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Impact of blood tube additives and timing of sampling on blood taurine concentrations in clinically healthy dogs

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KEYWORDS
Amino acids;
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Cardiomyopathy;
Canine;
Heart

Abstract *Introduction:* Dilated cardiomyopathy can be associated with taurine deficiency in dogs. Blood taurine concentrations can be analyzed in whole blood (WB) and plasma. The study objectives were to investigate agreement between taurine concentrations measured in WB, heparin plasma, and EDTA plasma, determine intraindividual variation in healthy dogs, and evaluate if time from feeding to sampling impacts concentrations.
Animals: Ten English Cocker spaniels and 10 dogs of various breeds.
Materials and methods: Dogs were fasted 12 h prior to initial blood sampling, and the blood was collected at five occasions over eight h. Food was offered immediately after first and one h after fourth sampling time point.
Results: Agreement between taurine concentrations in EDTA plasma and heparinized plasma was good (mean difference 4.5 nmol/mL, 95% confidence interval (CI) 36.8—45.8 nmol/mL). Whole blood concentrations were systematically higher than EDTA and heparin plasma concentrations (mean difference 132.7 nmol/mL, 95% CI 23.6—241.8 nmol/mL, and 127.6 nmol/mL, 95% CI 28.6—226.6 nmol/mL,

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respectively, all $P < 0.001$). Intraindividual daily variations in taurine concentration were seen in all additives, with largest variations in plasma ($P < 0.001$). Taurine concentration in heparinized plasma was higher at first and fifth sampling time points compared to the fourth ($P = 0.014$).
Discussion: Agreement was found between taurine concentrations measured in different additives, with expected higher concentration in WB than plasma. Taurine concentrations measured in heparinized plasma varied with sampling time point. Intraindividual daily variations were observed in all additives, but mainly in plasma samples.
Conclusion: Taurine concentrations in dogs with suspected deficiency should be interpreted with caution.

Abbreviations

BW	body weight
CI	confidence interval
CV	coefficient of variation
DCM	dilated cardiomyopathy
ECG	electrocardiogram
ECS	English cocker spaniel
IQR	interquartile ranges
WB	whole blood

Introduction

Taurine (two-aminoethanesulfonic acid) is the most abundant of the free amino acids in the body, and can be acquired from food or endogenously synthesized from dietary methionine and cysteine. Under normal dietary conditions, most mammals can synthesize taurine in sufficient quantities to meet their metabolic needs [1,2]. However, certain species, predominantly carnivores but also omnivores, may develop taurine deficiency if taurine is not provided in adequate amounts in the diet [1,3–6].

Chronic taurine deficiency is associated with alterations in several physiological processes, including myocardial and retinal function, development of the central nervous system, osmoregulation, immune response, conjugation of bile acids, and reproductive performance [1–3,7,8]. Development of a dilated cardiomyopathy (DCM) phenotype has been associated with low blood taurine concentrations in some dog breeds. Breeds reported to be predisposed to taurine deficiency-induced DCM are American cocker spaniels, golden retrievers, Dalmatians, Portuguese water dogs, and Newfoundland dogs [6,9–14].

English cocker spaniels (ECS) are known to be predisposed to primary and idiopathic DCM [15], but dogs of this breed have historically not been considered predisposed to the development of taurine deficiency. However, our clinical experience is that not only American cocker spaniels but also ECS are affected by taurine deficiency-induced DCM. Furthermore, in a recently published retrospective study, 13 of the 16 ECS (81%) presenting with a DCM-phenotype were found to have taurine concentrations below normal reference values [16]. In addition, the United States Food and Drug Administration is investigating whether taurine may have a role in the increase of nutritionally-induced DCM documented in dogs in recent years [17–19].

Taurine concentrations should ideally be analyzed in all dogs presenting with a DCM-phenotype [12,20]. Blood concentrations can be analyzed in whole blood (WB) and in plasma (EDTA or heparin). The analyses require sensitive and accurate methodologies [12,21–25]. Concurrent analyses of taurine concentrations in both plasma and WB are recommended to provide an accurate estimation of a patient's taurine status [12,20–22]. Whole blood taurine concentrations have been reported to be less variable than plasma concentrations, and to better reflect intracellular taurine concentrations [21,24]. Plasma, on the other hand, serves as a reservoir for cells with high affinity for taurine, and might, therefore, better reflect recent changes in taurine availability and requirements [21,24]. A recent study investigating differences in taurine concentrations in WB, plasma, and skeletal muscles in dogs-fed diets with different amounts of taurine precursors (methionine and cysteine) showed that plasma taurine concentrations might be a better indicator of muscle cell depletion than WB concentrations [26].

In many parts of the world, commercial veterinary laboratories are not offering analyses of taurine concentrations in WB. The knowledge of factors that might affect measured taurine concentrations has become important because many clinicians are left with the option to assess taurine concentrations solely in plasma. Both feeding statuses, as well as amount and quality of dietary protein, have been shown to affect plasma taurine concentrations in cats [27]. The association between feeding regimen and taurine concentrations in dogs appears to be more complex and is under investigation [28—30].

The objectives of the present study were to investigate: (1) agreement between taurine concentrations measured in heparinized WB, heparinized plasma, and EDTA plasma; (2) the intraindividual variation in concentrations; and (3) if time from feeding to sampling impacts taurine concentrations in a group of clinically healthy dogs.

Materials and methods

The study, which was approved by the Ethical Committee for Animal Welfare in Stockholm, Sweden (5.8.18—01548/2017), was performed at Anicura Albano animal hospital in Stockholm, Sweden. Written owner consent was obtained before inclusion.

Inclusion and exclusion criteria

The study was predetermined to include 10 healthy ECS and 10 healthy dogs of various breeds. Dogs were recruited via breeders or breeding clubs and from staff working at the investigating hospital. To be eligible, dogs had to have a body weight (BW) $\geq$5 kg, be $\geq$one year of age (no upper limit) and determined healthy based upon history, physical examination, systemic arterial blood pressure measurements, electrocardiographic (ECG) recording, echocardiographic examination, and blood analyses including complete blood count and serum biochemistry profile. Exclusion criteria included systemic or organ-related disease, or echocardiographic findings indicating congenital or acquired non-DCM related heart disease (trivial mitral and tricuspid insufficiencies were accepted). Dogs were also excluded if they had received any medications or taurine supplementation.

Diets

Diets were not standardized, and dogs were fed their regular type and amount of commercial dog food. The diets were then classified based on whether they were grain-free or grain-inclusive, and/or based on traditional (beef, pork, lamb, chicken, and fish) or non-traditional protein sources.

Schedule of events to be included in the study

Written consent and a detailed questionnaire, including information about the dog's health status and diet(s), were obtained from all the owners. At admission in the morning and after 12 h of fasting, all dogs underwent noninvasive blood pressure measurements, a complete physical examination, blood analyses (complete blood count, serum biochemistry, and baseline plasma taurine concentrations), echocardiographic examination, and an ECG recording.

Blood pressure measurements

Measurements were made using high-definition oscillometry[d], and followed a standardized protocol according to published guidelines [31]. Dogs were allowed 10 min of acclimatization prior to measurements. The cuff was placed on the tail, and blood pressure was measured until values reached a plateau, and an average was obtained from the last five consecutive measurements.

Echocardiography

All echocardiographic examinations were performed and assessed by one of two board-certified specialists in cardiology (IL and AT). Dogs were unsedated and gently restrained in right and then left recumbency during the examination. Two-dimensional examinations were performed under simultaneous ECG monitoring, with an ultrasound unit[e] using 5.0—8.5 MHz phased-array transducers. All echocardiographic measurements were made on three consecutive cardiac cycles, and a mean value was calculated for statistical analysis.

Measurements of the left atrial to aortic root ratio were performed on a right parasternal short-axis view, as previously described [32]. Left ventricular dimensions were measured using both two dimensional images and M-mode and obtained from right-sided parasternal short-axis views according to published guidelines [33,34]. Left ventricular internal dimensions at end-diastole

[d] Vet HDO Monitor S + B medVET GmbH, Babenhausen, Germany.
[e] EPIQ 7G; Philips Ultrasound, Bothell, WA, US.

and end-systole were normalized for BW using the Cornell formula [35]. Measurements of the left ventricular fractional shortening and E-point to septal separation were made on a right parasternal short-axis view [33,34]. Aortic, pulmonic, and mitral-inflow velocities were assessed using color and spectral doppler echocardiographic techniques.

Electrocardiogram

A 3-min standard six lead ECG recording[f] was obtained with the dog gently restrained in the right lateral recumbency. Recordings were interpreted by one of two board-certified specialists in cardiology (IL and AT).

Blood analyses

All blood samples were collected by cephalic venipuncture using a butterfly needle with Luer adapter (21 G), collecting blood directly into vacutainer tubes[g]. Each heparin and EDTA tube was carefully turned five times after collection.

Routine hematology and blood biochemistry

Four mL blood (3 mL serum and 1 mL EDTA) were collected and transported to the in-house laboratory and analyzed within one hour. Analyses included complete blood count and serum biochemistry profiles (creatinine, blood urea nitrogen, phosphate, alanine aminotransferase, alkaline phosphatase, bile acids, potassium, sodium, calcium, albumin, total protein, c-reactive protein, thyroid stimulation hormone, and thyroxine).

Schedule of events for included dogs

Dogs that met the inclusion criteria were further examined with repeated blood samplings, including pre and post prandial sampling, for taurine concentration analyses. The blood (WB, EDTA, and heparin) was collected on a total of five sampling time points over eight h, and dogs were fed twice during the study period to reflect different durations between feeding and sampling that may be encountered at veterinary examinations. All the collection of blood was performed during regular working hours (8 am—6 pm) to reflect the situation when sampling for assessment of taurine concentrations most commonly occurs. Dogs were fasted 12 h prior to admission, and the first blood sample was collected upon arrival at the hospital. Food was offered immediately after the first sampling time point, and the second blood sample was collected one h after the dog had finished the meal. The third and fourth blood samples were collected three and five h after the first meal, respectively. A second meal was served immediately after the fourth sampling time point, and the fifth blood sample was collected one h after the dog had finished the second meal (Fig. 1).

Taurine analyses

Six mL blood (4 mL heparin, and 2 mL EDTA) were collected at each sampling time point. Heparinized WB samples (1 mL) were immediately transferred into Eppendorf tubes (1 mL) and stored at −80 °C until shipping as a batch to an external laboratory. Plasma samples (heparinized and EDTA) were centrifuged at 1780 g for 5 min within 30 min from collection. Plasma (0.5 mL) was then transferred into Eppendorf tubes (1 mL) and stored at −80 °C until shipping in batches to external laboratories. The samples were treated and controlled according to published recommendations established by respective laboratories [36,37], and transported on dried ice with a registered temperature log to ensure a stable temperature during transport.

Idexx Laboratories in Germany analyzed 100 EDTA plasma samples using liquid chromatography with mass spectrometry[h]. University of California Davis Amino-Acid Laboratory in California, USA, analyzed 100 heparinized plasma samples and 100 WB samples, in accordance with previously described methods using ion exchange chromatography with post column derivatization with ninhydrin on a Biochrom 30 AA Analyzer[i] [27]. All results are reported in nmol/mL. Normal reference values for blood taurine concentrations were communicated from the laboratories as: 200—350 nmol/mL for WB, 60—120 nmol/mL for heparinized plasma[j], and 44—224 nmol/mL for EDTA plasma[k]. The lowest detection limit was 0.002 nmol/mL for WB,

[f] Televet 100-Veterinary ECG Device, Engel Engineering DServices GnbH, Heusenstamm, Germany.
[g] Greiner Bio-One GmbH, Kremsmünster, Austria

[h] http://www.ecs.umass.edu/eve/background/methods/chemical/Openlit/Chromacademy%20LCMS%20Intro.pdf
[i] Biochrom Ltd. Cambridge, England C, S
[j] https://www.vetmed.ucdavis.edu/index.php/labs/amino-acid-laboratory
[k] http://www.idexx.se/sv/veterinary/reference-laboratories/tests-and-services/

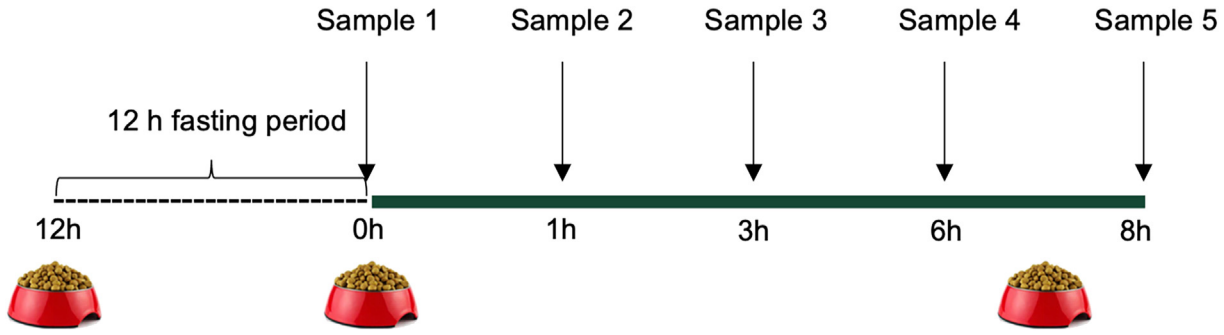

Fig. 1 Blood was collected at a total of five sampling time points over eight h. Food was offered immediately after the first sampling time point, and one h after the fourth sampling time point. The first sample was collected at arrival after approximately 12 h of fasting; the second sample was collected one h after the first meal served at the hospital; the third sample was collected three h after the first meal; the fourth sample was collected six h after the first meal; and the fifth sample was collected eight h after the first meal (i.e. one h after second meal served at the hospital).

0.002 nmol/mL for heparinized plasma, and 7.99 nmol/mL for EDTA plasma.

Statistical analyses

Statistical analyses were performed using a commercially available software program[l]. Continuous variables were presented as medians and interquartile ranges (IQRs). Continuous data were analyzed between ECS and other breeds using the non-parametric Wilcoxon signed rank test. Univariable and multivariable regression analyses were used to investigate for potential effects of dog characteristics (age, sex, BW, and cocker spaniel yes/no) on baseline (first sampling time point) taurine concentrations.

Group comparisons between the three additives were assessed by Bland Altman plots, in which the mean bias and 95% confidence intervals (CI) were calculated. For each additive, general linear model procedures (repeated measurements) were used to investigate the effects of sampling time point and dog identity. The coefficient of variation (CV) was calculated for each dog and additive, respectively, as $CV = \frac{s}{\bar{x}}$, where $\bar{x}$ and s are the mean and standard deviation of the five sampling time points for each dog. Friedman's non-parametric ANOVA with dogs as blocks was performed to check for differences between CV.

Results

Study group

A total of 20 dogs met the inclusion criteria. Eleven females (three neutered, eight intact) and nine males (two neutered, seven intact) were included. The median age was 5.3 years (IQR 2.8—8.3 years) and median BW was 14.1 kg (IQR 12.7—16.4 kg). The body condition score was assessed as ideal (4—5/9) for all dogs. The study sample included 10 ECS and 10 dogs of different breeds (Border Collies n = 3, Australian Kelpies n = 2, Labrador Retriever n = 1, Pointer n = 1, English Springer spaniel n = 1, Australian shepherd n = 1, and mixed breed n = 1).

All dogs were fed grain-inclusive diets based on non-exotic protein sources, thereby classified as traditional diets.

All echocardiographic variables were within normal reference values for the included dogs, and no differences were found when the various echocardiographic variables for each group (ECS and various breeds) were compared (Table 1). All dogs presented with a sinus rhythm and normal QRS-morphologies on the ECG recordings.

The total storage time from the collection of blood samples to taurine analyses was between 47 and 116 days for all dogs. The overall median taurine concentrations for all dogs were as follows: EDTA plasma Idexx 95.88 nmol/mL (IQR 71.91—119.85 nmol/mL); heparinized plasma 100.5 nmol/mL (IQR 81.25—128 nmol/mL); and WB 240 nmol/mL (IQR 210.25—272 nmol/mL). The median taurine concentrations and IQR for the two groups of dogs (ECS and various breeds) evaluated separately are shown in Table 1.

Taurine concentrations over time

Dog identity was associated with taurine concentrations for all additives over all sampling time points (all $P < 0.001$). Heparinized plasma taurine concentrations were higher at the first and fifth sampling time points (performed after 12 h of fasting and one h after second meal, respectively)

[l] SAS Institute Inc. (2017): SAS/Stat User's Guide. Version 9.4. Cary, N. C, US

Table 1 Summary of echocardiographic data and taurine concentrations measured in EDTA plasma, heparinized plasma, and whole blood in English cocker spaniels (n = 10) and dogs of various breeds (n = 10). Values are reported in median and interquartile ranges (IQR). Statistically significant differences are marked with an *.

	English cocker spaniels Median (IQR)	Other breeds Median (IQR)	P-value
LA/AO	1.35 (1.2—1.4)	1.35 (1.3—1.4)	0.29
LVIDd (cm)	3.43 (3.33—3.54)	3.48 (3.24—3.68)	0.07
LVIDs (cm)	2.53 (2.33—2.63)	2.51 (2.42—2.71)	0.6
LVIDdn	1.59 (1.56—1.69)	1.52 (1.42—1.65)	0.053
LVIDsn	1.07 (0.94—1.14)	1.09 (1.03—1.17)	0.08
EPSS (cm)	0.3 (0.3—0.4)	0.35 (0.2—0.4)	0.09
FS (%)	26.5 (25.2—30.5)	28.5 (25.1—29.8)	0.76
EDTA (nmol/mL)	95.88 (27.97*—115.86)	103.87(79.9—103.87)	0.02*
Heparin (nmol/mL)	92 (23*—116)	109.5 (95.5—139)	0.002*
Whole blood (nmol/mL)	233.5 (195—280)	240 (219.5—266.75)	0.43

Abbreviations: EPSS: E-point to septal separation; FS: fractional shortening; IQR: interquartile range; LA/AO: left atrial to aortic ratio; LVIDd: left ventricular internal dimension at end-diastole; LVIDdn: left ventricular internal dimension at end-diastole normalized for body weight; LVIDs: left ventricular internal dimension at end-systole; LVIDsn: left ventricular internal dimension at end-systole normalized for body weight.

compared to the fourth sampling time point (performed at six h after first meal at the hospital) (P=0.014) (Fig. 1). No association between taurine concentrations and sampling time points was found for the other additives (Fig. 2).

Intraindividual variation

Intraindividual taurine concentrations varied between sampling time points in all dogs. The results varied from below reference range to normal concentrations in one or more additives in seven (30%) of the dogs within the day of the examination (Fig. 3). Heparinized and EDTA plasmas were the additives demonstrating the largest variations in concentration (Fig. 3). The mean intraindividual variations, expressed as CV, in our study were as follows: EDTA plasma Idexx 26.4% (5.9—111.8%), heparin plasma 23% (12.6—83.9%), and heparinized WB 8.8% (3.6—26.9%). Pairwise comparisons of all 20 dogs showed that the CV

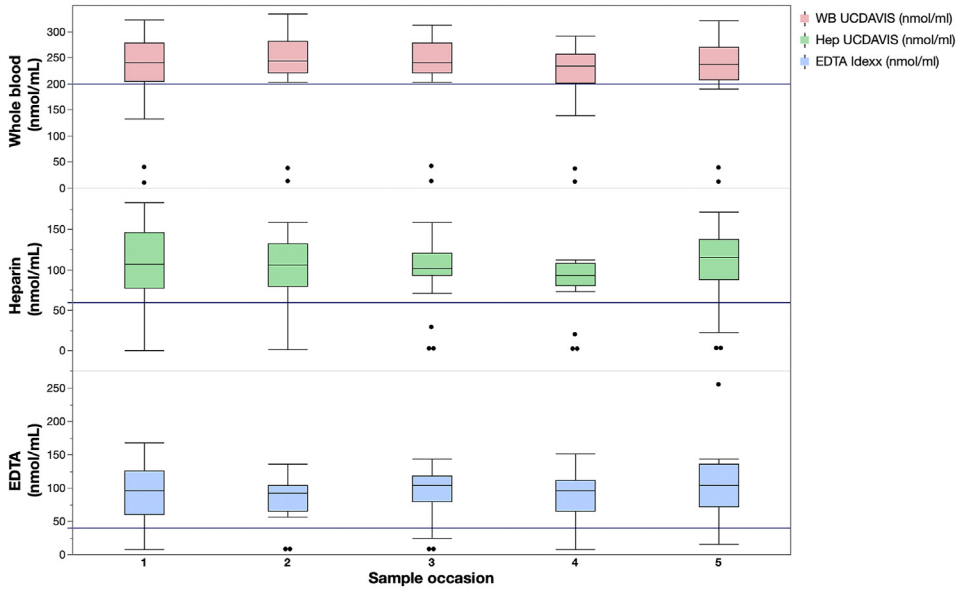

Fig. 2 Box and whiskers plots demonstrating median taurine concentrations for each sampling time point (n = 5) measured in whole blood, EDTA plasma, and heparinized plasma. The boxes (top, bottom, and central line) correspond to the 75th percentile, the 25th percentile, and 50th percentile (median), respectively. Whiskers corresponds to 10th and 90th quantiles. Horizontal dark blue lines show normal lower reference values for each additive. Outliers are shown as dots.

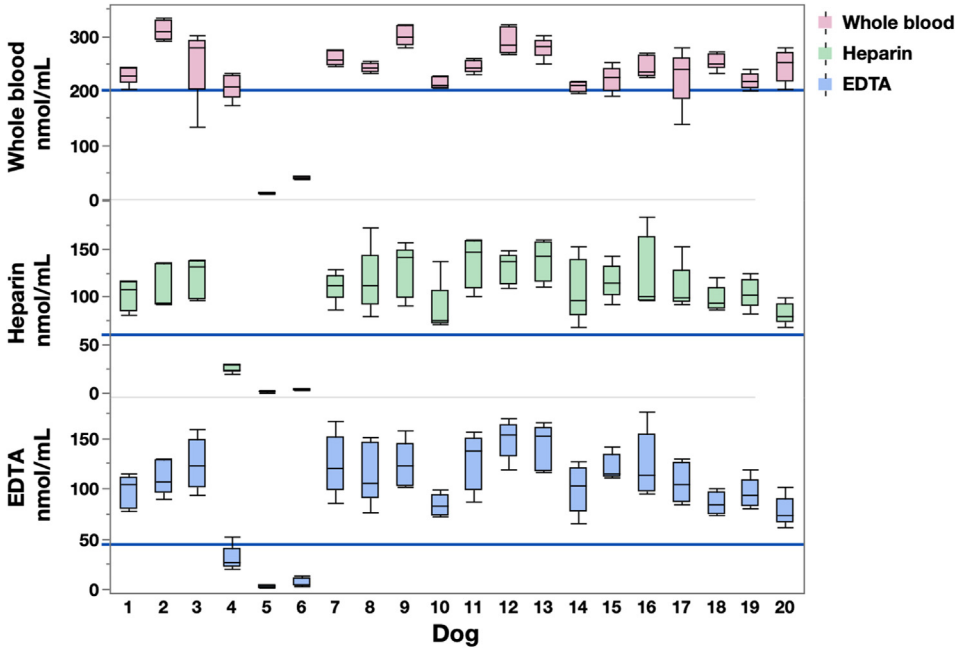

Fig. 3 Box and whiskers plots demonstrating intraindividual variation in taurine concentrations measured in whole blood, EDTA plasma and heparinized plasma at five sampling time points during an eight hour-period. Dark blue horizontal lines show normal lower reference values for each additive. Of the 20 dogs in the study, seven had taurine concentrations below reference range in one or more additive and at one or more sampling time points during the sampling period. Four of these were English cocker spaniels. Notice that dog number five and six were the only dogs having consistently low concentrations in all additives. Dog number four had consistently low concentrations in heparin plasma whereas concentrations in EDTA plasma and whole blood varied between below and within normal reference range. Dogs three, 14, 15, and 17 had low concentrations in whole blood at occasional sampling time points.

for WB was significantly smaller than the CV for the other two additives ($P<0.0001$).

Univariable and multivariable regression analyses

Univariable and multivariable regression analyses analyses showed that the cocker spaniels had lower baseline (first sampling time point) taurine concentrations in EDTA and heparin plasma ($P=0.049$ and $P=0.007$, respectively) but not in WB.

Agreement between methods to estimate taurine concentrations

Assessment of Bland—Altman plots showed good agreement between the two plasma analyses in the group of dogs evaluated (Fig. 4). Whole blood concentrations were systematically higher than concentrations in the two plasma additives, with an absolute (and percentage) difference of 55.7 nmol/mL (84%) compared to EDTA plasma and 50.5 nmol/mL (37%) compared to heparinized plasma (all $P<0.001$).

Discussion

Whole Blood taurine concentrations were systematically higher than plasma taurine concentrations in our study population. This was an expected result due to the presence of taurine-rich platelets and white blood cells in WB samples, and standard reference values at analyzing laboratories are adapted for these differences [21,23]. Taurine concentrations evaluated in the two plasma additives (EDTA and heparin) showed an overall agreement, although both analyzing methods and recommendations of anticoagulation additives differed between the two commercial veterinary laboratories used in the present study. Differences between plasma additives used for the analysis of taurine concentrations are rarely discussed, and previous studies on the effect of different anticoagulation additives recommended for taurine analyses show conflicting results. Heparinized plasma concentrations have been shown more variable than EDTA plasma concentrations in a human study investigating intraindividual variations in taurine concentrations analyzed over a

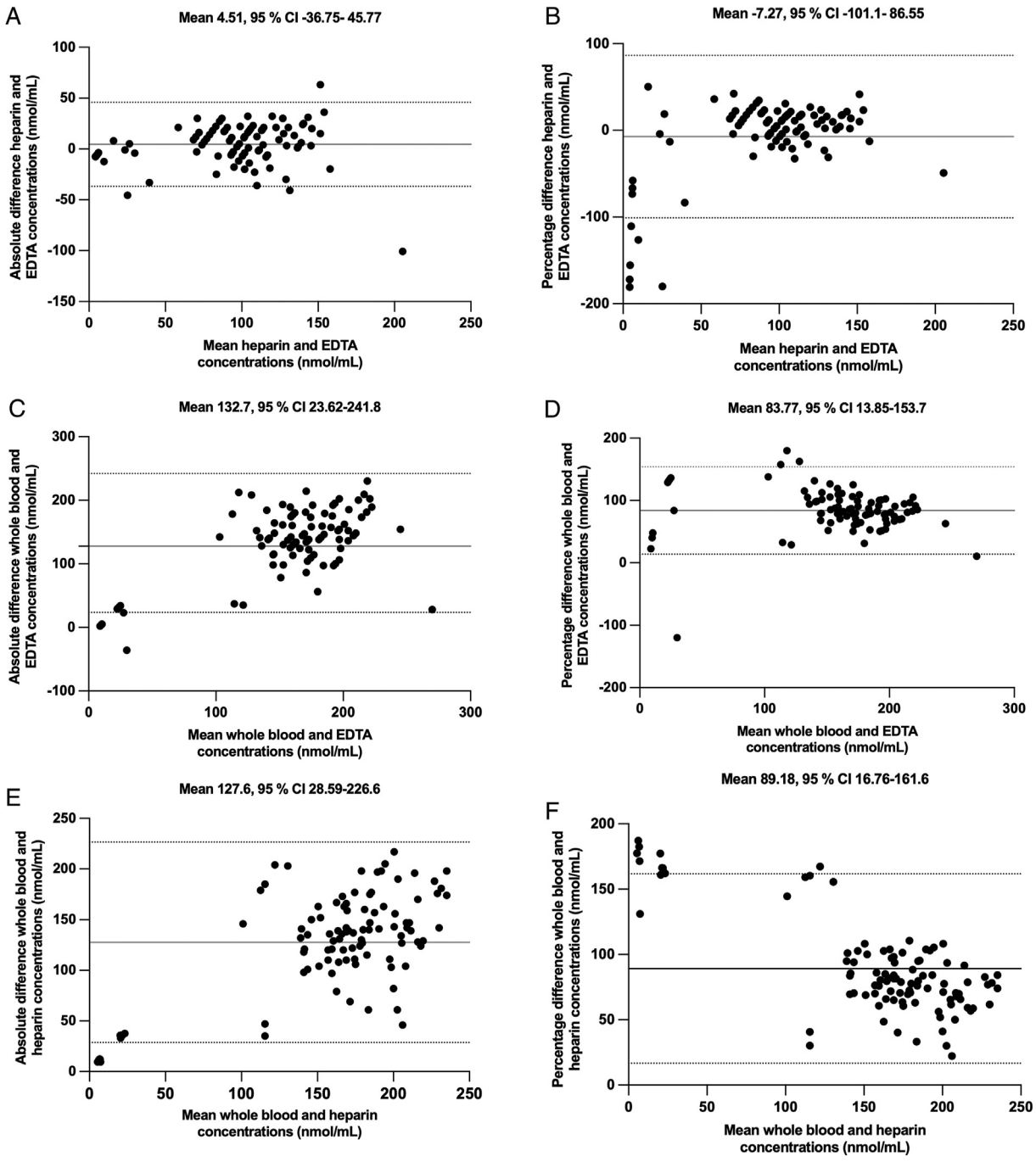

Fig. 4 Bland Altman plots showing mean and 95% confidence intervals of absolute differences (A, C, and E), and differences expressed as percentage of the mean concentrations (B, D, and F) in 100 samples obtained at five occasions from 20 dogs. A systematic absolute (and percentage) difference of 55.7 nmol/mL (84%) and 50.5 nmol/mL (37%), respectively (all $P<0.001$) was found between WB and the two plasma additives (EDTA and heparinized plasma). Abbreviations: CI: confidential interval; WB: whole blood.

three-day period [22]. Other studies, on the other hand, have shown that the use of EDTAadditives might cause an over-estimation of taurine content due to ninhydrin-positive contaminants [22,38,39].

Taurine concentrations evaluated for each dog and sampling time point, separately, showed substantial intraindividual variation in some dogs, with significantly higher variability in plasma concentrations than in WB. Samples were collected during one single examination day and results varied between below and within normal reference range for seven out of 20 dogs in one or more

additive. Additionally, there was poor agreement between plasma and WB samples for some dogs, as shown in Figure 3. These variations highlight the difficulties in interpreting a result from a single measurement, and suggest that concurrent testing in different blood tube additives or repeated testing in the same blood tube additive may provide more reliable results.

Intra-assay variability was less than 10% for all the test results from both analyzing laboratories, whereas the intraindividual variation between the different sampling time points expressed as CV in our study was as high as 112% in plasma and 27% in WB. The stability of WB taurine concentrations compared to plasma taurine concentrations has been shown in several studies, and the intraindividual variation demonstrated in plasma concentrations in our dogs is consistent with a previous study, where intraindividual plasma concentrations in humans were shown to vary by more than or equal to, 100% [22].

Blood collecting procedures may alter taurine concentrations in plasma as hemolysis, damage to blood cells during blood sampling, prolonged clotting time, or disruption of the buffy coat during separation might falsely increase taurine concentrations in plasma [21—23]. Plasma samples should also preferably be deproteinized or stored in −80 °C immediately after collection to minimize hydrolysis of proteins that might affect taurine concentrations [21,25]. All samples in our study were handled and stored according to above recommendations, although not deproteinized. Deproteinization of plasma samples (EDTA and heparin) prior to transport is, however, not specified in sample handling instructions communicated from the analyzing commercial veterinary laboratories used in this study. Mild hemolysis or contamination from just a few platelets or other blood cells might have contributed to the high intraindividual variations observed in this study, despite careful handling. The risk for inaccurate results caused by technical errors has been reported to be less when assessing WB samples that are not separated prior to analyses of taurine concentrations [22]. Intraindividual variation, with taurine concentrations varying between normal reference values and below reference values, were, however, also found in WB samples in the present study; thereby, demonstrating that variation in taurine concentrations occur, to varying degree, regardless of additive.

An association was found between heparinized plasma and sampling time point with higher concentrations at the first and last sampling (performed after 12 h of fasting and one hour after second meal, respectively) compared to the fourth sampling (performed at one h after first meal). The increase in taurine concentrations one h after the second meal could be explained by the dietary intake of taurine and its precursors' methionine and cysteine [21]. The somewhat unexpected pattern showing higher taurine concentrations after 12 h of fasting time is more difficult to explain. One potential explanation could be that plasma taurine conservation during depletion can be achieved by increased kidney reabsorption, increased endogenous synthesis, or increased gastrointestinal absorption of taurine-rich bile acids [30,40—43]. The increase in taurine concentrations could also potentially be caused by a type one error, overestimating normal biological variation. The variations between sampling time points in our study underline the difficulty of excluding taurine deficiency in a dog tested at a single occasion and in a single additive. Furthermore, the results do not support the recommendation for fasting dogs before sampling for taurine estimation.

Different concentrations and composition of amino acids in diets have been shown to affect the daily variation of taurine concentrations in dogs [44—47], and this might also be part of the explanation to the daily variations seen in the present study.

The type and amount of food were not standardized for our study population, and food was not analyzed for taurine content as the purpose of the present study was to reflect a normal clinical situation, where dogs were fed their usual type and amount of food. All dogs were, however, fed traditional commercial diets (grain-inclusive and based on non-exotic protein sources). Fasting time prior to the first sampling time point was set to 12 h, based on general recommendations for fasting dogs prior to blood sampling in many clinics.

The ECS, in contrast to the American cocker spaniel, has not previously been considered predisposed to taurine-deficiency DCM [6]. However, four out of 10 clinically healthy ECS, included in the present study, had low blood taurine concentrations, and the ECS breed was associated with low taurine concentrations in the both the univariable and multivariable regression analysis. This is consistent with the results from a recent retrospective study on a total of 16 ECS with DCM-phenotype, where 13 (81%) of the affected dogs were found to have low blood taurine concentrations [16].

Study limitations

The small number of dogs included in the study constitutes a limitation of the present study. However, a total of 300 blood samples were analyzed for taurine concentrations, and the main limiting factor for not including more dogs in the study was financial restrictions.

The two groups of dogs were not matched regarding dog characteristics (age, BW, and sex). The intention of the study was to include 20 healthy dogs, of which 50% of the dogs should be ECS, and not two different groups of dogs for further comparison. When it later turned out that four out of 10 clinically healthy ECS had low taurine concentrations, a group comparison was deemed of interest even though the groups were not matched.

The present study used reference ranges for normal taurine concentrations communicated from the two laboratories, respectively. However, reference ranges for taurine concentrations in dogs have, to our knowledge, only been established for WB and heparinized plasma [48]. Reference ranges for EDTA plasma should therefore, preferably, be established in the future.

Finally, intraindividual variations in taurine concentrations can be caused by several factors such as analyzing methods. Some samples were analyzed by liquid chromatography mass spectrometry, and other samples by using ion exchange chromatography, with post column derivatization with ninhydrin. Intraindividual variations in taurine concentrations can also be caused by anticoagulating additive, sample handling, food content, storage time, and temperature during storage [22,38,49]. The purpose of the present study was, however, not to identify potential underlying causes to variations, but rather evaluate the impact of the use of different blood tube additives and of time from feeding on taurine concentrations analyzed at different commercial veterinary laboratories.

Conclusion

Plasma taurine concentrations showed an acceptable agreement between the two different plasma anticoagulation additives in the group of dogs investigated in our study. Whole blood concentrations were systematically higher than plasma concentrations, which was an expected finding.

Taurine concentrations were associated with sampling time point when measured in heparinized plasma but not in EDTA plasma or WB. These associations were seen both after 12 h of fasting and one h after a meal, indicating recommendation of fasting before taurine analyses to be redundant.

A substantial intraindividual variation was observed in taurine concentrations between various sampling time points for some dogs, with the largest variations seen in plasma concentrations (EDTA and heparin). To overcome some of these limitations, concurrent evaluation of taurine concentrations in both plasma and WB may be of value.

Conflicts of Interest Statement

Dr. Fascetti (AJF) is the Scientific Director and Dr. Yu is the Technical Director of the Amino Acid Laboratory at the University of California, Davis (UCD) that provides amino acid analysis on a fee for service basis. This did not lead to a conflict of interest or influence collection or interpretation of results. AJF advised Synergy Food Ingredients, Clorox, and received a grant from Nutro and remuneration for lectures, or as an advisor on behalf of Nestlé Purina PetCare, Mars Petcare, and the Pet Food and Mark Morris Institutes. A nutrition resident received funds from the Hill's Pet Nutrition Resident Clinical Study Grants program; AJF collaborated on the resulting research project. The Veterinary Medical Teaching Hospital at University of California, Davis receives partial support for a Nutrition Technician from Nestlé Purina PetCare and its veterinary nutrition program from Nestlé Purina, Mars Petcare and Hill's Pet Care.

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