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Enhancing Cognitive Functions in Old Dogs: A New Nutritional Approach

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Abstract

This study was designed to determine whether dietary supplementation with medium-chain triglycerides (MCT) can improve cognitive function in aged dogs by providing the brain with ketones as an alternative energy source. Aged dogs were randomized into two groups based on baseline cognitive tests and were maintained on either a control diet or an MCT diet for eight months. During the feeding trial, dogs were tested on a battery of cognitive test protocols to assess learning ability, memory and attention. Dogs fed the MCT diet showed significantly better performance on most of the cognitive tests than the control dogs.

Glossary of Abbreviations

AD: Alzheimer's Disease
ANOVA: Analysis of Variance
ATP: Adenosine Triphosphate
BHB: Beta-Hydroxybutyrate
CBC: Complete Blood Count
DNMP: Delayed Non-Matching-to-Position Task
MCT: Medium-Chain Triglycerides
MER: Maintenance Energy Requirements
PUFAs: Polyunsaturated Fatty Acids

Introduction

Decline in brain energy metabolism is a common feature associated with aging in animals including rats,[1] dogs,[2] humans,[3] and monkeys,[4] and may be partially responsible for age-dependent cognitive decline. For example, Milgram[5] reported that cognitive function decreased significantly in middle-aged Beagle dogs compared to young dogs, while a separate study indicated that cerebral glucose metabolism was significantly reduced in middle-aged Beagle dogs compared to 1-year-old dogs.[2] Interestingly, human studies showed that cerebral glucose metabolism was significantly lower in elderly patients with Alzheimer's disease (AD) compared to healthy elderly control subjects [6] or patients with mild cognitive impairment.[7] These data suggest that the reduction in brain glucose metabolism is a common feature associated with aging, that the process is progressive, starting around middle age, and that the decline in glucose metabolism may, at least partially, contribute to the cognitive decline associated with aging.

The brain accounts for only about 2 to 3% of body weight, but consumes 25% of whole body glucose utilization.[8] A tight coupling exists between neuronal activity and cerebral glucose utilization, and sustaining increased neuronal activity usually depends on increased adenosine triphosphate (ATP) production from glucose metabolism.[9] Although the brain utilizes glucose as the main energy source, the brain can utilize alternative energy sources to compensate for an insufficient supply of glucose. For example, ketone bodies (acetoacetate and β-hydroxybutyrate) are a natural endogenous energy source mainly produced by the liver from mobilization of endogenous body fat and utilized by extrahepatic tissues (brain, heart, kidney, muscle, etc). In fact, the brain is able to metabolize ketone bodies as energy substrates under the conditions of starvation or high fat diet.[10]

Henderson[11] proposed that dietary supplementation with MCTs can increase blood and brain levels of ketone bodies without the need for restricting dietary carbohydrates. MCTs are readily digested, and the resulting medium-chain fatty acids are converted to ketone bodies by the liver and, to a lesser extent, by astrocytes in the brain. The ketone bodies can then be used by neurons as an alternative energy source when there is a decline in brain glucose metabolism.

Reger et al.[12] reported that MCT supplementation to patients with Alzheimer's disease resulted in an improvement of cognitive function in a subset of subjects that were negative for the apopolipoprotein E ε4 allele, and that cognitive improvement correlated positively with blood levels of beta-hydroxybutyrate. More recently, Page et al.[13] found that hypoglycemia impaired cognitive function and that dietary supplementation of MCTs increased blood level of ketone bodies and reversed cognitive impairment caused by hypoglycemia in intensively treated type 1 diabetic patients. These findings provide further evidence that deficiency in the brain energy supply results in cognitive impairment, and MCTs can provide the human brain with ketone bodies as an alternative energy source to compensate for the deficiency of glucose supply.

The objective of this study was to determine whether dietary MCT supplementation could improve cognition in aged dogs.

To that end, cognition was measured by a battery of cognitive tests that assessed learning ability, visuospatial function and attention in aging dogs fed either a control diet or one containing 5.5% MCTs during an eight-month feeding trial.

Materials and Methods

Dogs and Diets

Twenty-four Beagle dogs (10 males and 14 females) of 7.5 to 11.6 (mean age±SD: 9.79±0.84) years of age were recruited in the study. The study was approved by the CanCog Technologies Institutional Animal Care Committee. During the baseline phase, all dogs were tested on a variable version of the delayed non-matching-to-position (DNMP) task,[14] a size discrimination learning task and a size discrimination reversal task.[15,16] Performance of the dogs on these three tests was ranked, and the ranking was used to assign dogs to two cognitively equivalent groups. One of the groups was then fed a standard control diet and the other a similar diet modified to contain 5.5% MCTs for eight months. Both diets, manufactured by Nestlé Purina PetCare (St. Louis, MO), were isoenergetic and contained the same levels of protein, fat and carbohydrates (Table 1). The dogs were offered food once daily for about an hour and fed to meet their maintenance energy requirements (MER) estimated by the formula "MER = 110 kcal/day * (BW0.75)."[17] Dogs had free access to water.

Cognitive Test Apparatus and Test Criterion

The cognitive testing apparatus consisted of a wooden box approximately 0.609 m × 1.15 m × 1.08 m.[18] The dogs were in the test apparatus only during the cognitive tests. The animal care technician was separated from the dog by a plastic partition containing a one-way mirror and a hinged door. The food reward tray was made of Plexiglas and contained either three or four equally spaced food wells, depending on the task. The food reward was approximately one gram of Purina® Pro Plan® brand Adult Chicken & Rice Entrée (wet dog food, manufactured by Nestlé Purina PetCare).

To pass one test and move on to the next test, dogs had to complete a two-stage criterion. The first stage was successfully completed when the dogs responded correctly in at least 9 of 10 trials or in 8 of 10 trials over two consecutive days. The second criterion stage was achieved when the dogs responded correctly in more than 70% of the total trials over three consecutive days.

Table 1. Nutrient Composition of Diets*

	Control	MCT
Nutrient Composition (% as fed)		
Moisture	7.41	7.10
Ash	6.32	6.24
Crude Protein	32.80	33.10
Crude Fat	18.5	18.8
Crude Fiber	2.86	2.47
Linoleic Acid (% of total fat)	10.1	10.2
Caprylic Acid (% of total fat)	<0.10	24.1
Capric Acid (% of total fat)	<0.01	1.33
Energy Content		
Calculated ME§(kJ/g)	17.86	18.06

*The table is modified from the original published in a previous paper.[22]
§Calculated based on the predictive equation for metabolizable energy in dog foods.[28]

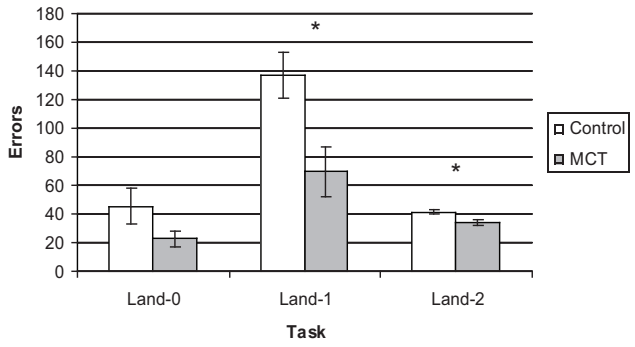
Cognitive Test Protocols and Schedule

After a one-week wash-in, all dogs were tested on a landmark protocol for up to 92 days. All the dogs were subject to an egocentric protocol test, starting on day 100 after the initiation of the study. The variable object oddity task protocol began on day 190 and continued for 35 days. During the whole study, dogs were tested once daily on one cognitive task at a time using a test schedule similar to previous studies.[19,20]

Dogs' learning ability and visuospatial function was assessed using a landmark discrimination protocol. The details of the landmark test protocol have been previously published.[21,22] Briefly, the landmark discrimination protocol included three separate tasks (land-0, land-1 and land-2), which are designed to assess allocentric spatial ability and require dogs to use external landmarks to localize objects in space. In all landmark protocols, dogs had to use a yellow peg (2 cm x 2 cm x 9 cm) as the external landmark to find the food reward. In land-0 test, two identical white coasters were used to cover food wells and the yellow peg was attached to one of the coasters. For land-1 test, the landmark was moved 1 cm medially and diagonally away from the edge of the coaster. For land-2 test, the landmark was moved 2 cm medially and diagonally away from the edge of the coaster. On each trial, the animal care technician placed the food reward in either the left or right food well and positioned the landmark accordingly. Dogs were able to obtain the food reward if they displaced the coaster attached to or closer to the yellow peg. In this and subsequent cognitive tasks, food inaccessible to the dogs was placed in the bottom of the coaster or objects associated with nonreward food well(s) in order to prevent the dogs from responding based on olfactory cues.

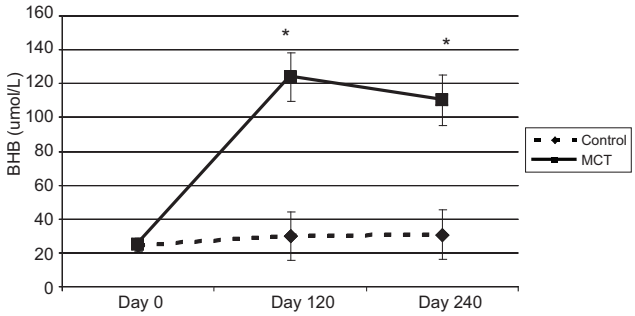
The egocentric protocol evaluates the animals' egocentric spatial learning ability to use a body-centered coordinate system to locate objects in space. The egocentric reversal protocol provides an additional measure of flexibility and executive function.[19] These protocols have been described in previous publications.[19,22] Briefly, the protocol first evaluated the ability of the dog to selectively respond to an object based on prox-

Figure 1



Effects of dietary MCT supplementation on blood BHB. The data are means ± SE, n = 12. *Mean values significantly differed (P<0.05). The figure is adapted from the data presented in a previous publication.[22]

Figure 2



Effects of dietary MCT supplementation on dogs' performance in the landmark tests. The data are means ± SE, n = 12. *Mean values significantly differed (P<0.05). The figure is adapted from the data presented in a previous publication.[22]

imity of the dog to its left or right side, and second, to reverse its original response. The egocentric protocol included three phases: a preference phase, an acquisition phase and a reversal phase. During the preference phase, the dog was presented with 10 discrete trials with identical objects covering both food wells containing food rewards over a single test day. The side chosen most frequently was selected as the preferred side and assigned to be the correct side for the acquisition phase. For dogs that responded five times to each side, a coin toss was used to determine the preferred side. All dogs were given two reversal tests (reversal 1 and reversal 2). The reversal phase was initiated on the day following completion of the acquisition phase. The reversal test procedure was identical to that of the acquisition phase except that the rewarded side was switched to the opposite side. Thus, if the dog's preferred side was right in the acquisition phase, the side closest to its left was rewarded in reversal 1 testing. Dogs that passed reversal 1 testing moved on to reversal 2 testing, which was identical to reversal 1 testing except that the rewarded side was switched to the opposite side of the reversal 1 testing.

The oddity discrimination protocol was designed to assess attention of the dogs. The details of the protocol were published in a previous paper.[22] Briefly, the task had three phases: acquisition, same distractor and different distractor. During the acquisition phase, the dog had to learn to selectively respond to a particular object to obtain a food reward. For the same and different distractor phases, dogs were presented with one, two, three or four objects, including the object they had been trained to respond to during the acquisition phase. The number of distractors varied from 0 to 3. Once a dog passed the acquisition phase, the dog moved on to the distractor phases. The first 7 sessions (1 to 7) had the same distractor phase, with the same distractors as in the acquisition phase. Sessions 8 to 14 covered the different distractor phase, with the reward object remaining unchanged but using new distractors.

Body Weight, Clinical Chemistry, Complete Blood Count (CBC) and Blood Ketone Bodies

Baseline blood samples were collected for measurements of beta-hydroxybutyrate (BHB), complete blood counts (CBC), and clinical chemistry. These measures were repeated after four and eight months of the feeding trial. Blood BHB samples were collected two hours after feeding. Samples for clinical chemistry, CBC and BHB were sent to Advance Vet Lab (Mississauga, Ontario) for analyses. Body weight was recorded at two-week intervals.

Statistical Analysis

Errors were used as the dependent measure and group comparisons were made using both Student's t-test and repeated measure analysis of variance (ANOVA). Values are means ± SEM except the cognitive data in the figures.

Results

Effect on Body Weight and Blood Levels of BHB, CBC and Clinical Chemistry

The MCT diet significantly increased blood ketone bodies as measured by BHB under fed conditions (Figure 1). Body weight did not change in either group. All the CBC and clinical chemistry parameters were within normal ranges for both groups at baseline and throughout the study (data not shown).

Effect on Performance of the Landmark Test.

The effects of MCT diet on landmark test were presented in Figure 2. The two groups were compared using repeated measure ANOVA, and the results showed that the MCT group differed significantly from controls, making fewer errors on the land-1 task (p=0.02), but not on the land-0 task (p=0.08).

The average days for the dogs fed the MCT diet to reach the test criterion in land-0 and land-1 tests were 15 days and 31.67 days after the initiation of the feeding trial, respectively. The average days for the dogs fed the control diet to reach the test criterion in land-0 and land-1 tests were 20.5 days and 50.17 days after the initiation of the feeding trial, respectively. Eleven dogs from each group completed the land-2 task. The groups were compared with Student's t-test, and the results showed that the MCT group made fewer errors than dogs in the control group on the land-2 task (p=0.0364).

Effect on Performance of the Egocentric Test

Multiple comparisons were performed to analyze the group effect (Fischer's least significant difference), and the results showed that the MCT diet significantly improved egocentric reversal 2 task (p=0.03, Figure 3). Dogs fed the MCT diet tended to make fewer errors to reach criterion in both acquisition and reversal 1 tests compared with control dogs, but the difference failed to reach statistical significance (data not shown).

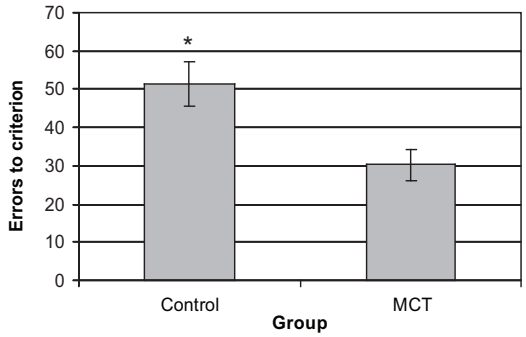
Effect on Performance of the Variable Object Oddity Test

Results from the acquisition phase of the oddity task showed that the dogs fed the MCT diet committed fewer errors than controls, but the differences were not significant ($p > 0.05$). During the zero distractor phase, all dogs performed at a very high level of accuracy leaving no room for improvement, and the data from this phase were not used in the statistical analysis. The data from 1-, 2-, and 3-distractor conditions were analyzed, and the results showed that dogs fed the MCT diet made significantly fewer errors per 168 trials than the control dogs (Figure 4).

Discussion

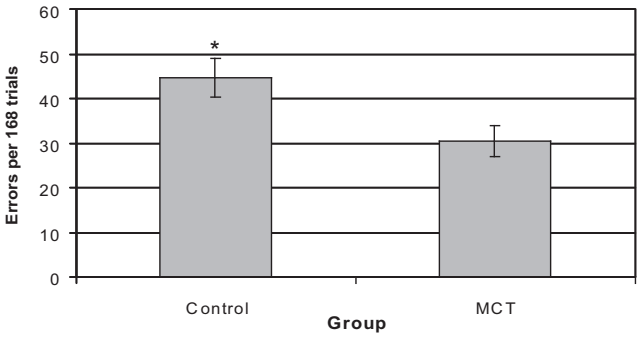
The objective of the present study was to evaluate whether dietary supplementation with MCTs can enhance cognitive function in aged dogs. Cognitive protocols designed to assess object discrimination learning and allocentric spatial ability showed improved function in the MCT-supplemented dogs. The average days for the dogs fed the MCT diet to reach the test criterion in land-0 and land-1 tests were 15 days and 31.67 days after the initiation of the feeding trial, respectively. These data indicated that on the average, the MCT supplementation tended to improve spatial learning and memory within two weeks after initiation of MCT supplementation and significantly improved spatial learning and memory in old dogs within one month after beginning supplementation. Dietary MCTs also significantly improved egocentric reversal learning and oddity task evaluations compared to the control dogs, indicating that the dietary MCT supplementation significantly enhanced spatial memory, executive functions and concept learning in old dogs, as well as improving attention ability. These results in dogs are comparable to those observed in humans.[12,13]

Figure 3



Effects of dietary MCT supplementation on dogs' performance in the egocentric reversal 2 task. The performance was expressed as error to criterion. The data are means ± SE, n = 12. *Mean values significantly differed (P<0.05). The figure is modified from the original presented in a previous publication.[22]

Figure 4



Effects of dietary MCT supplementation on dogs' performance in the oddity attention task. The performance was expressed as errors per 168 trials. The data are means ± SE, n = 11 for the control group, n = 9 for the MCT group. *Mean values significantly differed (P<0.05).

Blood levels of BHB at baseline, 120 and 240 days after initiation of the feeding trial were measured to determine the effects of MCT supplementation on the levels of ketone bodies in old dogs. Dogs fed the MCT diet had higher blood ketones, under fed conditions, confirming the ability of dietary MCTs to increase blood ketone levels under fed conditions and without restricting dietary carbohydrates. The resulting blood levels of ketone bodies were well-tolerated by dogs and were about four times lower than the levels of ketone bodies (0.5 mmol/L) in fasted dogs.[23]

All parameters of CBC and blood biochemistry were within normal physiological ranges at the end of the study in all dogs, indicating that the MCT diet was suitable to maintain the health of the dogs. In addition, an independent safety study confirmed the safety of 15% dietary MCT in dogs.[24]

Normally most of the ATP production in the neurons comes from glucose metabolism, and neurons maintain a tight coupling

between neuronal activity and cerebral glucose utilization. Increased neuronal activity usually requires increased glucose metabolism for more ATP production.[9] Since the brains of old animals have a reduced ability to metabolize blood glucose, they are not able to increase ATP production high enough to support increased neuronal activity, which may, at least partially, contribute to the decline in cognitive function in old animals. Reduced brain glucose metabolism is also observed in elderly humans[3]; it is highly possible that dietary MCT supplementation may be able to improve brain function in elderly people with and without dementia symptoms.

In addition to providing an alternative energy source, dietary MCT may have other beneficial effects on brain function. For instance, poor cognitive function is associated with loss of polyunsaturated fatty acids (PUFAs) in the brains of old animals.[25] Animal studies showed that dietary MCT resulted in enhanced concentrations of PUFAs in the brains of rats[26] and dogs.[27] These data suggest that dietary MCT may indirectly enhance brain function by increasing brain levels of PUFAs.

In summary, this study shows that dietary MCT supplementation can significantly increase blood ketone body concentrations under fed conditions and improve cognitive function in old healthy dogs. The MCT diet had no adverse effects on CBC and blood chemistry.

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Q&A Discussion

Q: Dr. Wouter Hendriks, Utrecht University: Can you elaborate a little bit on the allocation procedure of the dogs to the various treatments? Were the dogs equally intelligent in both groups before you started?

A: Dr. Pan: Yes, at the beginning we used two tests based on cognition, DNMP and the size discrimination test, then we randomized based on cognition to two groups, so they have equal cognitive functions between two groups at the beginning.

Q: Dr. Esther Plantinga, Utrecht University: I was wondering how do the data of the MCT-supplemented old dogs compare to young dogs relative to their cognitive function.

A: Dr. Pan: The MCT helped improve cognition, but not close to the young dogs, maybe 70% or 60% of young dogs' performance. The older dogs fed MCTs were intermediate between the young dogs and the untreated control dogs.

Q: Dr. Ernie Ward, Seaside Animal Care: I have a different interest in MCTs from an athletic performance standpoint. But in humans we've known that MCTs have some effects, and I've got a couple of little follow-up points to this. No. 1, the MCTs can suppress appetite. While this mechanism isn't totally understood, did you see that in any of your dogs?

A: Dr. Pan: No. On the contrary, study dogs actually ate more in the MCT group compared to the control group. They did not gain weight but actually ate more to maintain body weight.

Q: Dr. Ernie Ward, Seaside Animal Care: In humans it's been reported MCTs can cause nausea and GI discomfort. Did you see any of that?

A: Dr. Pan: Not in our study, and in addition, we actually sponsored a second study to look at safety and other parameters. They found that even with 15% MCT in their diet, there was no impact on all those parameters. That study has already been published.

Q: Dr. Ernie Ward, Seaside Animal Care: From a manufacturing standpoint, many dry dog foods are going through an extrusion process, in which they are flooded by thermal stress heat. And an issue with MCTs in humans is that it oxidizes when you start getting at high temperatures, above about 150 degrees. So how did you accommodate for this?

A: Dr. Pan: We have a patent on this to incorporate MCTs into dry food. Our food scientists figured out a way to put in and without oxidation and without influencing palatability. We use a natural source of MCTs from coconut oil.

Dr. Dottie Laflamme, Nestlé Purina Research: Ernie, to your point, one of the things that people often don't understand about commercial pet food extrusion is that it's not actually a high-temperature cooking process. It's actually a very short-term, relatively low-temperature process. It's about 120 to 140 degrees centigrade. So, it's not like the fryer stuff where you have really overcooked, rancid fat. And some people just don't understand that aspect of commercial pet food.

Q: Dr. Richard Hill, University of Florida: My question was going to be how you controlled for energy intake. You substituted MCTs with triglycerides, is that right? Or what was your substitution?

A: Dr. Pan: We reduced the beef tallow in exchange for MCTs.

Q: Dr. Richard Hill, University of Florida: Well, of course, beef tallow contains more calories per gram than MCTs do, and I believe MCTS increase the metabolic rate a bit. So you have to eat more of it, so they would have to eat more. So you fed to maintain body weight, presumably. This means your treatment dogs consumed two things more as a consequence. One would be protein, and the other one would be more glycerol, which would be a source of glucose. So how do you distinguish between your effect from giving more glucose versus glycerol and giving more protein?

A: Dr. Pan: We actually measured the blood glucose in our dogs. There are no differences between two groups in the blood glucose. But, I don't think the major benefit would be from the glucose. We think the major benefit is from ketone bodies produced from the MCTs. The whole purpose is to give the MCTs because the neurons are less capable of metabolizing glucose. So even if we increase the glucose intake a little bit, I don't think it will make a difference because the blood glucose level didn't change that much. I think the major benefit still comes from the ketone body production because we actually see the correlation between the blood ketone body concentration and performance in the dogs.

Q: Dr. Richard Hill, University of Florida: Well, certainly you had more beta-hydroxybutyrate, but that could be due to increased production or it could be due to reduced utilization. And, so there may be two aspects to that.

A: Dr. Pan: Yes, it's possible. I think the glucose also plays an important role. Actually, maybe 80% of the energy soup comes from glucose, maybe only 20% from ketone bodies. So we are not denying that glucose is an important part of this benefit, but the MCT makes the difference because we saw the correlation between the ketone body concentration and cognition in the model.

Q: Dr. Aulus Carciofi, University Sao Paulo: I have no doubts about this study. I would like your comments about when we put a dog in a weight loss program; we see that they become more alert. We see improvement in behavior. I understand that this improvement in behavior may have several causes, but could you comment about the possibility that some of these improvements in alertness and the behavior of the dog could be attributed to better hydroxybutyrate and more ketones in the blood of these dogs?

A: Dr. Pan: Yes, it could be, but when you have weight loss, you usually see improvement in glucose tolerance. What happens is chronically obese dogs usually have a glucose intolerance, not only for muscle, liver, but I think the brain also has problems using glucose. When you have weight loss, you not only increase ketone bodies, you also improve the glucose sensitivity in different tissues that actually helps the brain. Actually, some people suggest that dementia is a type 3 diabetes condition, so you can imagine if you induce weight loss for the obese dogs, definitely you will see the benefit.

Q: Dr. Bob Backus, University of Missouri: I just have two questions. One is how close was the control diet to the test diet? Was it just the fat substitution? The other was sometime back I remember reading that humans may be unique in the brain being able to utilize ketone bodies for energy. Is there work to show that the dog can substitute ketone bodies for glucose like the human?

A: Dr. Pan: The only difference between the diets was we substituted some beef tallow with the MCTs. I haven't seen any *in vivo* study for dogs. But today, people look at dog milk because they have MCTs in the milk. Dogs are born to have the ability to metabolize MCTs and ketone bodies. So my speculation is that dogs must be able to shift metabolism from glucose to ketone bodies to sustain the brain functions to keep them alive. Bill, do you know any studies to confirm the dogs actually metabolize ketone bodies?

Dr. Bill Milgram. CanCog: I'm not sure if there have been any studies. Before we started this work, there was a myth that dogs don't even create ketone bodies. And we were told by members of the faculty at Guelph University that this couldn't possibly work because they don't produce ketones. And so the first step was to show that they produce ketones. There, obviously the beta-hydroxybutyrate is removed from the blood, but the actual mechanistic work hasn't been done. It hasn't been done with people either. It's a very difficult thing to do.

Dr. Richard Hill, University of Florida: It's some time since I read it and I don't read Dutch, so I only read the English version, but De Bruijne looked at starvation and ketone turnover

in dogs and found it twice the rate of that in people. And it actually didn't increase much with starvation.

Dr. Joe Millward, University of Surrey: Just a quick comment about the uniqueness of humans in using ketones in the brain. It is my understanding that a lot of work on the regulation of the interaction between glucose and ketones in the brain was done in rats. There was some nice work done in the 1970s showing that ketones specifically blocked the oxidation of glucose in rat brains, so it's not unique to humans. I've always worked on the assumption that all or most species can utilize ketones.