Plasma amino acid and whole blood taurine concentrations in cats eating commercially prepared diets

Cailin R. Heinze, VMD; Jennifer A. Larsen, DVM, PhD; Philip H. Kass, DVM, PhD;
Andrea J. Fascetti, VMD, PhD

Objective—To establish comprehensive reference ranges for plasma amino acid and whole blood taurine concentrations in healthy adult cats eating commercial diets and to evaluate the relationships of age, sex, body weight, body condition score (BCS), dietary protein concentration, and dietary ingredients with plasma amino acid and whole blood taurine concentrations.

Animals—120 healthy adult cats.

Procedures—Blood samples and a complete health and diet history were obtained for each cat, and reference intervals for plasma amino acid and whole blood taurine concentrations were determined. Results were analyzed for associations of age, breed, sex, body weight, BCS, use of heparin, sample hemolysis and lipemia, dietary protein concentrations, and dietary ingredients with amino acid concentrations.

Results—95% reference intervals were determined for plasma amino acid and whole blood taurine concentrations. A significant difference in amino acid concentrations on the basis of sex was apparent for multiple amino acids. There was no clear relationship between age, BCS, body weight, and dietary protein concentration and amino acid concentrations. Differences in amino acid concentrations were detected for various dietary ingredients, but the relationships were difficult to interpret.

Conclusions and Clinical Relevance—This study provided data on plasma amino acid and whole blood taurine concentrations for a large population of adult cats eating commercial diets. Plasma amino acid and whole blood taurine concentrations were not affected by age, BCS, or body weight but were affected by sex and neuter status. Dietary protein concentration and dietary ingredients were not directly associated with plasma amino acid or whole blood taurine concentrations. (*Am J Vet Res* 2009;70:1374–1382)

The past 4 decades have been a time of dramatic advances in knowledge of feline nutrition, especially the relationships between protein metabolism and numerous disease states. Blood amino acid concentrations have been used for years to aid in the assessment of nutritional and protein status of cats and have a pivotal role in the diagnosis of specific medical conditions. The ability to measure whole blood and plasma taurine concentrations in cats aided in the discovery that taurine deficiency was a major cause of central retinal degen-

ABBREVIATIONS

BCS	Body condition score
NRC	National Research Council
SSA	Sulfosalicylic acid

eration[1] and dilated cardiomyopathy[2] in cats. These discoveries have saved many cats from these debilitating and potentially fatal diseases. Without the ability to analyze blood amino acid concentrations, these important connections may never have been made. Amino acid analysis has also been of benefit in investigating many other disease processes, including liver disease,[3] diabetes mellitus,[4,5] heart disease,[6] and even brain injury,[7] in humans and other animals, but comparatively little of this research has focused on cats.

Blood amino acid concentrations are dynamic and can be reflective of the most recently consumed diet when samples are obtained during the immediate postprandial period.[8,9] Alternatively, they can reflect the mean amino acid concentrations in protein-malnourished animals that eat a constant diet, thus allowing detection of severe and chronic amino acid deficiencies.[10]

Despite a long history of the use of amino acid concentrations for diagnostic and research purposes, there

are few reports of typical amino acid concentrations in plasma and whole blood of adult cats eating commercial diets. Although plasma amino acid concentrations for adult cats eating commercial diets have been published in 2 studies,[6,11] neither of those studies was designed to establish representative reference ranges; therefore, data were reported for only small numbers of healthy cats (29 and 24 cats, respectively) used as control animals in those studies. Additionally, neither study specifically addressed the diets being fed to the cats.

Currently, published reference values for feline amino acid concentrations have come primarily from studies[12–15] in which investigators evaluated amino acid requirements and protein metabolism in growing kittens consuming purified diets. Those diets were formulated with crystalline amino acids or protein concentrates (such as casein and soy protein), and the bioavailability of amino acids in such diets is extremely high.[16,17] In contrast, commercial foods undergo processing that may negatively impact the bioavailability of some amino acids, such as lysine, tryptophan, methionine, and cysteine.[10,18–20] Additionally, those studies[12–15] used only small numbers of growing kittens that were closely related and lacked the genetic diversity inherent in the general feline population. The potential problem with the use of data obtained from genetically similar animals consuming purified diets to develop reference ranges and nutritional recommendations was addressed in 2006 in the recent version of an NRC publication.[10] That publication acknowledged the differences between diet types and provided nutrient requirement recommendations based on the availability of nutrients in ingredients commonly used in foods commercially available for pets.

Our intent in the study reported here was to establish reference ranges for plasma amino acid and whole blood taurine concentrations in cats. We hypothesized that there would be associations between amino acid concentrations and dietary protein concentrations and ingredients. The first objective was to obtain samples from a large number of cats to facilitate the creation of new reference ranges to more accurately reflect the general feline population. A second objective was to analyze the collected data for relationships between plasma amino acid concentrations and signalment, body weight, BCS, dietary protein, and dietary ingredients. Other studies have not adequately addressed how sex, age, body weight, and BCS affect plasma amino acid concentrations, and such information allows for better interpretation of plasma amino acid values in clinically affected animals. In another study[21] conducted by our laboratory group, we detected a relationship between dietary ingredients and plasma amino acid concentrations, particularly taurine, methionine, and cysteine, in dogs. This relationship may also be relevant in cats because there has been a shift toward increasing the use of plant-based protein sources in commercially available pet foods. These ingredients can have lower concentrations of essential amino acids that may also be preferentially impacted by processing, which can result in a decrease in digestibility and bioavailability.[22–25]

Materials and Methods

Animals—Blood samples were obtained from 120 cats consuming commercially prepared feline diets. All cats were free of apparent systemic illness; obesity was not considered grounds for exclusion. The study population consisted of pet cats belonging to students, faculty, and staff of the School of Veterinary Medicine at the University of California-Davis, as well as a lesser number of university-owned cats housed in 2 separate colony facilities. Owners of participating pet cats provided written consent for use of their cats in the study. This study was reviewed and approved by the Institutional Animal Care and Use Committee of the University of California-Davis.

Development of reference intervals—All owners were instructed to feed their cats 3 to 5 hours before blood collection because blood amino acid concentrations are affected by meals and food deprivation.[26] Age, sex, neuter status, breed, body weight, BCS (9-point scale),[27] and current health status (detection of vomiting, diarrhea, sneezing, or coughing or changes in appetite, body weight, urination, or water intake) were recorded at the time of sample collection. One investigator (CRH) assigned a BCS to each privately owned cat and the cats from one of the university facilities. A staff veterinarian assigned the BCS for each cat at the second university facility. Both of these people used the same criteria[27] to assign each BCS and were experienced with the technique. Some university-owned cats were fed ad libitum, and samples were obtained at various intervals after eating (estimated time ranged from the immediate postprandial period up to 5 hours after eating).

Collection of blood samples—A blood sample (1 to 2 mL) was obtained from each cat with a heparinized or unheparinized syringe via jugular or medial saphenous venipuncture. Heparin supplies were unexpectedly interrupted because of a major recall[28] during the sample collection period; thus, heparin was not available for all samples (heparin was available at the beginning and end but not during the middle of the sample collection period). Regardless of whether the syringe was heparinized, all blood samples were immediately transferred to lithium heparin blood tubes; tubes were then gently inverted several times. When ≥ 1.5 mL of blood was collected, 0.5 mL of the sample was placed in a separate tube and frozen at –80°C for determination of whole blood taurine concentration. The remainder of the blood sample was centrifuged within 1 hour after collection. After centrifugation, plasma was immediately harvested and placed in labeled 1.5-mL microcentrifuge tubes. Plasma from most of the pet cats and cats from 1 university colony (n = 94) was subjectively assessed for lipemia and hemolysis by 1 investigator (CRH), who assigned a grade of mild, moderate, or severe. Two hundred microliters of plasma was removed, and an equal volume of 6% SSA (with a norleucine internal standard) was added to precipitate protein in the sample. All samples were maintained at –80°C until analysis. Interval between sample collection and analysis ranged from 1 to 60 days.

Assessment of amino acids—Plasma amino acid and whole blood taurine concentrations were analyzed

as described elsewhere.[29] Briefly, an automated amino acid analyzer[a] was used to perform cation-exchange high-pressure liquid chromatography separation and ninhydrin-reactive colorimetric detection. Complete plasma amino acid analysis (of 24 amino acids) was performed for all pet cats. One of the colony facilities mandated specific husbandry and security protocols that precluded immediate treatment of the samples with SSA; thus, cysteine concentrations could not be determined for all cats.[30] Furthermore, whole blood taurine concentration was only determined in cats when ≥ 1.5 mL of blood was obtained.

Evaluation of the effect of signalment, body weight, BCS, and diet on amino acid concentrations— All owners of pet cats completed a questionnaire on diet history. The questionnaire included information on the diet or diets fed, amount fed per day, number of times fed per day, amount of time fed the current diet or diets, food storage method, treats, treat amounts, treat frequency, supplemental products, amount of supplemental products, frequency of supplemental products, access to other animals' food, any medications, and exercise frequency. Information on diet and health history was obtained from university records for all university-owned cats. Only cats fed a single diet were included in the analysis to determine whether diet affected plasma amino acid concentrations. Dietary protein content (in g/100 kcal) was obtained from the diet manufacturer or calculated from the guaranteed analysis by use of modified Atwater factors.[10]

Statistical analysis—Statistical analysis was performed with computer software programs.[b–d] Results for tests of normality as well as the mean; median; SD; 0, 2.5th, 50th, 97.5th, and 100th percentile values; and 95% reference intervals based on percentiles were determined for each amino acid.

Multiple linear regression was used to assess relationships of age, body weight, and BCS with plasma amino acid concentrations. Population measures and dietary data were described by use of median, mean, and SD when appropriate. Kruskal-Wallis tests were used to evaluate potential relationships between sex-neuter status and plasma amino acid concentrations. Mann-Whitney tests were used to investigate the potential association of heparin use during blood collection with amino acid concentration, and Jonckheere-Terpestra tests were used to assess the association between ordinal categories of lipemia and hemolysis with amino acid concentration. Values of $P \leq 0.05$ were considered significant. Values of $R^2 > 0.5$ were considered indicative of a strong linear correlation.

Relationships between dietary ingredients and plasma amino acid concentrations were investigated by use of Kruskal-Wallis tests followed by pairwise dietary comparisons with results adjusted for multiple comparisons to preserve a nominal type 1 error rate of 5%. Significant pairwise comparisons were reported as $P \leq 0.05$.

Results

The majority (83/120) of cats were pets. The remaining 37 cats were university-owned animals. Age

ranged from 8 months to 16 years (median, 3 years; mean ± SD, 4.4 ± 3.13 years). Body weight ranged from 2.55 to 8.7 kg (median, 4.51 kg; mean, 4.76 ± 1.33 kg), and BCS ranged from 2.5 to 8 (median, 5.5; mean, 5.6 ± 1.06). Eighteen (15%) cats were considered obese (BCS ≥ 7). Fifty-six cats were neutered males, 29 were spayed females, 7 were sexually intact males, and 28 were sexually intact females. All of the sexually intact cats were university-owned animals. Breeds represented included domestic shorthair (n = 79), domestic medium-hair (10), mixed-breed cat (12), domestic longhair (7), Persian (7), Bengal (2), Siamese (1), Ragdoll (1), and Manx (1). The Persian and Bengal cats were part of a university colony, whereas the Siamese, Ragdoll, and Manx were pet cats. Domestic shorthair cats were represented in both university and pet cat populations. There was not a sufficient number of breeds represented to enable assessment of the relationship between breed and plasma amino acid concentrations.

University-owned cats were part of 2 separate colony facilities. The 2 facilities had no blood lines in common. At 1 facility (n = 13 cats), there were several sets of siblings and some parent-offspring pairs as well as unrelated cats from which blood samples were collected. At the second facility (n = 24 cats), there were siblings, half-siblings, and 1 parent-offspring pair from which blood samples were collected. There were some sibling groups in the pet cats and probably some parent-offspring pairs as well; however, these relationships were harder to assess because parentage information was not collected for the pet cats.

Insufficient blood samples (< 1.5 mL) were obtained from 31 cats; thus, whole blood taurine concentrations were determined for only 89 cats. Similarly, some samples obtained from university-owned cats were not treated with SSA immediately after collection; therefore, cysteine concentrations were determined for only 96 cats. Samples were collected into both heparinized (n = 54 samples) and unheparinized (66) syringes prior to being placed in lithium heparin tubes. There was a significant (range of P values, < 0.001 to 0.048) difference between heparinized syringe and unheparinized syringe samples for concentrations of the amino acids arginine, citrulline, glutamic acid, glycine, isoleucine, leucine, methionine, ornithine, threonine, tryptophan, and valine. Mean amino acid concentrations typically were lower in heparinized samples, although this was not the case for all amino acids, and not all concentrations differed significantly.

Increases in degree of lipemia had a significant (P = 0.008) positive association with threonine concentrations. Increases in degree of hemolysis had a significant positive association with the concentrations of isoleucine (P = 0.040), tryptophan (P = 0.030), and valine (P = 0.021) and a significant (P = 0.013) negative association with glutamic acid concentrations. The majority (17/25) of the amino acids assayed did not have a normal distribution, so nonparametric methods based on percentiles were used to determine the 95% reference intervals (**Table 1**).

Concentrations of arginine, asparagine, aspartic acid, cysteine, glutamic acid, glycine, hydroxyproline, isoleucine, lysine, ornithine, serine, threonine, trypto-

phan, and valine differed significantly (range of *P* values, < 0.001 to 0.038) among the 4 sex classifications (sexually intact male, castrated male, sexually intact female, and spayed female). Sexually intact females had higher concentrations of arginine, isoleucine, and valine, compared with the 3 other groups (all *P* values < 0.001). There were no significant differences between spayed females and castrated males; however, sexually intact females had significantly higher amino acid concentrations of arginine, isoleucine, tryptophan, and valine (range of *P* values, < 0.001 to 0.038) and significantly lower concentrations of aspartic acid (*P* = 0.019) than sexually intact males. Although there was a significant negative linear relationship between age and amino acid concentration for arginine (*P* = 0.019), glutamic acid (*P* = 0.035), and ornithine (*P* = 0.029), the linear correlation was weak (*R*² = 0.045, 0.037, and 0.040, respectively). As body weight increased, there was a significant (*P* = 0.032) positive linear relationship for plasma concentrations of histidine, whereas there was a significant (range of *P* values, < 0.001 to 0.032) negative relationship for concentrations of alanine, arginine, glutamic acid, glycine, hydroxyproline, isoleucine, lysine, ornithine, serine, and valine. Arginine, glutamic acid, glycine, hydroxyproline, isoleucine, lysine, ornithine, serine, and valine concentrations had a significant (range of *P* values, < 0.001 to 0.049) positive linear relationship with increases in BCS, whereas methionine concentrations had a significant (*P* = 0.007) negative relationship. The linear correlation was weak for concentrations of all amino acids for body weight (range of *R*², 0.039 to 0.123) and BCS (range of *R*², 0.033 to 0.139).

Fifty-four (39 dry, 13 canned, and 2 frozen-raw) diets were fed to the cats. Twelve diets were widely available at grocery and discount stores, 12 were veterinary prescription diets, and the other 30 were available from large pet supply chains or specialty stores. The 12 prescription diets included weight management diets (n = 6 diets), dental diets (2), renal diets (2), a urinary diet (1), and a gastrointestinal diet (1). Both renal diets were fed in addition to maintenance diets to healthy cats because other cats in the households had renal disease. The urinary diet was fed to a cat without recent or current clinical signs of urinary tract disease. The gastrointestinal diet had been prescribed to treat a cat with diarrhea 6 years prior to the study, and the owner opted to continue feeding it despite resolution of the problem.

University-owned cats were fed 6 diets; 10 cats were the most that were fed one of these diets. Pet cats were fed 48 diets; 13 cats were the most that were fed one of these diets. All the diets used in this study exceeded the NRC recommended allowance for dietary crude protein concentration for adult feline maintenance (5 g/100 kcal)[10] and had passed feeding trials or were formulated to meet Association of American Feed Control Officials minimum nutrient profiles for adult maintenance. All but 7 cats had been fed the same diet or combination of diets for at least 1 month before the study, with many cats being fed the same diet for 1 year or more prior to the study. All 7 cats with a more recent dietary change obtained most of their daily calories from the same diet for at least 1 week before blood samples were collected.

Twenty-three cats were fed specific amounts at each meal, 58 cats were fed ad libitum, and the remaining 39 cats were fed unknown or varying amounts, often because > 1 cat shared a feed bowl in multiple-cat households. Twenty-six of the 120 cats were fed > 1 diet, and many of the owners did not know the amount of each diet fed and sometimes did not know the diet or diets being fed at the time of sample collection. These 26 cats were excluded from analysis of the effects of diet on plasma amino acid and whole blood taurine concentrations.

None of the cats were fed supplemental products. Twenty-eight (23%) cats were fed treats, either commercial treats created for cats or treats in the form of foods consumed by humans. Of these 28 cats, only 9 were reportedly fed treats daily. The remaining 19 cats received treats from several times a week to once a month or less often. For 7 of the 9 cats that received treats daily, the treats did not provide a substantial (< 10%) portion of daily caloric intake. The other 2 cats that received treats daily obtained approximately 13% of their daily calories from treats. Because of the inconsistency of treat administration and the low percentage of daily calories provided by treats, this information was not included in the analysis to investigate potential associations between dietary protein and ingredients and plasma amino acid and whole blood taurine concentrations.

Data from the 94 cats eating only 1 diet were used to determine the relationship between diet and plasma amino acid concentrations. Of the 30 diets fed to this group of cats, 26 were dry expanded kibble, 2 were frozen-raw, and 2 were canned diets. Seven diets (6 dry and 1 canned) were veterinary prescription diets. Nineteen of the 94 cats received treats, with only 6 of the 19 cats receiving treats daily. Two cats obtained approximately 13% of their daily calories from commercial treats. Twenty-five of the 30 diets were supplemented with taurine, 14 were supplemented with methionine, 9 were supplemented with lysine, and 1 was supplemented with tryptophan.

Because of a lack of specific food intake information, protein intake could not be determined for most of the cats. Therefore, the protein content of the diet on a caloric basis (rather than the actual intake of each cat) was used for analysis. Dietary protein concentration ranged from 7.3 to 23.1 g/100 kcal (median, 9.32 g/kcal; mean ± SEM, 11.31 ± 5.19 g/kcal), with all but 3 diets containing between 7.3 and 12.0 g/100 kcal (the protein concentration for those 3 diets ranged from 18.6 to 23.1 g/100 kcal).

Protein concentration of the diet had a significant (range of *P* values, < 0.001 to 0.030) effect on amino acid concentrations for 15 amino acids. There were positive correlations between dietary protein concentration and plasma concentrations of arginine, glutamic acid, glycine, hydroxyproline, isoleucine, lysine, ornithine, serine, and valine, whereas plasma concentrations of aspartic acid, histidine, methionine, phenylalanine, proline, and taurine were negatively correlated with dietary protein concentration. However, none of these relationships had a strong linear correlation (range of *R*², 0.043 to 0.403). When only diets with ≤ 12 g of protein/100 kcal were examined, there were no significant

Table 1—Plasma amino acid and whole blood taurine concentrations in 120 adult cats eating commercially prepared diets.

Amino acid	95% reference interval (nmol/mL)	Median (nmol/mL)	Mean (nmol/mL)	SD	SEM	P value for test of normality
Alanine	270–925	425	462	160	15	< 0.001*
Arginine	46–200	84	95	38	3	< 0.001*
Asparagine	52–143	88	91	25	2	0.099
Aspartic acid	8–67	26	28	12	1	< 0.001*
Citrulline	9–30	17	18	6	1	0.031*
Cysteine†	12–42	24	26	9	1	< 0.001*
Glutamine	430–953	648	664	134	12	0.129
Glutamic acid	25–160	62	73	38	4	< 0.001*
Glycine	217–975	323	398	279	26	< 0.001*
Histidine	68–164	115	116	24	2	0.817
Hydroxyproline	21–145	60	63	31	3	< 0.001*
Isoleucine	30–141	55	63	29	3	< 0.001*
Leucine	78–278	135	146	49	5	< 0.001*
Lysine	44–282	89	108	61	6	< 0.001*
Methionine	20–128	61	64	28	3	0.003*
Ornithine	7–55	17	21	12	1	< 0.001*
Phenylalanine	38–103	71	70	15	1	0.615
Proline	104–423	248	258	76	7	0.572
Serine	92–413	159	179	85	8	< 0.001*
Taurine	37–252	108	118	55	5	< 0.001*
Whole blood taurine‡	275–701	455	457	103	11	0.154
Threonine	77–287	169	173	54	5	0.244
Tryptophan	30–104	57	60	17	2	0.003*
Tyrosine	31–86	56	57	15	1	0.236
Valine	85–302	148	164	62	6	< 0.001*

*Values of $P \leq 0.05$ were not compatible with a normal distribution; nonparametric methods based on percentiles were used to determine all 95% reference intervals. †Represents results for 96 cats. ‡Represents results for 89 cats.

associations between dietary protein concentration and plasma amino acid concentrations.

Supplementation of diets with taurine and methionine was significantly ($P = 0.007$ and $P = 0.015$, respectively) correlated with higher plasma concentrations of these amino acids. However, taurine supplementation was not significantly ($P = 0.360$) correlated with whole blood taurine concentrations, and lysine supplementation was not significantly ($P = 0.440$) correlated with plasma lysine concentrations. An analysis of plasma tryptophan concentrations was not conducted because only 1 diet was supplemented with tryptophan.

Seventy-four cats were fed diets that contained an animal-source product as the first ingredient listed. The remaining 20 cats were fed diets with a plant-source product as the first ingredient listed. The second ingredient listed was of plant origin in diets fed to 57 cats, whereas the second ingredient listed was of animal origin in diets fed to 37 cats. Twenty cats were fed diets that had animal products as the first 2 ingredients listed.

Regardless of the first 4 dietary ingredients listed, there were no significant associations between ingredients and plasma concentrations of aspartic acid, citrulline, cysteine, hydroxyproline, tryptophan, and tyrosine or whole blood concentrations of taurine. There were significant correlations with some amino acid concentrations and dietary ingredients, but they were not consistent throughout the ingredient list. For example, plasma concentrations of arginine, asparagine, glutamine, isoleucine, lysine, ornithine, proline, and valine were significantly (all P values, < 0.001) lower when chicken by-product meal was the first ingredient listed than when corn was the first ingredient listed. However, when chicken by-product meal was the second ingredient listed, plasma concentrations of histidine, leucine, lysine, ornithine, and proline were all significantly (all P values, < 0.001) higher than when the second ingredient listed was corn.

When the first ingredients listed were divided into plant-source versus animal-source products, there were significant (range of P values, < 0.001 to 0.031) differences in plasma concentrations of alanine, asparagine, aspartic acid, glutamine, histidine, leucine, methionine, phenylalanine, proline, taurine, threonine, and tyrosine. All of these amino acid concentrations were higher when the first ingredient listed was a plant-source product than when the first ingredient listed was an animal-source product. When the second ingredient listed was an animal-source product, as opposed to a plant-source product, plasma concentrations of alanine, arginine, glutamine, glutamic acid, glycine, hydroxyproline, isoleucine, leucine, lysine, ornithine, proline, serine, threonine, and valine were significantly (range of P values, < 0.001 to 0.023) higher.

Discussion

The objective of the study reported here was to develop reference ranges for plasma concentrations of amino acids and whole blood concentrations of taurine in healthy cats eating commercial diets and to determine the effect of age, sex, body weight, BCS, dietary protein, and dietary ingredients on these findings. A

power calculation determined that 120 animals were required to establish reliable reference intervals.[31] To our knowledge, we compiled data on amino acid concentrations from the largest population of adult cats eating commercial diets that has been reported to date (120 cats for all amino acids, except for whole blood taurine [89 cats] and cysteine [96 cats]).

This study population, although not an exact representation of the general cat population, is likely a close enough representation for these reference ranges to be meaningful. Twelve (10%) cats of the study population were purebred cats, which corresponded to the percentage of purebred cats in the general population in another report.[32] Unfortunately, these 12 cats did not represent a sufficient number of cats to assess differences in amino acid concentrations among breeds. The percentage of overweight and obese cats (BCS ≥ 6) in the study was 41% (49/120), which is higher than the 35% reported for the general cat population in another study.[33] The mean BCS was 5.6 for the cats in this study; only 18 of 120 (15%) cats had a BCS ≥ 7. It is possible that the subjective nature of assigning a BCS influences the proportion of overweight and obese cats reported by different sources.[34] Although some associations between body weight or BCS and plasma amino acid concentrations were significant, linear correlations were weak and no additional relationships could be discerned from the data. This finding was consistent with reported plasma and whole blood taurine concentrations in dogs[21] and plasma amino acid concentrations in adult cats.[11]

Castrated male cats outnumbered spayed female cats in a ratio of almost 2:1 in the study population, despite results of a recent survey[32] in which it was reported that female cats were kept as pets more often than are male cats. Because only pet cats with amiable dispositions were used in our study, it is possible that more female than male cats were adverse to procedures involved with collection of blood samples. Alternatively, this owner population may have specifically sought out male cats over female cats for other reasons. University-owned cats were included in the study population to introduce sexually intact cats into the study population because all the pet cats in the study population were spayed or neutered, whereas 87% of the overall pet cat population are spayed or neutered.[32] With the inclusion of the university-owned cats, 85 of 120 (71%) cats in the study population were spayed or neutered.

Significant differences among the 4 sex classifications were detected for concentrations of multiple amino acids. It was interesting that there were no significant differences between spayed females and neutered males, but there were differences between sexually intact males and females. These results suggest that hormonal differences between the sexes are likely affecting plasma amino acid concentrations. Studies in other species have also revealed an association between sex and plasma or serum amino acid concentrations. A study[35] in humans revealed that women have lower serum concentrations of proline, leucine, isoleucine, and tyrosine, compared with concentrations in men. In another study,[36] it was reported that elderly women have lower serum concentrations of essential amino acids, compared with concentrations in elderly men. A similar sex effect has been found in rats, with female rats having lower plasma concentrations of almost all amino acids than the concentrations in male rats.[37] It is unclear in these studies as to the factors responsible for the sex differences because each study examined a different population and not all populations involved hormonally active individuals. Further assessment of cats in all 4 sex classifications that are eating the same diet would be necessary to further clarify the true effect of sex and neuter status.

Although there was a significant relationship between age and amino acid concentration for several amino acids, the linear correlations were weak. In contrast, investigators in a study[6] of adult cats found an inverse relationship between plasma taurine concentration and age, but they did not examine all of the amino acids or the association with diet. However, that result was detected in a population of cats older than the population of cats in our study (mean ages, 8.3 and 8.9 years vs 4.4 years for the study reported here). Additionally, that study[6] was designed to assess plasma taurine concentrations in cats with heart disease, and it is possible that the correlation with age was more related to underlying disease than to age of the cats.

Only adult cats were used in the aforementioned study[6] and the study reported here. It is likely that actively growing kittens have plasma amino acid profiles that differ substantially from those of adult cats. Data from a study[13] in kittens indicated higher mean plasma concentrations of all amino acids, except citrulline, compared with the plasma concentration in our study population of adult cats. Investigators in another study[38] reported higher mean plasma concentrations for alanine, arginine, asparagine, cysteine, glutamic acid, glycine, histidine, lysine, methionine, ornithine, proline, threonine, and tryptophan, whereas the mean plasma concentrations of glutamine, isoleucine, phenylalanine, and tyrosine were lower, compared with the concentrations determined in our study. Mean concentrations of leucine and valine in that study[38] were similar to those in our study. However, an effect of diet cannot be ruled out because both of those previous studies[13,38] used animals fed purified diets. Because both of those studies used the same analytic methods as the study reported here, it is unlikely that this factor accounts for the differences observed.

Amino acid concentrations typically were lower for most amino acids in samples collected in heparinized syringes, compared with concentrations for samples collected in unheparinized syringes, which suggested that dilution of the samples may have been responsible for the difference. Because variable amounts of blood were collected from the cats, the effect of dilution was not consistent for all samples and could not be quantitatively tested. Although some of these concentration differences were significant, they were of small magnitude and unlikely to have a major clinical impact. It was unlikely to be a population bias contributing to these results because once heparin became unavailable, both cat populations (pet cats and university-owned cats) were affected equally. Amino acid concentrations were assayed in plasma rather than serum; thus, it was important that blood samples did not

clot prior to analysis. Therefore, the authors recommend the use of heparinized syringes to ensure that a plasma sample is obtained.

The effect of sample hemolysis on plasma amino acid concentrations in this study likely reflected the higher concentrations of amino acids in RBCs, compared with concentrations in plasma.[9] It is established that conditions that alter the concentration of cellular components in the blood affect plasma amino acid concentrations.[39] The finding that glutamic acid concentration was negatively correlated with hemolysis was unexpected because glutamic acid concentrations are higher in RBCs than in plasma.[9] It is possible that this result may have been artifactual. Because of the variability in plasma amino acid concentrations and the few moderately (6/94 [6%]) and severely (2/94 [2%]) hemolyzed samples, the effects of this sampling artifact could not be clearly defined. Additionally, the assessment of both hemolysis and lipemia in this study was subjective. Further investigation of the impact of hemolysis and lipemia via objective measurements of these changes (such as absorbance data from spectrophotometry) is needed.

Treats composed a small percentage of the daily caloric intake of the cats; thus, they were not included in the calculations of dietary protein or the ingredient comparisons. Twenty-three percent of the study cats received treats at least monthly. It has been reported[40] that approximately 26% of pet cats receive treats daily and 44% receive treats at least once per week.

It was impossible to calculate protein intake for most of the cats fed ad libitum or fed vague or varying amounts of food. In addition, many cats were fed > 1 diet. For these reasons, dietary protein concentration (rather than actual intake) was assessed, and only cats fed 1 diet were included in the analysis. Although the dietary protein concentration was examined on a caloric basis, it is still possible that cats may have had higher or lower energy requirements than expected and thus were consuming more or less protein than predicted. All of the diets exceeded NRC recommendations for feline maintenance of 5 g/100 kcal of protein, with protein concentrations ranging from 7.3 to 23.1 g/100 kcal. All diets, except for 3, provided protein concentrations of ≤ 12.0 g/100 kcal. Although dietary protein concentration was significantly correlated with plasma amino acid concentrations for 15 amino acids, these were weak linear correlations. When the data for the 3 diets containing the highest concentrations of protein (18.6 to 23.1 g/100 kcal) were excluded, the correlations were no longer significant.

The essential amino acid requirements for cats were originally determined with growth response curves as well as by comparing plasma amino acid concentrations obtained when feeding diets containing varying concentrations of the amino acid of interest.[10] In other species, it has been determined that plasma amino acid concentrations remain low until the requirement is met and then increase markedly.[41] Studies[42,e] have revealed that when lysine is provided in excess of dietary requirements in cats, plasma amino acid concentrations do not reliably predict the relative proportion of the excess. Data from these studies[41,e] and the study reported here suggest that diets providing amino acids and crude protein concentrations in excess of the recommended allowances for adult feline maintenance established by the NRC should not necessarily be expected to cause higher plasma amino acid concentrations than diets providing amino acids and crude protein concentrations closer to the minimal requirements. This effect may be especially relevant in cats consuming commercially available foods, compared with cats consuming purified diets.

The relationships of ingredients to amino acid concentrations were unexpected. In this study, amino acid concentrations, including many essential amino acids, were lower in cats consuming diets that contained animal protein as the first ingredient listed. Although plant proteins are poorer sources of taurine and other sulfur-containing amino acids, compared with many animal proteins, cats consuming diets containing a plant-source ingredient (usually a grain) as the first ingredient listed had higher mean taurine concentrations than cats consuming diets with an animal protein as the first ingredient listed. It is likely that other factors were involved because diets that contained higher proportions of protein from plant sources may be more consistently and aggressively supplemented with taurine. The concentrations of supplemental amino acids are not required to be reported on the label of feline diets, which makes it difficult to compare diets.

Taurine and methionine supplementation of diets was correlated with higher plasma concentrations of these amino acids. However, taurine supplementation was not correlated with whole blood taurine concentrations. This finding is not surprising because plasma taurine concentrations are thought to be more indicative of recent meals than are whole blood taurine concentrations.[26]

Lysine supplementation was not correlated with plasma lysine concentrations. The diets with added lysine may have been sufficiently limiting in this amino acid before supplementation such that additional lysine only brought them up to the concentrations in the unsupplemented diets. The lack of correlation between lysine supplementation and plasma lysine concentrations is likely to be a dietary factor rather than a biological factor unique to the cats eating the supplemented diets.

Whole blood taurine concentration is considered to be a more accurate measure of taurine status than is plasma taurine concentration[9] in cats and reflects skeletal muscle concentrations more accurately than do plasma taurine concentrations.[43] In the study reported here, 3 cats had plasma taurine concentrations that would be considered a risk factor for development of dilated cardiomyopathy (< 40 nmol/mL).[10] However, whole blood taurine concentrations in these cats were considered to be reflective of normal physiologic taurine status (> 200 nmol/mL).[10] It was later determined that 2 of the 3 cats had been placed under substantial caloric restriction (relative to calculated energy needs) by their owner in an attempt to maintain a lean body condition while feeding a diet designed for adult cats with normal energy requirements. Because nutrient requirements are established with the assumption of av-

erage energy needs, animals with lower than expected calorie requirements will consume less total nutrients from a diet designed to support a typical animal. This likely explains the low plasma taurine concentrations determined in these 2 cats. The findings in this study support the contention that whole blood taurine concentrations should always be assessed in addition to plasma taurine concentrations to determine true taurine status.

Alterations in plasma amino acids attributable to differences in dietary ingredients have been reported. Because of the association of dilated cardiomyopathy with taurine deficiency in dogs and cats, most studies have concentrated on this amino acid. The substitution of rice bran[44] and soy protein[29] for corn starch and casein in purified diets, respectively, can decrease plasma taurine concentrations in cats. In another study,[21] diets containing whole grain brown rice as the first plant ingredient listed were associated with lower whole blood taurine concentrations in dogs, compared with results for diets with ground corn as the first plant ingredient listed, whereas diets containing lamb meal and rice were associated with lower mean whole blood taurine concentrations than were diets containing other combinations of animal and plant ingredients. In the study reported here, we found no correlation between any of the first 4 ingredients listed and whole blood taurine or plasma cysteine concentrations.

In our study, we found that feeding taurine-supplemented diets resulted in significantly higher plasma taurine concentrations, independent of other dietary ingredients. However, dietary ingredients still had an impact on plasma taurine concentration. Plasma taurine concentrations were significantly higher when the first ingredient listed was corn instead of beef or chicken; all diets, except for 1, in which corn was the first ingredient listed were supplemented with taurine. However, plasma taurine concentrations were significantly lower when the second ingredient listed was rice or beef liver rather than chicken by-product meal, despite the fact that all of the diets, except for 1, with rice or beef liver as the second ingredient listed were supplemented with taurine. This discrepancy was likely attributable to the contribution of ingredient interactions and, possibly, other confounding factors such as variable amounts of supplemental taurine in the diets or variable caloric intake by the cats consuming them. Differences in associations between ingredients and amino acid concentrations between this study and the data reported for a study[21] in dogs may be attributable to differences in the metabolism of sulfur-containing amino acids in cats, compared with metabolism in dogs, or differences in study design. It is likely that diet and population factors also may explain the differences between the studies in cats that examined rice bran[44] and soy protein[29] and plasma taurine concentrations because those studies were performed under more controlled circumstances and used purified diets.

It is difficult to assess the ingredients in a commercial diet that provide the greatest proportions of nutrients because ingredients are listed by order of weight and can be further subdivided into constituent parts. Because of differences in moisture, a diet that has chicken as the first ingredient listed may provide less chicken protein than a diet that has chicken meal as the first ingredient listed. Similarly, an ingredient such as rice may contribute a substantial proportion of the nutrients to a diet despite being listed later in the ingredient list because this component is low in moisture and could appear as rice, brewer's rice, and rice flour in the same diet. Currently, manufacturers are not required to list the amounts of each ingredient in a diet, and this information is generally considered to be proprietary. In our study, the relationships between plasma amino acid concentrations and diet were difficult to interpret because of the number of diets, ingredients, and amino acids assessed. Many of the relationships were inconsistent with results reported in other studies or expected nutrient amounts of certain ingredients. Moreover, the results became more difficult to interpret as ingredients farther down the list (ingredients 3 through 7) were analyzed. This study illustrated the limitations of assessing the nutritional adequacy of diets by use of criteria such as the ingredient list. It is the authors' experience that diets are often assessed solely on the basis of ingredient lists, disregarding the legal definitions for ingredients as well as how ingredient lists are developed by manufacturers within the current regulatory framework.

The study reported here was the first large study to determine plasma amino acid concentrations in cats eating commercial diets. Despite some limitations, the plasma amino acid and whole blood taurine concentrations reported here are likely representative of the general pet cat population. This information will be of value in clinical assessment of patients and should encourage further research into alterations in amino acid concentrations in many disease processes and conditions in cats.

References

1. Hayes KC, Carey RE, Schmidt SY. Retinal degeneration associated with taurine deficiency in the cat. *Science* 1975;188:949–951.
2. Pion PD, Kittleson MD, Rogers QR, et al. Myocardial failure in cats associated with low plasma taurine: a reversible cardiomyopathy. *Science* 1987;237:764–768.
3. Strombeck DR, Rogers Q. Plasma amino acid concentrations in dogs with hepatic disease. *J Am Vet Med Assoc* 1978;173:93–96.
4. Albisser AM, Cheng DC, Yamasaki Y, et al. Changes in blood amino acids account for the insulin and glucagon responses to mixed meals in dogs. *Diabetes Res* 1985;2:49–55.
5. Hansen SH. The role of taurine in diabetes and the development of diabetic complications. *Diabetes Metab Res Rev* 2001;17:330–346.
6. McMichael MA, Freeman LM, Selhub J, et al. Plasma homocysteine, B vitamins, and amino acid concentrations in cats with cardiomyopathy and arterial thromboembolism. *J Vet Intern Med* 2000;14:507–512.
7. Borsheim E, Bui QU, Wolfe RR. Plasma amino acid concentra-

tions during late rehabilitation in patients with traumatic brain injury. *Arch Phys Med Rehabil* 2007;88:234–238.

8. Maggs DJ, Nasisse MP, Kass PH. Efficacy of oral supplementation with L-lysine in cats latently infected with feline herpesvirus. *Am J Vet Res* 2003;64:37–42.

9. Zicker SC, Rogers QR. Use of plasma amino acid concentrations in the diagnosis of nutritional and metabolic diseases in veterinary medicine, in *Proceedings*. IVth Cong Int Soc Anim Clin Biochem 1990;1–16.

10. National Research Council ad hoc Committee on Dog and Cat Nutrition. *Nutrient requirements of dogs and cats*. Washington, DC: National Academies Press, 2006.

11. Goldstein RE, Marks SL, Cowgill LD, et al. Plasma amino acid profiles in cats with naturally acquired chronic renal failure. *Am J Vet Res* 1999;60:109–113.

12. Strieker MJ, Morris JG, Rogers QR. Increasing dietary crude protein does not increase the essential amino acid requirements of kittens. *J Anim Physiol Anim Nutr (Berl)* 2006;90:344–353.

13. Rogers QR, Morris JG. Essentiality of amino acids for the growing kitten. *J Nutr* 1979;109:718–723.

14. Hargrove DM, Rogers QR, Calvert CC, et al. Effects of dietary excesses of the branched-chain amino acids on growth, food intake and plasma amino acid concentrations of kittens. *J Nutr* 1988;118:311–320.

15. Hammer VA, Rogers QR, Morris JG. Dietary crude protein increases slightly the requirement for threonine in kittens. *J Nutr* 1996;126:1496–1504.

16. Lewis AJ, Bayley HS. Amino acid bioavailability. In: Ammerman CB, Baker DH, Lewis AJ, eds. *Bioavailability of nutrients for animals: amino acids, minerals and vitamins*. San Diego: Academic Press Inc, 1995;35–52.

17. Morris JG, Rogers QR, O'Donnell JA. Lysine requirement of kittens given purified diets for maximal growth. *J Anim Physiol Anim Nutr (Berl)* 2004;88:113–116.

18. Rutherfurd SM, Rutherfurd-Markwick KJ, Moughan PJ. Available (ileal digestible reactive) lysine in selected pet foods. *J Agric Food Chem* 2007;55:3517–3522.

19. Rutherfurd SM, Moughan PJ. Determination of sulfur amino acids in foods as related to bioavailability. *J AOAC Int* 2008;91:907–913.

20. Hurrell RF, Finot PA. Food processing and storage as a determinant of protein and amino acid availability. *Experientia Suppl* 1983;44:135–156.

21. Delaney SJ, Kass PH, Rogers QR, et al. Plasma and whole blood taurine in normal dogs of varying size fed commercially prepared food. *J Anim Physiol Anim Nutr (Berl)* 2003;87:236–244.

22. Burns RA, LeFaivre MH, Milner JA. Effects of dietary protein quantity and quality on the growth of dogs and rats. *J Nutr* 1982;112:1843–1853.

23. Major EJ, Batterham ES. Availability of lysine in protein concentrates as determined by the slope-ratio assay with chicks and comparisons with rat, pig and chemical assays. *Br J Nutr* 1981;46:513–519.

24. Williams PA, Hodgkinson SM, Rutherfurd SM, et al. Lysine content in canine diets can be severely heat damaged. *J Nutr* 2006;136:1998S–2000S.

25. Ousterhout LE, Grau CR, Lundholm BD. Biological availability of amino acids in fish meals and other protein sources. *J Nutr* 1959;69:65–73.

26. Pion PD, Lewis J, Greene K, et al. Effect of meal-feeding and food deprivation on plasma and whole blood taurine concentrations in cats. *J Nutr* 1991;121:S177–S178.

27. Laflamme D. Development and validation of a body condition score system for cats: a clinical tool. *Feline Pract* 1997;25(5–6):13–18.

28. FDA Recall Information Web site. Baxter to proceed with recall of remaining heparin sodium vial products. Available at: www.fda.gov/oc/po/firmrecalls/baxter02_08.html. Accessed Aug 13, 2008.

29. Kim SW, Morris JG, Rogers QR. Dietary soybean protein decreases plasma taurine in cats. *J Nutr* 1995;125:2831–2837.

30. Torres CL, Miller JW, Rogers QR. Determination of free and total cyst(e)ine in plasma of dogs and cats. *Vet Clin Pathol* 2004;33:228–233.

31. Jennen-Steinmetz C, Wellek S. A new approach to sample size calculation for reference interval studies. *Stat Med* 2005;24:3199–3212.

32. American Pet Products Manufacturers Association. *2007–2008 APPMA National Pet Owners Survey*. Greenwich, Conn: American Pet Products Manufacturers Association Inc, 2008.

33. Lund EM, Armstrong PJ, Kirk CA, et al. Prevalence and risk factors for obesity in adult cats from private US veterinary practices. *Intl J Appl Res Vet Med* 2005;3:88–96.

34. Burkholder WJ. Use of body condition scores in clinical assessment of the provision of optimal nutrition. *J Am Vet Med Assoc* 2000;217:650–654.

35. Tomiya M, Fukushima T, Watanabe H, et al. Alterations in serum amino acid concentrations in male and female schizophrenic patients. *Clin Chim Acta* 2007;380:186–190.

36. Pitkanen HT, Oja SS, Kemppainen K, et al. Serum amino acid concentrations in aging men and women. *Amino Acids* 2003;24:413–421.

37. Chance WT, Grossman CJ, Newrock R, et al. Effects of electromagnetic fields and gender on neurotransmitters and amino acids in rats. *Physiol Behav* 1995;58:743–748.

38. Taylor TP, Morris JG, Willits NH, et al. Optimizing the pattern of essential amino acids as the sole source of dietary nitrogen supports near-maximal growth in kittens. *J Nutr* 1996;126:2243–2252.

39. Backus RC, Howard KA, Rogers QR, et al. Leukocytosis and thrombocytosis caused by consumption of a low magnesium and high calcium diet elevates whole-blood taurine concentration in cats. *J Nutr* 1998;128:2581S–2583S.

40. Laflamme DP, Abood SK, Fascetti AJ, et al. Pet feeding practices of dog and cat owners in the United States and Australia. *J Am Vet Med Assoc* 2008;232:687–694.

41. Zimmerman RA, Scott HM. Interrelationship of plasma amino acid levels and weight gain in the chick as influenced by suboptimal and superoptimal dietary concentrations of single amino acids. *J Nutr* 1965;87:13–18.

42. Fascetti AJ, Maggs DJ, Kanchuk ML, et al. Excess dietary lysine does not cause lysine-arginine antagonism in adult cats. *J Nutr* 2004;134:2042S–2045S.

43. Pacioretty L, Hickman MA, Morris JG, et al. Kinetics of taurine depletion and repletion in plasma, serum, whole blood and skeletal muscle in cats. *Amino Acids* 2001;21:417–427.

44. Stratton-Phelps M, Backus RC, Rogers QR, et al. Dietary rice bran decreases plasma and whole-blood taurine in cats. *J Nutr* 2002;132:1745S–1747S.