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Kinetics of taurine depletion and repletion in plasma, serum, whole blood and skeletal muscle in cats*

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Summary. The relationship between taurine concentrations of plasma, whole blood, serum and skeletal muscle during taurine depletion and repletion was investigated in cats, to identify the most useful indicators of taurine status. Sixteen cats were fed a purified diet containing either 0 or 0.15 g/kg taurine for 5 months. Treatments were then reversed and the taurine concentration was measured during repletion and depletion phases. Plasma taurine exhibited the fastest rate (slow component) of depletion ($t_{1/2}$ = 4.8 wk), followed by serum (5.3 wk), whole blood (6.2 wk), and skeletal muscle (11.2 wk). Whole blood taurine was the first to replete at a rate of 0.74 wk to $^1/_2$ maximal repletion, followed by serum (2.1 wk), skeletal muscle (3.5 wk), and plasma (3.5 wk). Whole blood more closely reflected skeletal muscle taurine concentrations than plasma during depletion, while plasma taurine concentrations appear to be the most valuable predictor of skeletal muscle taurine concentrations during repletion. This study suggests that the best clinical method to evaluate the taurine status of the cat is the determination and interpretation of both plasma and whole blood taurine concentrations.

Keywords: Amino acids – Taurine – Taurine depletion – Taurine repletion – Cats – Feline

Introduction

Taurine deficiency is associated with a number of specific pathological conditions in cats, including feline central retinal degeneration (Hayes et al., 1975;

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Schmidt et al., 1976), reproductive failure in queens with associated congenital abnormalities in kittens (Sturman et al., 1986; Sturman and Messing, 1991) and dilated cardiomyopathy (Pion et al., 1987). Studies in normal humans have shown that plasma taurine concentration is subject to wide variation (Trautwein and Hayes, 1990; Vinton et al., 1986) and that sampling technique can have a marked affect on resulting values (Laidlaw et al., 1987; Trautwein and Hayes, 1990). Sampling discrepancies occur from contamination of plasma with taurine from intracellular blood sources, including platelets or granulocytes, which can have 300–400 fold greater taurine concentrations. In addition, the relatively small plasma taurine pool rapidly exchanges with the larger tissue taurine pools, which can lead to marked fluctuations in plasma taurine concentration (Sturman et al., 1975; Matsubara et al., 1985). Whole blood, an easily obtainable tissue to sample, has been investigated as an index of taurine status (Laidlaw et al., 1987; Trautwein and Hayes, 1990) and may prove to be a superior indicator of the taurine status of an animal than plasma taurine. However, the correlation between plasma, whole blood and other tissue taurine pools has not been thoroughly investigated.

Kinetic studies examining taurine metabolism and turnover in humans (Sturman et al., 1975), rats (Sturman, 1973), and the rhesus monkey (Matsubara et al., 1985), demonstrate the existence of at least two pools of whole body taurine, a relatively small pool that has a rapid turnover rate and a much larger pool with a slow turnover rate. Tissues that comprise the rapidly exchanging pool include the liver, kidney, intestine, spleen and lung, while the slower pool is comprised of brain, heart and skeletal muscle. The differences in taurine kinetics between tissues complicate the determination of whole body taurine status in an individual animal. This study was conducted to determine the relationship between taurine concentrations of plasma, whole blood, serum and skeletal muscle during taurine depletion and repletion in cats to identify the most useful indicators of taurine status. Previous studies have measured the concentrations of taurine in plasma and various tissues of taurine depleted kittens (Sturman et al., 1978), but no studies have investigated the time-course of taurine concentration changes during depletion and none have evaluated changes during taurine repletion. Preliminary studies in our laboratory (unpublished) suggest that taurine kinetics during repletion and depletion are not the same in all taurine pools. Because cats may be fed diets varying widely in taurine content, an individual animal may be undergoing taurine depletion or repletion at the time taurine status is evaluated. This study examined pools of taurine that may be clinically assayed with the objective of defining the most appropriate indicator of taurine status for an individual cat.

Materials and methods

Animals

Sixteen, 9-wk old cats (1,050–1,180 g), from the Nutrition and Pet Care Center, University of California at Davis, were used as experimental animals. Cats were housed in individual

stainless steel cages with food and water available at all times, in a light-controlled (14 h light: 10 h dark) room at 21 ± 3°C. Care throughout the study was in compliance with the "Guide for The Use and Care of Laboratory Animals" developed by the Institute of Laboratory Animal Resources of the National Research Council. The experimental protocol was approved by the University of California at Davis, Animal Use and Care Administrative Advisory Committee, utilizing the guidelines provided by the American Association for Laboratory Animal Care.

Diets

Cats were fed two purified diets that were identical except for the taurine concentrations, 1,500 or 0 mg taurine/kg (Table 1). In the 0 mg taurine/kg diet, taurine was replaced by an equal weight of cornstarch. Food intake was measured daily.

Design

Cats were randomly assigned to two groups, with equal representation of males and females, and fed either the 1,500 or 0 mg taurine/kg purified diet for 5 mo. The dietary treatments were then reversed and the cats then fed the taurine-free diet were designated the depletion group and those fed the 1,500 mg taurine/kg diet were designated the repletion group. Changes in the concentration of taurine in plasma, whole blood, serum and skeletal muscle were then measured for 25 wk. Prior to the dietary change, 2 samples were taken from each cat at 4 day intervals with subsequent samples taken on d 1, 3, 6 and 13, and wk 5, 11, 17 and 25. Lean body mass was estimated at wk 24 by whole body counting of ^{40}K as previously described (Peacock et al., 1987).

Blood samples

Food was withheld from cats for 12 h and then 3 mL blood samples were collected by jugular puncture in a heparin-coated plastic syringe and in a non-heparinized plastic syringe. Each heparinized blood sample was divided into 2 sub-samples, one was centrifuged at room temperature (15 min at 3,900 × g), and the other remained as whole blood.

Table 1. Composition of diets

Dietary component	
	g/kg
High nitrogen casein[1]	340
Corn oil[2]	250
Cornstarch[3]	195
Glucose monohydrate[4]	150
Mineral Mix[5]	50
Vitamin Mix[6]	10
Choline chloride	3
Taurine	1.5

[1] U.S. Biochemical Corp., Cleveland, OH. [2] Mazola, CPC International Inc., Englewood Cliffs, NJ. [3] Melojel, Bridgewater, NJ. [4] International Corp., Englewood Cliffs, NJ. [5,6] For composition see Williams et al., 1987.

The non-heparinized sample remained at room temperature for 1 h to allow clot formation (in plastic tubes) and was then centrifuged (20 min at 3,900 × g) to separate the serum. All samples were stored at −20°C until analysis.

Muscle samples

Skeletal muscle samples were obtained under light anesthesia. The average length of anesthesia prior to sample collection was 5 minutes, a length of time unlikely to cause changes in taurine pools. Cats were pre-medicated with 0.5 mg acetylpromazine (Acepromazine, Tech America, Elwood, Kansas) and 0.12 mg atropine (Elkins-Sinn, Inc., Cherry Hill, New Jersey), subcutaneously, and anesthetized with 40 mg of intravenous ketamine hydrochloride (Vetalar, Parke-Davis, Morris Plains, New Jersey). Using aseptic technique, a 5 to 24 mg (mean 11.4 mg) sample of semitendinosus muscle was obtained percutaneously using a biopsy needle (Tru-Cut, Travenol Inc., Deerfield, IL). Biopsies were taken alternately from the left and right muscles and the sampling site located approximately 5 mm proximally with each sampling to avoid previous biopsy sites.

Sample analysis

Taurine concentration was determined in all samples by amino acid analysis (Beckman models 121MB or 7300, Fullerton, CA) after deproteinization with an equal volume of aqueous sulfosalicylic acid (60 g/L). Before deproteinization, whole blood was lysed by freezing and thawing twice and an equal volume of distilled water was mixed with each sample resulting in a 1:1 dilution. Muscle samples were weighed and diluted with 400–750 μL of distilled water, depending on sample size, and homogenized with a glass homogenizer (Duall 20, Kontes, Vineland, New Jersey). Following homogenization, each sample was sonicated for three seconds (Biosonik IV sonicator, Bronwill Scientific) to ensure total cell disruption. Samples were then deproteinized by the same procedure as for plasma, serum and whole blood.

Statistical analysis

Differences in weekly body weight and food intake over time were assessed using a repeated measures analysis of variance for unbalanced data (SAS – General Linear Model, Version 6, SAS Institute, Cary, NC). Taurine depletion data were analyzed using a least squares curve fitting and decision making program (Brown and Manno, 1978). A nonlinear least squares analysis was used to assess taurine repletion kinetics (IMTEC, 1983, Bowie, Maryland). In order to assess the relationship of taurine concentrations in whole blood, plasma and serum to muscle, a simple linear regression was performed with muscle taurine concentration as the dependent variable. Correlation coefficients were used as an index of association for each of the three independent variables with muscle taurine. The statistical procedures used to determine linear regressions were as described (Snedecor and Cochran, 1980). Values in the text are means ± SEM, n = 8. Differences between means were considered statistically significant at $p < 0.05$.

Results

The mean body weight of cats in the repletion group increased from 2.5 to 2.9 kg, while the mean body weight of cats in the depletion group remained unchanged at 2.7 kg. Maximum body weight was attained at 17 weeks for the repletion group. Despite the small difference between groups in mean body

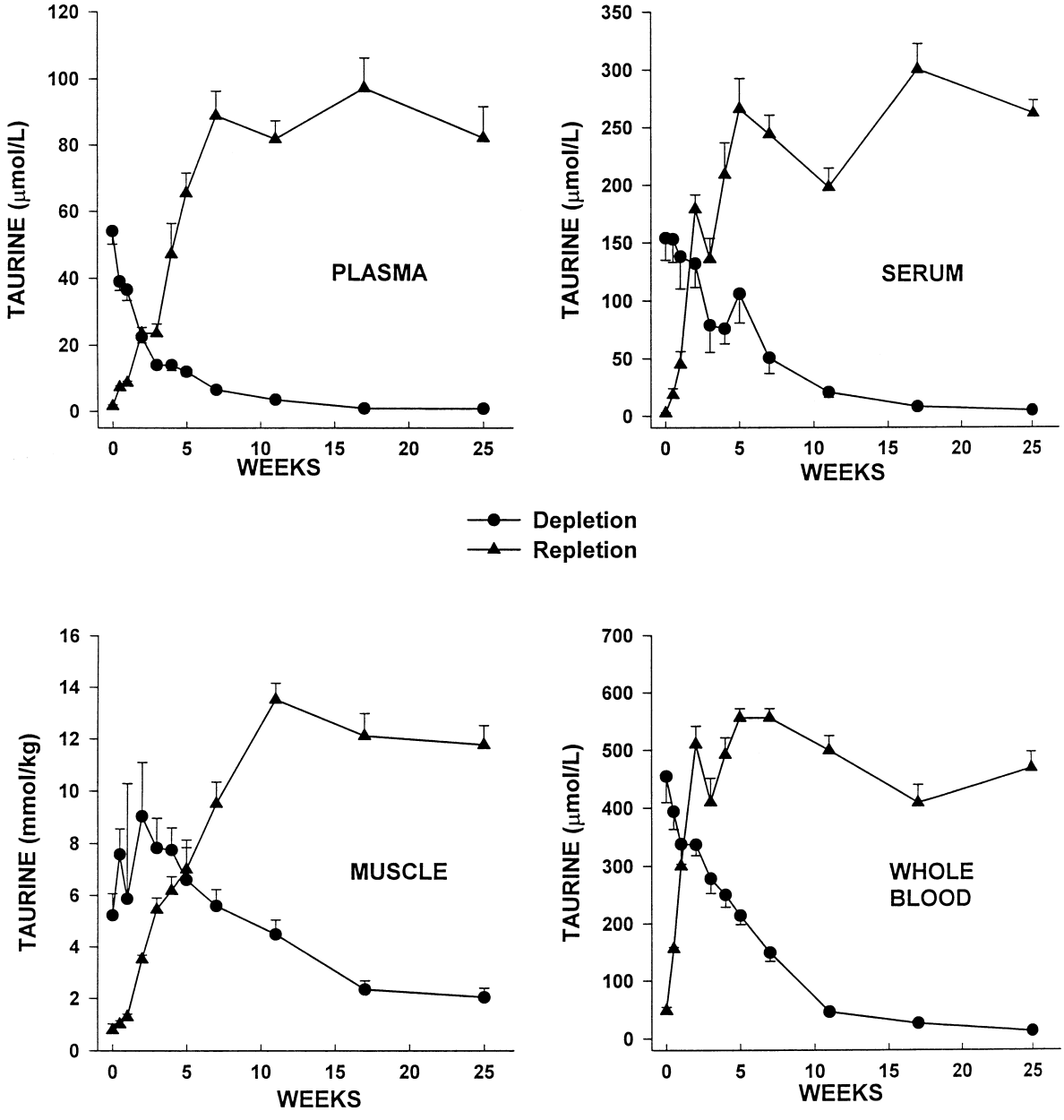

Fig. 1. Time course of taurine concentrations in plasma, serum, whole blood and skeletal muscle of cats during taurine depletion or taurine repletion. Values are means ± SEM of 8 cats

weight, there was no difference in lean body mass as determined by percentage body potassium (taurine-repleted cats, 0.19 ± 0.01% compared to 0.18 ± 0.005% for the taurine-depleted cats). Food intakes were not different between the two groups following the dietary switch, nor did food intake change over the course of the experiment.

Kinetics of taurine depletion in plasma, serum and whole blood exhibited two components (Fig. 1). The rapid component of plasma taurine depletion had a half-life of 0.76 wk, depleting at a rate of 1.26 μmol/L/wk from an initial

Table 2. Taurine concentration, depletion rate and half-life in plasma, serum, whole blood and skeletal muscle of kittens[1]

Tissue	Initial taurine	Rapid component		Slow component	
		Depletion rate	$t_{1/2}$	Depletion rate	$t_{1/2}$
	μmol/L, *μmol/kg (wet weight)	μmol/L/wk	wk	μmol/L/wk	wk
Plasma	59.3 ± 4.6[b]	1.26 ± 0.31	0.876 ± 0.15[a]	0.15 ± 0.01[b]	4.77 ± 0.43[a]
Serum	172 ± 18[c]	1.11 ± 0.60	1.98 ± 0.41[ab]	0.13 ± 0.01[b]	5.3 ± 0.41[a]
Whole blood	477 ± 84[d]	1.53 ± 1.18	2.97 ± 0.69[b]	0.12 ± 0.01[b]	6.16 ± 0.58[a]
Skeletal muscle*	9,800 ± 1,300[a]			73 ± 10[a]	11.2 ± 1.78[b]

[1] All values are mean ± SEM, n = 8. Means within each column not sharing a common superscript letter are significantly different at $p < 0.05$.

concentration of 59.3 μmol/L (Table 2). The rapid components of serum and whole blood taurine depletion had half-lives of 1.98 and 2.97 wk, respectively and the rates of depletion for these two pools were not different from plasma. Although the rates (μmol/L/wk) of taurine depletion did not differ, the half-life of the rapid component of plasma depletion was less than the half-life observed in whole blood taurine. No significant difference was observed between the half-life of the rapid component of plasma and serum, or serum and whole blood ($p > 0.05$).

Skeletal muscle taurine concentration exhibited only one component of depletion and actually appeared to increase early in the depletion period (Fig. 1). The rate of depletion and the half-life of skeletal muscle taurine was, therefore, determined from the last five samples, beginning 5 wk into the depletion period. The mean half-life for this period was 11.2 wk, with a depletion rate of 73 μmol/wk/kg (wet weight) from an initial concentration of 9,800 μmol/kg wet weight. During depletion the quantity of taurine lost from the muscle pool was greater than the other pools, because of the total size of the muscle pool. However, the half-life of the muscle pool was longer than the half-lives of the slow components of taurine depletion of the plasma, serum and whole blood pools.

The time for plasma taurine to replete to half-maximal concentration was 3.72 wk (Table 3), which was longer than the time for both serum and whole blood to reach half-maximal concentrations. The time to achieve half maximal repletion in skeletal muscle (3.49 wk), was similar to plasma (3.72 wk), but the maximum taurine concentration was much higher, 12,600 μmol/kg wet weight in muscle compared to 96.4 μmol/L in plasma. In addition, muscle size has several fold the volume of plasma.

The depletion characteristics of skeletal muscle (beginning 2 weeks into the depletion period) were significantly correlated with those of whole blood ($r = 0.95$), serum ($r = 0.88$) and plasma ($r = 0.91$) as illustrated in Fig. 2. While the depletion characteristics of skeletal muscle were highly correlated with those of whole blood, serum and plasma, the repletion characteristics of skeletal muscle most closely resembled plasma ($r = 0.94$). The relationship between the repletion of serum and skeletal muscle taurine, and between

Table 3. Taurine repletion variables in plasma, serum, whole blood and skeletal muscle in kittens[1]

Tissue	Estimated maximal taurine concentration	Repletion rate	Time of half maximal repletion
	μmol/L, *μmol/kg wet wt	μmol/L/wk	wk
Plasma	96.4 ± 10.2[b]	0.39 ± 0.10[a]	3.72 ± 0.2[c]
Serum	273 ± 9.9[c]	0.70 ± 0.08[b]	2.06 ± 0.1[b]
Whole blood	512 ± 21[d]	1.95 ± 0.06[c]	0.74 ± 0.1[a]
Skeletal muscle*	12,560 ± 800[a]	410 ± 10[a]	3.49 ± 0.2[c]

[1] All values of mean ± SEM, n = 8. Means in a column not sharing a common superscript letter are significantly different at $p < 0.05$.

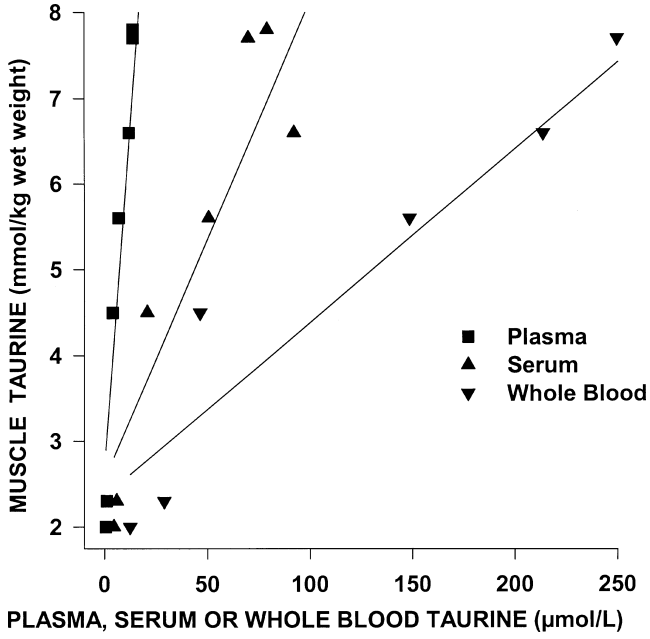

Fig. 2. Relationship between taurine concentrations of plasma ($r = 0.91$, $P < 0.001$), serum ($r = 0.88$, $P < 0.01$), whole blood ($r = 0.95$, $P < 0.001$) and taurine concentration of skeletal muscle during taurine depletion in cats. Each point represents mean data from 8 cats at each time point during depletion (from 2–25 weeks)

whole blood and skeletal muscle taurine were not as highly correlated, $r = 0.80$ and $r = 0.59$, respectively (Fig. 3). The low correlation between whole blood and skeletal muscle appears to be due to the rapid repletion of whole blood taurine compared to skeletal muscle.

Discussion

The major objective of this study was to compare the rates of taurine depletion and repletion and correlate the taurine concentrations of plasma, serum,

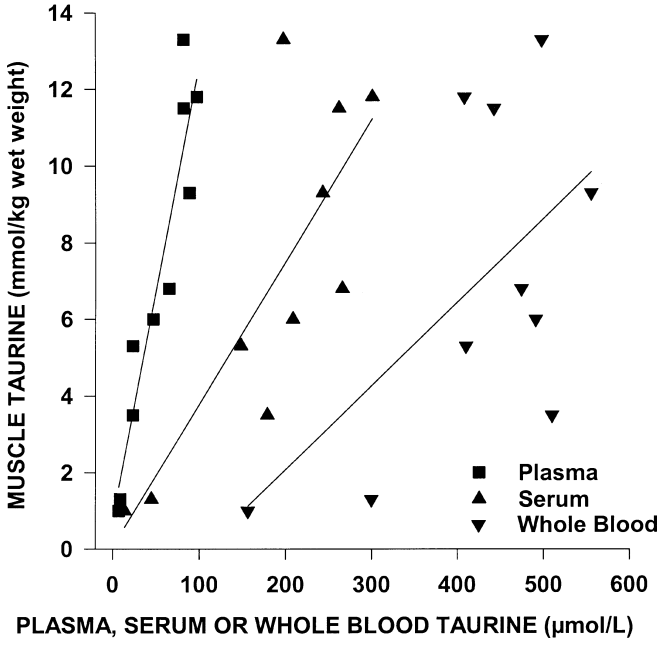

Fig. 3. Relationship between taurine concentrations of plasma ($r = 0.94$, $P < 0.001$), serum ($r = 0.80$, $P < 0.01$), whole blood ($r = 0.59$, $P < 0.1$) and taurine concentration of skeletal muscle during taurine repletion in cats. Each point represents mean data from 8 cats at each time point during repletion (from 0–25 weeks)

whole blood and skeletal muscle in order to identify the most useful clinical index of taurine status. The kinetics of depletion appear to be similar for plasma, serum and whole blood taurine concentrations with a more rapid decrease initially, followed by a slower phase of decline. In contrast, skeletal muscle taurine concentrations increased for the first 5 weeks of depletion and then demonstrated only a slow decrease. This is consistent with the findings of Sturman et al. (1978), who reported a greater half-life of taurine in feline gastrocnemius muscle than plasma, 10 d compared to 2.5 d, respectively. They also found that during taurine depletion, the half-life of taurine in gastrocnemius muscle remained unchanged while that of plasma increased to 10 d, similar to the half-life observed in gastrocnemius muscle.

A change in plasma half-life during depletion could explain the initial rapid and slow terminal components of plasma taurine depletion observed in this study, with the slow component of plasma taurine depletion reflecting the increased $t_{1/2}$ of taurine. It is not known if the half-life of taurine in serum and whole blood changes during depletion, however, both of these fluids exhibit two components of taurine depletion similar to plasma.

The physiological role of taurine in skeletal muscle is unknown, but undoubtedly muscle taurine serves as a large reserve pool and supplies taurine to other tissues during depletion. Skeletal muscle might also be expected to replete at a slower rate, after full repletion of tissues having a higher affinity for taurine. This pattern of repletion was observed in skeletal muscle, with

rates of repletion significantly lower than whole blood or serum. The physiological make-up of the semitendinosus muscle may also influence the results due to fiber type and innervation (Iwata et al., 1986). "Normal" taurine concentrations in the semitendinosus muscle as determined in this study are consistent with the taurine concentrations in gastrocnemius muscle reported in previous feline studies (Knopf et al., 1978; Sturman et al., 1978, 1986; Sturman and Messing, 1991).

Repletion characteristics of plasma taurine were similar to that of skeletal muscle and suggest that plasma may also replete only after taurine provision to other tissues. In contrast, whole blood repleted rapidly compared to plasma and skeletal muscle and depleted more slowly than plasma. This likely reflects differences in active transport systems for taurine (Ahtee et al., 1974; Jacobson et al., 1986) and may be related to the important functions of taurine demonstrated in blood cells such as leukocytes and platelets (Hayes et al., 1989; Schuller-Levis et al., 1990). Changes in whole blood taurine during repletion and depletion reflect changes in the taurine content of leukocytes and platelets and not in erythrocytes.

Serum taurine concentrations were more closely correlated with whole blood taurine concentrations during depletion than plasma, but serum may be of questionable clinical value because of the variation in the time of clotting and method of separation of serum at various times and in various laboratories. Taurine is extruded from platelets during platelet aggregation and a prolonged clotting time may allow for a greater extrusion by the platelets. The rate of clot formation is dependent on many factors including temperature and type of tube used, variables which were controlled in this study. In previous studies, the variability observed in serum taurine concentrations was greater than that in plasma taurine concentration (unpublished observations). Because of these limitations and since serum is not a true compartment, we do not recommend the use of serum taurine concentration as an index of the taurine status.

It can be concluded from the comparison of depletion and repletion rates that whole blood taurine concentrations more closely reflects skeletal muscle taurine concentrations than plasma during depletion. However, plasma taurine concentrations appear to be the better predictor of skeletal muscle taurine concentrations during repletion. These findings suggest that the cytoplasmic membranes of muscle, leukocytes and platelets have the ability to retain taurine against a concentration gradient in plasma, a common feature of many cells containing active transport systems. However, the mechanism whereby different tissues sense and maintain appropriate intracellular taurine concentrations is unclear. The differential ability of tissues to extract taurine from plasma and to retain taurine against a concentration gradient during depletion may be related to the affinities of the membrane transporters for taurine, differences in number of transporters or differential rates of passive diffusion of taurine out of cells.

The concentration of taurine in soft tissues, whole blood and organs is greater than that of plasma, and their depletion and repletion kinetics differ. These differences make it difficult to evaluate, using a single clinical sample,

the taurine status of the cat. However, this study suggests that the best clinical method to evaluate taurine status may be the determination and interpretation of both plasma and whole blood taurine concentrations. When cats are undergoing active repletion or depletion, a single sample may give misleading results, either overestimating or underestimating body taurine. Therefore, we recommend that clinicians submit both plasma and whole blood taurine samples for analysis to evaluate taurine status. In cases where either plasma or whole blood values are not normal, serial samples should be monitored to ensure that cats are maintaining adequate taurine status. Until both plasma and whole blood taurine concentrations are normalized, cats should be considered at risk for taurine deficiency related diseases.

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