In vivo proton magnetic resonance spectroscopy for the evaluation of hepatic encephalopathy in dogs

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Objective—To investigate clinical use of proton magnetic resonance spectroscopy (1H MRS) and to compare metabolic brain bioprofiles of dogs with and without hepatic encephalopathy.

Animals—6 dogs with hepatic encephalopathy and 12 control dogs.

Procedures—Conventional MRI and single-voxel 1H MRS were performed with a 3-T magnet. Images for routine MRI planes and sequences were obtained. Single-voxel 1H MRS was performed with a point-resolved sequence with a short echo time (35 milliseconds) and voxel of interest placement at the level of the basal ganglia. Metabolites of interest included the glutamine-glutamate complex (sum quantification of glutamate and glutamine), myoinositol, N-acetyl aspartate, total choline, and creatine. Data were analyzed with post-processing fitting algorithm software, and metabolite concentration relative to water and ratios with creatine as the reference metabolite were calculated.

Results—Compared with control dogs, dogs with hepatic encephalopathy had specific changes, which included significantly higher concentration relative to water of the glutamine-glutamate complex and significantly lower concentration of myoinositol. Choline and N-acetyl aspartate concentrations were also slightly lower in dogs with hepatic encephalopathy than in control dogs. No differences in creatine concentration were detected between groups.

Conclusions and Clinical Relevance—1H MRS aided in the diagnosis of hepatic encephalopathy in dogs, and findings supported the assumption that ammonia is a neurotoxin that manifests via glutamine-glutamate complex derangements. Use of 1H MRS may provide clinically relevant information in patients with subclinical hepatic encephalopathy, equivocal results of bile acids tests, and equivocal ammonia concentrations or may be helpful in monitoring efficacy of medical management. (Am J Vet Res 2014;75:818–827)

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Hepatic encephalopathy is a neurologic condition associated with failure of the liver to detoxify inhibitory neurotoxins. Hepatic encephalopathy in dogs is most often seen with congenital portosystemic vascular abnormalities, but it may also be seen with hepatopathies associated with acquired portosystemic shunts or with acute fulminant hepatic failure. The pathogenesis of hepatic encephalopathy is complex and there are multiple contributing factors, including ammonia, short-chain fatty acids, γ-aminobutyric acid, aromatic amino acids, endogenous benzodiazepines, and manganese. There is general consensus that ammonia plays the central role in this pathogenesis. Ammonia is produced primarily in the gastrointestinal tract by intestinal urease-positive bacteria and amino acid metabolism. Typically, a high amount of ammonia is extracted by the liver via the urea cycle. Blood ammonia concentrations increase and cross the blood-brain barrier in cases of hepatic dysfunction or portosystemic shunting, which causes neurotoxicosis. Astrocytes are the site for ammonia detoxification in the brain. Ammonia is eliminated by the amidation of glutamate to glutamine by the enzyme glutamine synthetase.

Two hypotheses have been proposed to explain the pathogenesis for hepatic encephalopathy: the osmotic gliopathy theory and the Trojan horse theory. The osmotic gliopathy theory proposes that glutamine synthesis is stimulated in response to hyperammonemia, which leads to accumulation of glutamine within the astrocytes and subsequent swelling of astrocytes.

ABBREVIATION

1H MRS Proton magnetic resonance spectroscopy
according to the Trojan horse theory, glutamine acts as a carrier and transfers ammonia across the mitochondrial membrane. In the mitochondria, glutamine is hydrolyzed by phosphate-activated glutamine synthetase to glutamate and ammonia. In this manner, excessive amounts of glutamine are transported from the cytoplasm to the mitochondria and serve as a carrier of ammonia. The glutamine-derived ammonia within the mitochondria interferes with mitochondrial function, which leads to excessive production of free radicals and induction of mitochondrial permeability transition (a Ca<sup>2+</sup>-dependent process associated with collapse of the inner mitochondrial membrane potential as a result of opening of the permeability transition pore). These 2 phenomena lead to mitochondrial and astrocyte dysfunction, including cell swelling and energy failure.8,14

There are numerous consequences of ammonia in the CNS. Deleterious effects include amino acid disturbances, alterations in neurotransmission, cerebral energy disturbances, alterations of nitric oxide synthesis and oxidative stress, impairments of axonal and dendritic growth, and alterations in signal transduction and channel transporter activities.2,5,9,10,15

Clinical signs of hepatic encephalopathy in dogs include those of diffuse cerebral disease, such as disorientation, seizures, stupor, and coma.1 In humans, hepatic encephalopathy is divided into 3 categories depending on the nature of the hepatic dysfunction, with further categorization depending on the pattern and severity of clinical signs.16 Type A encephalopathy is associated with acute liver failure, type B with portosystemic bypass without intrinsic liver disease, and type C with liver cirrhosis. Hepatic encephalopathy can be further described clinically as episodic, persistent, or minimal. The latter is associated with cognitive dysfunction but, if misdiagnosed, can lead to overt encephalopathy with decreased survival rate, poor response to medical management, and irreversible brain damage.17

Proton magnetic resonance spectroscopy allows for determination of the biochemical composition of the brain in vivo and for accurate identification and quantification of metabolites in localized regions.18,19 The metabolites detected in physiologically normal conditions in the brain with long echo time sequences (typically ≥ 144 milliseconds) are N-acetyl aspartate, choline, and creatine. With short echo time sequences (typically 35 milliseconds), other smaller metabolites such as glutamate, glutamine, and myoinositol can be detected.10

N-acetyl aspartate resonates at 2.01 ppm. N-acetyl aspartate is considered a neuronal marker, and it is present only in neurons, axons, and dendrites. The choline signal represents a composite peak that consists of contributions from the trimethyl amine, groups of glycerophosphocholine and phosphocholine, and a small amount of free choline. Choline (which resonates at 3.20 ppm) is involved in membrane synthesis and degradation. Creatine (which resonates at 3.03 ppm) is a composite peak that consists of creatine and phosphocreatine, which are compounds involved in energy metabolism via the creatine kinase reaction to generate ATP. Myoinositol (which resonates at 3.5 to 3.6 ppm) is a pentose sugar, which is part of the inositol triphosphate intracellular second-messenger system. Glutamate and glutamine are key compounds in brain metabolism. Glutamate is an excitatory neurotransmitter that plays a role in mitochondrial metabolism, and glutamine plays a role in detoxification and regulation of neurotransmitter activity. At 1.5 T, there is almost complete overlap of the glutamate and glutamine peaks, and the composite peak is often referred to as the glutamine-glutamate complex peak (which resonates at 3.75 ppm and between 2.1 and 2.5 ppm). At 3 T, glutamate and glutamine can be reliably determined with an appropriate pulse sequence (short echo time) and curve fitting methods.20 In pathological conditions, there may be abnormal concentrations (total absence of or lower or higher concentrations) of these metabolites, and other metabolites (eg, lipids or lactate) not typically present in healthy brain tissue may be evident. Proton magnetic resonance spectroscopy is widely used in human medicine for the diagnosis of several intracranial diseases, such as tumors, inflammatory diseases, psychiatric disorders, and metabolic or biochemical analysis.

The use of 1H MRS for the diagnosis and investigation of pathophysiologic mechanisms of hepatic encephalopathy in humans and other animals has been reported.1,20–38 The main finding in those studies was the increased glutamine-glutamate complex peak and reduced myoinositol peak, which reflect amino acid disturbances and osmoregulation, respectively. The use of 1H MRS in veterinary medicine is not widespread. Recently, 2 studies39,40 have reported reference values for clinically normal Beagles. There are some reports41–44 of experiments in dogs. However, clinical use of 1H MRS has not been extensively reported in veterinary medicine.

The purpose of the study reported here was to investigate changes in the brains of dogs with hepatic dysfunction attributable to a portosystemic shunt, chronic cirrhosis, or acute liver disease by means of 1H MRS with single-voxel and short echo time sequences and to evaluate the clinical use of 1H MRS for the evaluation of dogs with hepatic encephalopathy. We hypothesized that dogs with hepatic encephalopathy would have brain bioprofiles that differed from those of control dogs.

Materials and Methods

Animals—Six dogs with a portosystemic shunt, chronic fibrotic liver disease, or acute liver failure confirmed by CT, surgery, and evaluation of biopsy specimens were included in the study (hepatic encephalopathy group). Dogs were included if they had neurologic signs and examination of the brain with MRI and 1H MRS. Patient evaluation included physical and neurologic examinations, a CBC, serum biochemical analysis, and determination of blood ammonia and bile acids concentrations. The study was approved by the Cantonal Veterinary Office of Zurich (Switzerland).

A control group of 12 client-owned dogs was evaluated. Control dogs had a history of acute (< 4 days' duration) intervertebral disk disease attributable to acute intervertebral disk extrusion or fibrocartilaginous embolism. No dogs in the control group had a history of CNS disease or had clinical evidence of CNS disease during the initial evaluation, and no control dogs had historical evidence of hepatic disease. Informed con-
sent was obtained from owners prior to MRI evaluation of the vertebral column and MRI and 1H MRS evaluation of the brain. Analysis of CSF was performed to rule out myelitis and meningoencephalitis. Dogs were included in the control group if they had results of hematologic analysis within respective reference ranges, a morphologically normal brain on MRI evaluation, and results for CSF analysis within reference limits (total protein concentration, < 25 mg/dL; total nucleated cell count, < 5 cells/μL).

**MRI and 1H MRS**—Each dog was premedicated with methadone (0.2 mg/kg, IV) and midazolam (0.1 mg/kg, IV). Anesthesia was induced by the administration of propofol (4.5 mg/kg, IV). Endotracheal intubation was performed, and dogs were mechanically ventilated with sevoflurane in oxygen.

Dogs were positioned in dorsal recumbency. Magnetic resonance imaging was performed with a 3-T MRI scanner with a 15-channel receive-transmit head coil. Conventional MRI sequences were obtained before 1H MRS and included, at a minimum, standard sequences obtained at a slice thickness of 2.5 mm with a 2-mm slice gap, field of view of 180 mm, and matrix of 356×260. Sequences included dorsal T1-weighted (recovery time, 320 milliseconds; echo time, 8 milliseconds; number of signal averages, 240; spectral bandwidth, 2,000 Hz) with a minimum voxel dimension of 10×10×10 mm^3 to 10×13×20 mm^3, which was applied in the area of the left basal ganglia (Figure 1). Dimensions and orientation of each voxel were adjusted to match the size and shape of the targeted anatomic area; the investigator was careful to avoid CSF and lipid contamination. For all dogs included in the study, placement of the volume of interest in the left basal ganglia area and parameters of the 1H MRS procedures were the same. The 1H MRS images were obtained prior to contrast administration.

Before image acquisition for 1H MRS, field homogeneity was optimized with a second-order automatic pencil-beam shim, which was followed by use of water suppression techniques (excitation). A water-suppressed image was acquired to serve as a reference for quantifying metabolite concentrations. Acquisition time for 1H MRS was 8 minutes and 3 seconds, plus the shimming time (mean, 2 minutes); thus, mean total time for 1H MRS was < 11 minutes. Exclusion criteria for 1H MRS data were an unstable baseline, line width > 15 Hz, signal-to-noise ratio < 4, and presence of artifacts or lipid contamination; however, no dogs were excluded on the basis of 1H MRS data.

**Processing**—Metabolite peak areas were estimated by means of automated data processing spectral fitting algorithms in a software program. This program automatically adjusted the phase and chemical shift of the spectra, estimated the baseline, and performed eddy current correction. Relative metabolite peak areas and their uncertainties were estimated by fitting the spectrum to a basis set of spectra acquired from known solutions of metabolites. The basis set included 17 metabolites (alanine, aspartate, glucose, creatine, phosphocreatine, glutamine, glutamate, glycerocephosphocholine, phosphocholine, lactate, lipids, myo-inositol, N-acetyl aspartate, N-acetylaspartylglutamate, scyllo-inositol, glutathione, and taurine).

The software program quantified metabolite peak areas of reference to the peak for the water-suppressed image. Briefly, peak signals of metabolites from the water-suppressed spectrum were divided by those from a water-suppressed spectrum, while applying corrections (differences in T1 and T2 relaxation times between the metabolite and water and the number of 1H nuclei contributing to the metabolite and water peaks). This resulted in a molar concentration of the metabolite. Quantitative results were reported as error estimates, which are the Cramer-Rao lower bounds. Cramer-Rao lower bounds are the lowest possible SDs of all unbiased model parameter estimates obtained from the data. Consequently, they provide insight into the potential performance of quantification estimators. In other words, they are estimated SDs expressed as a percentage of the estimated concentrations. Cramer-Rao lower bounds > 50% indicate that the metabolite concentration could range from zero to twice the estimated concentration; thus, the metabolite is practically undetectable. Cramer-Rao lower bounds of approximately 20% indicate that only changes of approximately 40% can be detected with reliability. Cramer-Rao lower bounds < 20% provide acceptable reliability. Cramer-Rao lower bounds are directly related to the signal-to-noise ratio and linewidth of the spectra, so they are parameters that should be considered as criteria of spectral quality. Only those metabolites with Cramer-Rao lower bounds < 20% were evaluated in the present study.

The MRI and 1H MRS data were reviewed by one of the investigators (IC). All MRI images were assessed, with specific attention to evaluation of the following abnormalities: presence of widened cerebral gyri, thinned interthalamic adhesion, enlarged ventricular system, and T1-weighted hyperintensity of the lentiform nuclei. Changes in T2-weighted signal intensity in white and gray matter were recorded, if present. Metabolite peak areas of the 2 groups of dogs were compared. Metabolites assessed included N-acetyl aspartate, total choline, creatine, the
glutamine-glutamate complex, and myoinositol. Ratios of total choline to creatine, N-acetyl aspartate to creatine, the glutamine-glutamate complex to creatine, and myoinositol to creatine were also calculated.

Statistical analysis—Statistical tests were performed with statistical software. Descriptive analyses were performed for continuous variables (ie, age and metabolite peak area), and values (including mean ± SD and median) were reported. Normal distribution of data was tested with the 1-sample Kolmogorov-Smirnov test. Mean age was compared between the groups by use of a Wilcoxon test. All metabolite peak areas measured by use of the 1H MRS data of the 2 groups (hepatic encephalopathy group vs control group) were compared by use of the Mann-Whitney U test. A nonparametric test was chosen because of the small sample size. Cramer-Rao lower bounds > 20% were excluded from further evaluation. Values of P ≤ 0.05 were considered significant.

Results

Seven dogs with hepatic encephalopathy were initially examined; however, 1 was excluded because of incomplete data for the hematologic analysis. Thus, 6 dogs were included in the hepatic encephalopathy group. There were 3 females and 3 males, with a median age of 25 months (range, 16 to 81 months; mean ± SD, 40.17 ± 24.28 months). Breeds included Bernese Mountain Dog, Border Terrier, Cocker Spaniel, Hovawart, Maltese, and Shih Tzu.

Dogs had clinical signs for at least 2 weeks. Clinical signs included circling and compulsive walking (n = 2), compulsive walking and blindness (1), behavioral changes (1), epileptic seizures and behavioral changes (1), and head pressing, stupor, and focal seizures (1). Preprandial bile acids concentration was measured in 5 dogs, and values ranged from 8.7 to 108.8 µmol/L (median, 90.0 µmol/L; reference range, < 6.5 µmol/L). Postprandial bile acids concentration was measured in 4 dogs, and values ranged from 40.0 to 239.5 µmol/L (median, 145.9 µmol/L; reference range, < 25 µmol/L). Ammonia concentration was measured in all 6 dogs, and values ranged from 73.2 to 362.8 mmol/L (median, 108.2 mmol/L; reference range, < 70 mmol/L).

The diagnosis for the condition that caused the hepatic encephalopathy was chronic hepatitis with fibrosis (n = 2), single extrahepatic portocaval shunt (2), single extrahepatic portohepatic shunt (1), and single extrahepatic portophrenic shunt (1). All 6 dogs had aberrant portosystemic shunts confirmed with CT angiography of the abdomen. Four dogs had single congenital extrahepatic anomalous portosystemic vasculature, which was confirmed during surgery. Two dogs had multiple acquired extrahepatic shunts secondary to portal hypertension (one was confirmed ultrasonographically, but the other was not definitively confirmed). In these 2 dogs, hepatic cirrhosis was confirmed by histologic evaluation of biopsy specimens.

Four of 6 dogs with hepatic encephalopathy had mild brain atrophy, which was defined as widening of
the cerebral gyri (from the transverse images), decreased size of the interthalamic adhesion (from the transverse images), and ventriculomegaly. Mild ventriculomegaly was evident in 2 dogs (Cocker Spaniel and Border Terrier), and moderate ventriculomegaly was evident in 2 other dogs (Shih Tzu and Maltese). Hyperintense T1-weighted lentiform nuclei were observed in 2 dogs. In 1 dog, T2* weighting was performed, but it did not reveal evidence of hemorrhage. The T2-weighted hyperintensities of the gray and white matter were not evident in 2 dogs with hepatic encephalopathy, and no MRI abnormalities were observed.

The control group included 12 dogs that did not have evidence of neurologic signs compatible with intracranial disease. There were 6 females and 6 males, with a median age of 49 months (range, 12 to 90 months; mean ± SD, 44.08 ± 28.83 months). Mean age did not differ significantly (P = 0.2) between the hepatic encephalopathy and control groups. Breeds included Bernese Mountain Dog (n = 1), Border Terrier (1), Chihuahua (2), French Bulldog (1), German Shepherd Dog (1), Labrador Retriever (3), Rottweiler (2), and Spitz (1). All control dogs had no abnormal results for CSF analysis or hematologic analysis. Concentrations of bile acids or ammonia were not determined for the control dogs.

All 1H MRS data had a signal-to-noise ratio between 17 and 9, line width between 3.96 and 5.87 Hz, and volume of interest between 1.0 and 2.6 cm3. All spectra obtained had good quality, and no dogs were rejected because of a low-quality spectrum. Only 1 dog had lipid contamination, but this did not influence quality of the spectrum (Figure 2). The spectrum of that dog was compared with the spectrum of another dog with a high amount of lipids, which negatively influences reliability of the measurements. Metabolites that had Cramer-Rao lower bounds < 20% were included in the statistical analysis. Thus, total N-acetyl aspartate (the sum of N-acetyl aspartate and N-acetylaspartylglutamate), total choline (predominantly glycerophosphocholine and phosphocholine), total creatine (the sum of creatine

Figure 2—Single-voxel short echo time MRI spectra of a dog with hepatic encephalopathy attributable to a portosystemic shunt (A) and a dog with massive lipid contamination as a result of incorrect positioning (overlying bone) of the volume of interest (B). In panel A, the peak at 1.5 to 1.2 ppm represents the presence of lipids. Notice that the N-acetyl aspartate peak is well defined and clearly separated from the lipid peak and the increased width of the remaining metabolites, which makes the spectrum less reliable. Cho = Choline. Cr = Creatine. Glx = The glutamate-glutamine complex. Lip = Lipids. mI = Myo-inositol. NAA = N-acetyl aspartate.

Figure 3—Single-voxel short echo time MRI spectra of a dog with hepatic encephalopathy attributable to a portosystemic shunt (A) and a control dog of the same age (B). Notice the high peak for the glutamine-glutamate complex and the lower peak for myo-inositol in the dog with hepatic encephalopathy, compared with results for the control dog. See Figure 2 for remainder of key.
and phosphocreatine), myoinositol, and the glutamine-glutamate complex (sum of glutamate and glutamine) were statistically analyzed.

The $^1$H MRS spectra of the dogs with hepatic encephalopathy and control dogs were evaluated (Figures 3 and 4). Dogs with hepatic encephalopathy consistently had abnormalities, compared with results for the control group. Relevant results were summarized (Figure 5; Tables 1 and 2). Metabolite data were normally distributed, as determined by results of a 1-sample Kolmogorov-Smirnov test. However, because of the small sample size, a nonparametric test (Mann-Whitney U test) was used.

Two main differences were detected. First, the spectral regions around 3.75 ppm and 2.1 to 2.5 ppm were elevated in the hepatic encephalopathy group. These findings were consistent with increases in metabolite concentration relative to water of the glutamine-glutamate complex. Median ± SD metabolite concentration relative to water of the glutamine-glutamate complex for the dogs with hepatic encephalopathy was 20.90 ± 3.32 mMol/L. That value differed significantly ($P = 0.001$) from the metabolite concentration relative to water of the glutamine-glutamate complex for the control dogs (11.72 ± 1.24 mMol/L). As determined by use of the software program, glutamine was the main metabolite responsible for the increased peak area of the glutamine-glutamate complex in all dogs with hepatic encephalopathy, given that glutamine had Cramer-Rao lower bounds < 20% (between 16% and 7%). For glutamate in the control group, Cramer-Rao lower bounds were > 20% (between 21% and 61%). For glutamate, Cramer-Rao lower bounds were < 20% in both groups. Median ± SD glutamate concentration relative to water was 8.10 ± 0.92 mMol/L in the hepatic encephalopathy group and 7.96 ± 0.69 mMol/L in the control group. Thus, the concentration of this metabolite remained constant, and glutamine was responsible for the increase in the glutamine-glutamate complex peak.

Second, the myoinositol peak at 3.56 ppm was reduced in the hepatic encephalopathy group, with a median ± SD of 3.00 ± 1.14 mMol/L. The myoinositol concentration relative to water for the control dogs was significantly ($P = 0.001$) higher (6.09 ± 0.89 mMol/L). Concentrations relative to water of N-acetyl aspartate ($P = 0.002$) and total choline ($P = 0.006$) differed significantly between the 2 groups. Total creatine concentration did not differ significantly ($P = 0.640$) between the groups.

Median metabolite ratios for dogs with hepatic encephalopathy were as follows: N-acetyl aspartate to creatine, 0.90 ± 0.15; total choline to creatine, 0.33 ± 0.05; the glutamine-glutamate complex to creatine, 2.75 ± 0.50; and myoinositol to creatine, 0.34 ± 0.20. Median metabolite ratios for the control dogs were as follows: N-acetyl aspartate to creatine, 1.04 ± 0.13; total choline to creatine, 0.39 ± 0.05; the glutamine-glutamate complex to creatine, 1.65 ± 0.14; and myoinositol to creatine, 0.85 ± 0.14. Metabolite ratios differed significantly between the hepatic encephalopathy and control groups for the glutamine-glutamate complex to creatine ($P = 0.001$), myoinositol to creatine ($P = 0.001$), choline to creatine ($P = 0.003$), and N-acetyl aspartate to creatine ($P = 0.006$).

Because there were significant differences in myoinositol and the glutamine-glutamate complex between
Hepatic encephalopathy and control dogs. The most significant differences were region between dogs with hepatic encephalopathy and of the molecular spectral profiles of the basal ganglia noninvasively confirming the presence of the disease and abnormal ammonia metabolism within neural cells while.

The present study revealed substantial differences of the molecular spectral profiles of the basal ganglia region between dogs with hepatic encephalopathy and control dogs. The most significant differences were high concentrations relative to water for the glutamine-glutamate complex and low peak areas for myoinositol, which is in agreement with results of previous studies in humans with hepatic encephalopathy and animals with experimentally induced conditions. It is difficult to differentiate glutamine from glutamate because of the almost identical peak locations, which is why the composite peak is referred to as the glutamine-glutamate complex. This is caused by phase distortion of multiplets produced by coupled spin systems and to fast transverse relaxation (T2 relaxation) that leads to signal reduction, which prevents detection of small peaks in the spectrum. However, by use of adequate postprocessing fitting programs, the peaks for glutamate and glutamine can be resolved. There was a reliable concentration relative to water of glutamate (Cramer-Rao lower bounds < 20%) for the control dogs of the present study, whereas glutamine had Cramer-Rao lower bounds > 20%, which made those values less reliable. Glutamate peak areas were extremely similar between the hepatic encephalopathy and control groups. However, compared with the metabolic concentration for the control dogs, the concentration relative to water of glutamine for the dogs with hepatic encephalopathy was reliably increased, given the low value (> 20%) for Cramer-Rao lower bounds. This is in agreement with a study in which rodents with hyperammonemia had increased glutamate concentrations with constant or decreased glutamate concentrations. The proposed cause for the increase of glutamine is the glutamine synthetase reaction, which is the predominant pathway for ammonia metabolism. Glutamine synthetase activity is predominantly localized in astrocytes. This is the cause of the underlying sensitivity of astrocytes to ammonia, which leads to osmotic stress, astrocyte swelling, and finally brain edema.

Myoinositol peak areas were lower in dogs with hepatic encephalopathy than in control dogs. Historically, this finding in humans is presumed to be attributable to a compensatory mechanism astrocytes use to buffer ammonia-induced increases in glutamate, and the depletion of myoinositol may be a response to osmotic alterations. Lower myoinositol concentrations are detected in humans with chronic liver failure, whereas the change in myoinositol concentrations is not evident in humans with acute liver failure. This may be a result of the time it takes for the compensatory shift toward homeostasis of astrocyte volume to occur. Furthermore, decreases in the myoinositol peak appear to be the best discriminator for determining the severity of hepatic encephalopathy.

### Table 1—Descriptive analyses of the ages and metabolite concentrations relative to water content for dogs with hepatic encephalopathy and control dogs. (n = 6)

<table>
<thead>
<tr>
<th>Group</th>
<th>Age (mo)</th>
<th>N-acetyl aspartate</th>
<th>Total choline</th>
<th>Creatine</th>
<th>Glutamine-glutamate complex</th>
<th>Myoinositol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatic encephalopathy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>40.17 ± 28.83</td>
<td>6.33 ± 0.74</td>
<td>2.33 ± 0.20</td>
<td>7.19 ± 0.91</td>
<td>20.90 ± 3.32</td>
<td>3.00 ± 1.14</td>
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<tr>
<td>Median</td>
<td>25.00</td>
<td>6.52</td>
<td>2.28</td>
<td>7.36</td>
<td>19.81</td>
<td>3.04</td>
</tr>
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<td>Range</td>
<td>16.00–81.00</td>
<td>5.34–7.30</td>
<td>2.09–2.61</td>
<td>6.08–8.18</td>
<td>17.89–26.80</td>
<td>1.34–4.25</td>
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<tr>
<td>Control (n = 12)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>47.08 ± 24.28</td>
<td>7.72 ± 0.48</td>
<td>2.71 ± 0.33</td>
<td>7.10 ± 0.53</td>
<td>11.72 ± 1.25</td>
<td>6.09 ± 0.89</td>
</tr>
<tr>
<td>Median</td>
<td>49.00</td>
<td>7.55</td>
<td>2.71</td>
<td>7.11</td>
<td>11.84</td>
<td>6.35</td>
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<tr>
<td>Range</td>
<td>12.00–90.00</td>
<td>7.06–8.69</td>
<td>2.51–3.48</td>
<td>6.27–8.26</td>
<td>9.05–12.29</td>
<td>4.46–7.26</td>
</tr>
</tbody>
</table>

Values reported are mean ± SD. Metabolite concentrations are reported in mMol/L.

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Hepatic encephalopathy Mean ± SD</th>
<th>Control Mean ± SD</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>N-acetyl aspartate</td>
<td>6.32 ± 0.73</td>
<td>7.72 ± 0.48</td>
<td>0.002</td>
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<tr>
<td>Total choline</td>
<td>2.53 ± 0.20</td>
<td>2.70 ± 0.32</td>
<td>0.006</td>
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<tr>
<td>Creatine</td>
<td>7.19 ± 0.91</td>
<td>7.10 ± 0.52</td>
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<tr>
<td>Glutamine-glutamate complex</td>
<td>20.90 ± 3.32</td>
<td>11.72 ± 1.24</td>
<td>0.001</td>
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<tr>
<td>Myoinositol</td>
<td>3.00 ± 1.14</td>
<td>6.09 ± 0.89</td>
<td>0.001</td>
</tr>
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</table>

Values reported are mean ± SD. Metabolite concentrations are reported in mMol/L.

*Values were considered significant at P ≤ 0.05.

### Table 2—Comparison of metabolite concentrations relative to water content between 6 dogs with hepatic encephalopathy and 12 control dogs.

The most significant differences were region between dogs with hepatic encephalopathy and low peak areas for myoinositol, whereas the change in myoinositol concentrations is not evident in humans with acute liver failure. This may be a result of the time it takes for the compensatory shift toward homeostasis of astrocyte volume to occur. Furthermore, decreases in the myoinositol peak appear to be the best discriminator for determining the severity of hepatic encephalopathy.
Concentrations of N-acetyl aspartate remain within reference ranges in humans and other animals with hepatic encephalopathy.\textsuperscript{31,32,36,38} The present study revealed significant, albeit mild, decreases in N-acetyl aspartate concentrations. N-acetyl aspartate is considered a neuronal and mitochondrial marker in gray and white matter, and concentrations of N-acetyl aspartate typically are decreased when there is neuronal loss or neural dysfunction, such as with tumors or inflammation.\textsuperscript{39} The most common histopathologic feature of hyperammonemia is the presence of enlarged astrocytes with a watery nuclei and cytosol (type II Alzheimer cells), with little or no changes apparent in the neurons.\textsuperscript{12,33-35} Because hepatic encephalopathy was a chronic (clinical signs > 2 weeks) condition in all dogs in the present study, it is possible that the prolonged episodes of cerebral edema could have partially damaged the function of astrocytes. Histopathologic confirmation was not performed in any of the dogs. To the authors’ knowledge, this is the first report of the use of single-voxel \textsuperscript{1}H MRS to investigate neurovascular metabolic profiles in dogs with hepatic encephalopathy. Conventional MRI findings for dogs with hepatic encephalopathy include brain atrophy, widened sulci, ventriculomegaly, and hyperintense T1-weighted lentiform nuclei.\textsuperscript{36,37} Four of 6 dogs in the present study had MRI evidence of ventriculomegaly; however, they were small-breed dogs in which dilation of the ventricular system may be considered a normal anatomic variation.\textsuperscript{36} Two dogs with hepatic encephalopathy had hyperintense T1-weighted lentiform nuclei. This feature has been attributed to accumulation of manganese.\textsuperscript{36,37} Another differential diagnosis for hyperintense T1-weighted lentiform nuclei is laminar necrosis. Hemorrhage was an extremely unlikely cause given the lack of signal in T2* sequences. Lipids, mineralization, and melanin may cause hyperintense signals in T1-weighted images, but these substances cause an altered signal intensity pattern in T2-weighted sequences, which did not correlate with our findings (ie, there were no abnormalities on T2-weighted images for the dogs with hepatic encephalopathy). Two of the 6 dogs had normal morphological images. Hence, patients with hepatic encephalopathy and normal morphological images highlight the importance of the use of a noninvasive method for confirming the presence of hepatic encephalopathy, especially for patients with low or equivocal blood ammonia concentrations. We also propose that \textsuperscript{1}H MRS has value in the diagnosis, management, and treatment of subclinically affected patients.

Investigators have recently reported\textsuperscript{59} the limited value of single-voxel spectroscopy in the brain of dogs. However, for the present study, we clearly provided valid, accurate results with \textsuperscript{1}H MRS quality control (shimming techniques), the described single-voxel protocol, and application of the described fitting algorithm.

The present study had a few limitations. These included the low number of dogs with hepatic encephalopathy, the inability to perform histologic evaluation of the brains of study dogs, the lack of measured copper and iron concentrations in hepatic parenchyma of dogs with hepatic encephalopathy, and the lack of measured ammonia concentrations and evaluation of liver biopsy specimens in the control dogs. Diagnosis of hepatic encephalopathy in the hepatic encephalopathy group was justified on the basis of results of clinical findings, CT, and surgical exploration to evaluate for portosystemic shunts; gross hepatic appearance; histologic evaluation of hepatic biopsy specimens; and ammonia and bile acids concentrations. Combined findings supported the high likelihood of hepatic encephalopathy in that group of dogs.

The authors could not definitively rule out that the seizures in some of the dogs with hepatic encephalopathy were caused by another disease entity, such as idiopathic epilepsy or other neurodegenerative disease. The lack of previous illness and lack of abnormal biochemical profiles in the control dogs made it unlikely that these dogs had primary hepatic disease. Dogs with hepatic encephalopathy and control dogs did not differ significantly in age. However, metabolite alterations were reported among age groups of clinically normal Beagles.\textsuperscript{36} In that study, the ratio of choline to creatine was higher in young and geriatric dogs and the ratio of N-acetyl aspartate to creatine was lower in geriatric dogs, compared with those ratios in adult dogs.\textsuperscript{39} That study was performed with a 1.5-T magnet and a long echo time point-resolved spectroscopy sequence that resulted in poor resolution of peaks of interest (ie, the glutamine-glutamate complex and myo-inositol). Future studies that use short echo time point-resolved spectroscopy are needed to further investigate age-related differences in brain bioprofiles in domestic animals.

Despite these limitations, the results of the study reported here were quite promising for future investigations of the deleterious effects of hepatic dysfunction on the CNS and neuromolecular bioprofile. We believe that \textsuperscript{1}H MRS is a quantitative and qualitative tool for the in vivo assessment of metabolic derangements such as hepatic encephalopathy, and it can be used to support a diagnosis of hepatic encephalopathy in patients with equivocal blood ammonia concentrations.

\begin{itemize}
  \item a. Philips Ingenia scanner, Philips AG, Zurich, Switzerland.
  \item b. dStream Headspin coil solution, Philips AG, Zurich, Switzerland.
  \item c. LCModel, version 6.3, S Provencher, Oakville, ON, Canada.
  \item d. SPSS Statistics, version 21.0.0.0, 64-bit edition, IBM, Chicago, Ill.
\end{itemize}

\textbf{References}


