenuation difference was higher \((P = 0.015)\) in the liver imaged through a left paraxiphoid window in experimental dogs by day 3. Significantly \((P < 0.05)\) greater attenuation persisted in the liver of experimental dogs throughout the experimental period. Mean backscatter ratio was significantly increased \((P = 0.02)\) by day 10. Uncorrected pixel intensity of the liver in 2 experimental dogs was approximately equal to that of the spleen on day 10 and greater than that of the spleen on day 14.

Conclusion—Administration of prednisone to dogs results in increased acoustic backscatter and attenuation in the liver.

Clinical Relevance—The video signal method is a sensitive technique for detecting subtle acoustic changes in the liver of dogs. (Am J Vet Res 1996;57:1690–1694)

Focal lesions of the liver are easy to sonographically differentiate from surrounding normal tissues. However, diagnosis of diffuse disease conditions is usually more difficult. Extensive research has been devoted to quantitative imaging of diffuse liver disease in human beings. To a large extent, this has been an attempt to more accurately diagnose cirrhosis sonographically. There is evidence that quantitative imaging of the liver may provide a more sensitive diagnosis than does conventional B-mode ultrasonography.

Current clinical veterinary methods are subjective, or at best semiquantitative, and evaluate principally the display intensity (echogenicity) of the liver. Image intensity is influenced by various instrument and operator factors, making unlikely the duplication of results without an identical imaging system. In the study reported here, experimentally induced steroid hepatopathy was investigated as a model for diffuse liver disease, and analysis of an in vivo video signal method of estimating attenuation and backscatter in dogs was tested.

Materials and Methods

Dogs—Ten adult, sexually intact male Beagles were entered into the project on the basis of normal clinical findings, CBC, serum biochemical analyses (including alkaline phosphatase and alanine transaminase), ACTH stimulation test, and liver histologic findings. Dogs were randomized to experimental \((n = 5)\) and control \((n = 5)\) groups without knowledge by personnel performing the imaging, analyzing the signal data, or interpreting the histopathologic findings. Steroid hepatopathy was induced by daily injections of prednisone \((2.0 \text{ mg/kg of body weight, IM})\) for 14 days. Imaging and percutaneous liver biopsy were performed on days 0, 3, 7, 10, and 14.

Ultrasonography—Imaging was performed, using a 7.5-MHz mechanical transducer and ultrasound machine. Left paraxiphoid (paragastrial plane, caudal to the costal arch) and right intercostal (transverse plane) windows were used to image left and right liver regions, respectively. Three images were collected from each window on each day. Dogs were sedated with acepromazine \((0.2 \text{ mg/kg, IV})\) to decrease discomfort and speed the imaging and biopsy procedures. Dogs were euthanized at conclusion of the study. The body wall was then opened, allowing in situ imaging of the liver and removal of the liver for gross and microscopic analyses.

Images of the liver in all dogs were evaluated subjectively in an attempt to predict which dogs were in the experimental group. Evaluation was based on liver size, shape of the liver margins, and echogenicity. In addition, 2 quantitative echo analysis methods were applied.

Video echo signal method—The video signal output from the ultrasound machine was digitized by use of a frame grabber board, recorded, and analyzed by image analysis software. Scanner settings, including receiver gain, time-gain compensation, transmit power, and dynamic range, were standardized. After scanning of the liver, 3 images were collected from a reference ultrasound phantom.
during the XIX World Biometrics Congress in Edinburgh, Scotland.

**New dean named at Florida**

*Dr. Joseph A. DiPietro* has been named the dean of the University of Florida College of Veterinary Medicine.

Presently an administrator at the University of Illinois College of Veterinary Medicine, DiPietro is known for his scientific contributions to the epidemiology, control, and treatment of parasitic diseases of large farm animals.

DiPietro will begin his new position at Florida on February 1. He will succeed Dean Richard Dierks, who is retiring in December. Dean Dierks came to Florida from the University of Illinois seven years ago after serving as dean there for 13 years.

"Dr. DiPietro is one of the world's leading experts on the treatment of parasitic disease in animals," David Challoner, vice president for health affairs at UF's Health Science Center, said. "He has helped advance new drug therapies that have saved hundreds of millions of dollars in the production of food animals and large sports animals."

DiPietro served as assistant and associate professor of veterinary clinical medicine and veterinary pathobiology at the University of Illinois from 1980-1990. From 1990-1993, he was assistant dean or acting associate dean for research, and from 1993-1994, assistant director of the Agricultural Experiment Station. Since 1994, he has been associate dean for research.

"I bring to this job my enthusiasm for the profession and a renewed view of the importance of the DVM curriculum, as well as a strong interest in research and extension activities," Dr. DiPietro said.

**Ground broken for poultry facility addition**

Ground was broken October 1 for a $6.5 million addition and renovation to the University of Georgia College of Veterinary Medicine's Poultry Diagnostic and Research Center. During the ceremony, university and industry leaders spoke about the contributions of the Department of Avian Medicine's faculty to the world's poultry industry and the significance of the renovation.

The two-story addition will contain 10 office and laboratory rooms. "This addition will give our faculty the space to conduct their research in a more efficient way," Dr. Stanley Klevens, department head of avian medicine, said. Since the original building was built in 1958, the number of faculty and staff has increased from about 15 to over 75.

A new necropsy laboratory also will be built, replacing the temporary facilities built in the early 1970s. Both building projects are expected to be completed in early fall 1997.

**Students receive awards**

During the Leadership Program for Veterinary Students at Cornell University, four students received veterinary medical prizes for their research. Instone Swaney, University of Tennessee, received the Veterinary Medicine prize for research identifying risk factors associated with equine protozoal myeloencephalitis and evaluating the accuracy of current diagnostic methods. Tamara Gull, Tufts University, was awarded the Cell Biology prize for research relating to the polymorphism in donkey MHC class-I cell surface antigens. Polly Peterson, Washington State University, received the Program prize for research relating to canine parvovirus infection. Margaret Fleischl, University of Wisconsin, was awarded the Molecular Biology prize for research related to the expression of the p53 tumor suppressor gene in two canine osteosarcoma cell lines.

The leadership program is an intensive educational experience for students pursuing research careers. Twenty-four students from the United States and other countries are selected for the 10-week program. Students present their findings at the conclusion of the program.

**Invitations**

**Nutrition abstracts invited**

The Waltham Centre for Pet Nutrition in Leicestershire, England, is interested in receiving abstracts for the Waltham International Symposium 1997, May 26-29 at the Hyatt Grand Cypress Hotel in Orlando, Fla. The symposium will follow the American College of Veterinary Internal Medicine Forum.

The symposium theme is Pet Nutrition and Health in the 21st Century. The first section will be devoted to new developments in pet nutrition and nutritional aspects of activity, growth, and aging. The second section will feature topical issues of dietary management.

Individuals interested in presenting short communications of original work are asked to convey their interest without delay to Dr. Ivan Burger; phone, 44 01664 415400; fax, 44 01664 415440; or e-mail, mailbox@wcpn.demon.co.uk.

**Candidates for equine research award requested**

Nominations for the 1997 Equine Applied Research Award in equine reproduction may be made by boards of equine veterinary associations affiliated with the World Equine Veterinary Association. Sponsored by Schering-Plough Animal Health, the award recognizes outstanding equine research performed during the past five years. A $5,000 prize and plaque will be presented to the winner during the 1997 WEVA Congress in Padua, Italy.

Nominations should include a brief account of the candidate's qualifications and career history, a listing of research articles published between 1992 and 1996, and a short explanation why the proposed candidate was chosen. Information for each category should be limited to a single printed page and must be submitted before Dec 25, 1996.

Send nominations to Dr. A. Atlock, secretary/treasurer, World Equine Veterinary Association, UAE Equestrian and Racing Federation, PO Box 3234, Abu Dhabi, United Arab Emirates; fax, 971 2 655 700.
phantom, along with a gray-scale mapping table derived for the scanner, was used to quantify absolute echo values for the liver. At 7 MHz, the approximate center frequency of the transducer, the attenuation coefficient in the reference phantom, is 4.1 dB/cm.11

A rectangular region of interest with subjectively uniform texture was chosen near the midline of the image. The region of interest started 2 cm beneath the skin, was 2.5 cm deep (4.5 cm from the skin surface), and approximately 1 cm wide. The width was varied to exclude regions with vessels or artifacts. Average pixel intensity for each horizontal row was plotted against depth. Profiles from several parallel scanning planes were averaged to reduce statistical fluctuations. Using the same criteria, regions of interest were drawn and mean pixel intensity versus mean depth was measured in the reference phantom. For each depth increment, the mean pixel value for the liver was subtracted from the mean pixel value at the same depth in the phantom. The pixel value differences were converted to relative echo signal differences in decibels by applying a gray-scale mapping table derived for the scanner. Because the reference phantom images are obtained using the same instrument settings and transducer, determining the echo value in the tissue relative to that of the reference phantom should "correct" for instrument dependencies on the pixel value versus depth profiles.11

The result was a mean echo value relative to the echo value in the phantom. Because the reference phantom’s acoustic attenuation and backscatter were known, the attenuation and scattering values in the liver could now be measured.

The depth-dependent echo value from the liver was expressed relative to that of the reference phantom. The slope and y-intercept, derived from linear fitting, were related to the attenuation and backscatter of the liver and reference phantom. If the attenuation in the liver is the same as that of the reference phantom, the slope of this line is zero. A positive or negative slope (in units of dB/cm) quantifies the attenuation relative to that of the reference phantom. Likewise, the zero depth (y) intercept of the relative echo value quantifies the echo scattering value (backscatter) in the tissue. For homogeneous materials, if the backscatter in the sample is the same as that in the reference phantom, the relative echo value is zero at depth = 0 cm; higher or lower backscatter values are reflected in the echo value being positive or negative at 0 cm. Results can be given either relative to the attenuation and backscatter in the reference phantom, as in this report, or, alternatively, they can be computed absolutely, using the known values for the phantom.11 Data from experimental dogs were compared with those from control dogs, using a two-tailed Student t-test.13

Gray-scale mapping table—The pixel value (brightness) of an ultrasound image is related to the echo signal amplitude. However, the actual dependence of the pixel value on echo amplitude is distorted by logarithmic amplification and by other signal and image processing functions in the scanner. A gray-scale mapping table for the system-transducer combination was generated to convert mean pixel values between the liver and reference phantoms to absolute echo amplitude differences in decibels. This minimizes the dependence of the relative image data on the scanner itself.

With the transducer scan plane and all system settings fixed, serial images were recorded from a tissue-mimicking phantom at transmit power settings of 0, −3, −6, −9, −12, −16, and −20 dB. The scanner was equipped with special connector jacks that allowed the actual (linear) echo values changes (in dB) for the nominal transmit power variations to be determined. Subsequent analysis of regional pixel value changes (ΔPV) corresponding to actual echo value changes (ΔdB) resulting from the transmit power changes allowed us to construct the required lookup table.

Liver-spleen comparison—Because of availability of a second imaging system,7 liver and spleen echogenicity comparison was performed on only the second cohort of 2 control and 2 experimental dogs. A 7.5-MHz linear transducer was used to collect images of liver and spleen from dogs prior to sedation. Analysis of pixel intensity was performed, using the aforementioned image analysis software in 0.6 × 0.6–cm regions of interest beginning 1.5 cm beneath the skin surface. Three areas were sampled in the liver and spleen. Regions were chosen on the basis of lack of artifacts or blood vessels in the image area. The mean pixel intensity of the spleen was subtracted from that of the liver and, because of the logarithmic scale of pixel intensity, this represents a liver-to-spleen ratio. Owing to the small number of dogs tested, statistical evaluation was not performed.

Liver histologic examination—Biopsy specimens were analyzed to correlate histopathologic findings with quantification of liver acoustic properties. Two percutaneous biopsy specimens were obtained by use of 14-gauge biopsy needles and an automatic biopsy instrument” from the liver in the left and right imaging window sites. One specimen was bisected; half was placed in 100% ethanol and half was frozen unfixed at −70 ºC. The remaining specimen was placed in buffered 10% formalin. Formalin- and alcohol-fixed specimens were processed in routine manner, serially sectioned, stained with either H&E or periodic acid-Schiff (PAS; with and without diastase), and evaluated for histologic changes, including presence of glycogen. Glycogen content was estimated by comparison with specimens from day 0. The frozen specimens were sectioned and stained with oil red-O and evaluated for lipid content. Specimens were graded for severity of hepatocellular vacuolization on a semiquantitative scale: 0 = < 10%, 1 = 10 to 40%, 2 = 41 to 70%, and 3 = 71 to 100% of hepatocytes containing vacuoles. A similar method was used to estimate lipid and hepatocellular glycogen content.

Results

Control and experimental dogs had normal hepatic histomorphometry with equivalent low amounts of hepatocellular vacuolization (grade 0) and of lipid vacuoles (grade 0) on day 0. Intracytoplasmic granular glycogen was present in > 70% of hepatocytes (grade 3) of all dogs. Values in control dogs maintained all these levels throughout the experimental period.

Figure 1—Photomicrograph of a section of liver from a treated dog on day 3 after induction of steroid hepatopathy. Notice pale diffuse staining vacuoles in hepatocytes. H&E stain.
The 5 experimental dogs developed histologic changes consistent with steroid hepatopathy. On day 3, small vacuoles were detected in most hepatocytes (Fig 1, grade 3). These vacuoles were predominantly PAS and oil red-O negative, indicative of water accumulation (hydropic degeneration). Increased amounts of oil red-O-positive lipid outside of the previously mentioned vacuoles were seen in experimental, compared with control, dogs.

On day 7, a discrete midzonal lobular (acinar zone 3) distribution of larger vacuoles (Fig 2, grade 2) was seen. Oil red-O- and PAS-positive material was seen in some of the medium to large vacuoles randomly scattered throughout liver specimens. This zonal distribution and minimal glycogen staining of hepatocellular vacuolization was maintained for the remainder of the experimental period. At time of necropsy, dogs in the experimental group (6.7 ± 0.93%) had significantly (P = 0.0001) increased mean (± SD) liver weight, expressed as percentage of body weight, compared with dogs in the control group (3.7 ± 0.42%).

Increased acoustic attenuation was measured in dogs of the experimental group, using the video signal method. The attenuation of liver, relative to the attenuation in the reference phantom, measured in the left window, increased on day 3, was significantly greater than that in control dogs (P ≤ 0.05), and maintained at this high level and significance throughout the experimental period (Fig 3). Compared with the reference phantom, untreated livers had just slightly lower attenuation, as did the livers of experimental dogs on day 0. However, the attenuation of livers in experimental dogs increased by 0.8 dB/cm to approximately 4.9 dB/cm by day 3. Data from the liver measured in the right window of experimental dogs was less consistent and did not indicate significance, compared with that in control dogs.

A trend of gradually increasing mean backscatter ratio was measured in experimental dogs, using the video signal method. A significantly (P ≤ 0.05) greater mean backscatter ratio was measured on days 10 and 14 (Fig 4), where the backscatter of the liver in ex-
Experimental dogs was approximately 5 dB greater than that in control dogs. Similar to measured attenuation, data from the liver measured in right window was less consistent and did not indicate a significant trend.

On day 0, the uncorrected pixel intensity in the spleen was greater than that in the liver in control and experimental dogs. The image intensities of the liver and spleen were approximately equivalent on day 7 and the uncorrected pixel value of the liver in experimental dogs was greater than that of the spleen on days 10 and 14 (Fig 3).

Subjective interpretation of the images was unable to discriminate between the liver of experimental and control dogs from days 0 to 7. A unanimous consensus was reached on day 10. This was based principally on hepatomegaly, round liver margins, and increased echogenicity. Retrospective review of the predictions revealed 100% accuracy.

Discussion

Administration of corticosteroids to dogs resulted in a characteristic pattern of hepatocellular vacuolization.13-18 Steroid hepatopathy in dogs is characterized by midzonal distribution of eccentric large nonstaining vacuoles. This is caused by cortisol excess attributable to endogenous or exogenous causes. Controversy exists as to the contents of the vacuole. Studies have variously hypothesized the contents to be water13,17,18 or glycogen.14,15 In this study, the major component of the vacuole content was predominately PAS- and oil red-O-negative material, consistent with water. Evidence of smaller amounts of lipid and glycogen in vacuoles also was seen. Steroid hepatopathy in this experimental model appeared to be characterized by vacuolar degeneration, disorientation of fat and glycogen metabolism. The effect of type of corticosteroid and dose regimen on contents of the vacuole are not known. Similarly, duration of the changes after cessation of cortisol administration and rate of return to normal have not been fully investigated.13

After 14 days of prednisone administration, the liver weight in experimental dogs, expressed as a function of percentage of body weight, was significantly greater than that in control dogs. This finding is consistent with reports of gross anatomic,19 radiographic,19 and sonographic20 evidence of hepatomegaly in dogs with hyperadrenocorticism.

Lack of significant data collected in the right window for backscatter and attenuation measurements was thought to be a result of increased statistical fluctuation associated with smaller, viewable liver or signal distortions occurring during transmission through the intercostal space. The typical depth of viewable liver on the right side of experimental and control dogs was approximately 3 cm, compared with 6 to 10 cm on the left side in control dogs. In experimental dogs, the depth of viewable liver in the left window often exceeded 10 cm by day 14.

The video signal method provided a sensitive measure of liver backscatter and attenuation. In the left window, significantly increased attenuation was measured 3 days after initiation of prednisone administration. At that time, the liver of experimental dogs appeared subjectively normal, even after retrospective evaluation of stored images. The increased attenuation persisted throughout the remainder of the experimental period.

Attenuation is the loss of signal intensity with increased depth as sound travels through a tissue. This occurs by a combination of absorption, scattering, and reflection. Absorption of sound is usually the dominant effect on attenuation in soft tissues.21

In steroid hepatopathy, increased attenuation appears to be the earliest detectable acoustic change. On day 3, there was no evidence of a substantially increased number of lipid-filled vacuoles and we concluded that the hyperattenuation was caused by increased numbers of diffusely distributed, small, water-filled vacuoles in the liver of experimental dogs. From days 7 to 14, increased size, more regional distribution, and increased numbers of lipid-filled vacuoles did not result in a significant change in attenuation. Previous studies20 have indicated that the amount of lipid affects attenuation in the liver of human beings. Further studies to evaluate the effect of distribution and size of vacuoles on attenuation may be useful.

Backscatter increased gradually in experimental dogs during the 14-day period, as measured by the video signal method. Significantly increased mean backscatter ratio was measured in experimental dogs on days 10 and 14 in the left window. The initial histopathologic change (day 3) of uniformly distributed, small, water-filled vacuoles resulted in no significant changes in measures of backscatter. Increased backscatter in dogs with experimentally induced steroid hepatopathy is hypothesized to be associated with increased size and density of vacuole distribution. The increased amount of lipid may also contribute to increased scattering.23

Backscatter coefficient is the parameter used to quantify the scattering properties of a volume of tissue and is defined as the ratio of the power scattered 180° to the incident sound beam. Using the video signal method, the mean backscatter coefficient is estimated by calculating the y-intercept of the slope of signal loss in the tissue relative to the reference phantom.

Attenuation and backscatter are major determinants of pixel intensity. Highly scattering tissue appears bright (hyperechoic) in the portion of the image closest to the transducer. If the tissue does not cause a large amount of attenuation (hypotattenuating), the portion of the image deeper in the same tissue (further away from the transducer) will remain bright. However, as is the situation in steroid hepatopathy in dogs, if the tissue is hyperattenuating, the same tissue will appear darker in deep than in superficial locations. Owing to the effects of attenuation, pixel intensity may be a poor measure of scattering in the tissue.

Although the screen brightness value (pixel intensity) is an indicator of the backscatter, this value is affected by a host of other factors. Some of these, such as attenuation, are tissue-related factors. Others are related to the individual machine, such as time-gain compensation, power output, pre- and postprocessing, gain, and contrast settings. These settings are virtually impossible to standardize between identical machines, much less different machine models/manufacturers. Any method that attempts to quantify acoustic parameters must account for and normalize all machine variables. The video signal method attempts to account for various machine and transducer effects. A potential weakness in the method, subject to further testing, is the effect of different machines and transducer types. A potential ef-
fect, seemingly not causing an effect in this model, is
temperature. As a broad band width sound beam
passes through a tissue, the signal will be differentially
attenuated, with the higher frequency sound attenuated
more than the lower end of the spectrum. In the me-
techanical transducer system evaluated at 0.1 cm depth
in a reference phantom, 7.5 MHz effectively had com-
ponent frequencies extending from 4.75 to 8.5 MHz. At
5 cm, the range was 4.75 to 6.5 MHz.4

Subjective comparison of liver and spleen echo-
genicity (pixel intensity) is currently the accepted
method of determining diffuse echogenicity changes in
either organ.23 On the basis of the previous discussion,
it is important that the comparison be performed on
images at the same distance from the transducer and
within the body wall of similar depth and with similar
acoustic parameters. In clinical imaging, these fac-
tors may be difficult to control. Variable thickness of
fat, body wall musculature, and dense fascial connec-
tive tissue may result in differing amounts of
attenuation. Optimally, the sonographer should strive
to produce an image that has both organs in the same
field to minimize anatomic variations in body wall
composition.23

Liver-spleen echogenicity was compared between
control (n = 2) and experimental (n = 2) dogs. From
days 0 to 7 in experimental and control dogs, the liver
had lower mean pixel value (hypoechoic) than did the
spleen. On day 10, the liver mean pixel values were
equivalent (isoechogenic) to those of the spleen. Finally,
on day 14 in experimental dogs the liver mean pixel values
were higher (hyepoechoic) than those in the spleen.
This correlates well with the clinical sonographic im-
pression of steroid hepatopathy in dogs, associated with
hyperadrenocorticism, causing hyepoechoic hepatomeg-
aly.24 Variably increased cortisol concentration may
cause variable degrees of hyepoechoicnecity. Therefore,
in addition to echogenicity, appreciation of the subjec-
tive appearance of the effects of increased attenuation
may be valuable in the clinical sonographic diagnosis of
steroid hepatopathy.

On the basis of the results of this study, the video
signal method may be a clinically useful tool to provide
estimates of acoustic properties, backscatter and attenu-
ation. Increased attenuation is an earlier and possibly
more sensitive indicator of steroid hepatopathy in the
liver of dogs. Whether measured quantitatively or evalu-
ated subjectively, appreciation of attenuation may
make the sonographer more sensitive to steroid hep-
atopathy and, possibly, other diffuse diseases of the
liver. Although the video signal method is sensitive for
steroid hepatopathy in dogs, further studies are neces-
sary to determine the specificity and compare the acous-
tic changes associated with additional liver diseases,
such as lipidosis, lymphoma, cirrhosis, or infection.

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**References**