Regional variations and age-related changes detected with magnetic resonance spectroscopy in the brain of healthy dogs

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Objective—To investigate age-related and regional differences in estimated metabolite concentrations in the brain of healthy dogs by means of magnetic resonance spectroscopy (MRS).

Animals—15 healthy Beagles.

Procedures—Dogs were grouped according to age as young (n = 5; all dogs were 2 months old), adult (5; mean age, 4.5 years), or geriatric (5; all dogs were 12 years old). Imaging was performed by use of a 1.5-T MRI system with T1- and T2-weighted spin-echo and fluid-attenuated inversion recovery sequences. Signal intensity measurements for N-acetyl aspartate, creatine, choline, and lactate-alanine (the spectroscopic peaks associated with alanine and lactate could not be reliably differentiated) were determined with MRS, and areas under the spectroscopic peaks (representing concentration estimates) were calculated. Ratios of these metabolite values were compared among age groups and among brain regions with regression analysis.

Results—The choline-to-creatine ratio was significantly higher in young dogs, compared with other age groups. The N-acetyl aspartate-to-choline ratio was significantly lower in young dogs and geriatric dogs than in adult dogs. When all age groups were considered, the choline-to-creatine ratio was significantly higher and N-acetyl aspartate-to-choline ratio was significantly lower in the frontal lobe than in all other regions. The N-acetyl aspartate-to-creatine ratio was significantly lower in the cerebellum than in other regions.


Magnetic resonance spectroscopy is an MRI technique that allows noninvasive evaluation of metabolism in various tissues and can be used to detect quantities of amino acids, neurotransmitters, osmolytes, and other products. In human medicine, quantitative evaluation of these substances with MRS is often used for differential diagnosis of brain lesions, such as infarction, inflammation, edema, and tumors. Substances analyzed with MRS of brain tissue include N-acetyl aspartate, creatine, choline, lactate, and alanine.

In brain tissue of humans and other animals, N-acetyl aspartate exists only in neurons, making it useful in the evaluation of neuron density. Regional concentrations of N-acetyl aspartate can decrease following injury. The MRS peak of N-acetyl aspartate at 2.02 ppm is higher than spectroscopic peaks corresponding to creatine, choline, lactate, and alanine. Creatine is used as an indicator of cellular energy metabolism and has a spectroscopic peak at 3.03 ppm that reflects the sum of creatine and phosphocreatine signals; the creatine concentration can also serve as an internal reference for MRS because it remains relatively stable in patients with metabolic disease. Choline is involved in cell membrane turnover (ie, synthesis and degeneration) and has a spectroscopic peak at 3.2 ppm; astrocytes are known to have higher choline concentrations than do neuronal cells. Lactate is detected in brain tissue under conditions associated with anaerobic metabolism or failure of oxidative phosphorylation (eg, mitochondrial disease, ischemia, inflammation, or tumors), but concentrations are below the lower limit of detection of MRS under normal conditions. Lactate has an MRS peak at 1.32 ppm that appears as a doublet and is inverted below the baseline. Alanine is also
not detectable in the brain under normal physiologic conditions, but it is commonly found in the presence of meningiomas and abscesses. Alanine has an MRS peak at 1.5 ppm that also appears as an inverted doublet below baseline. Metabolite concentrations can be estimated through measurement of MRS signal intensity where absolute quantitation cannot be performed. In some MRS studies, metabolite ratios (e.g., N-acetyl aspartate-to-creatine, choline-to-creatine, and N-acetyl aspartate-to-choline ratios) have been used to investigate metabolic changes in human brain tissue under various conditions. For example, the N-acetyl aspartate-to-choline ratio is considered an indicator of neuronal activity because N-acetyl aspartate is specific to neurons and choline is regarded as a marker of cellular density.  

In human medicine, regional differences and metabolic changes associated with neuronal brain development and aging have been described. In particular, differences in specific MRS signals have been found among different age groups (e.g., young, adult, and old age). For example, the N-acetyl aspartate-to-choline ratio increases from childhood to the early 20s and then decreases gradually from the late 20s to old age. It is believed that the increasing ratio observed during development corresponds to the myelination of axons and that the decreasing ratio during aging corresponds to membrane breakdown. During active myelination (in early childhood), choline concentrations are somewhat high (relative to adult values) and are associated with increased membrane turnover; after myelination is complete (during the early 20s), choline concentrations are reduced to adult values (e.g., a mean \[ SD \text{ value of } 2.03 \pm 0.39 \text{mM in the white matter has been reported.} \] It has also been reported that pathological changes associated with aging lead to increased choline concentrations in the brain.  

Understanding the changes that are detectable via MRS is important for the diagnosis of some brain diseases. In veterinary medicine, use of MRS to measure various metabolites in brain tissue of dogs has been reported, but to our knowledge, there are no reports describing use of this methodology to evaluate regional differences or developmental and age-related changes in canine brain tissue. A recent study demonstrated the feasibility of MRS at 3.0 and 7.0 T for examination of canine brain tissue and suggested that, for detection of metabolite concentrations in this tissue, MRS with a clinical 3.0-T scanner is more effective than with a 7.0-T scanner due to insufficient water suppression at 7.0 T. Currently, however, few small-animal practice facilities have access to a 3.0-T MRI system (these facilities typically have MRI systems with magnetic field strengths \( \leq 1.5 \text{T} \). The authors believe that fundamental MRS data from a 1.5-T MRI scanner can be used to provide useful information on metabolite concentrations in the brain.  

The purpose of the study reported here was to investigate age-related and regional differences in estimated concentrations of N-acetyl aspartate, creatine, choline, lactate, and alanine detectable by means of MRS of the brain of dogs with a 1.5-T MRI system. To evaluate differences in these metabolite values among dogs of different ages and among regions of interest in the brain, we calculated and compared metabolite ratios in these tissues.  

**Materials and Methods**  

**Animals**—Fifteen Beagles with no abnormalities detected on physical and neurologic examination were included. Dogs were classified into 3 groups on the basis of age as follows: young (n = 5 [1 male and 4 females]; all dogs were 2 months old), adult (5 [1 male and 4 females]; mean age, 4.5 years [range, 3 to 5 years]), or geriatric (5 [3 males and 2 females]; all dogs were 12 years old). The experimental protocol was performed in accordance with the Guide for the Experimentation of Animals produced by the College of Bioresource Sciences, Nihon University.  

**Imaging protocol**—Magnetic resonance imaging and MRS were performed in dogs under general anesthesia. An ECG tracing, blood pressure, and body temperature were monitored and results were confirmed as normal in all dogs. Each dog was premedicated with atropine sulfate (0.04 mg/kg, SC) and IV infusion of a mixture of midazolam hydrochloride (0.2 mg/kg) and butorphanol tartrate (0.2 mg/kg). General anesthesia was induced with propofol (4.0 mg/kg, IV). After endotracheal intubation, the dogs were mechanically ventilated with isoflurane in a mixture with 100% oxygen. During MRI and MRS examination, end-tidal carbon dioxide concentration and oxygen saturation as measured via pulse oximetry were monitored in addition to the ECG tracing and blood pressure. During anesthesia, all dogs received an IV infusion of lactated Ringer’s solution, supplemented with 2.6% glucose, at a rate of 3 mL/kg/h.  

A 1.5-T MRI scan system equipped with a quadrature knee coil was used for MRI and MRS. T1-weighted, T2-weighted, and fluid-attenuated inversion recovery images were obtained to rule out physical abnormalities of the brain. The T1-weighted images were obtained with a standard spin-echo sequence, repetition time of 600 milliseconds, and echo time of 15 milliseconds. The T2-weighted images were obtained with a fast spin-echo sequence, repetition time of 4,500 milliseconds, and echo time of 105 milliseconds. Fluid-attenuated inversion recovery images were obtained with a repetition time of 10,000 milliseconds and echo time of 108 milliseconds. For all sequences, sagittal, transverse, and dorsal plane images were acquired.  

Magnetic resonance spectroscopy was performed after the presence of brain abnormalities was ruled out with MRI. To perform MRS, an approximately 3.4-cm voxel, for which size was determined in a preliminary experiment, was placed on the frontal lobe, occipital lobe, and cerebellum on 3-way T2-weighted images (Figure 1). For measurements in the frontal lobe, the voxel was placed on the midline just rostral to the cerebral parenchyma from the sulcus cruciatus. For the occipital lobe, the voxel was placed on the midline of the cerebral parenchyma just above the tentorium cerebelli. For the cerebellum, the voxel was placed on the center of the cerebellum, avoiding the fourth ventricle as much as possible. During placement of voxels in each
region, interference by the skull and cerebral ventricles was avoided as much as possible. Global and localized shimming of the water protons with the image-based shimming method and optimization of water suppression were performed. Spectra were obtained with a point-resolved spectroscopy sequence (repetition time, 2,000 milliseconds; echo time, 136 milliseconds) with averaging (number of repetitions, 256) for improvement of the signal-to-noise ratio. The time required to obtain MRS data was 8.53 min/region. We judged the spectrum to be reliable within the following parameters: voxel positioning (as described), predicted signal-to-noise ratio, full width at half maximum of the peak for water (< 0.20 ppm), and absence of disturbance in the baseline of the spectrum. Predicted signal-to-noise ratio is a rough indication of the quality of the spectrum, and the protocol suggested by the manufacturer (to obtain the best signal-to-noise ratio) was used.

After imaging, isoflurane administration was discontinued. Monitoring was continued as described for each dog until spontaneous breathing was detected, and then respiratory rate was also monitored. Dogs were extubated when palpebral reflex and jaw tension were detectable. After extubation, oxygen was supplied via face mask and monitoring was continued. When dogs recovered to an apparently normal level of consciousness and could walk unassisted, they were returned to their housing.

Data analysis—The spectroscopic display shows MRS-acquired data as an image, with the signal intensity (i.e., size of the peak) representing an estimate of metabolite concentration. Areas under the spectroscopic peaks were measured for N-acetyl aspartate at 2.02 ppm, choline at 3.2 ppm, creatine at 3.03 ppm, and lactate-alanine at 1.3 to 1.5 ppm. The spectroscopic peaks associated with alanine and lactate could not be reliably differentiated, these were reported together. Results for individual metabolite measurements could not be directly compared because the absolute value of each metabolite changes with every measurement and is dependent on receiver gain and shimming; thus, ratios of the measured values (which have arbitrary units) for N-acetyl aspartate and creatine, choline and creatine, and lactate-alanine and creatine were used for semiquantitative estimation of brain metabolism. In addition to these ratios, in which creatine was used as an internal reference standard, the N-acetyl aspartate-to-choline ratio was also evaluated.

To investigate factors potentially affecting each metabolite ratio among the 3 age groups and 3 regions of the brain, multiple regression analysis was performed with statistical software. The dependent variables were the 4 metabolite ratios, and the independent variables were age group and anatomic region.

Table 1—Mean ± SD area under the MRS peak (arbitrary units) of 4 metabolites in the frontal and occipital lobes and cerebellum in healthy Beagles grouped according to age.

<table>
<thead>
<tr>
<th>Group and anatomic region</th>
<th>N-acetyl aspartate</th>
<th>Creatine</th>
<th>Choline</th>
<th>Lactate-alanine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young Frontal lobe</td>
<td>105.37 ± 7.03</td>
<td>70.93 ± 6.35</td>
<td>164.21 ± 10.21</td>
<td>7.04 ± 7.04</td>
</tr>
<tr>
<td>Occipital lobe</td>
<td>115.85 ± 4.24</td>
<td>55.51 ± 1.88</td>
<td>129.91 ± 8.21</td>
<td>ND</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>100.29 ± 5.03</td>
<td>74.03 ± 5.18</td>
<td>122.82 ± 3.11</td>
<td>ND</td>
</tr>
<tr>
<td>Adult Frontal lobe</td>
<td>62.43 ± 3.76</td>
<td>36.74 ± 4.22</td>
<td>69.13 ± 2.86</td>
<td>1.80 ± 1.24</td>
</tr>
<tr>
<td>Occipital lobe</td>
<td>90.17 ± 6.34</td>
<td>60.64 ± 2.94</td>
<td>73.67 ± 3.30</td>
<td>2.54 ± 2.54</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>98.65 ± 3.84</td>
<td>71.37 ± 3.46</td>
<td>84.11 ± 8.57</td>
<td>ND</td>
</tr>
<tr>
<td>Geriatric Frontal lobe</td>
<td>44.28 ± 8.26</td>
<td>30.66 ± 4.37</td>
<td>59.48 ± 7.81</td>
<td>ND</td>
</tr>
<tr>
<td>Occipital lobe</td>
<td>80.70 ± 6.58</td>
<td>56.74 ± 0.40</td>
<td>76.90 ± 4.57</td>
<td>ND</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>81.4 ± 5.03</td>
<td>62.26 ± 8.50</td>
<td>71.38 ± 7.46</td>
<td>9.66 ± 4.31</td>
</tr>
</tbody>
</table>

ND = Not detected.

Dogs were categorized as young (n = 5; all dogs were 2 months old), adult (5; mean age, 4.5 years [range, 3 to 5 years]), or geriatric (5; all dogs were 12 years old). The spectroscopic peaks associated with alanine and lactate could not be reliably differentiated because of their close proximity and similar forms and directions; values for peaks occurring at 1.3 to 1.5 ppm were reported as lactate-alanine.
brain region. Normal distribution of the variables and the distribution condition were assessed with a Shapiro-Wilk test that was performed before the analysis. A logarithmic transformation was applied to the variables that did not fit a normal distribution (N-acetyl aspartate-to-creatine, choline-to-creatine, and lactate-alanine-to-creatine ratios). Dummy variables were applied to data of nominal scale (age groups and brain regions). A stepwise variable selection method was used. This method selects and shows only independent variables that have significant association with dependent variables from among diverse independent variables. Standardized and unstandardized coefficients were automatically calculated by the described software. Results were presented as mean and SD.

**Results**

Areas under the MRS peak for each metabolite, indicating signal intensity (used as an estimate of metabolite concentration), were summarized (Table 1). Representative MRS results for each region of the brain in each group (young, adult, and geriatric dogs) are provided (Figure 2). Ratios of the measured values for metabolites were calculated, and values for the 3 age groups and brain regions of interest were summarized (Table 2). The results of multiple regression analysis for significant associations among age group, region of the brain, and metabolite ratios are also reported (Table 3). Evaluation of the correlation matrix table revealed that no variables were correlated with each other (|r| > 0.9); thus, we included all of the variables in the analyses. Unstandardized and standardized coefficients were determined; the P values are reported for unstandardized coefficients.

**Metabolite ratio differences among age groups**—The choline-to-creatine ratio was significantly (P = 0.000) higher for the young group of dogs (unstandardized coefficient, 0.16) than for other groups (Table 3). The young and geriatric groups had significantly lower N-acetyl aspartate-to-choline ratios (unstandardized coefficients, –0.33 [P = 0.000] and –0.12 [P = 0.041], respectively), compared with adult dogs. There were no significant differences in the N-acetyl aspartate-to-creatine and lactate-alanine-to-creatine ratios among age groups.

**Regional metabolite ratio differences**—The cerebellum had a significantly (P = 0.018) lower N-acetyl aspartate-to-creatine ratio than did other regions, with an unstandardized coefficient of −0.07 (Table 3). The frontal lobe had a significantly (P = 0.000) higher choline-to-creatine ratio, with an unstandardized coefficient of 0.16, compared with other regions. The frontal lobe also had a significantly (P = 0.000) lower N-acetyl aspartate-to-choline ratio, with an unstandardized coefficient of −0.31, compared with other regions. There were no significant differences in lactate-alanine-to-creatine ratio among regions of the brain.

It was not possible to obtain separate measurements for gray matter and white matter with the 1.5-T MRS unit used in the study. The MRI images were sent to the com-

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**Table 1:** Results of metabolite ratio analysis (mean ± SD) for 3 regions of the brain in 3 groups of healthy Beagles.

<table>
<thead>
<tr>
<th>Group and anatomic region</th>
<th>N-acetyl aspartate-to-creatine ratio</th>
<th>Choline-to-creatine ratio</th>
<th>N-acetyl aspartate to choline ratio</th>
<th>Lactate-alanine-to-creatine ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young</td>
<td>1.52 ± 0.25</td>
<td>2.39 ± 0.53</td>
<td>0.64 ± 0.07</td>
<td>1.41 ± 0.14</td>
</tr>
<tr>
<td>Frontal lobe</td>
<td>2.11 ± 0.34</td>
<td>2.35 ± 0.35</td>
<td>0.91 ± 0.15</td>
<td>ND</td>
</tr>
<tr>
<td>Occipital lobe</td>
<td>1.37 ± 0.14</td>
<td>1.70 ± 0.33</td>
<td>0.81 ± 0.10</td>
<td>ND</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>1.82 ± 0.66</td>
<td>2.07 ± 0.95</td>
<td>0.90 ± 0.10</td>
<td>0.05 ± 0.03</td>
</tr>
<tr>
<td>Adult</td>
<td>1.50 ± 0.18</td>
<td>1.21 ± 0.06</td>
<td>1.23 ± 0.11</td>
<td>0.05 ± 0.05</td>
</tr>
<tr>
<td>Frontal lobe</td>
<td>1.40 ± 0.21</td>
<td>1.18 ± 0.23</td>
<td>1.21 ± 0.24</td>
<td>ND</td>
</tr>
<tr>
<td>Occipital lobe</td>
<td>1.43 ± 0.11</td>
<td>2.00 ± 0.25</td>
<td>0.74 ± 0.06</td>
<td>ND</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>1.42 ± 0.11</td>
<td>1.34 ± 0.07</td>
<td>1.08 ± 0.11</td>
<td>ND</td>
</tr>
</tbody>
</table>

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![Figure 2](image-url)
has been reported that the number of noradrenergic neurons decreases in the brains of aged dogs with cognitive impairments.26–31 Furthermore, it has been reported that the number of noradrenergic neurons decreases in the brains of aged dogs with cognitive impairments, compared with the number in young dogs, but that obvious neuronal loss is not observed in the brains of healthy aged dogs. In that study, the stereologically estimated number of neurons in the brains of aged dogs with cognitive impairments was up to 23% lower than that of aged dogs without such impairments. In our opinion, neuronal loss may not have occurred in geriatric dogs in the present study because they were found to be healthy and had no detectable cognitive impairments. This may suggest that MRS could potentially be used as a means to help diagnose cognitive impairments in dogs because a decreased N-acetyl aspartate-to-creatine ratio (similar to that observed in humans) is expected in the brains of dogs with cognitive impairments.32 However, this could not be evaluated in our study, and further research is needed to evaluate the method for such use.

A significantly higher choline-to-creatine ratio (considered indicative of higher choline concentrations) was observed in the brain of young dogs, compared with that in other age groups. We believe that these high choline concentrations were associated with myelination because this metabolite is involved in cell membrane turnover. The significantly lower N-acetyl aspartate-to-choline ratio in the young group, compared with that in the adult group, was also likely associated with brain development for the same reason. The significantly lower N-acetyl aspartate-to-choline ratio in the geriatric group, compared with that in the adult group, might have been attributable to age-related changes, such as demyelination, which can also increase choline concentrations.1,10 In addition, astrogliosis potentially could have affected this ratio in the geriatric group because of the high choline concentrations found in astrocytes.7

The cerebellum had a significantly lower N-acetyl aspartate-to-creatine ratio, compared with other brain regions of interest. Recent studies have indicated that estimated creatine concentrations in the cerebellum are higher than in other brain regions in humans and rats and that it is possible to underestimate metabolite-to-creatine ratios. Higher concentrations of creatine in the cerebellum might imply a higher energy demand, compared with other regions.33 Thus, local increases in estimated creatine concentrations could have contributed to low N-acetyl aspartate-to-creatine ratios in the cerebellum of dogs in the present study. Furthermore, choline-to-creatine ratios in the cerebellum could be similarly affected, although values for dogs in this study did not differ between

Table 3—Results of multiple regression analysis to identify significant associations between metabolite ratios and age groups or regions of the brain.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Adjusted $R^2$</th>
<th>Unstandardized coefficient (95% confidence interval)</th>
<th>Standardized coefficient</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Associations with age group</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Choline-to-creatine ratio</td>
<td>0.44</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Young</td>
<td></td>
<td>0.16 (0.09 to 0.22)</td>
<td>0.523</td>
<td>0.000</td>
</tr>
<tr>
<td>N-acetyl aspartate-to-choline ratio</td>
<td>0.81</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Young</td>
<td></td>
<td>−0.33 (−0.44 to −0.21)</td>
<td>−0.057</td>
<td>0.000</td>
</tr>
<tr>
<td>Geriatric</td>
<td></td>
<td>−0.12 (−0.24 to −0.01)</td>
<td>−0.057</td>
<td>0.041</td>
</tr>
<tr>
<td>Associations with region</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N-acetyl aspartate-to-creatine ratio</td>
<td>0.16</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Cerebellum</td>
<td></td>
<td>−0.07 (−0.12 to −0.01)</td>
<td>−0.341</td>
<td>0.018</td>
</tr>
<tr>
<td>Choline-to-creatine ratio</td>
<td>0.44</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Frontal lobe</td>
<td></td>
<td>0.16 (0.09 to 0.22)</td>
<td>0.513</td>
<td>0.000</td>
</tr>
<tr>
<td>N-acetyl aspartate-to-choline ratio</td>
<td>0.61</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Frontal lobe</td>
<td></td>
<td>−0.31 (−0.40 to −0.20)</td>
<td>−0.049</td>
<td>0.000</td>
</tr>
<tr>
<td>Intercept</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N-acetyl aspartate-to-creatine ratio</td>
<td>0.22</td>
<td>(0.18 to 0.26)</td>
<td>—</td>
<td>0.000</td>
</tr>
<tr>
<td>Choline-to-creatine ratio</td>
<td></td>
<td>0.11 (0.07 to 0.15)</td>
<td>—</td>
<td>0.000</td>
</tr>
<tr>
<td>N-acetyl aspartate-to-choline ratio</td>
<td></td>
<td>1.22 (1.13 to 1.31)</td>
<td>—</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Only regions and age groups with a relationship to each metabolite ratio as analyzed with a stepwise method are reported. $P$ values are shown for the unstandardized coefficient. Regression was modeled as $Y = a + bX(C) + b(C) + ..., where Y is the dependent variable (metabolite ratio), a is the intercept, b is the unstandardized coefficient, and C is a dummy variable assigned to each age group or region, with a value of 1 for the age group or region of interest and 0 for all other groups or regions.

— = Not applicable.
the cerebellum and occipital lobe. Therefore, caution should be exercised when interpreting MRS results with metabolite ratios for the cerebellum.

In healthy human adults, it has been reported that choline concentrations are higher in the frontal lobe than in the other lobes of the brain. The reason for this regional variation remains unclear, but it presumably reflects the cellular composition of the different brain regions in humans (eg, glial cells are known to have higher choline concentrations than do neuronal cells). 7 The upper and lower concentrations were found in the frontal lobe, compared with other regions of interest, and this may have been attributable to relatively high concentrations of choline in these regions.

In the present study, significantly higher choline-to-creatine ratios were found in the frontal lobe than in other regions likely had the same cause, and the finding that the N-acetyl aspartate-to-creatine ratio in this region was not significantly different from that in the occipital lobe supported this hypothesis.

The choline-to-creatine ratios in the present study (range, 1.18 to 2.39) were higher than those previously reported for healthy humans (range, 0.26 to 0.50). Although it has been reported that MRS results can be influenced by the anesthesia protocol, it has not been specifically reported that measurement of absolute or estimated choline concentration is affected. However, influence of the anesthesia protocol cannot be ruled out in the present study. In addition, this difference may possibly have been influenced by differences in MRI equipment, although to our knowledge, this has not been reported.

Lactate is a product of anaerobic glycolysis and is detected in diseased brain tissue, which is oxygen starved in many conditions, including stroke, tumor, inflammation, lactic acidosis, recovery from cardiac arrest, and neonatal hypoxia. Alanine is frequently considered a specific marker of meningioma and abscess, and alanine concentrations may be increased by a unique transamination pathway associated with these diseases. At 1.5 T, the MRS system used in this study could not provide reliable differentiation of the peaks associated with alanine and lactate. If both metabolites exist in parallel, a joint spectral signal appears as a downward triplet located at approximately 1.5 and 1.3 ppm. Our results were reported as lactate-alanine, and limited signal intensities for one or both substances were detected in some regions. It was possible that CSF in the ventricle was inadvertently included in these voxels. It has been reported that a small amount of lactate can be detected from ventricles because of an equilibrium in lactate concentrations between serum and CSF.

In human medicine, it has been reported that MRS is useful for the diagnosis of diseases that often can be difficult to diagnose with a single application of conventional MRI, such as degenerative disease (eg, Alzheimer disease, in which patients have a decreased N-acetyl aspartate-to-creatine ratio in brain tissue; both sensitivity and specificity are significantly increased when MRS is used in combination with MRI) and metabolic disorders (eg, hepatic encephalopathy, in which a decreased choline-to-creatine ratio is found).

In dogs, the existence of similar diseases is known, and, thus, MRS is expected to become a useful tool for the diagnosis of these types of diseases. Additionally, MRS data can change with treatment in some of these diseases, making it potentially useful in the future for follow-up assessments of dogs.

The MRS protocol used in the present study allowed detection of spectra from the frontal lobe, occipital lobe, and cerebellum in dogs. Because the brain of a dog is smaller than that of a human, in some regions (eg, the frontal lobe), a voxel may include tissue other than the brain, such as bone or subcutaneous fat, or cavities (eg, the nasal cavity or frontal sinus) when attempts are made to place the voxel at the center of a targeted region. If this occurs, the signal is disturbed, with resulting noise affecting detection of a suitable spectrum and requiring reexamination. This creates a need to move the voxel by a small amount from the center of the intended region in some cases; however, this movement of the voxel can also cause other regions of the brain to be included and result in a loss of measurement accuracy. In this situation, interference by other brain regions can be prevented by placing the voxel within the intended region just below the border between the brain and non–brain tissues (eg, the internal table of the frontal sinus).

In the present study, we placed voxels for MRS in a manner intended to avoid inclusion of non–brain tissues and deviation from the intended region on the basis of described landmarks. Caution is needed in voxel positioning when MRS is performed in young dogs. Because of their smaller brain sizes, it was more likely that regions other than the intended region were included in voxel selection in the young group, which comprised 2-month-old puppies. The 3.4-cm³ voxel used in this study was the maximum size possible for placement in each evaluated region for dogs in this group. To obtain a good spectrum with a small voxel, the number of repetitions used for averaging must be increased, and this results in an extension of measurement times that is likely not reasonable in clinical practice. For example, for a 1.7-cm³ voxel, the time required for a scan is 17 min/region. Measurable regions are also likely to be limited in the brains of small-breed dogs that have brains smaller than those of Beagles.

Careful attention was paid to the imaging conditions in the present study (voxel position, predicted signal-to-noise ratio, full width at half maximum of the peak for water, and untroubled spectrum). Therefore, there were likely no artifacts caused by the presence of these structures.

Another limitation of the present study was that we analyzed various metabolite-to-creatine ratios, rather than absolute concentrations. Because creatine concentrations in the brain are considered stable, relative to other metabolites, metabolite-to-creatine estimated concentration ratios are used as evaluation methods in many studies. However, although this could not be evaluated statistically, our MRS results suggested that each age group (young, adult, and geriatric) in the study had higher creatine concentrations (as estimated via MRS) in the cerebellum than in the supratentorial...
region. This result is similar to those in previous reports\textsuperscript{17,18,19} describing MRS results in children, adults, and the elderly. A recent study\textsuperscript{18} suggested that gray matter contains more creatine than does white matter in the brain of healthy humans. This difference is believed to be caused by greater energy demands and neuronal mitochondrial cell density of gray matter, compared with that of white matter. This could also be true in dogs; however, the 1.5-T MRS system used in our study could not measure metabolites in gray matter and white matter separately, and higher magnetic field systems would be required. Although not evaluated statistically, the percentage of gray matter determined by analysis of voxels appeared higher in voxels used for measurements in the cerebellum of the young group than in the adult and geriatric groups. Therefore, high gray matter content in the region of voxel placement could have influenced creatine concentrations in the cerebellum of dogs in the young group. However, we consider that the difference in energy demand is the main factor affecting these values because the adult and geriatric groups also had subjectively higher estimated creatine concentrations in the cerebellum than in other regions. To assess variability of creatine concentrations in the brain of dogs accurately, quantitative software should be used; otherwise, biochemical quantitation of metabolites would be needed. Without this information, the metabolite ratios used in this study should be interpreted with care. In addition, it would be desirable to conduct large-scale in vivo MRS studies with a larger number of dogs to confirm the reproducibility of our results. Concentrations of various metabolites can be estimated with different echo times. In addition to N-acetyl aspartate, choline, creatine, and lactate-alanine, short echo-time MRS can be used to detect myoinositol and glutamine, which are markers for astrocytes, as well as the excitatory neurotransmitter glutamate, and a short echo time has been used to distinguish between glioma and other tumors.\textsuperscript{20} In this study, a long echo time was adopted to distinguish between lactate and lipids. Lactate and lipids have the same position on the MRS spectrum, but at an echo time of 136 milliseconds, these metabolites create peaks in different directions.\textsuperscript{1–4}

References