J. Anim. Physiol. a. Anim. Nutr. 87 (2003), 359–372
© 2003 Blackwell Verlag, Berlin
ISSN 0931–2439

Receipt of Ms.: 15. 04. 2003
Accepted: 14. 05. 2003

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Taurine status in normal dogs fed a commercial diet associated with taurine deficiency and dilated cardiomyopathy

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Summary

Taurine (Tau) deficiencies have been associated with the feeding of commercial lamb-meal and rice diets to dogs. We hypothesized that the poor digestibility of some lamb-meals may limit sulphur amino acids availability for Tau synthesis and/or increase of Tau degradation in the gut. Growing dogs were fed either a lamb-meal-based (Diet A) or poultry by-product-based (Diet B) commercial diet. Plasma, whole blood and urinary Tau were measured for 22 weeks. Plasma and whole blood Tau concentrations were similar between the groups throughout the study. Urinary excretion of Tau in dogs fed diet A was 3.2 times greater than that from dogs fed Diet B, suggesting greater renal reabsorption and the need for conservation of Tau in the Diet A group. Food restriction affected Tau status as indicted by a positive correlation of food intake and urinary Tau. Dogs fed Diet A were given antibiotics to inhibit bacterial activity in the gut. Increases in breath hydrogen, indicative of increased bacterial activity, correlated negatively with urinary Tau. Urinary Tau increased by 54% when methionine (Met) was supplemented to Diet A, supporting the suggestion of a low bioavailability of sulphur amino acids and/or an increased fecal loss of Tau in dogs consuming Diet A.

Introduction

Taurine (Tau) is not considered an essential dietary amino acid in domestic canine species (Pion et al., 1998). With the exception of what appears to be breed specific defects (Kittleson et al., 1997), dogs are able to synthesize sufficient Tau when its sulphur amino acid precursors, methionine (Met) and/or cysteine (Cys), are present in adequate amounts in the diet. Consequently, diet-induced Tau deficiency has not been considered a problem in dogs. However, several cases of diet-induced Tau deficiency have been recently reported in dog breeds (Fascetti et al., in press; Sanderson et al., 2001; Backus et al., in press) not traditionally associated with Tau deficiency (Gavaghan and Kittleson, 1997). The clinical manifestation of the Tau deficiency in these reports was a dilated cardiomyopathy similar to that classically described in cats (Pion et al., 1987).

In foxes with Tau deficiency, dietary Met and Cys concentrations were found to be low; thus the observed Tau deficiency appears to be a consequence of inadequate provision of precursors for Tau synthesis (Moise et al., 1991). Tau deficiency has been associated with the feeding of commercial lamb-meal and rice diets to dogs (Fascetti et al., in press; Backus et al., in press). The nutritional and metabolic basis for these cases of Tau deficiency was not clear. The commercial diets consumed by the dogs in these reports met or exceeded nutrient profiles recommended by the Association of American Feed Control Officials (AAFCO, 1999) and have passed an AAFCO feeding protocol.

Dietary protein that is either low in quality or abundant in quantity increases the dietary requirement for Tau in cats (Backus et al., 1994; Kim et al., 1995; Backus et al., 1998). This effect appears to be caused by undigested protein reaching the lower gut, where it serves as a substrate for bacterial growth. Several species of gut bacteria utilize protein and produce cholylhydrolase (Mandl et al., 1957; Nair et al., 1965; Batta et al., 1984; Eyssen and Robben, 1988). This enzyme releases Tau from taurocholic and other bile acids that are normally conserved in the enterohepatic circulation. It has been hypothesized that proteins in the Tau-depleting commercial lamb-meal and rice diets caused an extraordinary gastrointestinal loss of Tau that in turn caused Tau depletion (Backus et al., in press).

The inclusion of rice bran in diets formulated for cats increases the dietary Tau requirement (Stratton-Phelps et al., 2002a). The mechanism for this appears to be either stimulation of gut bacterial growth or sequestration of bile acids containing Tau (Stratton-Phelps et al., 2002b). Similar to the mechanism proposed for the commercial diets, dietary rice bran may contribute to the loss of body Tau in dogs that is in excess of Tau synthesized from precursors in the diet.

In the present study, the commercial lamb-meal and rice diet most frequently associated with Tau deficiency was fed to dogs of similar ages and genetic backgrounds to evaluate the hypothesized mechanisms of diet-induced Tau deficiency in dogs. The effects of diet, food intake, antibiotic feeding and Met supplementation were evaluated.

Materials and methods

Animals

Twelve healthy, intact male Beagles (age 5–5.5 months) were used in this study. During the experiments, dogs were housed individually in metabolism cages in temperature (22 ± 3 °C) and light controlled (12 h light/12 h dark) rooms (Animal Resource Service, University of California, Davis, CA, USA). Body weights were determined weekly. Water was always available during the experiments. Jugular venous blood samples (~3 ml) were collected by routine venipuncture. Sampling frequency varied with experiment, but was no more frequent than once every 2 weeks. To avoid haemolysis of the samples, the dogs were immobilized during blood collections with isoflurane (2.5 to 3%) delivered by face mask. Housing and treatment of the dogs met recommendations of the Guide for the Care and Use of Laboratory Animals (National Research Council (NRC), 1996) and were approved by the UC Davis Animal Use and Care Advisory Committee.

Diets

For 6 weeks prior to initiating the experiments, the dogs were fed one of two purified diets with casein and soya bean protein isolate as protein sources. The diets were complete and balanced (NRC, 1985), and contained either 23% crude protein + 0.3% L-Met (0.7% Met + Cys by calculation), or 41% crude protein with no supplemental L-Met (1.3% Met + Cys by calculation) (see Hill et al., 2001 for compositions). The dogs were then fed one of two nationally distributed commercial dry-type (extruded) diets: Diet A, Nutro's Natural Choice Lamb-meal and Rice Formula (Nutro Products, City of Industry, CA, USA); and Diet B, Purina Dog Chow (Nestle Purina, St Louis, MO, USA). Package labeling indicated that the diets passed minimum AAFCO feeding protocol tests for all life stages. The manufacturer's guaranteed analyses, as well as the crude protein, crude fat, Met, Cys and Tau concentrations determined by an independent laboratory for each diet are listed in Table 1. The first six ingredients, in order of their appearance on Diet A package label, were lamb-meal, ground rice, rice bran, rice flour, sunflower oil and rice gluten, whereas, those on Diet B package label were ground yellow corn, poultry by-product meal, corn gluten meal, soya bean meal, beef tallow and brewers rice.

Table 1. **Reported[a] and measured[b] nutrient contents of commercial diets based on lamb-meal and rice (A)[c] and poultry by-product meal (B)[d]**

	Diet (%)			
	A		B	
	Reported	Measured	Reported	Measured
Crude protein	21	21.9	21	22.9
Crude fat	12	13.5	10	11.5
Crude fibre	5	–	4.5	–
Moisture	10	7.6	12	7.0
Met	–	0.41	–	0.43
Cys	–	0.38	–	0.41
Tau	–	0.02	–	0.02

[a] Guaranteed analysis values listed on package labels
[b] 'As fed' concentrations determined by independent laboratory
[c] Nutro's Natural Choice Lamb-meal and Rice Formula (Nutro Products, City of Industry, CA, USA)
[d] Purina Dog Chow (Nestle Purina, St Louis, MO, USA)

Laboratory methods

Blood samples were collected into syringes containing heparin and mixed by inversion of the syringes. Plasma from a portion of each blood sample was separated by centrifugation and stored in ice until extraction in 6% sulphosalicylic acid. The acid extractions reduced storage loss of Cys due to oxidation (Tôrres and Rogers, 2001). Details of procedures used in the processing of plasma, whole blood and urine samples for free amino acid analyses are described elsewhere (Kim et al., 1995). Amino acid concentrations in plasma and urine samples were determined with automated analysers (Model 121-MB and System 7300; Beckman Instruments, Fullerton, CA, USA).

Creatinine concentrations in urine samples were determined using a commercial kit (No. 555; Sigma, St Louis, MO, USA). Faecal bile acid concentrations were determined using a kit (No. 450-A; Sigma). Breath samples were collected through a face mask fitted with a flow dividing valve that was temporarily (2–3 min) placed over the muzzles of the dogs (Washabau et al., 1986). A rubber membrane on the mask base formed an air-tight seal. Expired air was collected in a 2-l rubber anaesthetic bag, from which 60 ml samples were taken for hydrogen gas determinations (Model 12 MicroLyzer; QuinTron Instruments, Milwaukee, WI, USA).

Experiment 1

Dogs were assigned to one of the two dietary treatments ($n = 6$) for 22 weeks. Blood samples were collected from each dog immediately before beginning the experiment, after they had been fed purified diets for 6 weeks. Blood samples were collected monthly thereafter. Food was continuously presented during the experiment, one group receiving Diet A and the other group receiving Diet B. Food intakes were determined once a month for four successive days, beginning 1 week after presentation of the diets. One of the dogs fed Diet B refused to eat the diet unless it was moistened with warm water. This dog ate only enough diet to maintain its body weight, whereas the other dogs consumed diet sufficient to support the weight gain of normal growth. Food intake observations on that dog were omitted from statistical analysis. Faecal and urine collections were conducted during the days when food intake was measured. Collected urine was acidified to pH 2 with concentrated hydrochloric acid to prevent Tau loss from bacterial growth. The urine

was pooled, its total weight determined and the aliquots were stored frozen at –20 °C until later analysis. Apparent digestibilities were determined from faecal dry-matter excretion and food intake observations.

All results are expressed as mean ± SEM (standard error of the mean). Tau concentrations in plasma, whole blood and urine; Tau excretion; Tau : creatinine ratios and plasma Met and Cys concentrations were compared between dog groups and over time using a two-way ANOVA repeated measures (Systat 9.0; SPSS Inc. Chicago, IL, USA). Bonferroni *post hoc* analysis was used to determine if p-values approached significance levels equal to or lower than 0.05.

Experiment 2

Two new dietary treatment groups, each containing six dogs, were formed. One group was food-restricted and the other fed *ad libitum*. Group assignment of dogs was randomized except for the requirement that each group included three dogs fed Diet A and three dogs fed Diet B in experiment 1. Each dog continued to receive the same diet they were fed in experiment 1. The degree of food restriction imposed on each dog in the restricted group was 75% of their average daily *ad libitum* food consumption determined during the final food intake period of experiment 1. The restriction was continued for 12 weeks, the duration of experiment 2. After 40 days, food restriction was discontinued in one dog that lost more than 20% of its initial body weight and had a body condition score of 3 on a nine-point scale, where 5 is ideal (LAFLAMME, 1993).

Blood samples were collected 2, 4, 8 and 12 weeks after initiating the experiment. Urine was collected on four successive days, once a month, beginning 4 weeks after the last collection period in experiment 1.

Group differences in food intakes, body weights, plasma and whole blood Tau concentrations, and urinary Tau excretions and Tau to creatinine ratios were determined using a Student's *t*-test for statistical analyses.

Experiment 3

Immediately following the food restriction trial, two new weight-balanced groups ($n = 6$) were formed. All of the dogs were fed Diet A in experiment 3. As some dogs were fed *ad libitum* during experiment 2 and became overweight, diet was presented to all of the dogs in experiment 3 for only 30 min/day in a restricted amount, 17 g/kg body weight [60 kcal/kg, estimated metabolizable energy (AAFCO, 1999)]. The destruction of Tau in the gastrointestinal tract of cats and chickens is substantially reduced when penicillin procaine and tetracycline are added to commercial diets (FEIGNER and DASHKEVICZ, 1987; KIM et al., 1996). Penicillin G procaine (Aquacillin; VEDCO, St Joseph, MO, USA) and tetracycline hydrochloride (Sigma, St Louis, MO, USA) were dissolved in water and added daily to aliquots of Diet A so that concentrations of the antibiotics in the diet were 1.5 and 5 g/kg, respectively. For homogenous application, the antibiotics were sprayed on and mixed into the diet each day immediately before feeding. One group received antibiotics applied to the diet, whereas the other group was given the diet alone. Food intake was determined daily. A post-feeding blood sampling (3–5 h) and a 5-day period of urine and faecal collection were conducted during weeks 2 and 4 of the experiment. During the last day of the experiment, breath collections for hydrogen determinations were obtained immediately before feeding and hourly for 12 h after diet presentation. The significance of group differences in food intake, peak and area-under-the-curve (*AUC*) breath hydrogen concentrations, plasma Tau concentrations and urinary Tau excretion between groups was determined using student's *t*-test for statistical analyses. Linear regression analysis was used to evaluate the significance of the relationship between *AUC* breath hydrogen concentrations and urinary Tau excretion.

Experiment 4

Two new dietary treatment groups ($n = 6$) were formed. The groups were weight-balanced and each group included three dogs previously receiving antibiotics in their food and three dogs fed diet alone. Diet A was also fed in experiment 4. One group received the diet supplemented with Met, whereas the other group was not given the supplement. When Diet A was supplemented with 1 g/kg L-Met (Ajinomoto, Raleigh, NC, USA), the Met was dissolved in warm water and applied to the diet in same manner as that described for the antibiotics. For the 3-week duration of the experiment, food intake was determined daily. During the last five experimental days, urine was collected for determination of Tau excretion. Concentrations of Tau, Met and Cys were determined in plasma extracted from post-feeding (3–5 h) blood samples.

The statistical significance of group differences in food intake and changes in plasma Tau, Met and Cys concentrations, and urinary Tau excretion was determined using a Student's *t*-test.

Results

Experiment 1: Diet A vs. Diet B

During the week preceding experiment 1, when the dogs were still receiving the purified diets, mean plasma Tau concentrations (± SEM) were 109 ± 8 and 115 ± 7 μmol/l for dogs to be fed Diets A and B, respectively (Fig. 1). Mean plasma Tau values decreased ($p = 0.001$) after 5 weeks of feeding both Diets. After 10 weeks, mean plasma Tau concentrations reached minimums of 40 ± 4 and 45 ± 7 μmol/l in dogs consuming Diets A and B, respectively. At 22 weeks, the end of the experiment, the plasma Tau concentration for dogs consuming Diet A was 51 ± 8 μmol/l, whereas that for dogs fed Diet B was 61 ± 7 μmol/l. Plasma Met and Cys concentrations did not change with time, nor did they differ between the two dietary groups during any of the weeks evaluated. Plasma concentrations of Met and Cys were 44 ± 2 and 54 ± 3 μmol/l, respectively, for dogs fed Diet A and 44 ± 2 and 51 ± 2 μmol/l, for dogs fed Diet B.

Initial whole blood concentrations of Tau for dogs consuming Diet A (262 ± 18 μmol/l) and Diet B (291 ± 25 μmol/l) were not significantly different. For both dietary groups, initial whole blood Tau concentrations were greater than all successive whole blood concentrations determined for each group. During week 22, whole blood Tau concentrations for dietary groups A and B were 222 ± 10 and 226 ± 11 μmol/l, respectively. At no time during experiment 1 were whole blood Tau concentrations significantly different between the groups.

All the dogs remained physically active and showed no signs of disease. Although dogs given Diet B had consistently lower mean body weights than dogs fed Diet A, body weights between the two groups were not significantly different throughout experiment 1. Food intake was similar between the groups of dogs until week 14 (data not shown). During weeks 18 and 22, four of six dogs given Diet B decreased their food intake for no apparent reason. This resulted in a non-significant decrease in mean body weight for the group receiving Diet B. The food intake of dogs given Diet A did not significantly change during the experiment. Dietary Met and Cys intake was not significantly different between the groups until week 22, when Met and Cys intake in the dogs given Diet B was lower ($p = 0.02$) than that in dogs given Diet A (2.2 ± 0.9 and 1.4 ± 0.2 g/day, respectively). The fractional dry-matter apparent digestibilities of groups A and B were 0.81 and 0.84, respectively, and not significantly different. Bile acid excretion in faeces was not different between the groups (93 ± 20 and 71 ± 26 μmol/day, for group A and B, respectively).

During all the weeks evaluated, dogs receiving Diet B excreted more Tau in their urine than dogs receiving Diet A (Fig. 1). The mean urinary Tau excretion in dogs fed Diet B

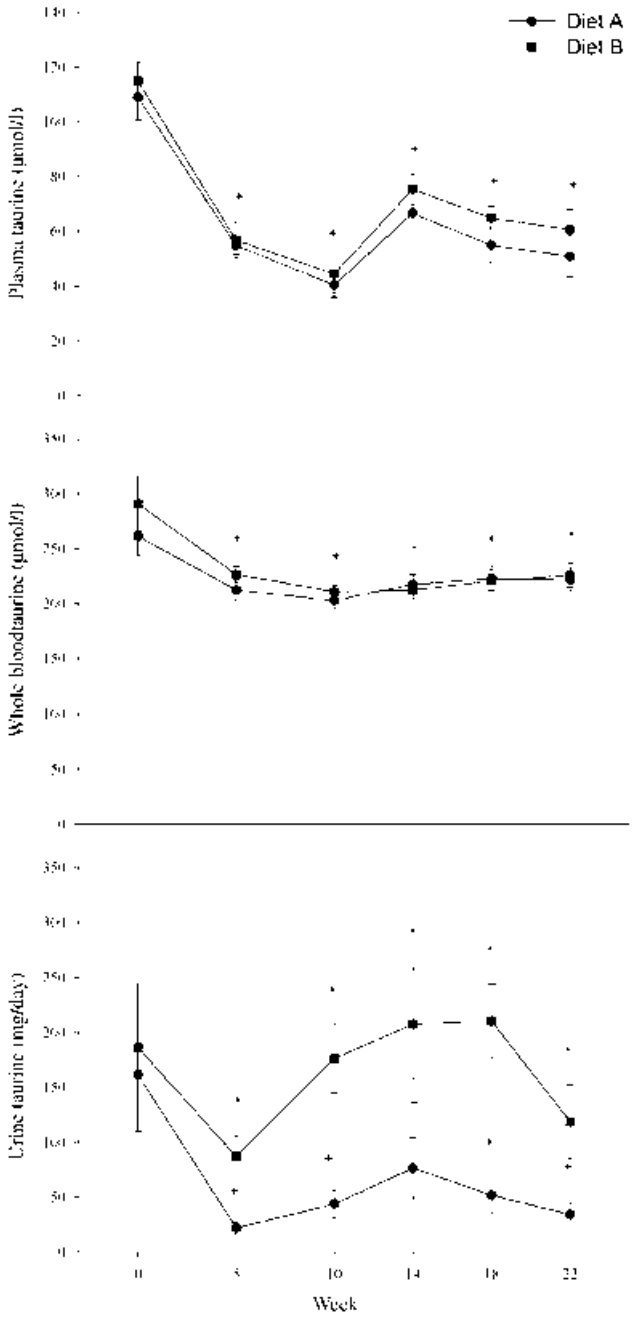

Fig. 1. Plasma and whole blood taurine concentrations and urinary taurine excretion in dogs fed commercial diets based on lamb-meal and rice (Diet A) and poultry by-product meal and corn (Diet B). Plot points represent mean ± SEM of six dogs. Values plotted for week 0 are from determinations on samples collected from the dogs while they were pre-fed purified diets based on casein and soya bean protein isolate. $^{+}p < 0.05$, each group compared with week 0; $^{*}p < 0.05$, group A compared with group B.

(138 mg/day) was 3.2 times greater than in dogs fed Diet A (46 mg/day). The quantity of urinary Tau excreted per day increased linearly ($p = 0.01$) with the ratio of Tau to creatinine in the urine (e.g. Week 14, $y = 0.0029x + 0.0448$, $R^2 = 0.83$; Fig. 2).

Experiment 2: Food restriction

Initial food intakes, body weights, plasma and whole blood Tau concentrations, urinary Tau excretion and Tau : creatinine ratios of the dogs in the restricted group were not different from the dogs in the *ad libitum* group. Plasma and whole blood Tau concentrations were not different between the groups at week 2.

During week 4, the mean food intake of dogs in the restricted group was 64% of that of dogs in the *ad libitum* group. The difference in food intake between the groups at week 4 was greater than expected because dogs in the *ad libitum* group had increased their food intakes above pre-trial consumption by a mean of 26% (Fig. 3). During this week, plasma Tau concentrations were significantly lower ($p = 0.02$) and whole blood Tau concentrations were reduced ($p = 0.07$) in the food-restricted dogs compared with those in the *ad libitum*-fed dogs. Linear regression of week 4 plasma Tau concentrations against food intake for all dogs ($n = 12$) revealed a positive linear relationship ($p = 0.0001$) (Fig. 4).

Plasma Met and Cys concentrations, urinary Tau excretion and Tau : creatinine ratios were not statistically different between the food-restricted and *ad libitum* groups at weeks 2 and 4. Bile acid excretion in faeces was not different between the groups (47 ± 14 and 87 ± 23 μmol/day, for the food restricted group and the control, respectively).

After week 4 of food restriction, one dog in the food restriction group was removed from the experiment because of weight loss and a low body condition score. Dogs in the *ad libitum*-fed group reduced their food intake during weeks 8 and 12 (Fig. 3). The cause for the reduction in food intake was not apparent and presumed to be an unrecognized environmental factor. Therefore, observations on the effect of food intake during weeks 8 and 12 are not reported.

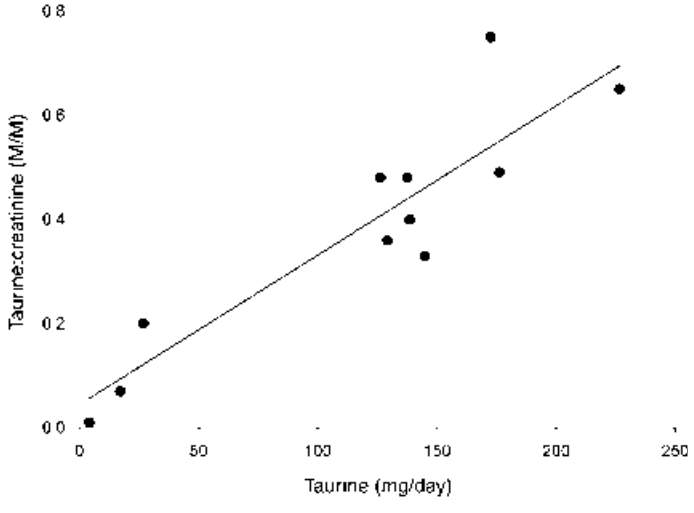

Fig. 2. Relationship between daily urinary taurine (Tau) excretion in dogs and the ratio in urinary Tau concentration to creatinine concentration (molar basis). The plotted line is the linear function derived from the regression of Tau : creatinine ratio on urinary Tau excretion [Tau : creatinine ratio (M/M) = 0.0029 × Tau excretion (mg/day) + 0.048, $R^2 = 0.83$, $p = 0.01$].

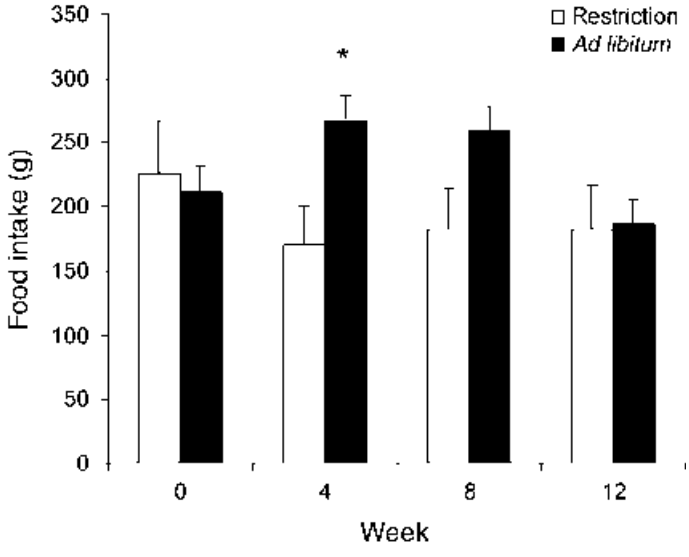

Fig. 3. Ad libitum and 75% of predetermined food intakes in dogs given commercial diets containing either lamb-meal and rice, or poultry by-product meal and corn. Plotted values are mean ± SEM food intakes of six dogs. *Significantly greater compared with restricted dogs during week 4, $p < 0.05$.

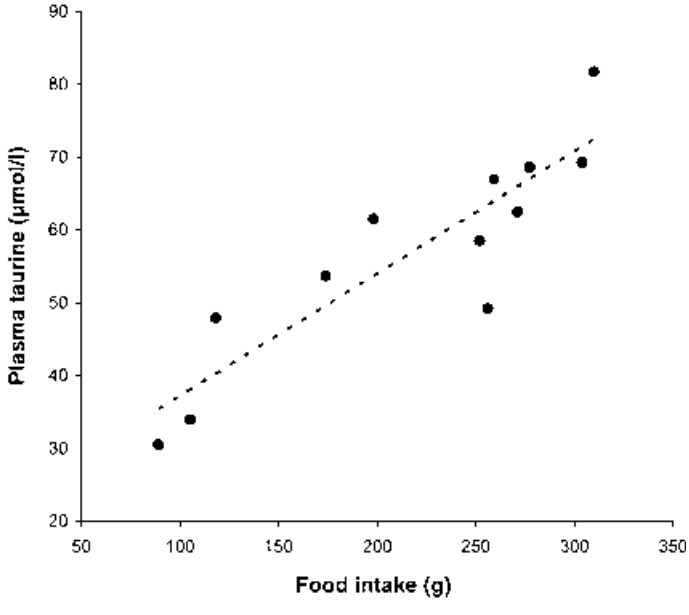

Fig. 4. Relationship between amount of food consumed and plasma taurine (Tau) concentration in dogs given commercial diets based on lamb-meal and rice ($n = 6$) and poultry by-product meal and corn ($n = 6$). Each plotted point represents the mean daily food intake in a dog determined over 5 days after 4 weeks of consuming one of the commercial diets. The plotted line is the linear function derived from regression of plasma Tau concentration on food intake [plasma Tau (μmol/l) = 0.17 × food intake (g) + 20, $R^2 = 0.79$, $p = 0.0001$].

Experiment 3: Antibiotic feeding

Daily food intakes of dogs given the diet containing antibiotics were not significantly different from control dogs. Breath hydrogen peak and *AUC* concentrations determined for one dog were omitted from statistical analyses because they were extraordinarily greater than peak and *AUC* concentrations found in all other dogs (greater than the mean values found of all dogs plus 4 × SEM). Peak breath hydrogen concentrations in dogs given antibiotics ($n = 6$) were greater ($p = 0.03$) than concentrations in dogs not given antibiotics ($n = 5$) (Table 2). *AUC* breath hydrogen concentrations in dogs given antibiotics tended to be greater ($p = 0.06$) than concentrations in dogs not given antibiotics. Among dogs of both groups ($n = 11$), urinary Tau excretion decreased ($p = 0.05$) with increasing peak breath hydrogen concentration (Fig. 5). Urinary Tau excretion tended to decrease ($p = 0.06$) with increasing *AUC* breath hydrogen concentration.

Plasma Tau concentrations at weeks 2 and 4 in dogs receiving antibiotics were not statistically different from the control group (Table 2). Daily urinary Tau excretion and apparent dry-matter digestibilities were not different between the groups (Table 2).

Experiment 4: L-Met supplementation

Food intakes in the dogs supplemented with Met were not significantly different from dogs fed the diet without supplementation. With dietary Met supplementation, plasma Met concentrations tended ($p = 0.09$) to increase; whereas plasma Cys concentrations were not significantly changed (Table 3). Plasma Tau concentrations tended ($p = 0.08$) to increase as a result of Met supplementation. Urinary excretion of Tau increased ($p = 0.03$) in dogs that received the Met by a mean of 54%. For the control group, significant changes were not observed in urinary Tau excretion or in plasma concentrations of Tau, Met or Cys.

Table 2. **Peak and area-under-the-curve (*AUC*) breath hydrogen concentrations, plasma taurine (Tau) concentrations, urinary Tau excretions and apparent dry-matter digestibilities of dogs given a commercial lamb-meal and rice diet with and without added antibiotics**[a]

	Antibiotics[b]		
	+	−	p[c]
Breath hydrogen concentration			
Peak (p.p.m.)[d]	3.8 ± 0.8	1.3 ± 0.1	0.03
AUC (p.p.m × h)	10.0 ± 2.4	3.9 ± 1.4	0.06
Plasma Tau concentration (μmol/l)			
Day −1	68 ± 7	57 ± 5	0.23
Day 14	72 ± 6	72 ± 8	1.00
Day 29	81 ± 8	94 ± 6	0.23
Daily urinary Tau excretion (mg/day)	52 ± 14	69 ± 9	0.33
Diet dry-matter digestibility (%)	78 ± 1	79 ± 1	0.76

[a] Values represent mean ± SEM for six dogs, except for the breath hydrogen values of dogs that received antibiotics, where $n = 5$
[b] Procaine penicillin G and tetracycline hydrochloride added to the diet at 1.5 and 5 g/kg, respectively
[c] Significance of *t*-test comparison between with (+) and without (−) antibiotic values
[d] Parts per million

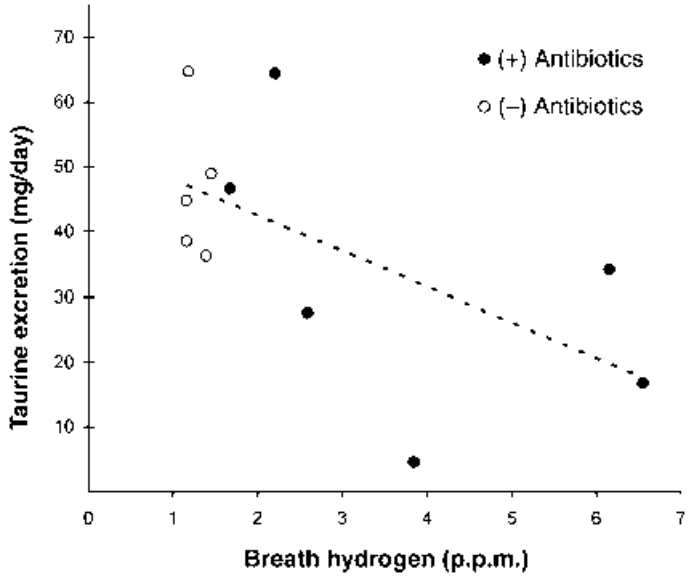

Fig. 5. Relationship between breath hydrogen peak concentration and daily urinary taurine (Tau) excretion in dogs given a commercial dry-type diet with ($n = 6$) and without ($n = 5$) added antibiotics (procaine penicillin G and tetracycline hydrochloride). The plotted line is the linear function derived from regression of daily urinary Tau excretion on peak breath hydrogen concentration [Tau excretion (mg/day) = −0.065 × hydrogen concentration (p.p.m.) + 5.2, $R^2 = 0.36$, p = 0.05].

Table 3. **Plasma methionine (Met), cysteine (Cys) and taurine (Tau) concentrations and daily urine Tau excretion in dogs given a commercial lamb-meal and rice diet with and without added dietary L-methionine (1 g/kg)[a]**

	Plasma (μmol/l)			
Group	Met	Cys	Tau	Urine Tau (mg/day)
–Met	75 ± 2	54 ± 3	75 ± 6	41 ± 8
+Met	85 ± 6	60 ± 4	83 ± 11	63 ± 6
p[b]	0.09	0.63	0.08	0.03

[a] Values represent mean ± SEM for six dogs
[b] Significance of *t*-test comparison between changes observed with (+) and without (–) addition of L-methionine

Discussion

The purpose of this study was to evaluate the hypothesized causes of Tau deficiency observed in dogs fed commercial lamb-meal and rice diets (Fascetti et al., in press; Backus et al., in press). As Tau is a dispensable amino acid, three hypotheses were considered: the commercial diet (i) induces low Tau synthesis, (ii) promotes excessive Tau loss, or (iii) produces a combination of (i) and (ii).

Our initial approach was to compare outcomes of dogs eating the Tau-depleting diet to those eating a non-Tau-depleting diet with similar nutrient compositions. In experiment 1,

we investigated whether dogs of similar genetic backgrounds (Beagles from a single source) fed the commercial diets *ad libitum* would differ in Tau status.

Before the initiation of experiment 1, all of the dogs were pre-fed nutritionally complete and balanced purified diets that did not contain Tau. Although the purified diets lacked Tau, plasma and whole blood Tau concentrations among the dogs were within the upper limit of reported normal ranges (Kramer et al., 1995; Kittleson et al., 1997). Plasma and whole blood Tau concentrations during the pre-feeding period were also significantly greater compared with those determined when the commercial diets were fed. These observations indicate adequate synthesis of Tau occurred in the dogs.

After feeding the commercial diets, a substantial decrease in plasma and whole blood Tau concentrations occurred in dogs in both dietary groups. Tau concentrations decreased to a minimum at week 14 and partially rebounded during the following weeks. These observations indicate that the commercial diets induced reductions in body Tau status to levels below those supported by the purified diets (Czarnecki et al., 1985). The observed increase in plasma and whole blood Tau concentrations that followed the initial decreases were probably reflective of changes in environmental conditions that affected food intake among the dogs or batch variations in the diets that affected Tau synthesis or loss. The reduction in body Tau status caused by feeding the commercial diets was not so severe that Tau deficiency signs would have been expected. Tau-deficiency dilated cardiomyopathy is reportedly observed in dogs with plasma Tau concentrations much lower than those presently observed, i.e. less than 44 μmol/l (Kramer et al., 1995).

Tau homeostasis is achieved primarily by the regulation of renal Tau excretion against a background of Tau synthesis supported by dietary precursors (Chesney et al., 1984; Park et al., 1989; Han et al., 1999). Therefore, the amount of Tau excreted in urine is a sensitive indicator of the adequacy of Tau provision, either from synthesis or dietary ingredients. In experiment 1, urinary Tau excretion decreased substantially when dogs were given the commercial lamb-meal and rice diet after a period of maintenance on a purified diet (Fig. 1). The decrease in Tau excretion occurred although concentrations of substrates for Tau synthesis in the lamb and rice diet were similar to those in the purified diet ($\sim$0.8 and 0.7% dry weight Met + Cys, respectively). A rapid decrease in urinary Tau excretion was also observed following the switch from a purified diet to the commercial poultry by-product meal and corn diet. However, after 5 weeks the decrease in Tau excretion was not as marked after feeding the poultry-meal and corn diet as that observed when changing from the purified to the lamb-meal and rice diet. Therefore, it would appear that sulphur amino acid precursors in the commercial diets were of low bioavailability relative to those in the purified diets. Although both commercial diets contained approximately 0.8% by dry-weight sulphur amino acids, it appears that: (i) sulphur amino precursors in the lamb and rice diet were less bioavailable than those in the poultry-meal and corn diet; and/or (ii) there was a substantially greater faecal loss of Tau when the lamb-meal and rice diet was fed.

Differences in sulphur amino bioavailabilities between the diets studied could result from differences in digestibilities of ingredient proteins. Johnson et al. (1998) have shown that ileal digestibility of amino acids in dogs depends upon the raw material sources and temperature used in the processing of feeds. These authors estimated that ileal digestibility of Met is similar between low-ash lamb-meals and low-ash poultry by-product meals (84 and 83%, respectively). However, they found that ileal digestibilities of Cys in the meals were 29 and 42%, respectively, and that Met and Cys concentrations are lower in tested lamb-meals compared with poultry by-product meals. If these observations are representative of commercially available meat meals, then poor ileal digestibility of Met and Cys might well account for the present finding of low urinary Tau excretion in dogs given the lamb-meal-based diet. Faecal bile acid excretion was similar between the two groups, suggesting that taurocholic acid losses were similar for both diets.

Although bioavailabilities of Met and Cys may have differed between the commercial diets presently studied, plasma Met and Cys concentrations were not different between the

dietary treatments. This finding probably reflects effectiveness of hepatic regulation of Met and Cys concentrations in the peripheral plasma during the absorptive period. One explanation for the increase of Tau in the urine would be that the liver acts as a buffer for sulphur amino acids delivered by the portal circulation first, and metabolites are then secreted into the peripheral circulation.

During experiment 4, when the effect of Met supplementation to the lamb-meal and rice diet was evaluated, plasma was sampled approximately 3 h after feeding. Among these samples, plasma Met concentrations in dogs that were supplemented tended to be greater than those of dogs not supplemented (Table 3). Although the Met supplementation increased the dietary sulphur amino acid content by only 13%, it increased urinary Tau excretion (+54%) and increased plasma Tau concentration. These observations support the hypothesis that the problem is not the result of inefficiency in the biosynthetic enzymes in the dogs.

Tau balance studies in cats fed commercial diets show that the primary route for Tau loss is through the gastrointestinal tract (Morris et al., 1994). These studies also show that in cats, gastrointestinal loss of Tau varies with diet (Backus et al., 1998) and the activity of gut bacteria. Accepting that breath hydrogen concentration is indicative of gut microbial population in dogs (Stone et al., 1994; Sasaki et al., 1999; Pelletier et al., 2001; Backus et al., 2002), our finding of decreasing urinary Tau excretion with increasing breath hydrogen concentration (Fig. 5) is consistent with gut microbial activity determining, in part, the extent of the need for Tau synthesis in dogs.

In cats, where Tau synthesis from sulphur amino acids is low, Tau must be present in the diet to meet tissue requirements for Tau. In experiment 3, it was assumed that, as in cats, dietary procaine penicillin and tetracycline at similar dose used for cats (Kim et al., 1996) would reduce Tau loss and improve Tau status in dogs given the lamb-meal and rice diet, if the Tau-depleting effect of the diet was primarily mediated by gut bacteria. This effect was not observed in this study. Dogs given the antibiotics had greater breath hydrogen concentrations than dogs not receiving antibiotics (Table 2). Therefore, it would appear the antibiotics stimulated, rather than reduced gut bacteria. The reason for this is unclear. However, given that four of the lowest urinary Tau excretion findings were in dogs that received antibiotics, a plausible explanation may be that the dietary antibiotics induced an overgrowth of gut bacteria resistant to penicillin G procaine and tetracycline hydrochloride.

The lamb-meal and rice diet evaluated in this study did not produce Tau deficiency in the dogs studied. However, in comparing indicators of Tau status in the dogs given the lamb-meal and rice diet to those in dogs given a commercial diet of similar nutrient composition, it appears that Tau synthesis supported by the lamb-meal and rice diet is marginal. The urinary Tau excretion responses of dogs given Met support the hypothesis of a reduced bioavailability of sulphur amino acids in the lamb-meal and rice diet. Whole body Tau status, as indicted by plasma Tau concentrations, increased with increasing food intake. This suggests that the metabolic energy requirement, which is coupled to food intake, may affect Tau status. Inherent in this statement is the assumption that Tau synthesis may not be related to energy needs. In previous reports, the dogs consuming this lamb-meal and rice diet that developed Tau deficiency and dilated cardiomyopathy were larger and older (Fascetti et al., in press; Backus et al., in press). Young adult, small breed dogs were presently studied. Such dogs, compared with older and larger dogs, have greater energy demands (Kienzle and Rainbird, 1991), and therefore may be less susceptible to developing Tau deficiency when given a diet of marginal sulphur amino acid content or bioavailability, or when fed a diet that increases faecal Tau loss.

Acknowledgements

This work was supported by Morris Animal Foundation and the Center for Companion Animal Health, School of Veterinary Medicine, University of California, Davis. Cristina L. Tôrres was supported by Hill's Pet Nutrition, Inc., USA.

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