Effects of dietary fat and L-carnitine on plasma and whole blood taurine concentrations and cardiac function in healthy dogs fed protein-restricted diets

Sherry L. Sanderson, DVM, PhD; Kathy L. Gross, PhD; Phillip N. Ogburn, DVM, PhD; Clay Calvert, DVM; Gil Jacobs, DVM; Stephen R. Lowry, PhD; Kathy A. Bird; Lori A. Koehler; Laurie L. Swanson

Objective—To evaluate plasma taurine concentrations (PTC), whole blood taurine concentrations (WBTC), and echocardiographic findings in dogs fed 1 of 3 protein-restricted diets that varied in fat and L-carnitine content.

Animals—17 healthy Beagles.

Design—Baseline PTC and WBTC were determined, and echocardiography was performed in all dogs consuming a maintenance diet. Dogs were then fed 1 of 3 protein-restricted diets for 48 months: a low-fat (LF) diet, a high-fat and L-carnitine supplemented (HF + C) diet, or a high-fat (HF) diet. All diets contained methionine and cystine concentrations at or above recommended Association of American Feed Control Officials (AAFCO) minimum requirements. Echocardiographic findings, PTC, and WBTC were evaluated every 6 months.

Results—The PTC and WBTC were not significantly different among the 3 groups after 12 months. All groups had significant decreases in WBTC from baseline concentrations, and the HF group also had a significant decrease in PTC. One dog with PT and WBT deficiency developed dilated cardiomyopathy (DCM). Taurine supplementation resulted in significant improvement in cardiac function. Another dog with decreased WBTC developed changes compatible with early DCM.

Conclusions and Clinical Relevance—Results revealed that dogs fed protein-restricted diets can develop decreased taurine concentrations; therefore, protein-restricted diets should be supplemented with taurine. Dietary methionine and cystine concentrations at or above AAFCO recommended minimum requirements did not prevent decreased taurine concentrations. The possibility exists that AAFCO recommended minimum requirements are not adequate for dogs consuming protein-restricted diets. Our results also revealed that, similar to cats, dogs can develop DCM secondary to taurine deficiency, and taurine supplementation can result in substantial improvement in cardiac function. (*Am J Vet Res* 2001;62:1616–1623)

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From the Department of Small Animal Medicine, College of Veterinary Medicine, University of Georgia, Athens, GA 30602 (Sanderson, Calvert, Jacobs); Hill's Pet Nutrition, PO Box 1658, Topeka, KS 66601 (Gross, Lowry); and the Department of Small Animal Clinical Sciences, College of Veterinary Medicine, University of Minnesota, St. Paul, MN 55108 (Ogburn, Bird, Koehler, Swanson).

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Taurine (2-aminoethanesulfonic acid) is a sulfur-containing β-amino acid. Unlike most other amino acids, taurine is not incorporated into proteins but rather remains as 1 of the most abundant free amino acids in the body. Taurine is found in highest concentrations in cardiac muscle, skeletal muscle, the CNS, and platelets.[1]

Taurine is involved in numerous metabolic processes, including antioxidation, retinal photoreceptor activity, stabilization of neural membranes, development of the nervous system, reduction in platelet aggregation,[2-7] reproduction,[8] and conjugation of bile acids.[1,9] Taurine also has an important role related to myocardial function. The mechanisms underlying the effect of taurine on the heart remain unknown; however, much of the available evidence supports the theory that modulation of tissue calcium concentrations and availability may be the major effect taurine has on cellular function in the heart.[1,10,11] In addition, taurine may inactivate free radicals and protect the heart by changing cellular osmolality.[12]

Taurine is an essential amino acid in cats, and taurine deficiency can cause **dilated cardiomyopathy (DCM)** in this species.[13] However, taurine is not considered an essential amino acid in dogs. The activity of cysteine sulfinic acid decarboxylase (the rate-limiting enzyme in the synthesis of taurine from cysteine and methionine) is high in dogs, compared with cats[14]; therefore, unlike cats, dogs can synthesize adequate amounts of taurine from precursor amino acids. It has been concluded in a previous study[15] that clinically normal dogs fed cereal- and soybean-based diets that contained little or no taurine were able to maintain plasma and whole blood taurine concentrations similar to those found in clinically normal cats. Another study in dogs revealed that feeding taurine-free diets or diets found to be taurine-depleting in cats[16] did not result in taurine depletion when fed to a group of 8 healthy Beagles.[15] In addition, results of an early study in dogs[15] (that were initiated soon after the relationship between taurine deficiency and DCM was discovered in cats), in which the use of taurine supplementation for treatment of DCM was evaluated, were unrewarding. As a result, it was concluded that taurine did not play a considerable role in the development of DCM in dogs. However, in 1989, taurine deficiency was linked to DCM in foxes,[17] which reopened its possible role in DCM in dogs. Recently, low taurine concentrations

were detected in Cocker Spaniels and Golden Retrievers with DCM,[15,18,19,a] and dogs with urate and cystine urolithiasis.[15,20]

Although the cause and effect relationship between low taurine concentration and dogs with DCM remains unknown, dogs, like cats, conjugate bile acids primarily with taurine, and even when dogs become taurine-depleted, they do not readily use glycine for bile acid conjugation.[21] Therefore, loss of taurine through bile acid conjugation is a possibility in dogs. Bile acid secretion into the intestinal lumen is stimulated by dietary fat and amino acids.[22] As a result, dietary fat and protein may affect taurine excretion via bile. Therefore, 1 objective of this study was to determine whether taurine concentrations in healthy dogs were affected by dietary fat concentration in protein-restricted diets. We hypothesized that a **high-fat (HF)** diet would cause decreased taurine concentrations in dogs and that a **low-fat (LF)** diet would not cause decreased taurine concentrations, because there would be a decreased demand for taurine to conjugate bile acids. We also hypothesized that a **HF diet supplemented with L-carnitine (HF + C)** would not result in decreased taurine concentrations, because methionine, which is a precursor amino acid for both taurine and carnitine synthesis, would not be needed for carnitine synthesis and, therefore, would be available to support additional taurine synthesis.

A second objective was to evaluate cardiac function in dogs fed 1 of the 3 aforementioned diets. Echocardiography was used for assessment of cardiac function. In addition, because some dogs with taurine deficiency and DCM have a concurrent carnitine deficiency,[15,18,20] plasma and cardiac muscle carnitine concentrations were also evaluated in those dogs that developed cardiac dysfunction.

Materials and Methods

Dogs—Eighteen Beagles were selected for the study (twelve 1-year-old spayed females, three 9-year-old spayed females, and three 2.5-year-old castrated males). One of the 9-year-old spayed females developed hypothyroidism and was excluded from the final analyses. Another 9-year-old spayed female died 36 months after initiation of the study (when she was 12 years old) approximately 6 hours after the cardiac biopsy procedure. A necropsy did not reveal a cause for her sudden death; therefore, this dog was also excluded from the analyses after 36 months. All Beagles were determined to be healthy at the beginning of the study on the basis of physical examination, ECG, and echocardiography findings, and results of CBC,[b] serum biochemical analyses (alkaline phosphatase, alanine transaminase, amylase, and aspartate transaminase activities, and albumin, total bilirubin, cholesterol, phosphorus, total protein, triglyceride,[c] carbon dioxide,[d] BUN, glucose, creatinine, calcium, sodium, potassium, and chloride concentrations), complete urinalysis, quantitative aerobic bacterial culture of urine samples collected by cystocentesis, and endogenous creatinine clearance. Dogs were housed in individual cages under conditions of controlled lighting and temperature, according to the principles outlined by the NIH.[23] The study was approved by the University Animal Care and Use Committee.

Diet—All dogs were fed a canned canine maintenance[e] diet (**Appendix**) for a minimum of 2 weeks prior to initial sampling. The diet contained 27.5% protein (**dry-matter basis [DMB]**); protein sources were chicken, meat by-products, cracked pearled barley, ground corn, soybean meal, and liver. The diet contained a methionine-cystine content of 0.9% DMB, which is higher than the **Association of American Feed Control Officials (AAFCO)**[24] recommended minimum requirements (0.43% DMB) for maintenance in adult dogs. The free methionine content of the diet was 0.52% DMB.

After initial sampling to obtain baseline values, the 18 dogs were randomly assigned to 1 of 3 equally matched age and sex groups. Each group was randomly selected to receive 1 of 3 protein-restricted diets (Appendix) for 48 months. The percentages of fat and protein (DMB) in the LF diet were 13.3 and 10.1%, respectively; in the HF + C supplemented diet, 24.1 and 10.5%, respectively; and in the HF diet, 24.2 and 9.9%, respectively. The protein content, which consisted of whole dried eggs, was similar for the 3 diets, with a calculated methionine and cystine content of 0.6% (DMB) for all 3 diets (or 1.3 g/1,000 kcal for the LF diet and 1.2 g/1,000 kcal for the HF + C and HF diets) The calculated free methionine content of the diets was 0.4% (DMB). Of the 2 dogs excluded from the study, the dog with hypothyroidism was in the HF + C diet group, and the dog that died after 36 months was in the HF diet group.

Feeding protocol—The amount of food fed was based on caloric requirements determined from ideal body weight by use of the following formula: maintenance energy requirement = $2(30 \text{ body } wt_{kg} + 70)$.[25] Caloric intake was adjusted as necessary to maintain a body condition score of 3 (on a scale of 1 [very thin] to 5 [obese]), and mean body weight and caloric intake were calculated for each diet group. Diets were fed once a day.

Blood collection—Blood samples were collected from the jugular vein approximately 8 hours after eating. Heparinized whole blood was harvested and chilled on ice for approximately 30 minutes until spun in a cold centrifuge. Plasma was then immediately separated from the cellular components; a small amount of plasma was left above the buffy coat to prevent contamination of the plasma with cells. All plasma and whole blood samples were frozen at –70 C until analyzed for taurine concentrations. Samples for plasma carnitine concentrations were processed in the same fashion.

Serum was obtained within 30 minutes of clot formation. Complete blood cell counts and serum biochemical analyses were performed within 8 hours after blood was obtained.

Percutaneous cardiac muscle biopsies—Dogs were monitored by continuous ECG and indirect blood pressure oscillometry. Cardiac muscle biopsy specimens were obtained in dogs with evidence of cardiac dysfunction. Cardiac muscle biopsies were performed while dogs were under general anesthesia, using a previously described endomyocardial biopsy technique.[26]

Cardiac muscle samples were immediately blotted with gauze to remove blood, wrapped in foil, and snap frozen in liquid nitrogen. Samples were stored at –70 C until analyzed for carnitine concentrations.

Echocardiography—Cardiac function was evaluated by use of echocardiography by a cardiologist blinded to the dietary treatments. Dogs were positioned in right lateral recumbency, and a 7.5-mHz transducer was used to evaluate the heart, using 2-dimensional and **motion-mode (M-mode)** techniques. All measurements were obtained from the M-mode.

Criteria for diagnosis of dilated cardiomyopathy—The criteria used to establish a diagnosis of dilated cardiomyopathy included: increased end-systolic and end-diastolic left ventricular diameters; fractional shortening reduced to < 20%; **E-point-to-septal separation (EPSS)** > 8 mm; and absence of a grade 4/6 or greater heart murmur. All 4 criteria must have been present before a diagnosis of DCM was made.

Established echocardiographic ranges based on body weight were used to establish normal ranges for each dog.[27,28]

Taurine assay—Plasma and whole blood was analyzed for taurine concentration by use of an amino acid analyzer[f] as described.[29,30] To ensure complete release of taurine from blood cells, whole blood was frozen and thawed twice to lyse the cells. After the second thaw, 200 µl of whole blood was mixed with 200 µl of distilled water. This was followed by protein precipitation with 3% sulfosalicylic acid before analysis for taurine. Normal **plasma taurine concentration (PTC)** and **whole blood taurine concentration (WBTC)** ranges were determined, using the mean ± 2 SD from 18 healthy Beagles.

Carnitine assay—Carnitine was measured in plasma and cardiac muscle as free carnitine, short-chain acylcarnitine, long-chain acylcarnitine, and total carnitine and expressed in plasma as nmol/ml and in cardiac muscle as **nmol/mg of noncollagenous protein (nmol/mg NCP)**. Modifications[31,32] of the methods of McGarry and Foster[33] and Parvin and Pande[34] were performed to run carnitine assays in plasma and cardiac muscle extracts, using L-carnitine as a standard. Duplicate samples were assessed. An aliquot of extract was added to a reaction mixture (0.55 ml) containing 50 m*M* HEPES-KOH buffer (pH 7.6), 0.25 m*M* N-ethylmaleimide, and 0.01 µCi of [l-^{14}C] acetyl coenzyme A.[g] The reaction was initiated by addition of 0.5 units of carnitine acetyltransferase,[h] followed by frequent mixing at 25 C for 30 minutes. The reaction was terminated by addition of charcoal in acidified alcohol,[34] followed by equilibration on ice for 30 minutes. After centrifugation, an aliquot of the supernatant was placed in scintillation fluid, and radioactivity was counted. In this assay, sample results were compared with results for standard solutions of L-carnitine.

Percentage of recovery, using this modified method, has been assessed for human plasma. Recovery of added L-carnitine (5.25 nmol/ml) to plasma was 96.2%. Recovery of added L-carnitine to extracted plasma was 99.3%.

Statistical analyses—Statistical evaluations were performed by use of commercially available software.[i] Response over time for PTC, WBTC, and echocardiographic variables among the 3 diet groups and within each diet group were assessed. A 2-way ANOVA and least significant difference test were used to analyze differences among the 3 groups. Linear regression analysis of PTC, WBTC, and echocardiographic variables was used to analyze differences within each group. Values of $P < 0.05$ were considered significant.

Results

Body weights and caloric intake—Mean ± SD beginning and ending body weights for the LF group were 9.7 ± 1.2 kg and 10.0 ± 1.3 kg, respectively; however, differences were not significant ($P = 0.57$). The dogs in this group were consuming a mean of 18.2 g of LF diet/kg of body weight/d (76.8 kcal/kg/d). Mean ± SD beginning and ending body weights for the HF + C group were 10.5 ± 0.8 kg and 11.2 ± 1.0 kg, respectively; this difference was significant ($P = 0.01$). Mean weight gain per dog in this group was 0.7 kg during the 48-month study period. The dogs in this group were consuming a mean of 14.3 g of HF + C diet/kg/d (68.4 kcal/kg/d). Mean ± SD beginning and ending body weights for the HF group were 10.7 ± 0.6 kg and 11.0 ± 1.4 kg, respectively; this difference was not significant ($P = 0.42$). The dogs in this group were consuming a mean of 12.2 g of HF diet/kg/d (58.4 kcal/kg/d).

Plasma and whole blood taurine concentrations—Significant differences were not detected in PTC among the 3 diet groups at baseline (0 months). Additionally, significant differences were not detected in WBTC between the HF + C group and the HF group at baseline (whole blood taurine samples were inadvertently not collected from the LF group at baseline; however, all dogs in this group had WBTC within reference range at 6 months). At 6 months, a significant difference was detected in PTC but not WBTC among the 3 diet groups. Results of the least significant difference test and diet group means (**Table 1**) revealed that PTC was significantly lower in the HF group than in the LF group. At 12 months, results of the least significant difference test and diet group means revealed that WBTC was significantly lower in the HF + C group than in the LF group. However, at 18, 24, 30, 36, and 48 months, results of 2-way ANOVA revealed no significant difference in either PTC or WBTC among the 3 diet groups.

Results of linear regression analysis revealed there was not a significant difference in PTC over time in the LF and the HF + C groups (**Fig 1**), but there was a significant decrease in PTC over time for the HF group. Three of 6 dogs in the LF group, 2 of 5 dogs in the HF + C group, and 5 of 5 dogs in the HF group had PTC below the reference range at 48 months. The WBTC decreased significantly over time in all 3 diet groups (**Fig 2**). Four of 6 dogs in the LF group, 5 of 5 dogs in the HF + C group, and 5 of 5

Table 1—The effect of 3 protein-restricted diets on plasma and whole blood taurine concentrations in dogs

	Low fat diet		High fat plus carnitine diet		High fat diet	
Time (mo)	Plasma taurine* (mean ± SEM [range])	Whole blood taurine† (mean ± SEM [range])	Plasma taurine* (mean ± SEM [range])	Whole blood taurine† (mean ± SEM [range])	Plasma taurine* (mean ± SEM [range])	Whole blood taurine† (mean ± SEM [range])
0	70.5 ± 5.2 (62–96)	ND	68.8 ± 8.5 ND (41–86)	254.2 ± 20.9 (188–299)	66.8 ± 7.4 (47–82)	258.3 ± 20.9 (208–339)
6	60.2 ± 5.6[a] (46–85)	229.2 ± 15.1 (169–277)	50.8 ± 11.9 (31–95)	196.8 ± 27.5 (116–263)	30.7 ± 6.5[b] (14–49)	196.7 ± 37.6 (105–328)
12	42.7 ± 8.3 (18–68)	196.2 ± 23.3[a] (144–293)	36.6 ± 12.5 (9–76)	112.4 ± 28.4[b] (28–181)	22.0 ± 5.9 (13–45)	135.8 ± 25.4 (51–187)
18	55.7 ± 12.8 (21–98)	102.3 ± 17.0 (63–166)	54.2 ± 17.4 (18–116)	122.2 ± 29.7 (54–223)	29.5 ± 4.6 (14–53)	135.7 ± 42.0 (52–281)
24	28.0 ± 6.2 (5–45)	120.0 ± 19.2 (75–204)	27.0 ± 9.7 (5–52)	93.4 ± 29.1 (30–181)	24.8 ± 4.9 (7–55)	122.7 ± 23.3 (45–189)
30	65.3 ± 21.3 (12–162)	171.2 ± 45.9 (53–387)	34.4 ± 12.3 (9–78)	98.8 ± 19.9 (67–173)	36.5 ± 10.4 (12–81)	126.5 ± 19.8 (53–227)
36	7.5 ± 9.7 (24–153)	113.2 ± 20.7 (6–52)	33.8 ± 8.6 (24–188)	103.4 ± 27.1 (5–28)	14.2 ± 4.7 (35–200)	110.3 ± 26.6 (2–64)
48	59.1 ± 19.8 (12.1–142.4)	115.5 ± 19.9 (39.0–175.6)	58.7 ± 12.2 (24.2–87.9)	115.1 ± 11.3 (78.0–146.3)	23.0 ± 3.5 (15.2–33.3)	80.0 ± 20.3 (29.3–136.6)

ND = Not done.
*Reference range for heparinized plasma taurine = 41–97 nmol/ml. †Reference range for heparinized whole blood taurine = 155–347 nmol/ml.
[a,b]Different superscripts in the same row indicate significant ($P < 0.05$) difference between the diet groups indicated.

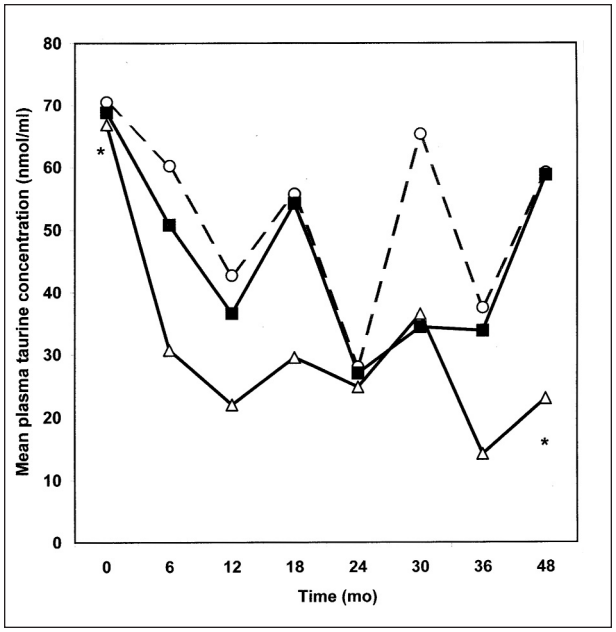

Figure 1—Within-group effect of 3 protein-restricted diets on plasma taurine concentrations in healthy Beagles. *Significant ($P < 0.05$) decrease over time within the high-fat diet group. Circles—Low-fat diet. Squares—High fat plus carnitine supplemented diet. Triangles—High-fat diet.

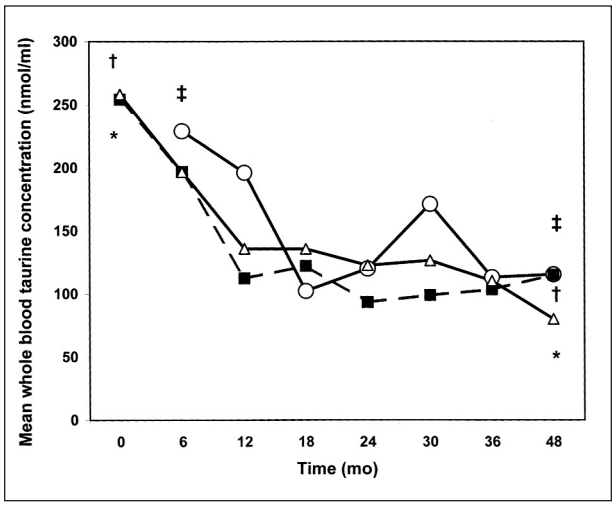

Figure 2—Within-group effect of 3 protein-restricted diets on whole blood taurine concentrations in healthy Beagles. *,†,‡Significant ($P < 0.05$) difference over time within the low-fat diet, high-fat plus carnitine supplemented diet, and high-fat diet groups, respectively. *See* Figure 1 for key.

dogs in the HF groups had WBTC below reference range at 48 months.

Echocardiographic findings—At baseline, significant differences were not detected among the 3 diet groups regarding **percentage of fractional shortening (FS%)**, EPSS, **left ventricular diameter at end systole (LVDs)**, and **left ventricular diameter at end diastole (LVDd)**. At 6 and 42 months, significant differences were detected in FS% among the 3 diet groups. Results of the least significant difference test and diet group means (**Table 2**) revealed that at 6 months, FS% was significantly higher in the LF group than in the HF + C and HF groups, and at 42 months, FS% was significantly higher in the LF group than in the HF + C group. Significant differences in LVDs were detected at 42 months among the 3 diet groups. Results of the least significant difference test and diet group means revealed that mean LVDs was significantly higher in the HF + C group than in the LF and HF groups. At 24 months, results of 2-way ANOVA revealed a significant difference in LVDd among the 3 groups. Results of the least significant difference test and diet group means revealed that LVDd was significantly higher in the HF + C group than in the LF and HF groups.

Results of linear regression analysis revealed no significant differences in FS%, EPSS, LVDs, and LVDd over time in the LF and HF groups. However, significant differences were detected in FS% and LVDs over time in the HF + C group; mean FS% decreased and mean LVDs increased over time in this group.

None of the dogs in the LF or HF diet groups had evidence of DCM by the end of the 48-month study. However, by the end of the study, 1 of 5 dogs in the HF + C diet group met all 4 criteria for the diagnosis of DCM (**Table 3**). At 12 months, echocardiography revealed mild left ventricular dilatation, suggestive of early DCM. By 42 months, all criteria established for the diagnosis of DCM were met. This dog developed PTC and WBTC below the reference range at 12 months, which persisted through 48 months. Because the dog was fed a carnitine-supplemented diet, plasma and cardiac muscle carnitine concentrations were above reference range limits. At 48 months, taurine supplementation (500 mg, PO, q 12 h) was initiated, and 3 months later, echocardiography revealed FS% and EPSS had normalized, with persistence of mild left ventricular dilatation during systole and diastole. The dog subsequently developed a thickened mitral valve (endocarditis).

A second dog in the HF + C diet group did not meet all the criteria for DCM but had a mildly decreased FS% (26.0%; reference range, 28 to 44%) and slightly increased LDVs (26.7 mm; reference range, 17.5 to 23.7 mm) that began at 42 months and persisted through 48 months. At 48 months, PTC was still within reference range limits (81.8 nmol/ml; reference range, 41 to 97 nmol/ml); however, WBTC was below reference range (126.8 nmol/ml; reference range, 155 to 347 nmol/ml). This dog was also fed a carnitine-supplemented diet, and, therefore, plasma and cardiac carnitine concentrations were above reference range limits. Plasma-free, short-chain acyl, long-chain acyl, and total carnitine were 99.9 nmol/ml (reference range, 9.0 to 36.0), 10.5 nmol/ml (reference range, < 7.0), 3.4 nmol/ml (reference range, < 2.0), and 113.8 nmol/ml (reference range, 12.0 to 40), respectively. Cardiac muscle free, short-chain acyl, long-chain acyl, and total carnitine were 16.9 nmol/mg NCP (reference range, 3.5 to 11.5), 4.0 nmol/mg NCP (reference range, < 5.0), 0 (reference range, < 0.66), and 20.9 nmol/mg NCP (reference range, 4.5 to 14.0), respectively. Taurine supplementation was not initiated in this dog.

Table 2—Effects of 3 protein-restricted diets on mean echocardiographic measurements in dogs

Time (mo)	Low-fat diet				High-fat plus carnitine-supplemented diet				High-fat diet			
	FS%*	EPSS† (mm)	LVDd‡ (mm)	LVDs§ (mm)	FS%*	EPSS† (mm)	LVDd‡ (mm)	LVDs§ (mm)	FS%*	EPSS† (mm)	LVDd‡ (mm)	LVDs§ (mm)
0	35.5	3.5	31.8	20.4	37.5	4.0	34.0	21.2	38.1	4.0	30.7	19.0
6	39.0[a]	2.3	32.6	20.0	32.7[b]	3.0	34.1	23.1	34.8[b]	3.5	31.2	20.5
12	38.8	3.8	32.1	19.7	36.8	4.4	29.4	22.5	32.5	4.4	30.6	20.6
18	35.8	3.2	32.4	20.9	32.9	4.4	34.7	23.3	34.3	3.0	30.5	20.0
24	36.8	3.8	30.0[e]	19.1	33.5	3.8	35.5[f]	23.7	37.7	3.7	30.3[e]	18.9
30	37.3	3.5	32.2	20.2	32.1	4.4	34.8	23.7	35.6	3.0	29.8	19.2
36	39.2	3.0	31.5	19.1	33.0	4.2	35.9	24.2	32.1	3.5	29.8	19.9
42	40.5[a]	4.0	30.7	18.7[c]	27.4[b]	5.5	36.7	27.0[d]	33.2	4.4	29.4	19.7[c]
48	37.8	4.1	31.0	19.2	28.0	5.7	35.0	25.7	34.2	3.2	30.8	20.3

*FS% = Fractional shortening percent [normal range = 28–44%]. †EPSS = E-point-to-septal-separation [normal range = < 8.0 mm]. ‡LVDd = Left ventricular diameter in diastole [normal range = 29.2–36.2 mm for dogs weighing 10.0 kg and 34.7–39.5 mm for dogs weighing 15.0 kg]. §LVDs = Left ventricular diameter in systole [normal range = 17.5–23.7 mm for dogs weighing 10.0 kg and 22.2–26.4 mm in dogs weighing 15 kg]. Different superscripts in the same row indicate significant ($P < 0.05$) difference among the diet groups at the time indicated.

Table 3—Results from a Beagle that developed dilated cardiomyopathy secondary to diet-induced taurine deficiency

Variable	Reference range	Time (mo) 0	6	12	18	24	30	36	42	48	51*
FS (%)	28–44	39.5	28.3	30.0	28.9	24.6	28.2	25.7	14.0	12.0	32.9
EPSS (mm)	< 8.0	6.0	3.0	7.0	5.0	6.0	8.0	10.0	12.8	10.2	3.8
LVDs (mm)	22.2–26.4	23.0	24.5	28.0	27.6	31.0	28.8	32.7	40.7	40.3	28.1
LVDd (mm)	34.7–39.5	38.0	34.6	40.0	39.4	41.1	40.1	44.0	47.1	45.6	41.9
Plasma taurine (nmol/ml)	41–97	86	32	9	25	7	19	6	NA	24.2	187
Whole blood taurine (nmol/ml)	155–347	188	170	28	54	30	67	24	NA	78	230
Plasma carnitine (nmol/ml)	Free† = 9.0–36.0	NA	NA	NA	NA	NA	NA	NA	NA	72.4	NA
	SC Acyl = < 7.0	NA	NA	NA	NA	NA	NA	NA	NA	12.1	NA
	LC Acyl = 0.6–2.0	NA	NA	NA	NA	NA	NA	NA	NA	3.0	NA
	Total‡ = 12.0–40.0	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	**87.5**	**NA**
Cardiac muscle carnitine (nmol/mg NCP§)	Free† = 3.5–11.5	NA	NA	NA	NA	NA	NA	NA	NA	10.3	NA
	SC Acyl = < 5.0	NA	NA	NA	NA	NA	NA	NA	NA	6.1	NA
	LC Acyl = < 0.66	NA	NA	NA	NA	NA	NA	NA	NA	0.03	NA
	Total‡ = 4.5–14.0	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	**NA**	**16.43**	**NA**

FS% = Fractional shortening. EPSS = E-point-to-septal-separation. LVDs = Left ventricular diameter in systole. LVDd = Left ventricular diameter in diastole. SC Acyl = Short-chain acylcarnitine is carnitine bound to a short-chain fatty acid. LC Acyl = Long-chain acylcarnitine is carnitine bound to a long-chain fatty acid. NA = Not available.

*Receiving taurine supplementation (500 mg, PO, q 12 h) for 3 months. †Free carnitine is carnitine not bound to a fatty acid. ‡Total carnitine is the sum of all the individual carnitine fractions (free, short-chain acylcarnitine, and long-chain acylcarnitine). §Nmol/mg NCP = Nanomoles of carnitine per mg of noncollagenous protein.

Discussion

Results of our study revealed that diet can result in decreased taurine concentrations in healthy dogs, which is in contrast to previous studies in dogs.[15,16,19,35] Although PTC declined more rapidly in dogs on the HF diet than on the LF diet, by the end of the study there was no significant difference in PTC and WBTC among the 3 diet groups. However, by the end of the study, WBTC decreased significantly within each diet group from their respective baseline concentrations. In addition, PTC decreased significantly within the HF diet group but not within the LF diet group nor within the HF + C diet group. All 3 diets were similar in protein content and considered protein-restricted (approx 10% protein DMB). In addition, all 3 diets were similar in methionine-cystine content. However, because of the lower energy density of the LF diet, dogs in this diet group consumed a larger quantity of food (18.2 g/kg/d) than did dogs in the HF + C diet group (14.3 g/kg/d) and the HF diet group (12.2 g/kg/d). As a result, when dietary intake was compared on a g/kg/d basis, dogs in the HF diet group consumed less protein and less methionine-cystine, compared with dogs in the LF and HF + C diet groups. This may account for why there was a significant decrease in PTC from baseline concentration in the HF diet group and not in the LF or HF + C diet groups. However, because there were no significant differences in PTC and WBTC among the 3 diet groups, but there was a significant difference in WBTC from baseline concentration over time within each diet group, suggests that the protein restriction of all 3 diets played a role in the development of taurine concentrations below the reference range that were detected in all 3 diet groups. This is consistent to what was reported in a similar diet study[j] performed in dogs, in which dietary protein ranged from 18.8 to 10.0% DMB, and linear decreases in PTC and WBTC were observed.

Alternatively, dietary fat and L-carnitine supplementation did not appear to consistently affect PTC or WBTC. Dietary fat intake did not appear to affect WBTC, because dogs in all 3 diet groups developed decreased WBTC, regardless of the quantity of fat consumed in the diet. This is in contrast to what was reported.[j] In addition, the diet group that developed the most severe decrease in PTC and WBTC (HF diet

group) had a daily fat intake (2.8 g/kg/d) that was between the daily fat intake of the LF diet group (2.2 g/kg/d) and the HF + C group (3.2 g/kg/d), which does not support the hypothesis that dietary fat intake affects PTC and WBTC, at least at the concentrations fed to the dogs of this study.

When caloric requirements were evaluated for each group, the LF diet group consumed a larger quantity of food than did either the HF + C or the HF diet group. Of interest, though, was the observation that the dogs in the LF group also required a higher caloric intake/kg/d to maintain body weight than did the dogs in the HF + C and the HF group. Another observation was that the caloric density of the HF + C and the HF diets were similar (4.78 kcal/g and 4.79 kcal/g as fed, respectively); however, dogs in the HF + C diet group consumed more calories per kg of body weight than did the dogs in the HF diet group. Dogs in the HF + C group had significant increases in body weight during the study, and the dogs in the HF group did not. The difference in absolute quantities of protein and methionine-cystine intake between these 2 groups may in part explain the differences in the severity of decreases in taurine concentrations, as well as the number of dogs in each group that were affected. In the LF and HF + C groups, 3 of 6 and 2 of 5 dogs, respectively, had PTC below reference range at 48 months, versus 5 of 5 dogs in the HF group. In the LF, HF + C, and HF group, 4 of 6, 5 of 5, and 5 of 5 dogs, respectively, had WBTC below reference range at 48 months.

Dietary L-carnitine supplementation also did not appear to prevent the development of decreased WBTC, as originally hypothesized. This finding is also similar to what was reported.[j] In addition, dietary L-carnitine supplementation, in the amount present in the HF + C diet, did not appear to protect cardiac function in the presence of low taurine concentrations, because both the dog that developed DCM and the dog that developed signs compatible with early DCM were from the L-carnitine supplemented group.

It is well known that cats have a limited ability to synthesize adequate quantities of taurine from the precursor amino acids cystine and methionine because of low concentrations of the enzyme cysteine sulfinic acid decarboxylase[14,36,37] and possibly cysteine dioxygenase.[38] One study[14] in which the amount of decarboxylation of cysteine sulfinic acid between dogs and cats was compared revealed that dogs have approximately 70 times more hepatic enzyme activity than cats and approximately 40 times more renal enzyme activity. In contrast, cats have approximately one third more brain enzyme activity than dogs. However, overall, dogs have higher cysteine sulfinic acid decarboxylase activity than cats; as a result, dogs can synthesize greater amounts of taurine from precursor amino acids than cats. Because of this greater enzyme activity in dogs, taurine has not been considered an essential amino acid in dogs.[39] However, all 3 diets in this study contained methionine-cystine concentrations at (1.3 g/1,000 kcal for the LF diet and 1.2 g/1,000 kcal for the HF + C and HF diets) or above (0.6% DMB for all 3 diets) the AAFCO recommended minimums for adult maintenance.[24] It is unknown whether increasing the methionine and cystine content of the diets would have affected taurine concentrations in the dogs of this study. However, our results suggest that the AAFCO recommended minimum concentrations are inadequate for dogs fed protein-restricted diets or that taurine may become conditionally essential for dogs fed protein-restricted diets.

Dogs have 1 known risk factor in common with cats for developing taurine deficiency. Primary bile acids, cholic, and chenodeoxycholic acids are synthesized from cholesterol in the liver and then conjugated to an amino acid to increase their water solubility.[40] Dogs, like cats, preferentially use taurine as the amino acid for bile acid conjugation, and they cannot readily convert to use of glycine for bile acid conjugation even when taurine pools in the body are depleted.[21] Therefore, dogs also have an obligatory loss of taurine in bile acids.

Dietary fat, protein, and gastric hydrochloric acid stimulate chemoreceptors in the duodenum, which results in the release of **cholecystokinin (CCK)**.[40] Cholecystokinin causes gallbladder contraction, which results in the release of bile acids into the duodenum; therefore, increased CCK release would stimulate greater release of bile acids. Whereas stimulation of CCK release in rats requires intact proteins, CCK release in dogs is stimulated by a simple solution of 2 amino acids (phenylalanine and tryptophan) and fat.[39] Therefore, it appears easier to stimulate release of CCK in dogs than in rats. It has also been demonstrated that increased intake of dietary fat is associated with increased fecal excretion of bile acids in rats,[41] monkeys,[42] and humans.[43] However, the relationship between dietary fat intake and fecal bile acid excretion is not a linear one in all species. Other variables such as the type of fat in the diet (saturated vs polyunsaturated) can affect the concentration of fecal bile acids in humans.[44] Dietary polyunsaturated fat causes greater fecal bile acid excretion than dietary saturated fat. The diets fed to the dogs in our study contained animal fats and were predominantly saturated and monounsaturated fatty acids. In contrast to humans, plasma taurine in cats is not influenced by the source of dietary fat,[29] but it is not known whether the same is true in dogs. However, because dogs preferentially use taurine for bile acid conjugation, and it is easier to stimulate CCK release in dogs than rats, it would be predicted that diets high in fat would be more likely to deplete taurine stores than diets low in fat. Taurine can be lost in feces through unabsorbed dietary taurine, unabsorbed bile acids, or bacterial degradation.[45-47] It is not known whether these factors contributed to the difference observed in the severity of decreased PTC between the LF and the HF groups. One study[j] performed in dogs concluded that fecal taurine and fecal bile acid concentrations were unaffected by dietary intake of fat. Therefore, although it is not likely that intestinal bacterial degradation of taurine and increased bile acid secretion played a role in the decreased taurine concentrations in our dogs, it cannot be completely ruled out. Intestinal bacteria degrade taurine in the colon; therefore, ruling out excessive loss of taurine in the feces is difficult unless fecal samples are collected prior

to entering the colon.[48] For this reason, it is also difficult to quantitate taurine excretion in bile acids unless the common bile duct is cannulated.

Another potential cause for taurine deficiency is through renal losses. However, all dogs in this study had normal renal function, as assessed by measurement of endogenous creatinine clearance, serum urea nitrogen, and serum creatinine concentrations. In addition, it was concluded in another study[j] that the concentration of taurine in urine was unaffected by dietary fat. It is, therefore, unlikely that renal loss of taurine contributed to the decreased taurine concentrations observed in our study.

Another important finding in this study was that dogs, like cats, can develop DCM secondary to taurine deficiency. Beagles are not a breed predisposed to developing DCM,[49,50] and, therefore, the diagnosis of DCM in Beagles is significant. In 1 dog, we documented normal cardiac function and blood taurine concentrations at the onset of our study; this dog subsequently had a decrease in PTC and WBTC below reference range after being fed a taurine-deficient diet, followed by development of DCM, and then almost complete reversal of DCM after only 3 months of taurine supplementation alone (Table 3). Unfortunately, it is not known whether DCM would have reversed completely in this dog if it had been supplemented with taurine for a longer period, because the dog developed a murmur and was found to have mitral valve endocardiosis.

At 48 months, PTC and WBTC were below reference ranges in 10 of 16 dogs, and 5 of 16 had only WBTC below reference range. Only 1 of 16 dogs maintained PTC or WBTC within reference ranges, although the WBTC in this dog was at the low end of the reference range (156.1 nmol/ml). In a pilot study[k] in which we evaluated how well PTC and WBTC predict cardiac muscle taurine concentrations in dogs, we determined that concurrent plasma and whole blood taurine deficiency was a better predictor of cardiac muscle taurine deficiency than either plasma taurine deficiency or whole blood taurine deficiency alone. However, in the present study, only 1 of 10 dogs with both PTC and WBTC below reference range developed DCM, and only 1 of 5 dogs with only WBTC below reference range had echocardiographic changes suggestive of early DCM. Therefore, we concluded that not every dog that has low taurine concentrations will develop DCM within 48 months.

In summary, contrary to what has been reported, diet can induce decreased concentrations of plasma taurine and whole blood taurine in healthy Beagles fed protein-restricted diets. Therefore, taurine is a conditionally essential amino acid in dogs fed protein-restricted diets. The data also suggest that the AAFCO recommended minimum requirements for methionine-cystine are inadequate in dogs fed protein-restricted diets. In addition, dogs, like cats, can develop DCM secondary to taurine deficiency.

[a]Kittleson MD, Pion PD, DeLellis LA, et al. Dilated cardiomyopathy in American Cocker Spaniels—taurine deficiency and preliminary results of response to supplementation (abstr), in *Proceedings*. 9th ACVIM Forum 1991;879.

[b]Coulter S-Plus IV, Coulter Electronic Inc, Hialeah, Fla.

[c]Syncron CX3 clinical system, Beckman Instruments Inc, Brea, Calif.

[d]Astra-8 automated stat/routine analyzer, Beckman Instruments Inc, Brea, Calif.

[e]Hill's Science Diet Canine Maintenance, Hill's Pet Products, Topeka, Kan.

[f]121 MB, Beckman Instruments Inc, Fullerton, Calif.

[g]Acetyl coenzyme A, New England Nuclear, Boston, Mass.

[h]Carnitine acetyltransferase, Sigma Chemical Co, St Louis, Mo.

[i]Statview 4.0, Abacus Concepts Inc, Berkeley, Calif.

[j]Gross KL, Kirk CA. Dietary protein and fat but not L-carnitine affect taurine status in the dog (abstr). *FASEB J* 2000;14:A505.

[k]Sanderson S, Osborne C, Gross K, et al. Reliability of canine plasma and whole blood taurine concentrations as indicators of cardiac and skeletal muscle taurine concentrations (abstr). *J Vet Intern Med* 1998;12:224.

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Appendix

Proximate analysis* of maintenance and 3 protein-restricted diets

Nutrient	Maintenance diet†	Low-fat diet	High-fat plus carnitine diet	High-fat diet
Moisture	71.7	7.6	6.4	7.6
Protein	27.5	10.1	10.5	9.9
Fat	19.4	13.3	24.1	24.2
NFE	45.6	70.5	59.0	59.4
Fiber	1.4	2.3	2.4	2.5
Ash	6.0	3.8	4.1	4.0
Lysine	1.4	0.6	0.6	0.6
Methionine	0.52	0.4‡	0.4§	0.4‖
Methionine + cystine	0.9	0.6¶	0.6#	0.6**
Taurine (ppm or mg/kg)	655	< 100	< 100	< 100
Carnitine (ppm or mg/kg)	104	29	344	20
ME††	36.8 kcal/oz	4.22 kcal/g	4.78 kcal/g	4.79 kcal/g

NFE = Nitrogen-free extract. ME = Metabolizable extract.

*Moisture and caloric density are expressed as percent of diet as fed. All other components, except methionine and methionine-cystine (g/1,000 kcal) are expressed as percent dry weight. †Hill's Science Diet Canine Maintenance canned.® Methionine and methionine-cystine (g/1,000 kcal) are on an as-fed basis: ‡0.9 g/1,000 kcal; §0.8 g/1,000 kcal; ‖0.8 g/1,000 kcal. ¶1.3 g/1,000 kcal; #1.2 g/1,000 kcal; **1.2 g/1,000 kcal. ††ME calculated according to Association of American Feed Control Officials protocol.[24]