Acta Universitatis Agriculturae Sueciae

Doctoral Thesis No. 2026:38
Faculty of Veterinary Medicine and Animal Sciences

Taurine-responsive cardiomyopathy in English Cocker Spaniels

Nutritional and familial influences on cardiac phenotype

Karin Kriström

Taurine-responsive cardiomyopathy in English Cocker Spaniels

Nutritional and familial influences on cardiac phenotype

Karin Kriström
Faculty of Veterinary Medicine and Animal Sciences
Department of Clinical Sciences
Uppsala

SLU
SWEDISH UNIVERSITY OF AGRICULTURAL SCIENCES

DOCTORAL THESIS
Uppsala 2026

Acta Universitatis Agriculturae Sueciae
2026:38

Cover: Illustration generated by ChatGPT, developed by OpenAI, 2026. The image is AI-generated and does not have a traditional human author; usage follows OpenAI's terms of service.

ISSN 1652-6880
ISBN (print version) 978-91-8124-255-3
ISBN (electronic version) 978-91-8124-285-0
https://doi.org/10.54612/a.4ljnjimqe3

Swedish University of Agricultural Sciences, Department of Clinical Sciences, Uppsala, Sweden
Print: SLU Grafisk service, Uppsala 2026

Taurine-responsive cardiomyopathy in English Cocker Spaniels – nutritional and familial influences on cardiac phenotype

Abstract

Taurine-responsive cardiomyopathy (TauR-CM) is a potentially reversible heart disease that is increasingly recognized in dogs; however, its occurrence, risk factors, and clinical significance remain incompletely understood.

The aims of this thesis were to evaluate factors that may affect blood taurine concentrations (B-TauC) in dogs and to investigate B-TauC in English Cocker Spaniels (ECS), with a focus on the occurrence of low B-TauC (<B-TauC), clinical consequences, underlying causes (including dietary factors), potential heritability of <B-TauC, and the response to taurine supplementation.

In paper I, agreement between analytical methods and additives, intra-individual variation, and effects of time from feeding on B-TauC were assessed in 100 samples from 20 dogs. In paper II, occurrence of <B-TauC and its associations with clinical findings, dog characteristics, and diet were prospectively studied in 180 ECS. In paper III, dogs from paper II were followed longitudinally, and response to taurine supplementation in dogs with <B-TauC, B-TauC changes over time in dogs with normal B-TauC, and survival between groups were evaluated. In paper IV, genetic factors associated with <B-TauC in ECS were investigated.

Blood taurine concentrations showed acceptable agreement between methods and additives when analyzed for the group as a whole but showed substantial intra-individual variation between sampling occasions (paper I). Almost 30% of the 180 ECS had <B-TauC at enrollment (paper II), increasing to nearly 40% at follow-up (paper III). Among dogs with <B-TauC at enrollment, 25% had congestive heart failure (CHF). Taurine supplementation restored B-TauC to within the normal range in all dogs with <B-TauC. Dogs with CHF showed marked clinical and echocardiographic improvement, allowing discontinuation of diuretics in all dogs and of all cardiac medications in 60%. At follow-up (median 3.3 years), overall survival for all included dogs was high. Mortality was highest in the CHF group, although only two deaths were cardiac-related. Dietary protein source, dietary amino acid concentrations, and age were associated with <B-TauC. Genetic analyses adjusted for diet identified a suggestive signal on chromosome 17 (paper IV), indicating combined genetic and dietary influences on B-TauC.

In conclusion, <B-TauC was common in ECS, and dogs of this breed can develop TauR-CM, which appears to be reversible in many cases. Dietary factors and genetic predisposition may contribute to <B-TauC.

Keywords: Dogs, Heart disease, Taurine deficiency, Amino acids, Taurine supplementation, Diet-associated DCM

Taurinresponsiv kardiomyopati hos engelsk cocker spaniel - foderrelaterade och ärftliga faktorer med betydelse för hjärtats fenotyp

Sammanfattning

Taurinresponsiv kardiomyopati (TauR-CM) är en potentiellt reversibel hjärtsjukdom som i allt högre grad uppmärksammas hos hund. Kunskapen om dess förekomst, riskfaktorer och kliniska betydelse är dock fortfarande begränsad.

Syftet med denna avhandling var att utvärdera faktorer som kan påverka taurinkoncentrationer i blod (B-TauC) hos hund samt att undersöka B-TauC hos engelsk cocker spaniel (ECS), med fokus på förekomst av låga B-TauC (<B-TauC), kliniska konsekvenser, bakomliggande orsaker (inklusive foderrelaterade faktorer), möjlig ärftlighet av <B-TauC samt kliniskt svar på taurintillskott.

I studie I utvärderades överensstämmelsen mellan analysmetoder och blodrörstillsatser, intraindividuell variation samt effekten av tid från utfodring på B-TauC i totalt 100 prover från 20 hundar. I studie II undersöktes förekomsten av <B-TauC och dess samband med kliniska fynd, hundkaraktäristika och fodertyp i en prospektiv studie på 180 ECS. I studie III följdes dessa hundar longitudinellt för att undersöka effekten av taurintillskott hos hundar med <B-TauC, förändringar i B-TauC över tid hos hundar med normal B-TauC samt överlevnad mellan grupper. I studie IV undersöktes genetiska faktorer associerade med <B-TauC hos ECS.

Taurinkoncentrationer i blod visade en godtagbar överensstämmelse mellan analysmetoder och blodrörstillsatser när hundarna utvärderades som grupp (studie I), medan en stor intraindividuell variation sågs mellan olika provtagningstillfällen. Nästan 30 % av de 180 ECS hade <B-TauC vid inklusion (studie II), vilket ökade till nära 40 % vid uppföljningen (studie III). Av hundar med <B-TauC vid inklusion hade 25 % kongestiv hjärtsvikt. Taurintillskott normaliserade B-TauC hos samtliga hundar med <B-TauC. Hundar med samtidig hjärtsvikt uppvisade tydlig klinisk och ekokardiografisk förbättring, vilket möjliggjorde utsättning av vätskedrivande behandling hos alla hundar och av all hjärtmedicinering hos 60 %. Vid uppföljning (median 3,3 år) var den totala överlevnaden hög i hela gruppen. Dödligheten var högst i gruppen med hjärtsvikt, men endast två dödsfall var hjärtrelaterade. Fodrets proteinkälla och aminosyrainnehåll samt hundens ålder var kopplade till <B-TauC. Genetiska analyser justerade för foder visade en möjlig koppling till en region på kromosom 17 (studie IV), vilket tyder på att både genetiska faktorer och foder påverkar B-TauC.

Sammanfattningsvis var <B-TauC vanligt förekommande hos ECS, och hundar av denna ras kan utveckla TauR-CM som i många fall förefaller vara reversibel. Både foderrelaterade och genetiska faktorer kan bidra till <B-TauC.

Nyckelord: Hund, hjärtsjukdom, taurinbrist, aminosyror, taurintillskott, foderrelaterad DCM

Dedication

To Isak and Emy

Contents

List of publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I. Kriström K, Häggström J, Tidholm A, Yu J.Z, Fascetti A.J, Ljungvall I. (2023). Impact of blood tube additives and timing of sampling on blood taurine concentrations in clinically healthy dogs. *Journal of Veterinary Cardiology*, *45*, 59-70.

II. Kriström K, Häggström J, Fascetti A.J, Ström L, Dirven M, Yu J.Z, Sjödal Essén T, Tidholm A, Pion P.D, Ljungvall I. (2024). The association between taurine concentrations and dog characteristics, clinical variables, and diet in English cocker spaniels: The Canine taURinE (CURE) project. *Journal of Veterinary Internal Medicine*, *38*(5), 2620-2632.

III. Kriström K, Häggström J, Tidholm A, Dirven M, Nilsson C, af Sandeberg L, Yu J. Z, Pion P.D, Ljungvall I. (2026). Long-term follow-up of plasma taurine concentrations, cardiac function, and survival in English Cocker Spaniels with taurine-responsive cardiomyopathy (submitted).

IV. Kriström K, Häggström J, Strandberg E, Tidholm A, van Rumpt M, Rozendom. C, Kittleson M.D, Pion P.D, van Steenbeck F*, Ljungvall I*. (2026). Heritability and genetic risk factors for taurine deficiency in English Cocker Spaniels (manuscript).

I-III Description of contribution to paper I. Took part in planning the studies, Took major part in planning and execution of examinations and blood sampling performed, including arrangements of transports of blood samples to analyzing laboratories. Interpreted results together with supervisors and had the main responsibility for writing the manuscripts.

IV Description of contribution to paper IV. Took part in planning the study. Took major part in collecting data and was responsible for arrangements of transports of blood samples to analyzing laboratories. Interpreted results together with supervisors and had the main responsibility for writing the manuscript.

List of figures

Abbreviations

ACS	American Cocker Spaniel
CHF	Congestive heart failure
CM	Cardiomyopathy
DCM	Dilated cardiomyopathy
ECS	English Cocker Spaniel
GWAS	Left atrium
LA	Genome-Wide Association Studies
LA/Ao	Left atrium to aortic ratio
LV	Left ventricle
LVEDV	Left ventricular end-diastolic volume
LVEF	Left ventricular ejection fraction
LVEPSS	Left ventricular E-point to septal separation
LVESV	Left ventricular end-systolic volume
LVFAC	Left ventricular fractional area change
LVFS	Left ventricular fractional shortening
LVIDd	Left ventricular inner diameter in diastole
LVIDdn	Left ventricular inner diameter in diastole normalized to body weight
LVIDs	Left ventricular inner diameter in systole
LVIDsn	Left ventricular inner diameter in systole normalized to body weight
LVSI	Left ventricular sphericity index
nB-TauC	Normal blood taurine concentrations
RA	Right atrium
RV	Right ventricle
TauC	Taurine concentration
TauR-CM	Taurine-responsive cardiomyopathy
TauT	Taurine transporter
<B-TauC	Low blood taurine concentrations
<B-TauC:CHF+	Low blood taurine concentrations and congestive heart failure
<B-TauC:CHF–	Low blood taurine concentrations and normal heart

1. Introduction

1.1 General background

Taurine-responsive cardiomyopathy (TauR-CM) is a form of secondary dilated cardiomyopathy (DCM) that occurs in association with a deficiency of the amino acid taurine (1-4). In contrast to primary idiopathic DCM, which generally carries a poor prognosis, TauR-CM may be partially or fully reversible if identified and adequately treated (1-3).

Taurine-responsive CM was first described in dogs during the 1990s, with the American Cocker Spaniel (ACS) being one of the most frequently reported breeds(2, 5). Following these early reports, ACS has been regarded as predisposed to TauR-CM and taurine analyses and supplementation are now routinely included in diagnostic and treatment protocols when dogs of this breed develop signs of a DCM-phenotype (6, 7). In contrast, although the English Cocker Spaniel (ECS) is also known to develop DCM and is closely related to the ACS, the association between heart disease and blood taurine concentrations (B-TauC) has not previously been systematically investigated in a larger population of ECS.

The underlying mechanisms of taurine deficiency in dogs are not fully understood and may involve multiple interacting factors, including inadequate dietary intake or cellular uptake of taurine or its precursor amino acids, impaired intestinal absorption, and alterations in taurine metabolism (4, 8-10). Potential associations between dietary composition and taurine deficiency have been investigated by several research groups, and while various dietary components have been proposed as contributing causes, no single causative factor has been established (11-15). In addition, reported breed predispositions suggest that genetic susceptibility may contribute to the risk of developing taurine deficiency and TauR-CM in dogs.

2. Background

2.1 The normal heart

The heart is divided into four chambers: the right atrium (RA), right ventricle (RV), left atrium (LA), and left ventricle (LV), separated by cardiac valves that control the direction of blood flow during the cardiac cycle (16-18) (figure 1). The cardiac cycle consists of coordinated phases of relaxation (diastole), when the ventricles fill with blood, and contraction (systole), when the ventricles eject blood into the circulation. Ventricular systolic function reflects the ability of the ventricles to eject blood and depends primarily on myocardial contractility, but also on ventricular filling and myocardial stretch prior to contraction (preload) and the resistance against which the ventricles must eject blood (afterload). Alterations in any of these factors can affect ventricular performance and overall cardiac function (16-19).

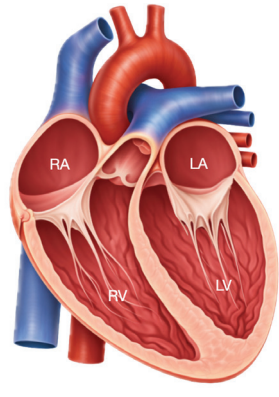


Figure 1. Deoxygenated venous blood from the systemic circulation enters the right side of the heart and is pumped to the lungs, where it is oxygenated, before entering the left side of the heart and being pumped into the systemic circulation, completing the circulatory cycle. LA: left atrium, LV: left ventricle, RA: right atrium, RV: right ventricle. Illustration: Christjan Wegner

The ventricular walls consist of three layers: the epicardium, myocardium and endocardium. The myocardium is the muscular layer responsible for generating the contractile force of the heart and is composed of cardiac muscle fibers arranged in a complex spiral orientation. This arrangement causes the ventricles to contract longitudinally and radially with a twisting motion, which helps eject blood more efficiently during systole (16-19).

The thickness of the myocardium varies between chambers, with the LV having thicker walls than the RV due to the higher pressures required for systemic circulation (16-18). Functional or anatomical abnormalities affecting any of these structures may lead to the development of cardiac disease, and the clinical presentation depends on where in the heart they occur (16-18). In dogs, most cardiac diseases primarily affect the structures of the left side of the heart (19, 20).

2.2 Dilated cardiomyopathy

Dilated cardiomyopathy is the most common myocardial disease in dogs and is characterized by left- or biventricular enlargement with systolic dysfunction that cannot be explained by other systemic or cardiac conditions, such as congenital or valvular diseases (20-23) (figure 2). The disease predominantly affects large and giant breed dogs, with the highest prevalence reported in the Doberman Pinscher, Great Dane, and Irish Wolfhound (6, 21, 24-27). An exception to this size predisposition is the Cocker Spaniel, that, despite being a small- to medium-sized breed, has been reported to develop DCM (6, 28).

Dilated cardiomyopathy is a heterogeneous disease that can result from both intrinsic and extrinsic factors affecting the myocardium. The disease can be divided into primary idiopathic and secondary forms, with clinical presentation varying between breeds, underlying causes, and disease stages (20, 21, 23, 24).

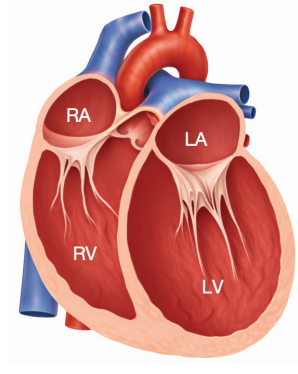


Figure 2. Dilated cardiomyopathy is characterized by left- or biventricular enlargement and systolic dysfunction, often with concurrent atrial enlargement. LA: left atrium, LV: left ventricle, RA: right atrium, RV: right ventricle. Illustration: Christjan Wegner

2.2.1 Primary idiopathic dilated cardiomyopathy

Primary idiopathic DCM is characterized by impaired myocardial contractility and myocardial atrophy, along with accumulation of interstitial collagen and fat, leading to ventricular dilation with eccentric hypertrophy, reduced stroke volume, and an increased risk of pathologic arrhythmias (20, 21, 23). A preclinical phase, in which dogs may have no clinical signs despite underlying systolic dysfunction, chamber dilation, or arrhythmias, commonly precedes the clinical phase of the disease. The preclinical phase can only be diagnosed by echocardiographic- or electrocardiographic (ECG) examinations and may last for months to years before progression to the clinical phase (20, 21, 24, 29). Clinical DCM is considered present when the dog begins to show clinical signs, such as lethargy, exercise intolerance, syncope, or signs of left- and right-sided CHF, including pulmonary edema and ascites (20, 21, 23, 24, 29). Tachyarrhythmias are also commonly seen in dogs with DCM, with atrial fibrillation and ventricular premature complexes most frequently observed. In some breeds episodes of ventricular tachycardia is a common complication and may result in syncope or sudden death also before overt cardiac disease develops (20, 21, 23, 29, 30).

2.2.2 Secondary dilated cardiomyopathy

Secondary DCM is characterized by systolic dysfunction and ventricular dilation that can be attributed to identifiable internal or external causes, such as nutritional deficiencies (11, 15, 31-33), exposure to drugs/toxins (34, 35), infectious agents (20, 36), certain endocrinopathies (37, 38), and persistent tachyarrhythmias (39, 40). In these cases, myocardial changes occur secondary to ongoing cellular injury, metabolic disturbance, or impaired myocardial energy utilization and may, unlike primary DCM, be partially or fully reversible if the underlying cause is identified and corrected (11, 20).

Diet-associated dilated cardiomyopathy

Diet-associated DCM is considered a form of secondary DCM linked to dietary factors, with many affected dogs reported to be fed so-called non-traditional or “Boutique, Exotic, or Grain-free (BEG)” diets that are often produced by smaller manufacturers and contain ingredients such as legumes (e.g., peas, lentils, and chickpeas), potatoes or sweet potatoes, and unconventional protein sources including kangaroo, venison, or bison (11, 12, 14, 41). In 2018, these observations led to an alert from the United States Food and Drug Administration regarding a potential association between certain diets and DCM. However, subsequent investigations did

not support a causal relationship between specific dietary ingredients or formulations and DCM, and the alert was later withdrawn (42, 43). Following this, several research groups have investigated associations between dietary composition and DCM, but findings have been inconsistent, and no clear causal relationship has been established.

Taurine-responsive cardiomyopathy

Taurine-responsive CM was first recognized in cats in the late 1980s, when taurine deficiency was identified as a cause of a DCM-phenotype that could be completely or partially reversed with taurine supplementation (1). Since then, taurine has been recognized as an essential amino acid in cats, and the implementation of nutritional guidelines specifying minimum requirements for taurine and its precursors in commercial feline diets has led to DCM now being a rare condition in cats (1, 44).

A similar association was later described in dogs in the mid-1990s, where taurine deficiency was identified in dogs presenting with a DCM-phenotype, with clinical and echocardiographic improvement observed following taurine supplementation (2, 5). Early studies suggested that certain breeds, including ACS (2, 5), Golden Retrievers (5, 45), and NewFoundland dogs (5, 46) were predisposed, but the condition was also observed in other breeds. These findings led to recommendations to measure B-TauC and supplement taurine in breeds considered predisposed to TauR-CM. As taurine has traditionally been considered a non-essential amino acid in dogs (5, 46-48), these recommendations did not include dogs of other breeds presenting with a DCM phenotype. More recent reports have, however, identified an increasing number of dogs of various breeds, including both atypical DCM breeds and those considered predisposed to the the primary idiopathic form, presenting with a DCM phenotype and concurrent <B-TauC (3, 49). The underlying mechanisms remain unknown, but this has resulted in taurine now being considered conditionally essential in dogs, with deficiency and secondary disease occurring under certain conditions (32, 47, 48, 50).

In a retrospective study of 115 Irish Wolfhounds, a breed generally considered predisposed to primary idiopathic DCM (20, 30, 51, 52), 53% of the dogs had <B-TauC, and of these, 41% had a DCM-phenotype (53). Although a similar proportion of dogs with normal B-TauC (nB-TauC) also had a DCM-phenotype and no clear difference between groups was observed, these findings suggest that different forms of DCM, including TauR-CM, may occur within the breed. Taurine-responsive CM has also been reported in ECS. In a retrospective UK study, 13 of 16 ECS with a DCM phenotype and CHF had <B-TauC and most dogs showed marked

improvement following taurine supplementation alongside standard DCM therapy (49).

3. Taurine

Taurine (2-aminoethanesulfonic acid) is a β-amino sulfonic acid that differs from standard α-amino acids by containing a sulfonic acid group instead of a carboxyl group, which means that it lacks the functional group required for peptide bond formation and is therefore not incorporated into proteins (4, 54, 55) (figure 3). At physiological pH, taurine occurs predominantly as a zwitterion, carrying both a negatively charged sulfonate group and a positively charged amino group (4, 55, 56). This contributes to the high solubility of taurine in water and facilitates its distribution in body fluids and tissues. However, due to its high polarity, taurine does not readily diffuse across biological membranes, and cellular uptake and transmembrane movement are therefore dependent on specialized transport systems (4, 55, 57-59).

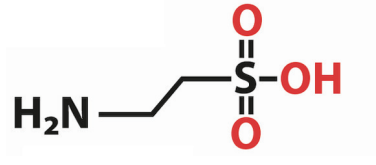


Figure 3. Taurine differs from other amino acids in that it contains a sulfonic acid group instead of a carboxyl group and therefore cannot form peptide bonds or be incorporated into proteins. The image above shows taurine in its neutral form. At physiological pH, the amino group is protonated (NH_3^+), and the sulfonic acid group is deprotonated (SO_3^-), forming a zwitterion that contributes to high water solubility and osmotic and membrane-stabilizing properties. Illustration: Christjan Wegner

3.1 Physiological functions of taurine

Taurine is present in all mammalian cells, with highest concentrations found in excitable and metabolically active tissues such as the heart, brain, retina, and skeletal muscle, where it supports processes including osmoregulation, antioxidant defense, intracellular calcium regulation, membrane stabilization, mitochondrial function, and bile acid conjugation (4, 8, 10, 60-62) (figure 4). Although a large proportion of studies on taurine physiology are based on experimental rodent models, the underlying mechanisms are considered largely conserved across mammalian species (4, 54).

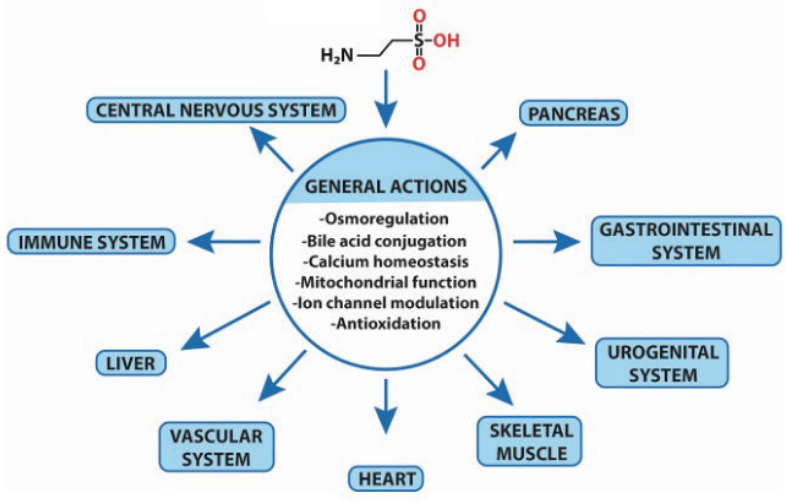


Figure 4. Taurine functions as an osmolyte, stabilizes cell membranes, modulates ion channels and intracellular calcium, and provides antioxidant, anti-inflammatory, and cytoprotective effects. It also contributes to bile acid conjugation, inhibitory signaling in the CNS, supports cardiac function and rhythm, promotes vasorelaxation and blood pressure regulation, supports immune function, enhances skeletal muscle function, supports fertility, and promotes insulin secretion in the pancreas.
Illustration: Christjan Wegner

3.1.1 Calcium handling

Intracellular calcium homeostasis is essential for normal cellular function, including muscle contraction, neurotransmitter release, enzyme activation, and cell survival (19, 60, 63). Taurine has been reported to modulate calcium fluxes across cellular membranes through effects on calcium channels and transporters, including the L-type Ca^{2+} channels and the Na^{+}/Ca^{2+} exchanger, and to support calcium re-uptake into the sarcoplasmic and endoplasmic reticulum by facilitating activation of the sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase calcium pump (SERCA) (4, 60, 63-65). These processes are particularly important in excitable cells, such as cardiomyocytes, skeletal muscle cells, and neurons, which depend on tightly regulated intracellular calcium concentrations and are sensitive to calcium overload (4, 9, 60, 63).

3.1.2 Cellular homeostasis and osmoregulation

Cellular homeostasis depends on osmotic balance, and taurine functions as an organic osmolyte that helps maintain cell volume in varying osmotic conditions (57, 62, 66). During hyperosmotic stress, taurine uptake

increases to promote intracellular accumulation and restore cell volume, whereas during hypoosmotic conditions, uptake instead decreases which prevents cell swelling (57, 62, 66). This mechanism is particularly important in tissues with frequent osmotic fluctuations, such as the kidneys, brain, heart, and skeletal muscle (57, 62, 66).

3.1.3 Mitochondrial function and cytoprotection

Taurine contributes to mitochondrial function primarily through modification of mitochondrial tRNA, which is essential for mitochondrial protein translation, oxidative phosphorylation, and ATP generation (64, 67, 68). It also helps maintain mitochondrial calcium homeostasis, preventing calcium overload and subsequent membrane disruption and apoptosis. Impaired mitochondrial function and reduced cellular energy metabolism is therefore especially detrimental in cells with high metabolic and oxygen demands, such as cardiomyocytes, neurons, and skeletal muscle cells (61, 64, 67).

The role of taurine as a mitochondrial antioxidant is well established, but the exact mechanisms remain unclear, as taurine has not been shown to neutralize common reactive oxygen species, such as superoxide and hydrogen peroxide (67, 69). In inflammatory states, taurine protects cells from oxidative stress by neutralizing hypochlorous acid (HOCl), a reactive oxidant produced by inflammatory cells, which limits tissue damage and modulates inflammatory responses (70-73).

3.1.4 Bile acid conjugation

Taurine has an essential role in bile acid conjugation, which increases bile acid solubility and facilitates the digestion and absorption of lipids and fat-soluble vitamins in the small intestine (64, 74, 75). Most conjugated bile acids are reabsorbed in the ileum and recycled via the enterohepatic circulation, with only a small proportion lost in the feces (76). In gastrointestinal disturbances this reabsorption may be reduced and if endogenous synthesis and dietary intake are unable to compensate, the increased fecal loss of taurine may result in <B-TauC (75, 77-79). Conjugation differs between species, and in dogs and cats bile acids are almost exclusively conjugated to taurine, which makes these species more dependent on taurine availability (74, 75).

3.2 Taurine homeostasis

Taurine homeostasis depends on a balance between three main processes: taurine biosynthesis, intestinal absorption, and renal handling. These processes are in turn influenced by factors such as dietary intake and various physiological and pathological conditions that may affect taurine availability and distribution (4, 54, 57, 58, 80).

3.2.1 Taurine biosynthesis

The liver is the primary site of taurine synthesis, although synthesis also occurs in other tissues, such as the brain, kidneys, pancreas, and skeletal muscle (4, 81-83). Taurine is synthesized from the sulfur-containing precursor amino acids methionine and cysteine via the cysteine sulfinic acid pathway. Synthesis is regulated by key enzymes, including cysteine dioxygenase (CDO) and cysteine sulfinic acid decarboxylase (CSAD), with vitamin B6 acting as an essential cofactor (4, 81-83) (figure 5).

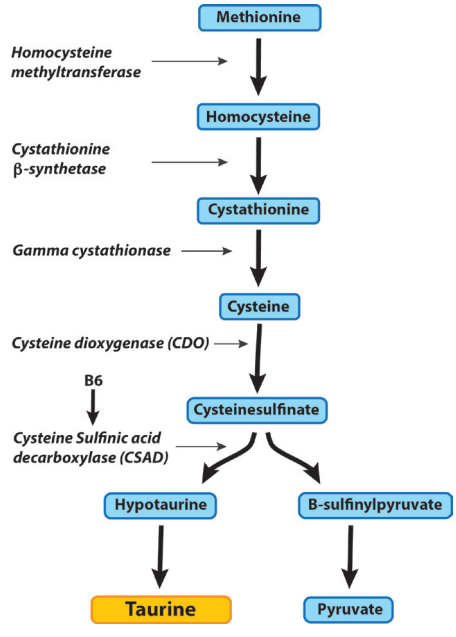


Figure 5. Taurine is synthesized via the cysteine sulfinic acid pathway, which is regulated by key enzymes, including cysteine dioxygenase (CDO) and cysteine sulfinic acid decarboxylase (CSAD). Illustration: Christjan Wegner

The rate-limiting step in taurine synthesis is generally considered to be the activity of CSAD, which has been reported to vary markedly between species, with for example, cats having significantly lower activity than rodents (54, 81, 83, 84). The CSAD activity has, to the best of our knowledge, not been evaluated in dogs.

3.2.2 Taurine uptake and transport

Taurine homeostasis depends not only on endogenous synthesis but also on efficient cellular uptake and transport. Because taurine is highly water-soluble and exists mainly in a zwitterionic form at physiological pH, it does not readily diffuse across lipid membranes. Therefore, both synthesized taurine and taurine obtained from the diet rely on specific transport systems for cellular uptake (4, 57, 58, 85). In mammals, cellular taurine uptake is mediated primarily by two transport systems: the taurine transporter (TauT; SLC6A6), a sodium- and chloride-dependent transporter with high affinity for taurine (figure 6), and the proton-coupled/pH dependent amino acid transporter 1 (PAT1; SLC36A1), which has lower affinity but higher transport capacity (57, 58, 85). The PAT1 can contribute to taurine transport, but it is not sufficient to maintain normal transport if TauT is malfunctioning (57, 58, 85).

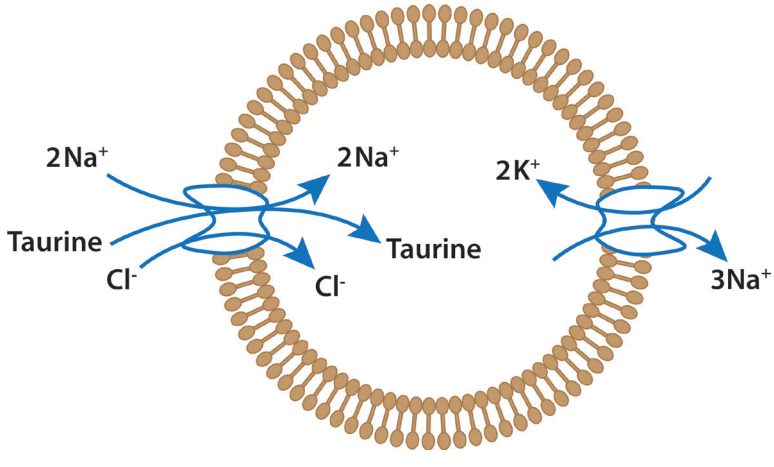


Figure 6. The taurine transporter (TauT) functions as a Na^+/Cl^--dependent cotransporter (2 Na^+:1 taurine:1 Cl^-) that uses the transmembrane chemical Na^+ gradient to drive cellular taurine uptake against a steep concentration gradient. The transmembrane gradient is maintained by the Na^+/K^+-dependent ATP-ase that actively exports sodium from the cell. Illustration: Christjan Wegner

The TauT activity is influenced by several factors, including extracellular TauC, electrochemical gradients, pH, and osmotic conditions (4, 57, 58). The limited membrane permeability of taurine, together with active TauT-mediated uptake, allow tissues with high metabolic demand to accumulate taurine against its concentration gradient, leading to intracellular taurine accumulation well above plasma concentrations (4, 57, 58).

3.2.3 Renal Handling

The kidneys play a central role in maintaining taurine homeostasis in mammals, as taurine is freely filtered by the glomerulus and, under normal conditions, almost completely reabsorbed in the proximal tubules (57, 80, 86). This reabsorption is mediated by TauT, located on the apical membrane of proximal tubular epithelial cells, and is driven by the Na^+ gradient (57, 80, 86). The reabsorptive capacity is highly adaptive to dietary intake and adjusts to taurine availability to maintain intracellular concentrations within the normal range (58, 80, 86).

3.3 Taurine deficiency and its effect on organ systems

Taurine deficiency occurs when endogenous synthesis and dietary intake fail to meet metabolic requirements. Because taurine is present at particularly high concentrations in tissues with high metabolic activity, organs such as the retina, central nervous system, reproductive system, and the heart are especially vulnerable to <B-TauC (4, 8, 10, 60, 64, 87).

3.3.1 Retina

Taurine constitutes a substantial proportion of the free amino acid pool in the retina and plays a key role in photoreceptor function by contributing to the regulation of cyclic GMP–gated channels involved in phototransduction (88-91). Taurine deficiency has been shown to cause degeneration of photoreceptor cells, likely involving increased susceptibility to oxidative stress and disturbances in calcium homeostasis (44, 89, 92, 93). Clinically, this is best described in cats, where taurine deficiency leads to feline central retinal degeneration, characterized by progressive loss of photoreceptors and irreversible blindness if not corrected (44, 91, 94, 95). In humans, central retinal degeneration has been observed in a family with biallelic SLC6A6 (TauT) variants, and taurine has also been proposed as a therapeutic candidate for human retinal degenerative diseases independent of taurine deficiency (96-99). Similar retinal changes have been reported in dogs included in studies investigating TauR-CM (46, 100), but, to the best

of our knowledge, no prospective studies investigating ocular abnormalities in taurine deficient dogs have been performed.

3.3.2 Reproductive system

Experimental studies in feline and rodent models have shown that taurine supports cellular proliferation and differentiation, as well as tissue growth during fetal development (101-103). Endogenous taurine synthesis is limited in the fetus, and taurine is actively transported across the placenta (101, 103). Fetal tissues therefore depend largely on maternal taurine supply, and low maternal B-TauC may consequently impair normal organ development and growth. Taurine deficiency has also been associated with reproductive abnormalities such as fetal resorption, abortion, stillbirth, or low birth weight in clinical cases in cats (104, 105).

3.3.3 Central nervous system

Taurine is one of the most abundant amino acids in the central nervous system, where it can act as a neuromodulator and contribute to inhibitory neurotransmission (87, 106-108). This inhibitory effect is mediated, at least in part, through membrane hyperpolarization and reduced neuronal excitability, and taurine deficiency may therefore result in decreased inhibitory tone and increased susceptibility to seizures (87, 106-108). Clinical and experimental studies in humans and rodents have reported that taurine supplementation may reduce seizure frequency in some patients with refractory epilepsy (106-109).

3.3.4 Taurine and cardiac function.

The underlying mechanisms for the development of TauR-CM are not fully understood but are thought to involve disturbances in calcium handling, reduced calcium sensitivity of contractile proteins, impaired myocardial energy metabolism, and a resulting loss of functional cardiomyocytes (110-114). These changes may lead to impaired myocardial contractility and LV or biventricular eccentric hypertrophy, with severity varying with disease stage.

In cardiomyocytes, contraction is triggered by electrical activation of the cell. When the membrane depolarizes, L-type Ca^{2+} channels open and allow a small influx of Ca^{2+} into the cell, which in turn triggers a larger release of Ca^{2+} from the sarcoplasmic reticulum. The resulting increase in cytosolic Ca^{2+} concentration activates the contractile proteins and leads to contraction (18, 19, 63, 110).

Relaxation is initiated by a decrease in cytosolic Ca^{2+} concentration, which occurs primarily through reuptake into the sarcoplasmic reticulum via the sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA) and through extrusion via the Na^{+}/Ca^{2+} exchanger. Enhanced Ca^{2+} uptake by SERCA during diastole will also increase the amount of Ca^{2+} available for the next contraction (18, 19, 63, 110) (figure 7).

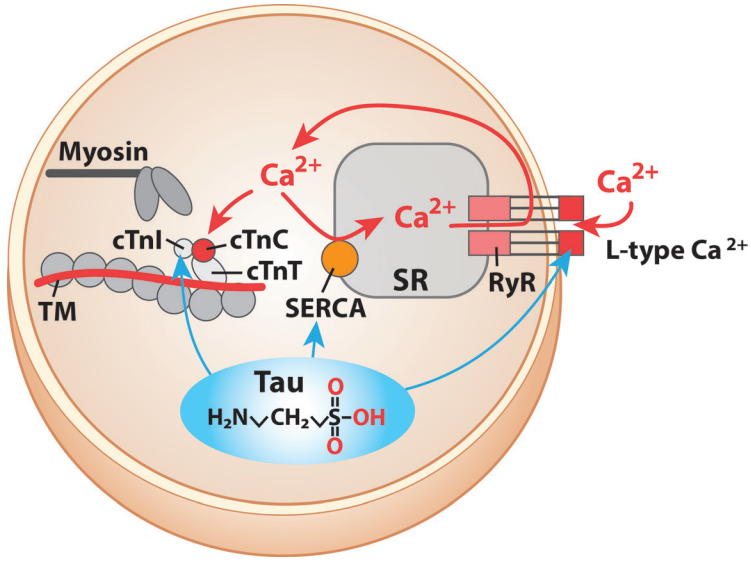


Figure 7. Excitation–contraction coupling in cardiomyocytes involves Ca^{2+} influx via L-type Ca^{2+} channels, Ca^{2+}-induced Ca^{2+} release from the sarcoplasmic reticulum (SR), and Ca^{2+} binding to troponin C within the actin–tropomyosin complex during systole, followed by Ca^{2+} reuptake by the sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA) and extrusion via the Na^{+}/Ca^{2+} exchanger during diastole. Taurine contributes to increased SERCA activity and myofilament sensitivity to Ca^{2+}, as well as modulation of the L-type Ca^{2+} channel activitycTnC: cardiac troponin C; cTnI: cardiac troponin I; cTnT: cardiac troponin T; RyR: ryanodine receptor; TM: tropomyosin.
Illustration: Christjan Wegner

Taurine supports intracellular Ca^{2+} regulation primarily through enhancement of SERCA activity, and reduced taurine concentrations may therefore impair Ca^{2+} reuptake into the sarcoplasmic reticulum, resulting in elevated cytosolic Ca^{2+} concentration and impaired myocyte relaxation and contraction (9, 110, 114, 115) (figure 7). Taurine has also been shown to modulate L-type Ca^{2+} channel activation as a function of extracellular Ca^{2+}

concentration, with reduced channel activity at higher concentrations, thereby potentially protecting cells from Ca^{2+} overload (9, 65, 115, 116) (figure 7). In addition, taurine increases the Ca^{2+} sensitivity of contractile proteins by facilitating dephosphorylation of troponin I within the actin-tropomyosin complex of the sarcomere, which allows cross-bridge formation at lower Ca^{2+} concentrations (60, 112) (figure 7).

Furthermore, taurine modulates the activity of ion channels essential for membrane function and excitability, thereby contributing to maintaining membrane potential and preventing abnormal electrical activity (60, 65, 115).

3.4 Predisposing factors

Several internal and external factors can influence the risk of developing taurine deficiency. These include diets with low concentrations or poor bioavailability of taurine and its precursors (11, 12, 31, 32, 78, 117, 118), genetic or environmental factors that may influence taurine synthesis or cellular uptake (57, 85), pathological conditions that increase taurine losses or demand (80, 119, 120), and physiological states with increased demand, including rapid growth, pregnancy, or lactation (8, 104, 105, 121).

3.4.1 Dietary factors

Dietary intake of taurine and its precursors is important in all mammals, regardless of synthesis capacity. As taurine is primarily found in animal tissues, the amount of protein, protein type and quality, as well as digestibility will have a direct effect on dietary taurine availability, although other ingredients such as carbohydrates and fiber may also influence amino acid bioavailability (15, 31, 32, 117, 118). Low protein content and reduced amino acid bioavailability have been associated with <B-TauC in dogs and cats in several studies (3, 15, 117, 118). Dietary taurine content varies between protein sources, with shellfish, fish, and poultry generally containing higher concentrations than red meats such as lamb, beef, and pork, whereas plant-based proteins, including soy, vegetables, and pulses, do not naturally contain taurine (122-125). In addition, taurine concentrations may vary depending on the animal part used in the diet; for example, chicken legs contain nearly twice as much taurine as chicken breast (122, 123, 125).

Dietary taurine availability also depends on overall diet composition, as certain ingredients may affect absorption, bile acid metabolism, and gastrointestinal microbial activity (75, 77, 78, 126, 127). High amounts of

soybean products, rice bran, and other dietary fibers, may, for example, alter the intestinal microbiota, which can result in microbial deconjugation of taurine-conjugated bile acids in the small intestine, and in turn reduce ileal bile acid reabsorption and increase fecal taurine loss (77, 128).

There are currently no official recommendations for minimum taurine content in dog food, and considerable variation in taurine concentrations has been reported between commercial diets (11, 124, 129, 130). In many cases, information regarding amino acid content or supplementation is also not disclosed on product labels, which makes it difficult to assess whether diets provide adequate concentrations. In addition, various heat and cooking processes during manufacturing of the diets may influence taurine bioavailability in the final product, meaning that taurine added or measured prior to processing may not represent its actual availability (123, 131-133).

3.4.2 Genetic factors

The overrepresentation of taurine deficiency in certain species and breeds suggests a potential genetic component, where variants affecting taurine synthesis, transport, or utilization may contribute to increased susceptibility. In humans, hereditary taurine deficiency has been associated with biallelic variants in SLC6A6*,* resulting in a dysfunctional or nonfunctional TauT (96, 134). These findings are supported by studies in mice, where targeted deletion of SLC6A6 and TauT resulted in severe taurine deficiency and secondary conditions such as CM, retinal degeneration, and impaired reproduction (85, 135). In dogs, genetic variants associated with taurine deficiency have, to the best of our knowledge, not previously been identified or proposed.

3.5 Diagnosis of taurine-responsive cardiomyopathy

Clinical suspicion of TauR-CM is often based on a combination of clinical signs, signalment, and dietary history, with diagnosis supported by echocardiographic findings together with <B-TauC.A definitive diagnosis in an individual does, however, require both improvement or normalization of myocardial function and normalization of B-TauC after taurine supplementation (1, 12, 136).

3.5.1 Blood analyses

Taurine concentrations are commonly measured using liquid chromatography-mass spectrometry (LC-MS) or ion-exchange chromatography. Both methods demonstrate high analytical precision, with

good sensitivity and specificity, and coefficients of variation (CV) well within accepted limits (<10–15%) (137, 138).

Measurements are typically performed in whole blood (WB), or plasma, and concurrent assessment of both sample types is recommended by some laboratories to improve reliability, as results may vary biologically and are sensitive to preanalytical errors (3, 139-141). Preanalytical errors are especially important in plasma samples, where hemolysis, cellular damage during sampling, or prolonged clotting time, may contaminate plasma and lead to falsely increased B-TauC (139, 142). Care should also be taken during separation to avoid the buffy coat (i.e. the layer between the blood cells and plasma containing platelets and white blood cells), as disruption of this layer may lead to contamination of plasma samples (138, 139, 142) (figure 8). The risk of inaccurate results due to technical error has been reported to be lower when assessing WB samples as they are not separated prior to analysis (141, 142).

Reference ranges for TauC in dogs have been established for heparin plasma and WB by the UC Davis Amino Acid Laboratory, whereas, to our knowledge, no established reference ranges exist for EDTA plasma (15, 118).

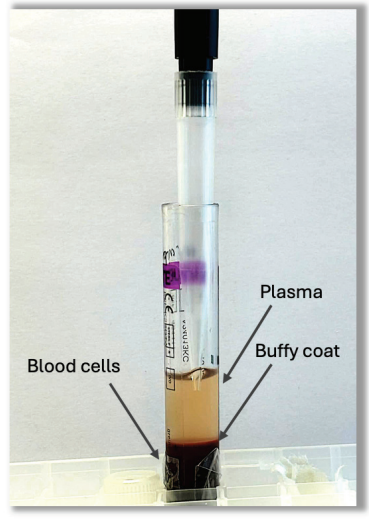


Figure 8. Plasma sample following centrifugation, with separation of blood components into plasma (upper layer), buffy coat (thin intermediate layer), and blood cells (lower layer). Care must be taken to avoid the buffy coat during separation to reduce the risk of plasma contamination. Photo: Leon af Sandeberg

3.5.2 Echocardiography

Echocardiographic diagnosis of a DCM-phenotype is characterized by increased LV end-diastolic and end-systolic internal dimensions (LVIDd, LVIDs) and volumes (LVEDV, LVESV), reduced LV fractional shortening (LVFS) and ejection fraction (LVEF), and increased LV E-point to septal separation (LVEPSS) (24, 29, 143) (figure 7). Additional variables, including interventricular septal and LV posterior wall thickness (IVSd, LVWd), LV sphericity index (LVSI), and LV fractional area change (LVFAC), may also be assessed to further evaluate ventricular size and function (24, 29, 143).

Primary idiopathic and secondary forms of DCM present with similar echocardiographic abnormalities although the phenotypic appearance may vary depending on the underlying cause and the duration of myocardial dysfunction (21, 24, 29, 143).

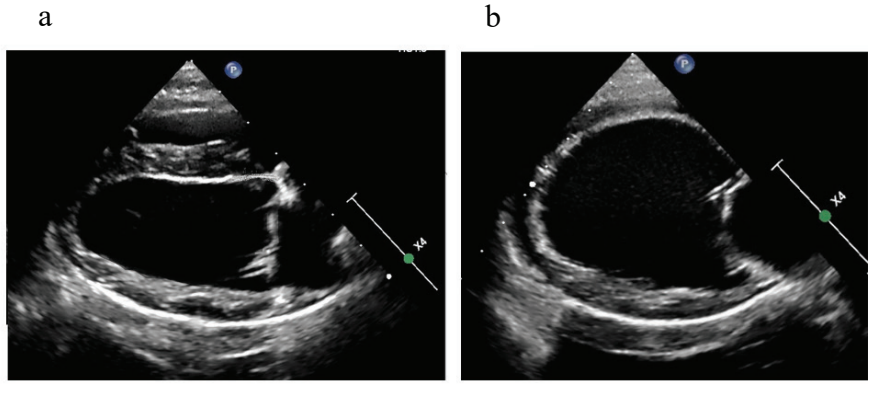


Figure 9. Echocardiographic images showing a normal heart (a) and a heart with a DCM-phenotype (b). The heart with the DCM-phenotype shows increased left ventricular diastolic dimensions, consistent with ventricular dilation.

3.5.3 Prevention and treatment

Taurine deficiency can be both prevented and treated with adequate dietary intake of taurine and its precursors (1, 2, 4, 8). Following the first reports of taurine deficiency in cats, taurine supplementation in commercial cat foods became regulated, which led to a marked reduction in disease prevalence, and DCM is now uncommon in cats (124, 130, 136). In dogs, taurine deficiency can most likely also be prevented through appropriate dietary management, but no specific dietary taurine requirement has yet been established (124, 130).

For individuals diagnosed with taurine deficiency, treatment consists of oral taurine supplementation and in cases with a concurrent DCM-phenotype, also standard DCM therapy (2, 3, 100, 117, 136). The optimal dosage for taurine supplementation has, however, not been established for dogs and published recommendations range from 500 to 6000 mg q24h per dog, divided into two to three daily doses depending on the dog's size (2, 3, 45, 117). No adverse effects have been reported for taurine supplementation at standard doses in dogs and cats (124, 130), and human studies have shown that doses of up to 10 g/day (approximately 20 times the recommended daily dose) are well tolerated without signs of toxicity (144).

3.6 The cocker spaniel

Cocker Spaniels originated from spaniel-type dogs considered to have been brought from Spain to England as early as 54-55 BC during Caesar's invasion and were later used as hunting dogs in the UK (145, 146). Initially, spaniels were not divided into distinct breeds but were instead classified based on size and function. Smaller dogs used to flush woodcock were known as Cocker Spaniels, while larger dogs used to spring birds from cover were referred to as Springer Spaniels, and dogs of intermediate size were described as Field Spaniels. Because this classification was based on size rather than pedigree, puppies from the same litter could be categorized differently, and individual dogs could be reclassified as they matured (145, 146).

During the early 19th century, the Cocker Spaniel was established as a breed and it was later also exported to the US, where breeding started to diverge from UK standards. In the UK, breeding remained focused on hunting ability and working traits, whereas in the US selection focused more on physical appearance and show qualities. This resulted in a smaller dog with a shorter muzzle, domed skull, longer coat, and more pronounced feathering. Over time, these differences became more pronounced, and in 1948 the ACS, and the ECS were recognized as separate breeds (145, 146).

4. Aims of the thesis

The overall aims of this thesis were to evaluate factors that may affect blood taurine concentrations (B-TauC) in dogs and to investigate B-TauC in English Cocker Spaniels (ECS), with a focus on the occurrence of low B-TauC (<B-TauC), clinical consequences, underlying causes (including dietary factors), potential heritability of <B-TauC, and the response to taurine supplementation. To address these aims, the thesis was divided into four studies with the following objectives:

1. Evaluate the agreement between B-TauC measured in WB and plasma (EDTA and heparin), assess intraindividual daily variation, and determine whether time from feeding to sampling had an influence on B-TauC in clinically healthy dogs.

2. Investigate the occurrence of <B-TauC in a Swedish population of ECS, and to identify associations between B-TauC and dog characteristics, clinical findings, and diet composition.

3. Prospectively follow ECS with both <B-TauC with or without concurrent CHF, and nB-TauC to:
    a) Assess the effects of taurine supplementation on B-TauC, as well as cardiac dimensions and function in <B-TauC dogs.
    b) Evaluate whether nB-TauC dogs develop <B-TauC over time.
    c) Compare survival outcomes between dogs with nB-TauC and <B-TauC.

4. Investigate the inheritance pattern of taurine deficiency within a population of ECS and to identify potential genetic variants associated with B-TauC, while accounting for dietary influences on the phenotype.

5. Material and Methods

This section summarizes the materials and methods used in the studies included in this thesis (papers I-IV). More detailed descriptions of the procedures are provided in the respective papers.

5.1 Dogs (papers I-IV)

All studies included in this thesis were approved by the Ethical Committee for Animal Welfare in Stockholm, Sweden (approval numbers 5.8.18-01548/2017, 5.8.18-21508/2021, and 5.8.18-04682/2020). Written informed owner consent was obtained prior to inclusion into all 4 studies included in this thesis. The number of dogs included in each study is summarized in figure 10.

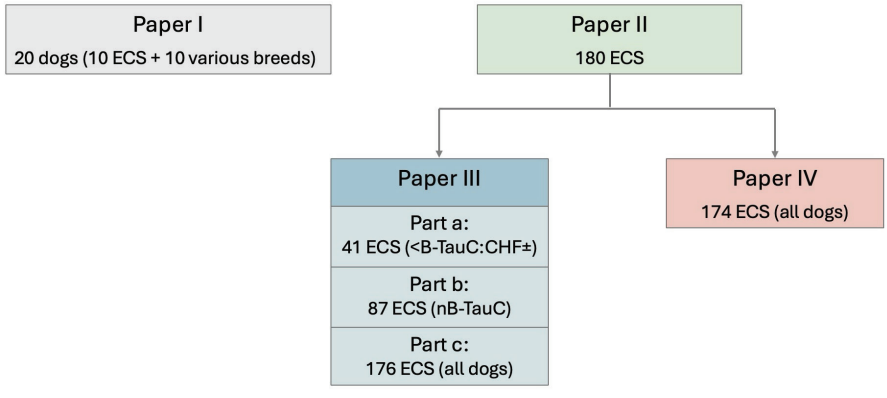


Figure 10. Overview of the study populations and overlap between studies. Paper I included 20 dogs. Paper II included 180 dogs and provided the dogs for paper III (part a: n = 41, part b: n = 87, part c: n = 176), and paper IV (n = 174). ECS: English Cocker Spaniel, < B-TauC:CHF±: Dogs with blood taurine concentrations below the normal reference range with or without echocardiographic findings consistent with a DCM-phenotype, and clinical and radiographic evidence of CHF, nB-TauC: dogs with both B-TauC and echocardiographic findings within normal variation.

All examinations were performed at the cardiology units of the University Animal Hospital, Swedish University of Agricultural Sciences (SLU), Uppsala, Sweden; AniCura Albano Animal Hospital, Danderyd, Sweden; and Evidensia Södra Djursjukhuset, Stockholm, Sweden (papers I–IV). Client-owned dogs were prospectively recruited via breeders, breed clubs, advertisements in breed magazines and social media, and through staff at the participating hospitals (papers I and II). In paper II, dogs were also recruited upon presentation with clinical signs of a DCM phenotype and CHF at the participating animal hospitals. Dogs included in papers III and IV were recruited from the population included in paper II.

In paper I, the study population was predetermined to include 10 ECS and 10 dogs of various breeds. Dogs were required to be clinically healthy, have a body weight ≥5 kg, and be ≥ one year of age.

In paper II, ECS were eligible from six months of age with no upper age limit and were either considered clinically healthy by their owners or presented with clinical signs of CHF (figure 11). Exclusion criteria included cardiovascular diseases unrelated to DCM and significant systemic or organ-related disease. Dogs receiving cardiac medical treatment or taurine supplementation were also excluded (papers I and II).

In paper III, dogs from paper II were followed prospectively and were excluded if their owners were unable or unwilling to attend scheduled follow-up examinations or administer daily taurine supplementation as part of the study protocol. Dogs that were no longer alive at the time of follow-up examinations remained in the survival analyses.

In paper IV, data and blood samples were derived from the dogs enrolled in papers II and III.

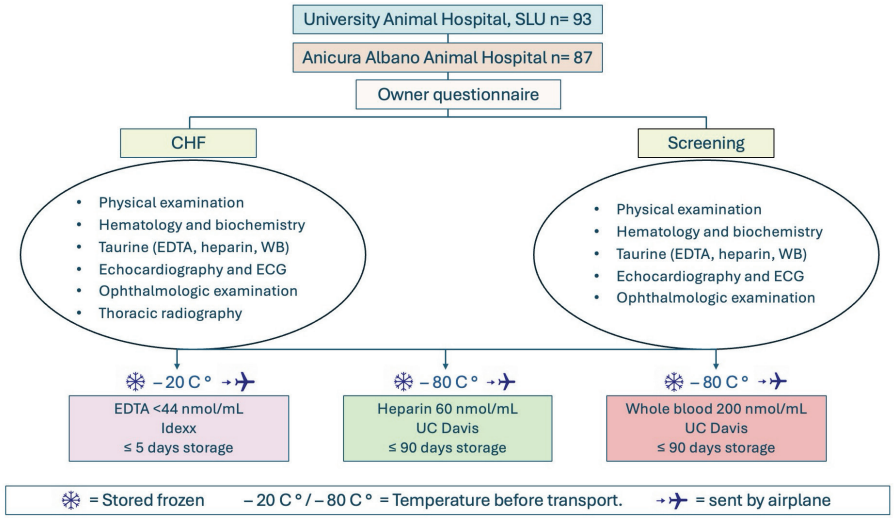


Figure 11. Dogs were recruited from two centers (SLU, n = 93; Anicura Albano Animal Hospital, n = 87) and presented either with congestive heart failure (CHF) or for screening. Clinical assessment included physical examination, hematology and biochemistry, taurine analysis (EDTA plasma, heparin plasma, whole blood), echocardiography and ECG, ophthalmologic examination, and thoracic radiography (CHF group only). Samples were stored frozen (−20 °C or −80 °C) prior to air transport. EDTA plasma samples were analyzed at IDEXX, whereas heparin plasma and whole blood were analyzed at UC Davis.

5.2 Procedures

5.2.1 Clinical examinations (paper I-IV)

At enrollment, all dogs underwent a complete physical examination, echocardiographic examination under simultaneous ECG monitoring (paper I-IV), noninvasive blood pressure measurements, and standard ECG recording (papers I and II).

5.2.2 Blood pressure measurements (paper I and II)

Blood pressure measurements were made using high definition oscillometry and followed a standardized protocol according to published guidelines (147). Dogs were allowed 10 minutes of acclimatization in the examination room 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 (147).

5.2.3 Electrocardiography (papers I and II)

Standard six-lead ECG recordings were obtained with the dogs gently restrained in right lateral recumbency. Recordings were interpreted by a board-certified specialist in veterinary cardiology or by a cardiology resident under supervision.

5.2.4 Echocardiographic examinations (papers I-IV)

Transthoracic echocardiographic examinations were performed with the dogs unsedated and gently restrained in right and left lateral recumbency. All examinations were conducted during simultaneous ECG monitoring.

Echocardiographic examinations were performed with an ultrasound unit using 5.0 to 9.2 MHz phased-array transducers (EPIQ 7G and CVx; Philips Ultrasound, Bothell, WA, USA) and included two-dimensional, M-mode, and Doppler echocardiography. Measurements of the left atrial to aortic root ratio (LA/Ao) were performed on a right parasternal short-axis view, as previously described (148). Left ventricular internal dimensions at end-diastole (LVIDd) and end-systole (LVIDs) were measured using two-dimensional images and M-mode from right parasternal short-axis views according to published guidelines (24, 29, 143, 149), and were normalized for body weight using the Cornell formula (150). Measurements of LV fractional shortening (LVFS) and LV E-point to septal separation (LVEPSS) were obtained from right parasternal short- and long-axis views (24, 29, 143, 149). Aortic, pulmonic, and mitral-inflow velocities were assessed using color and spectral Doppler echocardiographic techniques.

5.2.5 Ophthalmologic examination (paper II)

In paper II, dogs also underwent an ophthalmologic examination to evaluate potential ocular abnormalities associated with taurine deficiency. All examinations were performed by a board-certified specialist in ophthalmology, or a resident in veterinary ophthalmology under supervision. The examination included neuro-ophthalmic testing (menace response, dazzle reflex, pupillary light reflexes, and palpebral reflexes), slit-lamp biomicroscopy of the adnexa and anterior segment, rebound tonometry, and indirect ophthalmoscopy after pharmacologic mydriasis. Fundus photography was performed in dogs with detected retinal lesions, and images were reviewed by a board-certified veterinary ophthalmologist.

5.2.6 Questionnaires (papers II and III)

Owners completed a detailed questionnaire regarding the dog's health status, including previous diseases, medications, surgical interventions, reproductive history, and dietary information.

5.2.7 Grouping of dogs (papers II-IV)

Dogs were categorized at baseline (paper II) according to B-TauC and clinical, radiographic, and echocardiographic findings:

- Dogs with both B-TauC and echocardiographic findings within normal variation were classified as nB-TauC.
- Dogs with B-TauC below the reference range in one or more additive and echocardiographic findings within normal variation were classified as <B-TauC:CHF−.
- Dogs with B-TauC below the reference range in one or more additive, echocardiographic findings consistent with a DCM-phenotype, and clinical and radiographic evidence of CHF were classified as <B-TauC:CHF+.

5.3 Laboratory analyses

Blood samples were collected by cephalic (Paper I) or jugular venipuncture (Paper II-IV) via a butterfly needle with a Luer adapter (21G) (BD Vacutainer, Eysins, Switzerland) collecting blood directly into serum, heparin, and EDTA vacutainer tubes (Greiner Bio-One GmbH, Kremsmünster, Austria).

5.3.1 Hematology and biochemistry (papers I and II)

Laboratory analyses included complete blood count and serum biochemistry profiles including thyroid stimulating hormone, and thyroxine. Samples were analyzed either at the accredited veterinary laboratory at SLU or in the in-house laboratory at Anicura Albano Animal Hospital on the same day as collection.

5.3.2 Taurine analyses (paper I-IV)

Blood samples were collected in EDTA (plasma) and heparin (plasma and WB) tubes. Plasma samples were separated within 30 minutes of collection, and all samples were handled according to standardized protocols (138). EDTA plasma samples were stored in −20 °C for a maximum of five days

before being transported frozen to IDEXX Laboratories, Germany, where they were analyzed using liquid chromatography-mass spectrometry. Heparin plasma and WB samples were stored in −80 °C for a maximum of 90 days before being transported frozen as a batch to the University of California (UC) Davis Amino Acid Laboratory, US, where they were analyzed using ion exchange chromatography. Taurine concentrations are reported in nmol/mL.
According to reference ranges established by the UC Davis Amino Acid Laboratory, heparin plasma TauC <60 nmol/mL and WB TauC <200 nmol/mL were considered low, while concentrations <40 nmol/mL in heparin plasma or <150 nmol/mL in WB were considered critically low (i.e. concentrations associated with a risk of secondary disease). The lower reference range for EDTA plasma communicated by IDEXX Laboratories was <44 nmol/mL and the laboratory did not differentiate between low and critically low concentrations. The cut-offs used in papers I-IV were based on these reference ranges, except for the lower EDTA cut off in paper III and IV, where plasma reference ranges from the UC Davis Amino Acid Laboratory (<60 nmol/mL) were used. Dogs were classified as having <B-TauC if concentrations were below normal reference range in one or more additive.

5.3.3 Genetic analyses (paper IV)

Blood samples (1-2 mL EDTA blood) from each included dog were collected and stored at −80 °C until transported frozen to Utrecht University (the Netherlands) for genetic analyses.

5.3.4 Dietary evaluation (papers II-IV)

Information regarding each dog's diet at the time of enrollment and during the preceding three months was obtained from owner questionnaires (paper II-IV). Diet composition was determined from ingredient lists provided by the manufacturers. Protein sources were categorized into four groups: red meat, white meat, mixed red/white meat, and other protein sources such as plant-based ingredients or insects. A diet was classified as based on red or white meat only if all protein sources could be categorized as either red or white meat. Fish oil or animal fat were not taken into consideration in the categorization. Diets were categorized as grain-inclusive or grain-free based on the presence of grains or grain-derived ingredients, and as pulse/potato-inclusive based on the presence of potato, sweet potato, or legumes (peas, lentils, soybeans) among the first 10 ingredients on the ingredients list (paper II-IV). In paper II, samples of the dry diets consumed

by the dogs at baseline were collected and analyzed for taurine, methionine, and cysteine concentrations at Eurofin Laboratories, Lidköping (Sweden), and these data were used for diet classification in papers II-IV.

5.3.5 Heritability and genetic factors (paper IV)

Data and samples used in paper IV were derived from the ECS enrolled in papers II and III. Dogs were identified based on microchip identification and registered ownership records in the Swedish Kennel Club database. Pedigree information was retrieved and used to construct a relationship matrix, which was filtered to include only relatives of dogs with available taurine measurements.

Genetic analyses were performed on blood samples from 174 dogs enrolled in papers II and III at Utrecht University. Genome-wide association analyses (GWAS) were performed to identify genetic loci associated with <EDTA-TauC. Genomic DNA was extracted from EDTA blood samples and genotyped using a high-density SNP array. A subset (n = 25) of samples was additionally subjected to whole-genome sequencing, which was used as a reference panel for genotype imputation. The SNP positions were based on the canine reference genome (UU_Cfam_GSD_1.0) (151). Genotype data were quality controlled prior to analysis, including filtering of SNPs and samples with low call rates, as well as minor allele frequency and Hardy-Weinberg equilibrium thresholds. Imputation was performed using a Hidden Markov Model approach, and only variants with high imputation accuracy were retained.

5.4 Statistical analyses (papers I-IV)

Statistical analyses were performed using commercially available software (JMP Pro v. 16.0.0, Cary, NC, USA; R v. 4.1.1). Data were analyzed using descriptive as well as inferential statistics and presented as medians with interquartile ranges (IQR) or means with standard deviations (SD). A value of $P < 0.05$ was considered significant for the analyses, unless otherwise indicated.

Nonparametric Wilcoxon signed-rank tests were used to assess differences in continuous variables between groups (papers I-III). Chi-square and Fisher's exact tests were used to analyze association between categorical variables (paper II).

Univariable and multivariable regression analyses were used to assess associations between B-TauC, dog characteristics, and dietary factors (papers I-III). In the multivariable models, variables with $P < 0.2$ in the

univariable analyses were included. All variables were assessed as main effects only, and model performance was evaluated using adjusted R^2. Agreement between sample types was evaluated using Bland-Altman analysis (paper I).

Repeated-measures models (paper I) and linear mixed-effects models (paper III) were used to assess the effects of sampling and examination time, with dog included as a random effect. Variation was assessed using coefficients of variation and Friedman's nonparametric ANOVA (paper I). Groupwise comparisons of continuous variables were performed using the Tukey-Kramer test (paper III), and descriptive statistics were applied for long-term follow-up data (paper III).

In paper IV, Heritability of B-TauC was estimated based on concentrations in EDTA-plasma, heparin plasma and WB (EDTA-TauC, Hep-TauC, and WB-TauC), using mixed linear animal models. The models included dietary protein source (red meat or not) as a fixed effect and age (in days, rescaled to years) as a covariate. A random genetic effect of the individual dog was included, structured according to the pedigree-based relationship matrix. A bivariate version of the model was used to estimate genetic correlations between B-TauC.

For molecular genetic analyses, breed identity and population structure were assessed using genetic clustering and multidimensional scaling. Association analyses were conducted using B-TauC as a quantitative trait, with dietary protein source included as a covariate. Population stratification and relatedness were accounted for, and model performance was evaluated using standard diagnostic measures.

6. Results

This section summarizes the main findings from the studies included in the thesis. More detailed results are presented in the individual papers.

6.1 Variability and agreement of B-TauC (paper I)

Twenty dogs (11 females and nine males) were included. The population consisted of 10 ECS and 10 dogs of various 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).

6.1.1 Agreement of taurine concentrations (paper I)

Assessment of Bland–Altman plots showed good agreement between the two plasma analyses in the group of dogs evaluated, with a mean difference of 4.5 nmol/mL and a mean percentage difference of −7% (paper I, figure 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 TauC, and 50.5 nmol/mL (37%) compared to heparin plasma TauC.

6.1.2 Taurine concentrations over time (paper I)

No association was found between B-TauC and sampling time points, except for a small but statistically significant decrease in heparin plasma TauC at the fourth sampling time point (performed at six hours after the first meal), compared to the first and fifth (performed after 12 hours of fasting and one hour after the second meal, respectively) (paper I, figure 2, and figure 12).

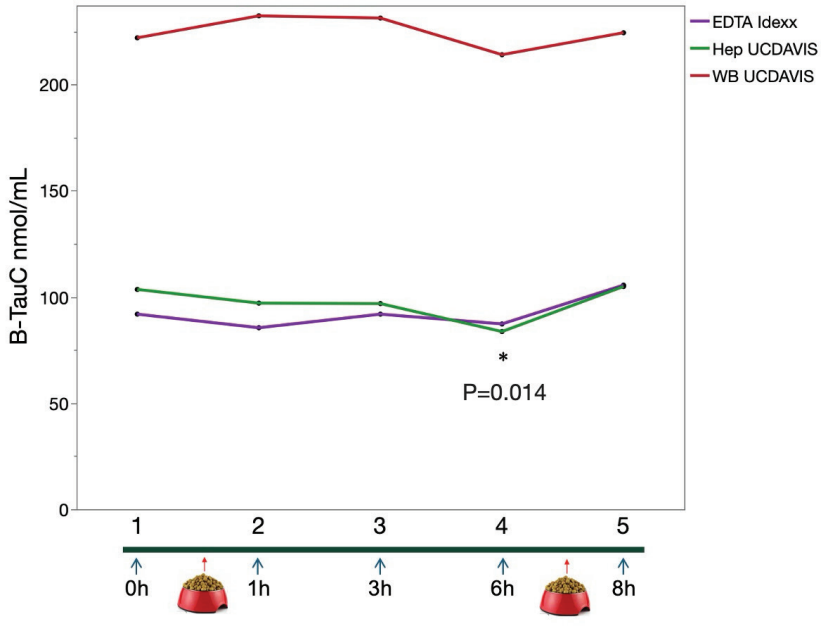


Figure 12. Taurine concentrations in 20 dogs measured in whole blood (red line), heparin plasma (green line), and EDTA plasma (purple line) at five time points (0, 1, 3, 6, and 8 h), with sampling performed in relation to feeding. No clear association between taurine concentrations and sampling time was observed. Heparin plasma concentrations were slightly higher at the first (12 h fasted) and fifth (1 h after the second meal) time points compared to the fourth time point (6 h after the first meal). B-TauC: blood taurine concentration.

6.1.3 Intraindividual variation (paper I)

In individual dogs, B-TauC varied considerably between sampling time points, with the largest variation observed in plasma samples, where intraindividual variability expressed as coefficient of variation (CV) were 26.4% (5.9–111.8%) for EDTA plasma, and 23% (12.6–83.9%) for heparin plasma, compared to 8.8% (3.6–26.9%) for WB. Pairwise comparisons of all 20 dogs showed that the CV for WB was significantly smaller than the CV for the two plasma additives ($P<0.0001$). Despite this, WB concentrations varied from below to within the normal reference range within the same day in seven (30%) of the dogs when the lower reference limit of 200 nmol/mL was applied. When the WB cut-off was set at 150 nmol/mL (i.e., critically low), this variation was observed in only two dogs.

A similar variation was observed in only one dog in EDTA plasma analyses and was not observed in any dogs in heparin plasma (paper I, figure 3).

6.2 B-TauC, clinical findings, and diet (paper II)

Of the 180 included ECS (109 females and 71 males), 167 (93%) were enrolled via screening of presumed healthy dogs, and 13 (7%) were enrolled as clinical cases, presenting with clinical and radiographic signs of CHF. Taurine concentration in EDTA-plasma was available for all 180 dogs and EDTA TauC was therefore used for comparisons and statistical analyses. The results for heparin plasma (n = 172) and WB (n = 175) are reported in paper II (table 1).

A total of 53 (29%) dogs had B-TauC below the normal reference range, and 38 (21%) of these dogs had B-TauC considered critically low (figure 13). In addition, all 13 dogs presenting with clinical and radiographic signs of CHF had B-TauC considered critically low (paper II, figure 1).

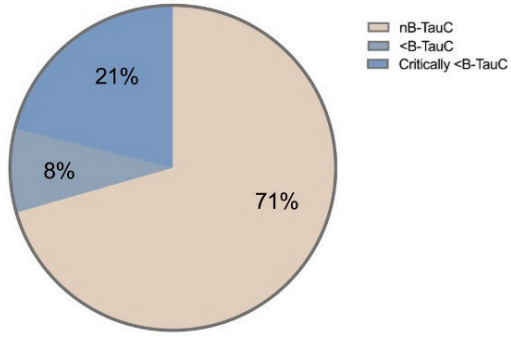


Figure 13. Distribution of blood taurine concentrations (B-TauC) in the study population of 180 English Cocker Spaniels (paper II). Twenty-nine percent of dogs had concentrations below the reference range, of which 21% were critically low.

The distribution between low and critically low concentrations was uneven across the different additives when reference ranges provided by the analyzing laboratories (EDTA <44 nmol/mL, heparin <60 nmol/mL, and WB <200 nmol/mL) were used. In addition, 10 dogs lacked taurine analyses in either heparin plasma or WB, or both, and four of these dogs had B-TauC considered critically low, which most likely skewed the distribution of low versus critically low B-TauC between additives. When

analyses were adjusted to include only dogs with taurine analyzed in all additives and to apply the same lower cut-off for both plasma methods (<60 nmol/mL), the distribution across the three additives showed good agreement (figure 14).

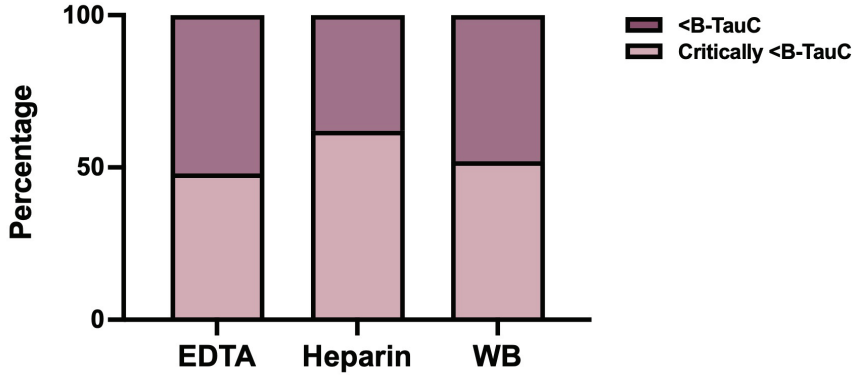
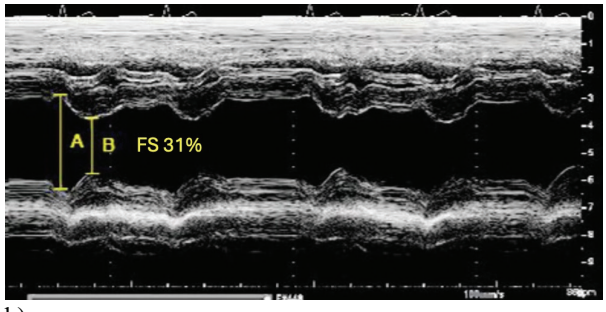


Figure 14. Stacked bars illustrating the distribution between low and critically low concentrations in the three additives (EDTA-plasma, heparin plasma and whole blood) (paper II), including only dogs with taurine analyzed in all additives (n=170) and using the same lower cut-off for both plasma methods (<60 nmol/mL). EDTA: EDTA-plasma concentrations, Heparin: heparin plasma concentrations, WB: whole blood concentrations, <B-TauC: low blood taurine concentrations,

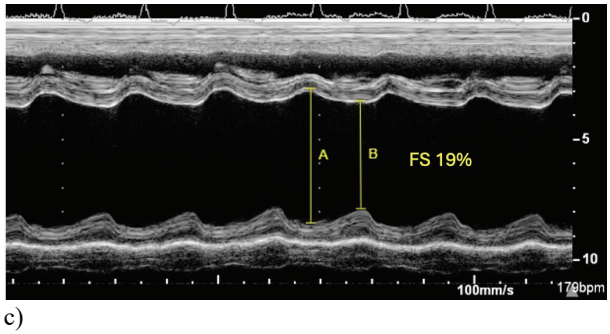
6.2.1 Echocardiographic findings (paper II)

All 13 dogs presenting with CHF had severely increased LV dimensional and voluminal variables, increased LVEPSS and decreased LVEF, all consistent with a DCM-phenotype. Left ventricular FS was, however, less affected and remained within or slightly below the reference range in 11 dogs and was <15% in only two dogs (paper II, table 1, and figure 15 a-c). The remaining 167 dogs (nB-TauC n=127, <B-TauC n=40) had echocardiographic dimensional, voluminal, and functional variables within normal reference ranges (paper II, table 1).

a)



b)



c)

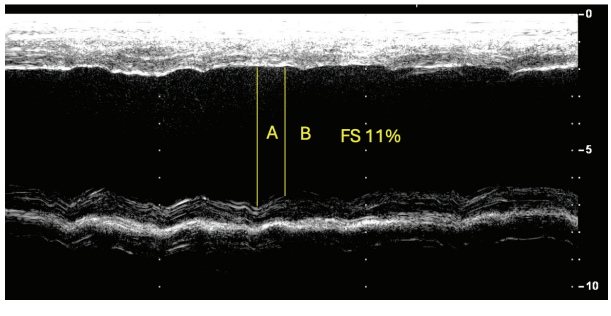


Figure 15. Echocardiographic M-mode measurements of the left ventricle (LV) from three English Cocker Spaniels. Line A: LV internal dimension in diastole (LVIDd), line B: LV internal dimension in systole (LVIDs). FS: fractional shortening.
a) M-mode of the LV in one of the ECS with normal systolic function.
b) M-mode of the LV in one of the ECS with a DCM-phenotype but relatively preserved LVFS.
c) M-mode of the LV in one of the ECS with a DCM-phenotype and severely decreased LVFS. This dog has a dyssynchronous wall motion (and no ECG tracing), making measurements less reliable, however, the image clearly illustrates reduced LV wall motion.

6.2.2 Ophthalmologic findings (paper II)

Bilateral, symmetrical, elliptical hyperreflective lesions in the area centralis, located dorso-temporal to the optic disc and consistent with retinal abnormalities described in taurine-deficient cats, were observed in six dogs; five with <B-TauC and one with nB-TauC (paper II, a-c).

6.2.3 Dietary information (paper II)

Almost all dogs were fed a commercial complete dry food and had regular access to treats, leftovers, or other animals' food (96% and 97% respectively). Each dog was reported to be fed one main diet, and in total, 68 different diets (60 dry foods and eight raw foods) from 29 manufacturers were recorded and classified based on protein source, grain-, and pulse/potato inclusion (paper II, figure 3).

Based on the classification of dietary protein sources, red meat–based diets were more common than diets based on white meat, mixed protein sources, or other protein sources (e.g., soy, insects, legumes) (figure 16 a). Despite this, a majority of dogs were fed a diet based on white meat (figure 16 b). The distribution of red-, and white-meat based diets containing grain or pulses/potato was similar (figure 17 a and b).

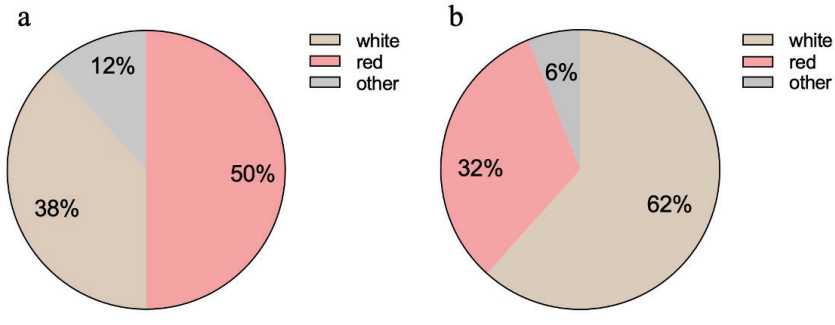


Figure 16. Distribution of diets by primary protein source (a) and of dogs fed diets with different primary protein sources (b) (paper II)
a) Distribution of protein sources in the 60 reported dry diets: 30 diets contained red meat (lamb, beef, pork, reindeer, and venison) as the main protein source, 23 diets contained white meat (poultry and fish), and seven contained mixed or other protein sources.
b) Distribution of dogs (n = 180) based on the protein source of their diet: the majority (n = 111) were fed diets based on white meat, while the remainder were fed diets based on red meat (n = 58) or mixed/other protein sources (n = 11).

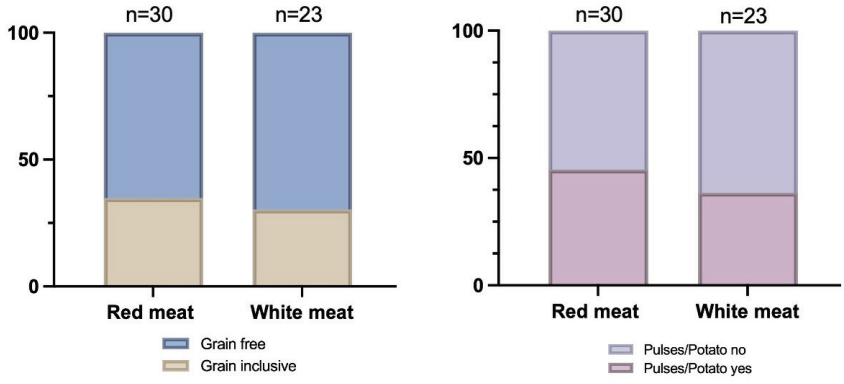


Figure 17. Grain and pulses/potato content of red-, and white-meat based diets (paper II).
a) Proportion of grain-free and grain-inclusive diets in red- and white-meat based diets.
b) Proportion of pulses/potatoes-free and pulses/potatoes-inclusive diets, in red- and white-meat based diets.
Similar distributions of grain and pulse/potato inclusion were observed between red- and white-meat based diets.

The 60 dry foods recorded were analyzed for taurine, cysteine, and methionine concentrations (dry matter), and a considerable variation was observed between the various diets for all three amino acids (paper II, tables S1 and S2). Red meat-based diets contained lower taurine, cysteine, and methionine concentrations compared with white-meat based diets (figure 18). Low dietary methionine concentrations were associated with low dietary taurine concentrations (figure 19). No differences were found between dietary amino acid concentrations in relation to grain or potato/pulses content in the diets.

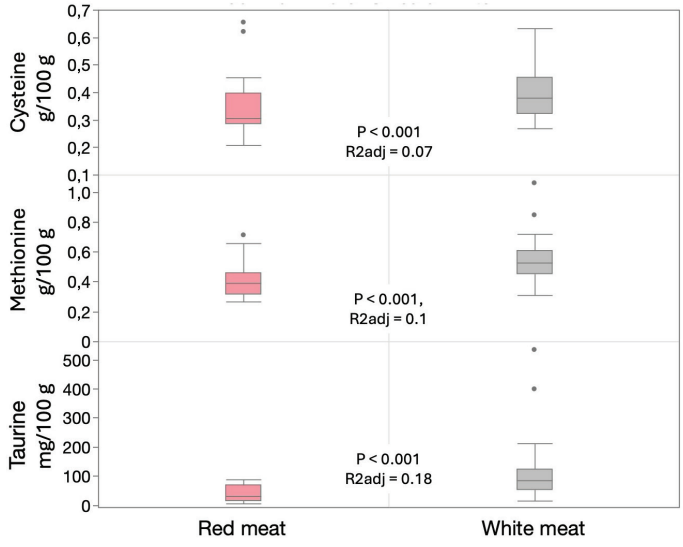


Figure 18. Amino acid concentrations in red- and white-meat based diet (paper II). Red meat-based diets (n=30) contained lower taurine, cysteine, and methionine concentrations, compared with white meat-based diets (n=23). R^2adj: adjusted R^2

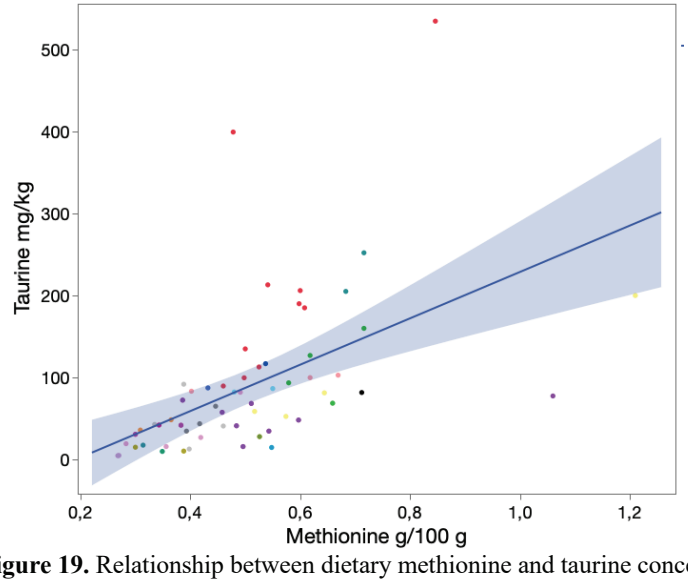


Figure 19. Relationship between dietary methionine and taurine concentrations in analyzed dog foods (n=60). Each dot represents a diets and dots with same colors were produced by the same manufacturer. The line represents linear regression and the shaded areas the 95% confidence interval. A positive association between methionine and taurine content was observed, with a stronger association at lower methionine concentrations and greater variability at higher concentrations.

The diets fed to the dogs were unevenly represented, with products from certain manufacturers fed to many dogs while others were only reported once. Two manufacturers stood out, with diets fed to 42 (23%) and 30 (17%) dogs, respectively, and these diets were all classified as white-meat-based with methionine and taurine concentrations in the higher end of the measured range (figure 20).

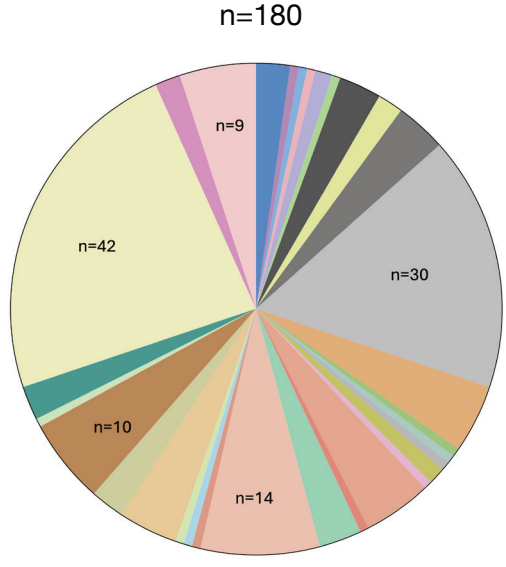


Figure 20. Pie chart illustrating the number of dogs out of total 180 ECS fed products from 29 different manufacturers in the study population (paper II). The distribution was uneven, with diets from certain manufacturers fed to multiple dogs, while others were fed to only one. Two manufacturers were overrepresented (n = 42 and n = 30), with all associated diets classified as white meat-based and with both dietary methionine and taurine concentrations in the higher end of the measured range. The number of diets is shown for manufacturers represented by nine or more dogs.

6.2.4 Factors associated with EDTA-TauC (paper II)

In the univariable analyses, diets with red meat as the main protein source were associated with lower EDTA-TauC compared with diets based on white meat. Grain-free diets and diets containing potatoes or pulses were also associated with lower EDTA-TauC, although these associations were weak. The association between red meat-based diets and lower EDTA-TauC remained regardless of grain content or inclusion of potatoes or pulses. EDTA-TauC was positively associated with dietary taurine and methionine concentrations. In addition, B-TauC decreased with increasing age.

In the final multivariable regression model, only dietary methionine concentration, protein source (red vs white meat), and age remained independently associated with EDTA-TauC ($P < 0.001$; adjusted R^2 0.39) (paper II, figure 4 a-c). Protein source showed the strongest effect, followed by dietary methionine concentration, whereas age had a weaker but significant effect (figure 21).

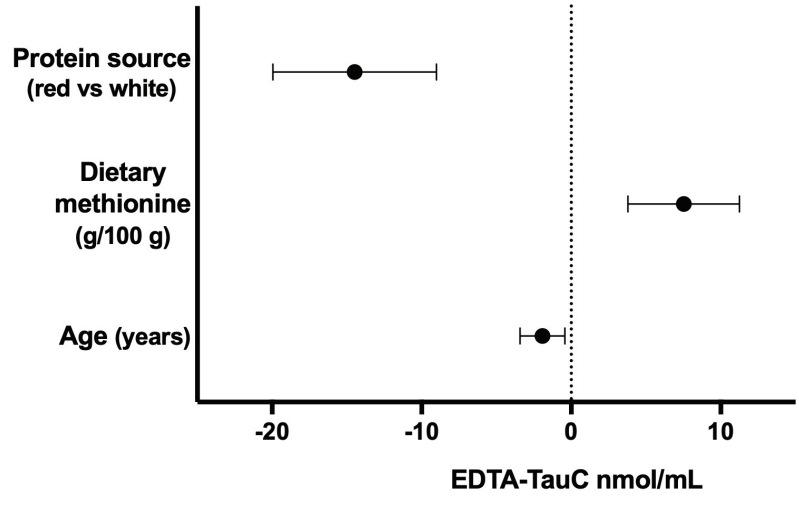


Figure 21. Multivariable linear regression model of factors associated with EDTA-TauC in 180 dogs. Points show estimated effect with 95% confidence intervals. Dietary methionine was associated with higher EDTA-TauC, whereas red meat and increasing age were associated with lower concentrations.

6.3 Response to taurine supplementation (paper III)

In total, 41 of the 53 dogs (77%) included in Paper II were followed longitudinally, including 30 dogs from the <B-TauC:CHF– group and 11 dogs from the <B-TauC:CHF+ group. The <B-TauC:CHF– dogs were examined at two time points (3- and 6-month follow-up). In the <B-TauC:CHF+ group, 11 dogs were examined at the 3-month follow-up. One dog was subsequently lost to follow-up, leaving 10 dogs that were examined at both the 6- and 12-month time points (figure 22).

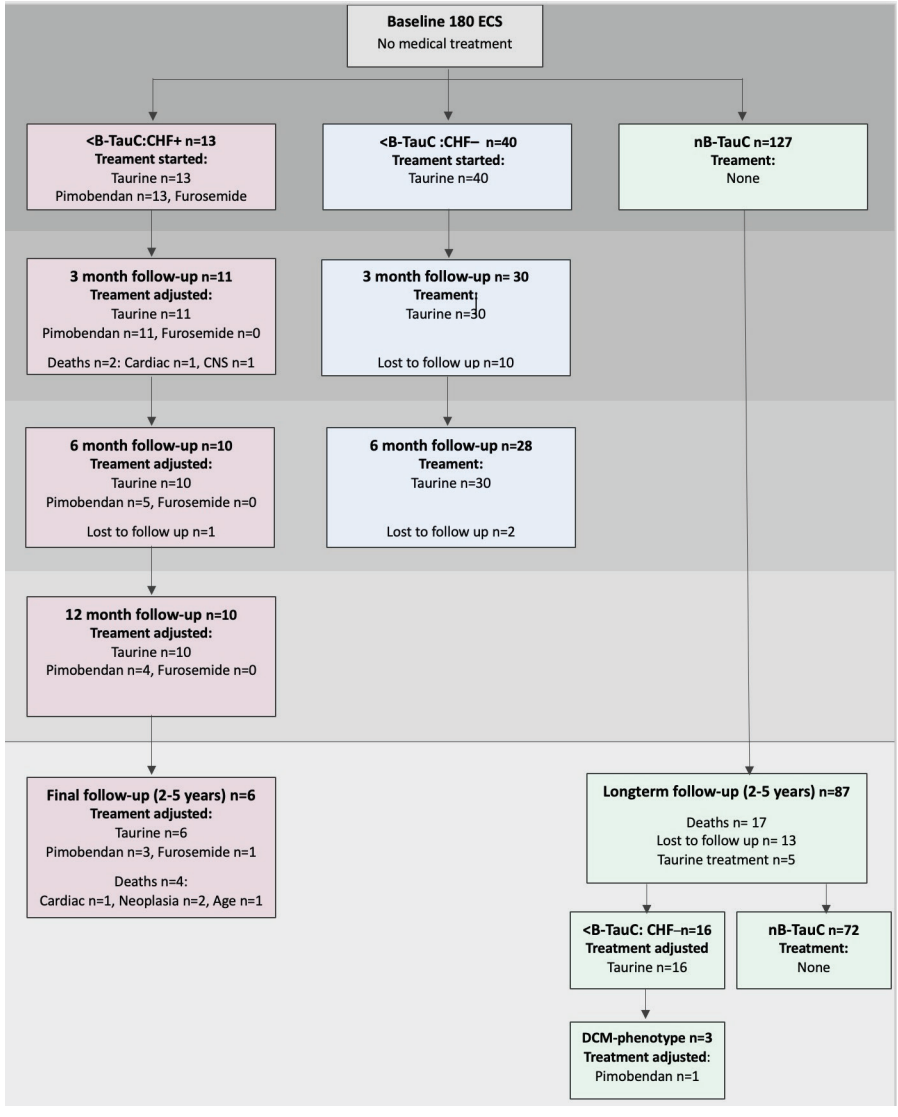


Figure 22. Flow chart of follow-up examinations in paper III. English Cocker Spaniels (n = 180) were classified at baseline (paper II) into three groups: <B-TauC:CHF+ (n = 13), <B-TauC:CHF– (n = 40), and nB-TauC (n = 127). <B-TauC:CHF+ dogs were followed at 3, 6, and 12 months and at 2–5 years; <B-TauC:CHF– dogs at 3 and 6 months; and nB-TauC dogs only long-term (2–5 years after enrollment).The diagram shows the number of dogs at each time point, treatment adjustments, loss to follow-up, and deaths. In the nB-TauC group, five dogs received taurine supplementation despite normal taurine concentrations and were excluded from the long-term follow-up but included in survival analyses. CHF: congestive heart failure.

6.3.1 Taurine concentrations in <B-TauC dogs (paper III)

Taurine supplementation restored B-TauC to within the normal range by the 3-month follow-up in all dogs in all additives, and concentrations remained within the normal range throughout follow-up (paper III; table 1, and figure 23). Data were available for 41 dogs at the three-month follow-up and 38 dogs at the six-month follow-up.

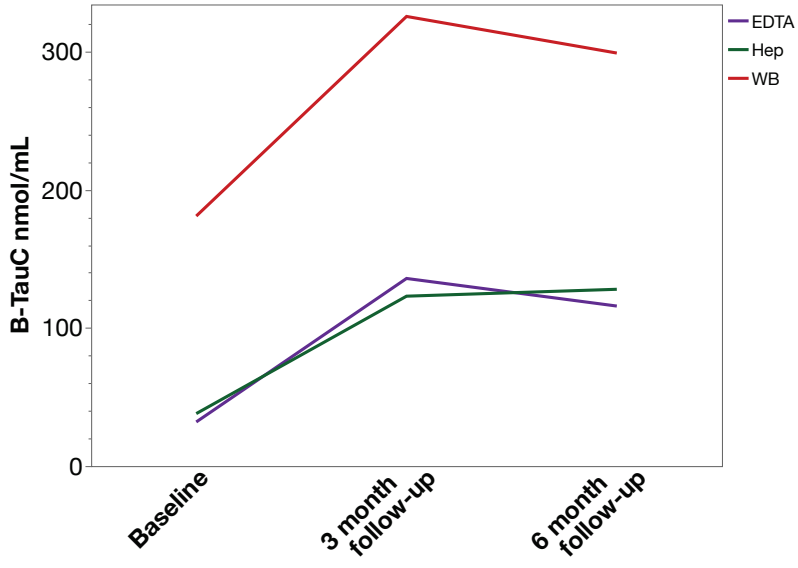


Figure 23. Median taurine concentrations (B-TauC) in dogs with low B-TauC with and without congestive heart failure measured in EDTA plasma, heparin plasma, and heparinized WB at baseline (n = 53), 3-month follow-up (n = 41), and 6-month follow-up (n = 38). Taurine supplementation restored B-TauC to within the normal range in all dogs across all additives, and concentrations remained within the normal range throughout follow-up.

6.3.2 Echocardiographic variables (paper III)

All 11 <B-TauC:CHF+ dogs showed a significant reduction in LA size by the three-month follow-up, and diuretic therapy could be tapered out and discontinued in all dogs (paper III, figure 2).
At the six- and 12-month follow-up examinations, six of 10 (60%) dogs showed normalization (n = 5) or marked improvement (n = 1) in LV size and systolic function, allowing discontinuation of pimobendan in five dogs at six months and in one dog at 12 months after diagnosis.

In the <B-TauC:CHF– group, all echocardiographic variables remained within normal reference ranges during the study period. In addition, LVIDs and LVEPSS decreased at the three-month follow-up compared with baseline (both $P < 0.01$). Echocardiographic results from follow-up examinations of dogs with <B-TauC are presented in paper III, table 1a-b.

The <B-TauC:CHF+ group was followed for a median time of 3.19 years [IQR 2.65–4.88], and all six dogs with normalized cardiac function remained clinically and echocardiographically normal at all subsequent cardiac examinations while receiving only taurine supplementation. Of the four dogs with persistent LV dilatation requiring continued pimobendan therapy, two remained stable, whereas two developed recurrent signs of CHF approximately two and three years after enrollment.

At the final follow-up, six (55%) <B-TauC:CHF+ dogs were alive and re-examined. Three had LV size and function within the normal variation, whereas three had persistent LV dilatation with systolic dysfunction and were still on pimobendan therapy. One of these dogs was also receiving furosemide (1.5 mg/kg q12h) but was clinically stable at the time of follow-up. One dog had been euthanized because of recurrent CHF two years and three months after diagnosis, and three dogs had died or been euthanized due to non-cardiac causes during the study period.

6.4 Longitudinal evaluation of nB-TauC dogs (paper III)

Ninety-two (72%) of 127 dogs (58 females and 34 males) from the nB-TauC group were re-examined in the long-term nB-TauC follow up study. Of these, five dogs had received taurine supplementation despite nB-TauC and were therefore excluded from the analyses. Time between inclusion and follow-up ranged from two years and 11 months to five years and one month after enrollment (median 3.7 [IQR 3.1-4.1 years]).

Taurine concentrations remained within the normal reference range in 71 of 87 dogs (82%), whereas 16 dogs (18%) had B-TauC below the reference range, including five dogs with critically low concentrations.

Echocardiographic variables remained within normal variation in 83 of 87 dogs (95%), whereas three dogs showed systolic dysfunction, all with <B-TauC. Two of these dogs were treated with taurine supplementation in combination with pimobendan, and one dog received taurine supplementation alone. In addition, one dog had developed myxomatous mitral valve disease.

Thirty-nine dogs (45%) were fed a different diet than at baseline. Statistical analyses of dietary composition between groups were limited due to the low number of dogs with <B-TauC. However, the overall patterns were

consistent with those reported in paper II. Diets classified as red meat-based, grain-free, and pulses/potato inclusive were more common in dogs with <B-TauC (including the three dogs with a DCM-phenotype), and dietary taurine and methionine concentrations were positively associated with EDTA-TauC (paper III, figure 3 and 4).

6.5 Survival and outcomes (paper III)

Owners of 176 of the 180 dogs (98%) included in paper II completed the questionnaire, which was conducted between two years and 10 months and four years and nine months after enrollment (median 3.25 years [IQR 2.93-3.85]). Overall survival was high, with 145 (82%) dogs alive at follow-up. Thirty-one dogs (18%) died or were euthanized during the study period, including 17/127 (13%) in the nB-TauC group, 7/40 (18%) in the <B-TauC:CHF– group, and 7/13 (54%) in the <B-TauC:CHF+ group.

Mortality was significantly higher in the <B-TauC:CHF+ group ($P = 0.001$), whereas no difference was observed between the <B-TauC:CHF– and nB-TauC groups. Causes of death in the <B-TauC:CHF+ group included neoplasia (n = 3), cardiovascular disease (n = 2), age-related conditions (n = 1), and central nervous system disorders (n = 1). Of the two dogs that died from cardiac causes, one died the day after inclusion, while the other died from recurrent CHF after 2 years and 3 months. Neoplasia and age-related conditions were also the most common causes of death across all groups.

6.6 Heritability and genetic analyses (paper IV)

Heritability analyses indicated a genetic influence on B-TauC, and heritability estimates were 0.186 (SE 0.197) for EDTA-TauC, 0.435 (SE 0.230) for Hep-TauC, and 0.000 (SE 0.178) for WB-TauC. For WB-TauC, all models converged to zero despite testing different starting values. The genetic correlation between EDTA-TauC and Hep-TauC was 0.79 (SE 0.28), whereas correlations involving WB-TauC could not be estimated. Dietary protein source was significantly associated with B-TauC across all additives ($P < 0.05$), while age was not significantly associated with Hep-TauC.

A total of 174 samples and 143,474 SNPs passed quality control following SNP genotyping. Whole genome sequencing in 25 samples resulted in 8,222,863 SNPs and 2,455,149 indels in the population cohort when compared to UU Cfam GSD 1.0. Imputation resulted in a total of

2,270,466 SNPs in 174 dogs. The mean imputation quality (R^2) of all non-sporadic SNPs (based on the whole imputed dataset) was 0.89.
Assessment of population structure using an MDS plot did not reveal clear stratification between cases and controls, which was supported by a genomic inflation factor of $\lambda = 1.06$.
The association analysis identified a region of interest on chromosome 17 after adjustment for diet. The most associated SNP (chr17:42375828) did not reach genome-wide significance ($P = 4.3 \times 10^{-6}$) but represented the strongest signal in the dataset.

7. General Discussion

7.1 Agreement and variation in B-TauC (paper I)

Overall, the different analytical methods and anticoagulant additives showed an acceptable agreement when B-TauC were evaluated in the group of dogs. Concentrations varied between sampling time points but showed a similar pattern for all additives, suggesting that at least part of the observed variation reflect biological variability rather than differences related to the analytical method or additive. The variations were not associated with time from feeding (paper I, figure 2, and figure 9), which is consistent with previous studies (48, 152), and suggests that fasting is probably not necessary prior to sampling.

When evaluated separately for each dog and sampling time point, B-TauC showed substantial intra-individual variation, with the greatest variation observed in plasma samples. Plasma samples are sensitive to preanalytical errors and require careful handling during collection, as hemolysis, cellular damage, prolonged clotting time, or disruption of the buffy coat during separation can result in contamination from taurine-rich cells and falsely increased taurine concentrations (139, 153, 154). Preanalytical factors may explain some of the intra-individual variation observed in our study but are unlikely to be the sole contributing factor. Samples were handled according to a standardized protocol (138), and all sample preparation was performed by the same two individuals to minimize variation in handling. In addition, substantial variation was observed also in WB concentrations, which are not separated before analysis and therefore not subject to the same preanalytical errors. Despite lower mean intra-individual variation in WB compared to plasma samples, WB-TauC still varied from below the reference range to normal within the same day in 30% of the dogs, whereas this was observed in only one dog in plasma samples (paper I, figure 3). This may indicate that the current lower WB reference limit of 200 nmol/mL is not sufficiently specific to distinguish between deficient and non-deficient dogs, and that concentrations near the lower limit may not be clinically relevant. This is supported by the fact that when the WB cut-off for critically low concentrations was applied, such fluctuations between normal and low were observed in only two dogs. Some of the observed intra-individual variation could also reflect analytical variability, as duplicate samples were not analyzed to assess intra-assay variation. However, intra-assay variability was reported to be less than 10% for all analytical methods used in this study (137, 138).

Importantly, dogs with low B-TauC (plasma <15 nmol/mL; WB <50 nmol/mL) showed minimal variation between sampling occasions across all additives, indicating that severe deficiency is consistently associated with low variability regardless of additive (paper I, figure 3).

7.2 Taurine deficiency and TauR-CM in ECS (paper II and III)

7.2.1 Blood taurine concentrations in ECS (paper II and III)

When results from both studies were combined, 38% of the 180 included ECS had been diagnosed with <B-TauC at either baseline or follow-up examinations, and 61% of these had critically low concentrations. These findings can be put into perspective by comparison with a previous prospective study investigating canine reference ranges for B-TauC, in which 11.5% of dogs of various breeds were identified with <B-TauC, and of which 15% had critically low concentrations (118). Given the substantial intra-individual variation in B-TauC observed in paper I, variation in concentrations close to the lower reference limit may have resulted in misclassification of some dogs in papers II and III as either <B-TauC or nB-TauC. However, the high proportion of dogs with critically low concentrations suggests that most dogs were correctly classified and that taurine deficiency in ECS may be more widespread than previously thought.

Taurine concentrations were restored to within the reference range in all dogs within three months after initiating supplementation and remained within normal range throughout the study period (paper III). Taurine supplementation dosing in dogs is not based on established nutritional requirements but rather on empirical use and previous clinical studies (2, 3, 155). The dose used in the present study (250 mg q12h) represents the lowest reported dose, and the consistent response observed in our study population indicates that this dose is sufficient for ECS with <B-TauC and may also be sufficient for other small- to medium-sized breeds.

7.2.2 Cardiac abnormalities (paper II and III)

All dogs with echocardiographic findings consistent with a DCM-phenotype presented with concurrent signs of CHF, and all these dogs also had critically low B-TauC. In contrast, no echocardiographic abnormalities were detected in dogs included via screening, despite 24 of 40 dogs having critically low B-TauC. A similar discrepancy between <B-TauC and

clinical findings has been reported in cats fed a taurine-depleted diet over two to four years, where only a subset developed myocardial failure despite critically low concentrations in all cats (100, 156). These variations suggest that progression from <B-TauC to deficiency and clinical disease differs between individuals and may depend on the duration of taurine deficiency and various individual factors affecting susceptibility.

All dogs with a DCM-phenotype showed markedly increased systolic and diastolic dimensions and volumes consistent with established echocardiographic criteria for a DCM-phenotype (24, 29, 143). Fractional shortening was, however, only mildly reduced or even within normal reference ranges in many of the dogs, which is not in line with current echocardiographic DCM guidelines (24, 29, 143). This may reflect limitations of LVFS as a measure of systolic function, as asymmetrical remodeling or regional wall motion abnormalities may be overlooked when relying on a single linear measurement. It is also possible that the observed variations reflect individual variability in the clinical presentation of TauR-CM.

In paper III, cardiac dimensions and systolic function improved in all <B-TauC:CHF+ dogs during the study period. A significant reduction in LA size was observed as early as three months after diagnosis, allowing discontinuation of diuretic treatment in all dogs. In addition, six of ten dogs had regained normal or near-normal cardiac size and systolic function and could be weaned off pimobendan within a year from diagnosis. All six remained stable for several years after enrollment while receiving only taurine supplementation. This degree of reversal of cardiac changes and discontinuation of diuretic therapy are very uncommon in dogs with CHF due to primary idiopathic DCM (6, 28, 30), and support that ECS, similar to ACS, are affected by TauR-CM.

Four dogs did, however, have persistent LV dilatation and systolic dysfunction at all follow-up examinations and two of these also developed recurrent CHF after approximately two and a half, and three years, respectively. Similar observations of persistent myocardial dysfunction, with long periods of stability before recurrence of CHF, have been reported in ACS and Golden Retrievers with TauR-CM (2, 12). Although ECS have been reported to have longer survival times than other breeds when affected by DCM (28), the time between the first and second CHF episodes in these dogs is unusually long, even for this breed.

Experimental studies in taurine-depleted mice and cats, have shown that cardiac changes in early stages of depletion are characterized by myocyte atrophy and eccentric remodeling without marked fibrosis, whereas later stages show more pronounced myocardial loss and fibrotic replacement

(113, 157, 158). Such variations could be an explanation for the variability in myocardial response in the ECS in paper III and indicate that TauR-CM has a favorable prognosis if identified and treated early.

7.2.3 Outcome and survival

The number of deaths was significantly higher in the <B-TauC:CHF+ group, compared to the other two groups. However, only two dogs in this group died from cardiac causes: one the day after diagnosis and the other from recurrent CHF after 2 years and 3 months, suggesting a relatively favorable prognosis for many dogs with TauR-CM if adequately treated. Neoplasia and old age were the most common causes of death in all three groups and the discrepancy in survival may partly be related to the <B-TauC:CHF+ dogs being older than dogs in the other two groups.

7.2.4 Ocular abnormalities (paper II)

Another interesting finding in paper II is the retinal abnormalities observed in five dogs with <B-TauC that corresponds to the focal hyperreflective lesions in the area centralis described in cats with taurine deficiency (paper II, figure 2 a-c) (44, 91, 94, 95). Reports of taurine deficiency-induced retinal degeneration in dogs are limited, but the characteristic thinning and loss of photoreceptor cells are well described in several species and suggest similar underlying mechanisms in dogs (62, 89, 91, 95, 97).

Retinal lesions were also observed in one dog with nB-TauC. These may be unrelated to taurine status, but as retinal lesions associated with taurine deficiency are irreversible, they may also reflect a previous period of taurine deficiency (44, 91, 94).

7.3 Potential causative factors (paper II and III)

7.3.1 Dietary factors (paper II and III)

Red meat-based diets and diets with low methionine concentrations were strongly associated with <B-TauC in our study population, and although dietary TauC did not remain significant in the multivariable analysis, an association between dietary taurine and methionine concentrations was also observed. In contrast to previous reports (11, 15, 32, 41, 45, 117, 118, 159), grain-free or pulse/potato inclusive diets were only weakly associated with B-TauC and the association with red meat persisted regardless of grain-, or pulse/potato inclusion.

Lamb and rice diets have been proposed as potential risk factors for <B-TauC in multiple previous studies, although it remains unclear whether the observed association was related to lamb as a protein source (reported to have lower taurine content than other meat sources), or to rice as a carbohydrate source that may affect taurine absorption and bioavailability (12, 15, 117). Almost 40 % of the red meat-based diets fed to our study population were based on lamb, with or without rice (paper II, figure 3). The red meat association did, however, remain even when the lamb diets were excluded from the analyses.

Although, red meat-based diets stood out as a potential cause of <B-TauC in our study population, the protein source alone is unlikely to explain the findings. The variations in dietary taurine concentrations were, so large in a few cases that they cannot possibly be explained solely by ingredient choice, but rather by taurine being added as a supplement during formulation. However, very few ingredient-lists or manufacturer information clarified whether taurine originated from raw ingredients or supplementation, which limited further interpretation.

In addition, the uneven distribution of diets in paper II, where more than 40% of the dogs were fed white-meat-based diets with high taurine content from the same two manufacturers, likely also influenced the observed associations although it is not possible to know in which direction.

7.3.2 Age (paper II)

Dogs in the <B-TauC:CHF+ group were older than those in the other two groups, a finding also reported in Newfoundland dogs with <B-TauC and which could suggest that age may play a role in disease progression. Age-related declines have also been described in mice and monkeys (160-162), and although the underlying mechanisms for this decline are currently unknown, experimental studies in rodents have shown decreased activity of cysteine dioxygenase (CDO), and reduced endogenous synthesis in aging individuals (163). Other potential contributing factors include age-related altered dietary intake and digestibility, increased losses, and changes in utilization and tissue distribution (164, 165). Decreasing B-TauC with increasing age has been reported in humans (161). However, more recent data suggest that this decline is not consistent and that B-TauC may instead vary substantially between individuals due to differences in diet, metabolism, and other factors (166).

7.4 Potential genetic background (paper IV)

The heritability analyses suggested that genetic factors likely contribute to <B-TauC in ECS, and that environmental or physiological factors, such as diet or age, likely influence how these genetic effects are expressed. Breed-associated occurrence of <B-TauC has also been reported in other breeds, including Golden Retrievers, Irish Wolfhounds and Newfoundlands, and, similar to findings in ECS, certain dietary compositions or ingredients appear to increase the risk of taurine deficiency also in these breeds (45, 46, 53).

The molecular genetic analyses further indicated a combined influence of genetic and dietary factors on B-tauC, as the signal observed on chromosome 17 was identified when dietary protein source was included as a covariate. Although the signal did not reach statistical significance and no clear candidate gene was identified, it does suggest that <B-TauC in ECS is influenced by genetic factors and that certain dietary compositions or ingredients can affect whether the deficiency becomes clinically relevant.

The lack of identifiable genes in this region may have several explanations, one of which could be that the canine genome is still not completely mapped and that this specific region may not be sufficiently characterized. Another explanation could be the presence of multiple variants at different loci affecting various functions that influence taurine bioavailability and homeostasis, or possibly that the region lies within a gene desert which is a region with no known genes but containing regulatory elements that affect the expression of nearby genes (167).

A similar situation has been described in genetic studies of primary DCM in Doberman Pinschers, where one of the first associated loci was observed on chromosome 5, but no candidate gene could be identified at the time of discovery (168). In more recent studies, this region has been better defined, and two different loci associated with disease development have now been reported on chromosome 5, and RNF207 and PRKAA2 have been proposed as candidate genes (169).

7.5 Limitations

Some limitations should be considered when interpreting the findings of this thesis.

7.5.1 General limitations paper I-IV

Diets were not standardized and all dogs were fed diets selected by their owners, resulting in considerable variation in dietary exposure (paper I-IV). This may add some uncertainty, but it may also make the findings more representative of clinical situations in which diets are rarely strictly controlled.

Dietary information was based on owner-reported data, which may not fully reflect exact feeding habits over time. Information may sometimes be incomplete or inaccurate, especially when more than one person is involved in feeding the dog. The frequent inclusion of treats and leftovers further complicated assessment of dietary effects.

In addition, diet composition was assessed based on information provided by the manufacturers, and only one batch per diet was analyzed for taurine, methionine and cysteine concentrations. Further studies of diet composition using more advanced analyses would most likely provide more detailed insights into the factors influencing taurine concentrations in dogs.

7.5.2 Study specific limitations

In paper I, the number of dogs included was limited, although a large number of blood samples were analyzed. Group comparisons were performed only after <B-TauC were identified in a subset of clinically healthy ECS, and dogs were therefore not matched with respect to age, body weight, or sex., which should be considered when interpreting these results.

In paper II, heparin plasma and WB TauC were not assessed in a subset of the of dogs presenting out of hours with CHF. EDTA-plasma concentrations were, however, markedly low in all these dogs (<8 nmol/mL), and this, together with the agreement of <B-TauC between the different additives observed in paper I, strongly suggests that B-TauC would also have been below the reference range in heparin plasma and WB samples.

In paper III, B-TauC was assessed using EDTA plasma only, and some dogs with potentially low concentrations in the other additives may therefore have been misclassified. However, the overall acceptable

agreement between additives in paper I suggests that this effect was probably limited and that dogs with critically low concentrations most likely were correctly identified.
Loss to follow-up and variation in follow-up duration should also be considered, as these factors may have affected assessment of long-term outcomes. In addition, all dogs with systolic dysfunction received both taurine supplementation and medical treatment, making it difficult to fully separate the effects of taurine from concurrent therapy. The absence of an untreated control group represents an additional limitation but was considered necessary for ethical reasons.

In paper IV, the main limitation was the relatively small study population that limited statistical power and contributed to uncertainty in the heritability estimates. The study population consisted of ECS enrolled in a previous observational study, and inclusion of dogs was limited by practical and financial factors, as all dogs underwent thorough clinical evaluation with multiple examinations, which are both time-consuming and costly.

Although heritability estimates were strongest when based on Hep-TauC, the molecular genetic analyses used EDTA plasma, which may have introduced some uncertainty in the association results. This choice was made to maximize sample size, as EDTA-TauC was available for all individuals. The good agreement between additives in paper I indicates that the use of different sample types is unlikely to have had a major impact on the results.

Taurine status was assessed based on single measurements, and as intraindividual variation in B-TauC can be substantial, some dogs may have been misclassified, particularly those with values near the lower reference limit.

8. Conclusions

- Taurine concentrations measured in WB and plasma showed acceptable agreement when dogs were assessed as a group. However, substantial intra-individual variation was observed, especially in plasma samples, while fluctuations from below to within the reference range within the same day were more common in WB. Markedly low concentrations were consistently identified across sample types, and time from feeding had no clinically relevant influence.

- Low B-TauC was observed in 29% of the included ECS, and of these, 25% presented with a DCM-phenotype and signs of CHF and 9% had retinal abnormalities. Dietary factors, particularly red meat-based diets and diets with low methionine concentration, were associated with <B-TauC. Dogs with <B-TauC were older than nB-TauC dogs.

- Taurine supplementation restored B-TauC to within normal reference range in all dogs and was associated with significant improvement in cardiac dimensions and systolic function in dogs with a concurrent DCM-phenotype. Long term outcome of dogs with a DCM-phenotype and CHF was good, although persistent cardiac changes and recurrence of signs of CHF occurred in some dogs with more advanced disease.

- Eighteen percent of dogs initially diagnosed with nB-TauC had <B-TauC at follow-up, and three dogs also had developed a DCM-phenotype. Red-meat based diets, grain free-, and pulse/potato inclusive diets, as well as diets with low taurine and methionine dietary concentrations were more commonly fed to dogs with <B-TauC.

- Overall survival was high, and the most common causes of death were neoplasia and age-related conditions. Mortality was highest in the <B-TauC:CHF+ group, but only two dogs died from cardiac disease during the study period.

- Both hereditary and genetic analyses suggested a possible association with taurine deficiency and indicate that dietary factors may have an impact on the phenotype in ECS with a genetic susceptibility.

9. Implications for future research

The results from the studies included in this thesis have identified multiple areas for future research.

- The large number of ECS with <B-TauC suggests that taurine deficiency in this breed is common and may indicate that other dog breeds could be similarly affected. Prospective studies investigating the occurrence of <B-TauC, underlying predisposing factors, and potential associations with cardiac disease or other secondary conditions in the general dog population would likely increase knowledge and help prevent disease development.

- The role of diet composition in the development of <B-TauC and TauR-CM in dogs, is intriguing and needs further investigation. Studies including detailed analyses of ingredients with respect to amino acid content, protein quantity and quality, as well as the impact of processing methods on nutrient availability and dietary taurine content are needed.

- The genetic signal on chromosome 17 suggests that this region may be associated to the development of taurine deficiency in ECS, and further studies in both ECS and other breeds would probably help assess this association in more detail.

- The variation in echocardiographic variables of systolic function in dogs diagnosed with TauR-CM could be further evaluated using additional advanced echocardiographic measures in both ECS and other breeds.

References

1. Pion PD, Kittleson MD, Rogers QR, Morris J. Myocardial failure in cats associated with low plasma taurine: a reversible cardiomyopathy. Science. 1987;237(4816):764–8.
2. Kittleson MD, Keene B, Pion PD, Loyer CG. Results of the multicenter spaniel trial (MUST): taurine-and carnitine-responsive dilated cardiomyopathy in American cocker spaniels with decreased plasma taurine concentration. Journal of veterinary internal medicine. 1997;11(4):204–11.
3. Fascetti AJ, Reed JR, Rogers QR, Backus RC. Taurine deficiency in dogs with dilated cardiomyopathy: 12 cases (1997–2001). Journal of the American Veterinary Medical Association. 2003;223(8):1137–41.
4. Huxtable R. Physiological actions of taurine. Physiological reviews. 1992;72(1):101–63.
5. Kramer GA, Kittleson MD, Fox PR, Lewis J, Pion PD. Plasma taurine concentrations in normal dogs and in dogs with heart disease. Journal of veterinary internal medicine. 1995;9(4):253–8.
6. Tidholm A. Canine dilated cardiomyopathy: A study of 189 cases in 38 breeds. Veterinary Quarterly. 1996;18(sup1):34–5.
7. Martin M, Stafford Johnson M, Celona B. Canine dilated cardiomyopathy: a retrospective study of signalment, presentation and clinical findings in 369 cases. Journal of Small Animal Practice. 2009;50(1):23–9.
8. Huxtable RJ, Michalk D. Taurine in health and disease: Springer Science & Business Media; 2013.
9. Schaffer SW, Ito T, Azuma J, Jong CJ, Kramer JH. Mechanisms underlying development of taurine-deficient cardiomyopathy. Hearts. 2020;1(2):86–98.
10. Schaffer SW, Ito T, Azuma J. Clinical significance of taurine. Amino Acids. 2014;46(1):1–5.
11. Freeman LM, Stern JA, Fries R, Adin DB, Rush JE. Diet-associated dilated cardiomyopathy in dogs: what do we know? Journal of the American Veterinary Medical Association. 2018;253(11):1390–4.
12. Kaplan JL, Stern JA, Fascetti AJ, Larsen JA, Skolnik H, Peddle GD, et al. Taurine deficiency and dilated cardiomyopathy in golden retrievers fed commercial diets. PloS one. 2018;13(12):e0209112.
13. Ko KS, Backus RC, Berg JR, Lame MW, Rogers QR. Differences in taurine synthesis rate among dogs relate to differences in their maintenance energy requirement. The Journal of nutrition. 2007;137(5):1171–5.
14. McCauley SR, Clark SD, Quest BW, Streeter RM, Oxford EM. Review of canine dilated cardiomyopathy in the wake of diet-associated concerns. Journal of animal science. 2020;98(6):skaa155.
15. Tôrres CL, Backus RC, Fascetti AJ, Rogers QR. Taurine status in normal dogs fed a commercial diet associated with taurine deficiency and dilated

cardiomyopathy. Journal of animal physiology and animal nutrition. 2003;87(9-10):359–72.
16. Falik R. Hurst's the Heart. Jama. 2009;301(8):884–5.
17. Standring S. Gray's Anatomy E-Book: Gray's Anatomy E-Book: Elsevier Health Sciences; 2021.
18. Ware WA, Bonagura JD. Cardiovascular disease in companion animals: dog, cat and horse: CRC Press; 2021.
19. Hall JE, Hall ME. Guyton and Hall Textbook of Medical Physiology E-Book: Elsevier Health Sciences; 2020.
20. Ware WA, Bonagura JD, Scansen BA. Myocardial diseases of the dog. Cardiovascular Disease in Companion Animals: CRC Press; 2021. p. 607–48.
21. Tidholm A, Häggström J, Borgarelli M, Tarducci A. Canine idiopathic dilated cardiomyopathy. Part I: aetiology, clinical characteristics, epidemiology and pathology. The Veterinary Journal. 2001;162(2):92–107.
22. Tilley L, Liu S. Cardiomyopathy in the dog. Recent advances in studies on cardiac structure and metabolism. 1975;10:641–53.
23. Borgarelli M, Tarducci A, Tidholm A, Häggström J. Canine idiopathic dilated cardiomyopathy. Part II: pathophysiology and therapy. The Veterinary Journal. 2001;162(3):182–95.
24. Bonagura J, Visser L. Echocardiographic assessment of dilated cardiomyopathy in dogs. Journal of Veterinary Cardiology. 2022;40:15–50.
25. Wess G, Schulze A, Butz V, Simak J, Killich M, Keller L, et al. Prevalence of dilated cardiomyopathy in Doberman Pinschers in various age groups. Journal of veterinary internal medicine. 2010;24(3):533–8.
26. Vollmar AC. The prevalence of cardiomyopathy in the Irish wolfhound: a clinical study of 500 dogs. Journal of the American Animal Hospital Association. 2000;36(2):125–32.
27. Meurs KM, Miller MW, Wright NA. Clinical features of dilated cardiomyopathy in Great Danes and results of a pedigree analysis: 17 cases (1990–2000). Journal of the American Veterinary Medical Association. 2001;218(5):729–32.
28. Martin M, Stafford Johnson M, Strehlau G, King J. Canine dilated cardiomyopathy: a retrospective study of prognostic findings in 367 clinical cases. Journal of small animal practice. 2010;51(8):428–36.
29. Wess G. Screening for dilated cardiomyopathy in dogs. Journal of Veterinary Cardiology. 2022;40:51–68.
30. Vollmar C, Vollmar A, Keene BW, Fox PR, Reese S, Kohn B. Dilated cardiomyopathy in 151 Irish Wolfhounds: characteristic clinical findings, life expectancy and causes of death. The veterinary journal. 2019;245:15–21.
31. Hickman MA, Rogers QR, Morris JG. Taurine balance is different in cats fed purified and commercial diets. The Journal of nutrition. 1992;122(3):553–9.
32. Ko KS, Fascetti AJ. Dietary beet pulp decreases taurine status in dogs fed low protein diet. Journal of animal science and technology. 2016;58(1):29.

33. Smith CE, Parnell LD, Lai C-Q, Rush JE, Freeman LM. Investigation of diets associated with dilated cardiomyopathy in dogs using foodomics analysis. Scientific Reports. 2021;11(1):15881.
34. Bright JM. Cardiotoxicity in Dogs. Journal of the National Cancer Institute. 1990;82(11):963.
35. Hantson P. Mechanisms of toxic cardiomyopathy. Clinical toxicology. 2019;57(1):1–9.
36. Woodruff J. Viral myocarditis. A review. The American journal of pathology. 1980;101(2):425.
37. Panciera DL. An echocardiographic and electrocardiographic study of cardiovascular function in hypothyroid dogs. Journal of the American Veterinary Medical Association. 1994;205(7):996–1000.
38. Fuentes-Mendoza JM, Concepción-Zavaleta MJ, Morón-Siguas JC, Muñoz-Moreno JM, Pérez-Reyes AI, Martinez-Galaviz R, et al. Cardiomyopathies of endocrine origin: A state-of-the-art review. World Journal of Cardiology. 2025;17(10):111462.
39. Shinbane JS, Wood MA, Jensen DN, Ellenbogen KA, Fitzpatrick AP, Scheinman MM. Tachycardia-induced cardiomyopathy: a review of animal models and clinical studies. Journal of the American College of Cardiology. 1997;29(4):709–15.
40. Umana E, Solares CA, Alpert MA. Tachycardia-induced cardiomyopathy. The American journal of medicine. 2003;114(1):51–5.
41. Freid KJ, Freeman LM, Rush JE, Cunningham SM, Davis MS, Karlin ET, et al. Retrospective study of dilated cardiomyopathy in dogs. Journal of veterinary internal medicine. 2021;35(1):58–67.
42. U.S.Food and Drug Administration (FDA). FDA investigation into potential link between certain diets and canine dilated cardiomyopathy 2019 [updated 19 February, 2019. Available from: https://www.fda.gov/animal-veterinary/cvm-updates/fda-provides-update-investigation-potential-connection-between-certain-diets-and-cases-canine-heart.
43. U.S.Food and Drug Administration (FDA). Questions & Answers: FDA's Work on Potential Causes of Non-Hereditary DCM in Dogs 2022 [updated 2024–08–19. Available from: https://www.fda.gov/animal-veterinary/animal-health-literacy/questions-answers-fdas-work-potential-causes-non-hereditary-dcm-dogs.
44. Hayes KC, Carey RE, Schmidt SY. Retinal degeneration associated with taurine deficiency in the cat. Science. 1975;188(4191):949–51.
45. Bélanger MC, Ouellet M, Queney G, Moreau M. Taurine-deficient dilated cardiomyopathy in a family of golden retrievers. Journal of the American Animal Hospital Association. 2005;41(5):284–91.
46. Backus RC, Ko KS, Fascetti AJ, Kittleson MD, MacDonald KA, Maggs DJ, et al. Low plasma taurine concentration in Newfoundland dogs is associated with low plasma methionine and cyst (e) ine concentrations and low taurine synthesis. The Journal of nutrition. 2006;136(10):2525–33.

47. Li P, Wu G. Amino acid nutrition and metabolism in domestic cats and dogs. Journal of Animal Science and Biotechnology. 2023;14(1):19.
48. Reilly LM, He F, Clark L, de Godoy MR. Longitudinal assessment of taurine and amino acid concentrations in dogs fed a green lentil diet. Journal of animal science. 2021;99(11):skab315.
49. Basili M, Pedro B, Hodgkiss-Geere H, Navarro-Cubas X, Graef N, Dukes-McEwan J. Low plasma taurine levels in English cocker spaniels diagnosed with dilated cardiomyopathy. Journal of Small Animal Practice. 2021;62(7):570–9.
50. McCauley SR, Clark SD, Leach SB, Quest BW, Streeter RM. Evaluation of taurine and carnitine concentrations in whole blood, plasma, skeletal muscle and cardiac muscle in dogs. Journal of Animal Physiology and Animal Nutrition. 2024;108(4):999–1015.
51. Simpson S, Dunning MD, Brownlie S, Patel J, Godden M, Cobb M, et al. Multiple genetic associations with Irish wolfhound dilated cardiomyopathy. BioMed research international. 2016;2016(1):6374082.
52. Philipp U, Vollmar A, Häggström J, Thomas A, Distl O. Multiple loci are associated with dilated cardiomyopathy in Irish wolfhounds. PLoS One. 2012;7(6):e36691.
53. Vollmar AC, Fox PR, Servet E, Biourge V. Determination of the prevalence of whole blood taurine in Irish wolfhound dogs with and without echocardiographic evidence of dilated cardiomyopathy. Journal of veterinary cardiology. 2013;15(3):189–96.
54. Ripps H, Shen W. taurine: a “very essential” amino acid. Molecular vision. 2012;18:2673.
55. Gupta R. Taurine analogues and taurine transport: therapeutic advantages. Taurine 6. 2006:449–67.
56. Donohue J. Hydrogen bonding and the zwitterion structure of taurine. Acta Crystallographica. 1964;17(6):761–.
57. Baliou S, Kyriakopoulos AM, Goulielmaki M, Panayiotidis MI, Spandidos DA, Zoumpourlis V. Significance of taurine transporter (TauT) in homeostasis and its layers of regulation. Molecular medicine reports. 2020;22(3):2163–73.
58. Han X, Patters A, Jones D, Zelikovic I, Chesney R. The taurine transporter: mechanisms of regulation. Acta physiologica. 2006;187(1-2):61–73.
59. Tappaz M. Taurine biosynthetic enzymes and taurine transporter: molecular identification and regulations. Neurochemical research. 2004;29(1):83–96.
60. Schaffer SW, Ju Jong C, KC R, Azuma J. Physiological roles of taurine in heart and muscle. Journal of biomedical science. 2010;17(Suppl 1):S2.
61. Schaffer SW, Shimada-Takaura K, Jong CJ, Ito T, Takahashi K. Impaired energy metabolism of the taurine-deficient heart. Amino Acids. 2016;48(2):549–58.
62. Schaffer S, Takahashi K, Azuma J. Role of osmoregulation in the actions of taurine. Amino acids. 2000;19(3):527–46.
63. Barry WH, Bridge J. Intracellular calcium homeostasis in cardiac myocytes. Circulation. 1993;87(6):1806–15.

64. Schaffer S, Kim HW. Effects and mechanisms of taurine as a therapeutic agent. Biomolecules & therapeutics. 2018;26(3):225.
65. Satoh H, Sperelakis N. Review of some actions of taurine on ion channels of cardiac muscle cells and others. General Pharmacology: The Vascular System. 1998;30(4):451–63.
66. Manolopoulos VG, Voets T, Declercq PE, Droogmans G, Nilius B. Swelling-activated efflux of taurine and other organic osmolytes in endothelial cells. American Journal of Physiology-Cell Physiology. 1997;273(1):C214–C22.
67. Jong CJ, Sandal P, Schaffer SW. The role of taurine in mitochondria health: more than just an antioxidant. Molecules. 2021;26(16):4913.
68. Seneff S, Kyriakopoulos AM. Taurine prevents mitochondrial dysfunction and protects mitochondria from reactive oxygen species and deuterium toxicity. Amino Acids. 2025;57(1):6.
69. Jong CJ, Azuma J, Schaffer S. Mechanism underlying the antioxidant activity of taurine: prevention of mitochondrial oxidant production. Amino acids. 2012;42(6):2223–32.
70. Shimada K, Jong CJ, Takahashi K, Schaffer SW. Role of ROS production and turnover in the antioxidant activity of taurine. Taurine 9. 2015:581–96.
71. Marcinkiewicz J, Kontny E. Taurine and inflammatory diseases. Amino acids. 2014;46(1):7–20.
72. Weiss SJ, Klein R, Slivka A, Wei M. Chlorination of taurine by human neutrophils: evidence for hypochlorous acid generation. The Journal of clinical investigation. 1982;70(3):598–607.
73. Kim C, Cha Y-N. Taurine chloramine produced from taurine under inflammation provides anti-inflammatory and cytoprotective effects. Amino acids. 2014;46(1):89–100.
74. Kaneko JJ. Bile acid composition of dog and cat gall-bladder bile. The Japanese Journal of Veterinary Science. 1990;52(2):423–5.
75. Herstad KMV, Rønning HT, Bakke AM, Moe L, Skancke E. Changes in the faecal bile acid profile in dogs fed dry food vs high content of beef: a pilot study. Acta Veterinaria Scandinavica. 2018;60(1):29.
76. Center SA. Serum bile acids in companion animal-medicine. Veterinary Clinics of North America: Small Animal Practice. 1993;23(3):625–57.
77. Backus RC, Rogers QR, Morris JG. Microbial degradation of taurine in fecal cultures from cats given commercial and purified diets. The Journal of nutrition. 1994;124:2540S–5S.
78. Tôrres CL, Biourge VC, Backus RC. Plasma and whole blood taurine concentrations in dogs may not be sensitive indicators of taurine deficiency when dietary sulfur amino acid content is reduced. Frontiers in Veterinary Science. 2022;9:873460.
79. Pezzali JG, Acuff HL, Henry W, Alexander C, Swanson KS, Aldrich CG. Effects of different carbohydrate sources on taurine status in healthy Beagle dogs. Journal of animal science. 2020;98(2):skaa010.

80. Chesney RW, Han X, Patters AB. Taurine and the renal system. Journal of biomedical science. 2010;17(Suppl 1):S4.
81. Knopf K, Sturman J, Armstrong M, Hayes K. Taurine: an essential nutrient for the cat. The Journal of nutrition. 1978;108(5):773–8.
82. Mahootchi E, Raasakka A, Luan W, Muruganandam G, Loris R, Haavik J, et al. Structure and substrate specificity determinants of the taurine biosynthetic enzyme cysteine sulphinic acid decarboxylase. Journal of Structural Biology. 2021;213(1):107674.
83. Miyazaki T. Identification of a novel enzyme and the regulation of key enzymes in mammalian taurine synthesis. Journal of Pharmacological Sciences. 2024;154(1):9–17.
84. Sturman JA. Taurine in development. The Journal of nutrition. 1988;118(10):1169–76.
85. Ito T, Kimura Y, Uozumi Y, Takai M, Muraoka S, Matsuda T, et al. Taurine depletion caused by knocking out the taurine transporter gene leads to cardiomyopathy with cardiac atrophy. Journal of molecular and cellular cardiology. 2008;44(5):927–37.
86. Silbernagl S. The renal handling of amino acids and oligopeptides. Physiological reviews. 1988;68(3):911–1007.
87. Huxtable RJ. Taurine in the central nervous system and the mammalian actions of taurine. Progress in neurobiology. 1989;32(6):471–533.
88. Voaden M, Lake N, Marshall J, Morjaria B. Studies on the distribution of taurine and other neuroactive amino acids in the retina. Experimental Eye Research. 1977;25(3):249–57.
89. Gaucher D, Arnault E, Husson Z, Froger N, Dubus E, Gondouin P, et al. Taurine deficiency damages retinal neurones: cone photoreceptors and retinal ganglion cells. Amino acids. 2012;43(5):1979–93.
90. Heller-Stilb B, van Roeyen C, Rascher K, Hartwig H-G, Huth A, Seeliger MW, et al. Disruption of the taurine transporter gene (taut) leads to retinal degeneration in mice. The FASEB journal. 2002;16(2):1–18.
91. Barnett K, Burger I. Taurine deficiency retinopathy in the cat. Journal of Small Animal Practice. 1980;21(10):521–34.
92. García-Ayuso D, Di Pierdomenico J, Martínez-Vacas A, Vidal-Sanz M, Picaud S, Villegas-Pérez MP. Taurine: a promising nutraceutic in the prevention of retinal degeneration. Neural Regeneration Research. 2024;19(3):606–10.
93. Militante J, Lombardini J. Pharmacological characterization of the effects of taurine on calcium uptake in the rat retina. Amino Acids. 1998;15(1):99–108.
94. Schmidt S, Berson E, Hayes K. Retinal degeneration in the taurine-deficient cat. Transactions Section on Ophthalmology American Academy of Ophthalmology and Otolaryngology. 1976;81(4 Pt 1):OP687–93.
95. Leon A, Levick WR, Sarossy MG. Lesion topography and new histological features in feline taurine deficiency retinopathy. Experimental eye research. 1995;61(6):731–41.

96. Preising MN, Görg B, Friedburg C, Qvartskhava N, Budde BS, Bonus M, et al. Biallelic mutation of human SLC6A6 encoding the taurine transporter TAUT is linked to early retinal degeneration. The FASEB journal. 2019;33(10):11507–27.
97. Ansar M, Ranza E, Shetty M, Paracha SA, Azam M, Kern I, et al. Taurine treatment of retinal degeneration and cardiomyopathy in a consanguineous family with SLC6A6 taurine transporter deficiency. Human molecular genetics. 2020;29(4):618–23.
98. García-Ayuso D, Martínez-Vacas A, Di Pierdomenico J, Vidal-Sanz M, Valiente Soriano FJ, Picaud S, et al. On the potential therapeutic role of taurine in retinal degenerative diseases. Acta Ophthalmologica. 2024;102.
99. Froger N, Moutsimilli L, Cadetti L, Jammoul F, Wang Q-P, Fan Y, et al. Taurine: the comeback of a neutraceutical in the prevention of retinal degenerations. Progress in retinal and eye research. 2014;41:44–63.
100. Pion PD, Sanderson SL, Kittelson MD. The effectiveness of taurine and levocarnitine in dogs with heart disease. Veterinary Clinics of North America: Small Animal Practice. 1998;28(6):1495–514.
101. Sturman J, Rassin D, Gaull G. Taurine in developing rat brain: maternal-fetal transfer of [35S] taurine and its fate in the neonate. Journal of neurochemistry. 1977;28(1):31–9.
102. Shivaraj MC, Marcy G, Low G, Ryu JR, Zhao X, Rosales FJ, et al. Taurine induces proliferation of neural stem cells and synapse development in the developing mouse brain. 2012.
103. Tochitani S. Functions of maternally-derived taurine in fetal and neonatal brain development. Taurine 10. 2017:17–25.
104. Sturman JA. Dietary taurine and feline reproduction and development. The Journal of nutrition. 1991;121:S166–S70.
105. Sturman JA, Messing JM. Dietary taurine content and feline reproduction and outcome. The Journal of nutrition. 1991;121(8):1195–203.
106. Jangra A, Gola P, Singh J, Gond P, Ghosh S, Rachamalla M, et al. Emergence of taurine as a therapeutic agent for neurological disorders. Neural regeneration research. 2024;19(1):62–8.
107. Kumar S, Goel RK. Taurine supplementation to anti-seizure drugs as the promising approach to treat pharmacoresistant epilepsy: A pre-clinical study. International Journal of Epilepsy. 2017;4(02):119–24.
108. Oja SS, Saransaari P. Taurine and epilepsy. Epilepsy research. 2013;104(3):187–94.
109. Junyent F, Utrera J, Romero R, Pallàs M, Camins A, Duque D, et al. Prevention of epilepsy by taurine treatments in mice experimental model. Journal of neuroscience research. 2009;87(6):1500–8.
110. Bkaily G, Haddad G, Jaalouk D, Gros-Louis N, Benchekroun MT, Naik R, et al. Modulation of Ca2+ and Na+ transport by taurine in heart and vascular smooth muscle. Taurine 2: Basic and Clinical Aspects: Springer; 1996. p. 263–73.

111. Bkaily G, Jazzar A, Normand A, Simon Y, Al-Khoury J, Jacques D. Taurine and cardiac disease: state of the art and perspectives. Canadian Journal of Physiology and Pharmacology. 2020;98(2):67–73.
112. Eley D, Lake N, Ter Keurs H. Taurine depletion and excitation-contraction coupling in rat myocardium. Circulation research. 1994;74(6):1210–9.
113. Lake N. Loss of cardiac myofibrils: mechanism of contractile deficits induced by taurine deficiency. American Journal of Physiology-Heart and Circulatory Physiology. 1993;264(4):H1323–H6.
114. Huxtable RJ. The regulation and function of taurine in the heart and other organs. Natural Sulfur Compounds: Novel Biochemical and Structural Aspects: Springer; 1980. p. 277–93.
115. Schaffer S, Solodushko V, Azuma J. Taurine-deficient cardiomyopathy: role of phospholipids, calcium and osmotic stress. Taurine 4: Taurine and Excitable Tissues. 2002:57–69.
116. Satoh H, Horie M. Actions of taurine on the L-type Ca2+ channel current in guinea pig ventricular cardiomyocytes. Journal of cardiovascular pharmacology. 1997;30(6):711–6.
117. Backus RC, Cohen G, Pion PD, Good KL, Rogers QR, Fascetti AJ. Taurine deficiency in Newfoundlands fed commercially available complete and balanced diets. Journal of the American Veterinary Medical Association. 2003;223(8):1130–6.
118. Delaney S, Kass P, Rogers Q, Fascetti A. Plasma and whole blood taurine in normal dogs of varying size fed commercially prepared food. Journal of animal physiology and animal nutrition. 2003;87(5-6):236–44.
119. Kathrani A, Fascetti AJ, Larsen JA, Maunder C, Hall E. Whole-blood taurine concentrations in cats with intestinal disease. Journal of veterinary internal medicine. 2017;31(4):1067–73.
120. Merheb M, Daher RT, Nasrallah M, Sabra R, Ziyadeh FN, Barada K. Taurine intestinal absorption and renal excretion test in diabetic patients: a pilot study. Diabetes care. 2007;30(10):2652–4.
121. Sturman JA, Hayes KC. The biology of taurine in nutrition and development. Advances in nutritional research: Springer; 1980. p. 231–99.
122. Pasantes-Morales H, Quesada O, Alcocer L, Olea RS. Taurine content in foods. Nutr Rep Int. 1989;40:793–801.
123. Laidlaw SA, Grosvenor M, Kopple J. The taurine content of common foodstuffs. Journal of parenteral and enteral nutrition. 1990;14(2):183–8.
124. Council NR, Earth Do, Studies L, Nutrition CoA, Dog So, Nutrition C. Nutrient requirements of dogs and cats: National Academies Press; 2006.
125. Johnson M, Parsons C, Fahey Jr G, Merchen N, Aldrich C. Effects of species raw material source, ash content, and processing temperature on amino acid digestibility of animal by-product meals by cecectomized roosters and ileally cannulated dogs. Journal of Animal Science. 1998;76(4):1112–22.

126. Backus RC, Rogers QR, Rosenquist GL, Calam J, Morris JG. Diets causing taurine depletion in cats substantially elevate postprandial plasma cholecystokinin concentration. The Journal of nutrition. 1995;125(10):2650–7.
127. Morris JG, Rogers QR, Kim SW, Backus RC. Dietary taurine requirement of cats is determined by microbial degradation of taurine in the gut. Taurine in Health and Disease. 1994:59–70.
128. Kim SW, Morris JG, Rogers QR. Dietary soybean protein decreases plasma taurine in cats. The Journal of nutrition. 1995;125(11):2831–7.
129. Ontiveros ES, Whelchel BD, Yu J, Kaplan JL, Sharpe AN, Fousse SL, et al. Development of plasma and whole blood taurine reference ranges and identification of dietary features associated with taurine deficiency and dilated cardiomyopathy in golden retrievers: A prospective, observational study. PloS one. 2020;15(5):e0233206.
130. Association of American Feed Control Officials. AAFCO Methods for Substantiating Nutritional Adequacy of Dog and Cat Foods. Champaign, IL: AAFCO. 2014.
131. Tran QD, Hendriks WH, van der Poel AF. Effects of extrusion processing on nutrients in dry pet food. Journal of the Science of Food and Agriculture. 2008;88(9):1487–93.
132. Harper JM. Effects of extrusion processing on nutrients. Nutritional evaluation of food processing: Springer; 1988. p. 365–91.
133. Spitze A, Wong D, Rogers Q, Fascetti A. Taurine concentrations in animal feed ingredients; cooking influences taurine content. Journal of Animal Physiology and Animal Nutrition. 2003;87(7-8):251–62.
134. Ullah M, Rehman AU, Shetty M, Allen MD, Ullah E, Signorini SG, et al. Early-Onset Retinopathy in Patients With Variants in SLC6A6 Leading to Impaired Taurine Transport. JAMA ophthalmology. 2026;144(1):70–8.
135. Schaffer S, Ramila K, Jong C, Ito T, Azuma J, editors. Role of protein phosphorylation in TauTKO cardiomyopathy. Int Taurine Symp; 2009.
136. Pion PD, Kittleson MD, Thomas WP, Delellis LA, Rogers QR. Response of cats with dilated cardiomyopathy to taurine supplementation. Journal of the American Veterinary Medical Association. 1992;201(2):275–84.
137. Idexx laboratories Germany 2026. Available from: https://www.idexx.de/de/veterinary/reference-laboratories/tests-and-services/#menu.IlGAf.
138. UC Davis Amino Acid Laboratory, 2026 Available from: https://www.vetmed.ucdavis.edu/labs/amino-acid-laboratory.
139. Trautwein EA, Hayes K. Taurine concentrations in plasma and whole blood in humans: estimation of error from intra-and interindividual variation and sampling technique. The American journal of clinical nutrition. 1990;52(4):758–64.
140. Trautwein EA, Hayes K. Evaluating taurine status: determination of plasma and whole blood taurine concentration. 1991.

141. Pacioretty L, Hickman M, Morris J, Rogers Q. Kinetics of taurine depletion and repletion in plasma, serum, whole blood and skeletal muscle in cats. Amino Acids. 2001;21(4):417–27.
142. Mou S, Ding X, Liu Y. Separation methods for taurine analysis in biological samples. Journal of Chromatography B. 2002;781(1-2):251–67.
143. Wess G, Domenech O, Dukes-McEwan J, Häggström J, Gordon S. European Society of Veterinary Cardiology screening guidelines for dilated cardiomyopathy in Doberman Pinschers. Journal of Veterinary Cardiology. 2017;19(5):405–15.
144. Shao A, Hathcock JN. Risk assessment for the amino acids taurine, L-glutamine and L-arginine. Regulatory toxicology and pharmacology. 2008;50(3):376–99.
145. Lloyd HS. The Popular Cocker Spaniel: Its History, Strains, Pedigrees, Breeding, Kennel Management: Popular Dogs Publishing Company, Limited; 1926.
146. Walsh JH. The dogs of Great Britain, America, and other countries, their breeding, training, and management in health and disease: Orange Judd; 1906.
147. Acierno MJ, Brown S, Coleman AE, Jepson RE, Papich M, Stepien RL, et al. ACVIM consensus statement: Guidelines for the identification, evaluation, and management of systemic hypertension in dogs and cats. Journal of Veterinary Internal Medicine. 2018;32(6):1803–22.
148. Hansson K, Häggström J, Kvart C, Lord P. Left atrial to aortic root indices using two-dimensional and M-mode echocardiography in cavalier King Charles spaniels with and without left atrial enlargement. Veterinary Radiology & Ultrasound. 2002;43(6):568–75.
149. Dukes-McEwan J, Borgarelli M, Tidholm A, Vollmar AC, Häggström J, Cardiomyopathy ETfCD. Proposed guidelines for the diagnosis of canine idiopathic dilated cardiomyopathy. Journal of veterinary cardiology. 2003;5(2):7–19.
150. Cornell CC, Kittleson MD, Torre PD, Häggström J, Lombard CW, Pedersen HD, et al. Allometric scaling of M-mode cardiac measurements in normal adult dogs. Journal of veterinary internal medicine. 2004;18(3):311–21.
151. JM K. dogmap: pipeline for dog WGS alignment 2023 [Available from: https://github.com/jmkidd/dogmap.
152. Gray K, Alexander L, Staunton R, Colyer A, Watson A, Fascetti A. The effect of 48-hour fasting on taurine status in healthy adult dogs. Journal of animal physiology and animal nutrition. 2016;100(3):532–6.
153. Paasonen M, Solatunturi E, Nieminen M. Blood platelets: accumulation and release of taurine. Progress in Clinical and Biological Research. 1985;179:225–33.
154. Fukuda K, Hirai Y, Yoshida H, Nakajima T, Usui T. Free amino acid content of lymphocytes nd granulocytes compared. Clinical chemistry. 1982;28(8):1758–61.
155. Plumb DC. Plumb's veterinary drug handbook: desk: John Wiley & Sons; 2018.

156. Pion PD, Kittleson MD, Rogers QR, Morris JG. Taurine deficiency myocardial failure in the domestic cat. Progress in clinical and biological research. 1990;351:423–30.
157. Pansani MC, Azevedo PS, Rafacho BPM, Minicucci MF, Chiuso-Minicucci F, Zorzella-Pezavento SG, et al. Atrophic cardiac remodeling induced by taurine deficiency in Wistar rats. PLoS One. 2012;7(7):e41439.
158. Novotny MJ, Hogan PM. Inotropic interventions in the assessment of myocardial failure associated with taurine deficiency in domestic cats. Taurine 2: Basic and Clinical Aspects: Springer; 1996. p. 305–14.
159. Freeman L, Rush J, Adin D, Weeks K, Antoon K, Brethel S, et al. Prospective study of dilated cardiomyopathy in dogs eating nontraditional or traditional diets and in dogs with subclinical cardiac abnormalities. Journal of veterinary internal medicine. 2022;36(2):451–63.
160. Kriebs A. Taurine levels modulate aging. Nature Aging. 2023;3(7):758–9.
161. Singh P, Gollapalli K, Mangiola S, Schranner D, Yusuf MA, Chamoli M, et al. Taurine deficiency as a driver of aging. Science. 2023;380(6649):eabn9257.
162. Gawryluk A, Cybulska-Klosowicz A, Charzynska A, Zakrzewska R, Sobolewska A, Kossut M, et al. Mitigation of aging-related plasticity decline through taurine supplementation and environmental enrichment. Scientific Reports. 2024;14(1):19546.
163. Eppler B, Dawson Jr R. Cysteine sulfinate decarboxylase and cysteine dioxygenase activities do not correlate with strain-specific changes in hepatic and cerebellar taurine content in aged rats. Mechanisms of ageing and development. 1999;110(1-2):57–72.
164. Eppler B, Dawson Jr R. The effects of aging on taurine content and biosynthesis in different strains of rats. Taurine 3: Cellular and Regulatory Mechanisms: Springer; 1998. p. 55–61.
165. Dawson Jr R, Eppler B, Patterson TA, Shih D, Liu S. The effects of taurine in a rodent model of aging. Taurine 2: Basic and Clinical Aspects: Springer; 1996. p. 37–50.
166. Fernandez ME, Bernier M, Price NL, Camandola S, Aon MA, Vaughan K, et al. Is taurine an aging biomarker? Science. 2025;388(6751):eadl2116.
167. Falconer DS. Introduction to quantitative genetics: Pearson Education India; 1996.
168. Mausberg T-B, Wess G, Simak J, Keller L, Drögemüller M, Drögemüller C, et al. A locus on chromosome 5 is associated with dilated cardiomyopathy in Doberman Pinschers. PloS one. 2011;6(5):e20042.
169. Niskanen JE, Ohlsson Å, Ljungvall I, Droegemueller M, Ernst RF, Dooijes D, et al. Identification of novel genetic risk factors of dilated cardiomyopathy: from canine to human. Genome medicine. 2023;15(1):73.

Popular science summary

Dilated cardiomyopathy (DCM) is the most common myocardial disease in dogs and is characterized by a gradual decline in systolic function and cardiac enlargement. Despite treatment, the prognosis is guarded, and the disease often progresses to congestive heart failure and, in some cases, sudden death due to severe arrhythmias. Certain breeds appear to be genetically predisposed to the primary form of the disease, although the underlying cause remains unknown in many cases. However, there are forms of DCM caused by treatable or reversible factors that often have a considerably better prognosis than primary DCM. One such form is taurine-responsive cardiomyopathy, which occurs as a result of deficiency of the amino acid taurine.

Taurine plays a central role in several fundamental physiological processes in mammals. In addition to taurine-responsive cardiomyopathy, prolonged taurine deficiency can lead to other diseases, of which the most reported are retinal degeneration with visual impairment, reproductive disorders and developmental abnormalities, as well as certain neurological conditions. The underlying causes of taurine deficiency are not yet fully understood, but several factors that may reduce endogenous synthesis, impair absorption, or increase losses of taurine are thought to contribute to the development.

The American Cocker Spaniel (ACS) is known to be predisposed to taurine-responsive cardiomyopathy, and taurine supplementation is therefore included in the standard treatment when dogs of this breed develop signs of DCM. The English Cocker Spaniel (ECS) is also known to develop DCM; however, despite its close relationship to the ACS, the association between heart disease and taurine concentrations has not previously been systematically investigated in a larger population of ECS.

The main aim of this thesis was therefore to investigate the occurrence of low taurine concentrations and taurine-responsive cardiomyopathy in ECS, as well as to evaluate underlying causes, including dietary and genetic factors, that may explain why some individuals are affected. In addition, the effect of daily taurine supplementation was evaluated in dogs with low concentrations, including dogs with concurrent DCM and congestive heart failure.

Of the 180 dogs included in the thesis, nearly 40% had low taurine concentrations, and approximately one fourth of these also showed signs of DCM, with all but three also having congestive heart failure. Daily taurine supplementation resulted in normalization of taurine concentrations in all

dogs with low concentrations, as well as marked improvement, and in some cases complete recovery, in dogs with concurrent heart disease. A clear association was observed between low taurine concentrations and certain dietary compositions, particularly diets based on red meat and diets with low concentrations of taurine and the amino acid methionine. Genetic analyses suggested that inherited factors may have an impact on an individual's susceptibility to developing taurine deficiency and that dietary factors may influence how these genetic effects are expressed.

In conclusion, the thesis demonstrates that low taurine concentrations are common in ECS and that some affected dogs develop a form of DCM that is often reversible. The results highlight the importance of measuring taurine concentrations in ECS with suspected DCM as correct treatment can markedly improve prognosis.

Populärvetenskaplig sammanfattning

Dilaterad kardiomyopati (DCM) är hundens vanligaste hjärtmuskelsjukdom och kännetecknas av hjärtförstoring samt en gradvis försämring av hjärtats pumpförmåga. Trots behandling är prognosen avvaktande, och sjukdomen leder ofta till utveckling av kongestiv hjärtsvikt och i vissa fall plötslig död till följd av allvarliga rytmrubbningar. Vissa raser förefaller vara genetiskt predisponerade för att utveckla den primära formen av sjukdomen, men orsaken är i många fall okänd. Det finns emellertid former av DCM som orsakas av behandlingsbara eller reversibla faktorer och som ofta kan ha en betydligt bättre prognos än primär DCM. En sådan form är taurinresponsiv kardiomyopati, som uppstår vid brist på aminosyran taurin.

Taurin har en central roll i flera grundläggande fysiologiska processer hos däggdjur. Långvarig taurinbrist kan, utöver taurinresponsiv kardiomyopati, även leda till andra sjukdomstillstånd, varav de mest rapporterade är näthinnedegeneration och synnedsättning, reproduktionsrubbningar och fosterskador samt vissa neurologiska tillstånd. De bakomliggande orsakerna till att en individ utvecklar taurinbrist är ännu inte helt klarlagda, men flera olika faktorer som kan leda till minskad kroppsegen produktion, försämrat upptag eller ökade förluster av taurin misstänks bidra till att brist uppstår.

Amerikansk cocker spaniel (ACS) anses vara predisponerad för taurinresponsiv kardiomyopati, och därför ingår taurinsupplementering i standardbehandlingen när hundar av rasen utvecklar tecken på DCM. Engelsk cocker spaniel (ECS) är också känd för att drabbas av DCM, men trots det nära släktskapet med ACS har kopplingen mellan hjärtsjukdom och taurinkoncentrationer inte tidigare undersökts systematiskt i en större grupp ECS.

Huvudsyftet med denna avhandling var därför att undersöka förekomsten av låga taurinkoncentrationer och taurinresponsiv kardiomyopati hos ECS, samt att undersöka bakomliggande orsaker, såsom foderrelaterade och ärftliga faktorer, som skulle kunna förklara varför vissa individer drabbas. Utöver detta undersöktes även effekten av daglig taurinsupplementering till hundar med låga koncentrationer inklusive hundar med samtidig DCM och kongestiv hjärtsvikt.

Av de 180 hundar som ingick i avhandlingen hade nästan 40 % låga taurinkoncentrationer, och av dessa uppvisade nära en fjärdedel tecken på DCM, varav alla utom tre även hade kongestiv hjärtsvikt. Daglig taurinsupplementering till hundar med låga värden resulterade i normaliserade koncentrationer hos samtliga individer samt en markant

förbättring, och i vissa fall fullt tillfrisknande, hos hundar med samtidig hjärtsjukdom. Det fanns en tydlig koppling mellan vissa fodersammansättningar och låga taurinkoncentrationer, där foder baserade på rött kött samt foder med lågt innehåll av taurin och aminosyran metionin utmärkte sig. De genetiska analyserna tydde på att ärftliga variationer kan påverka en individs känslighet för att utveckla taurinbrist och att olika fodertyper kan ha en inverkan på hur dessa genetiska effekter kommer till uttryck.

Sammanfattningsvis visade avhandlingen att låga taurinkoncentrationer är vanligt förekommande hos ECS och att vissa drabbade hundar utvecklar en, i många fall, reversibel form av DCM. Resultaten betonar vikten av att mäta taurinkoncentration hos ECS med misstänkt DCM, eftersom korrekt behandling avsevärt kan förbättra prognosen.

Acknowledgements

The present thesis was carried out at the **Department of Clinical Sciences, at the Swedish University of Agricultural Sciences**, Uppsala, Sweden. Examinations of client-owned cocker spaniels were performed at **University Animal Hospital (UDS)**, Uppsala, **AniCura Albano Small Animal Hospital**, Danderyd, and **Evidensia Södra Djursjukhuset**, Kungens Kurva, Stockholm.

The work included in this thesis was made possible by generous financial support provided by **The Greater Stockholm Veterinary Care Foundation, Agria and SKK Research Foundation,** and **Thure F och Karin Forsbergs Foundation**, as well as **AniCura Research Foundation and Svelands Research Foundation**.

This thesis would not have been possible without the help and support from many different people (and dogs), and I want to thank every single one deeply.

I would especially like to express my deep gratitude to:

Associate professor Ingrid Ljungvall - my main supervisor. Thank you for not only being an amazing guide through academic life, but also for becoming one of my closest friends in real life! This would SO never have worked without you!
There are so many things to say, but to summarize, I truly admire you. We have had so much fun during these years, but you have also carried me through some rough times, both professionally and privately and I am forever grateful for that. I very much look forward to all that is to come!

Professor Jens Häggström - my co-supervisor. Thank you for being such a brilliant, kind and generous person. You are truly impressive and set the standard for so many people around you. I am honored and proud to work with you. And last (but not least) “Brynäs är tillbaks”!

Associate professor Anna Tidholm - my co-supervisor and guiding star. in both life and work. Thank you for always being there for me! And thank you for everything you have taught me about being a good veterinarian, a cardiologist and a person! What would I do without you?

And to all three supervisors combined - thank you for giving in to my persistence and letting me do this!

Thank you **SLU and UDS** for welcoming me into your beautiful facilities and for making this possible! Thank you **Albano** for giving me the opportunity to start this dream and achievement, and thank you **Södran** for letting me fulfill it!

Thank you:

Mark Dirven for making me a cardiologist! And for being my very good friend and colleague. I look forward to celebrating you and Lieke in July!

Johanna Holmberg for making everything so much more fun when you are around! You are kindness personified and one of the smartest people I know. I will miss sharing academic life with you. So glad we have a "normal" life too.

Paul Pion for being our taurine guru, and for your generosity of both knowledge and friendship. Thank you for opening doors and letting me in.

Frank van Steenbeek for standing steady with patience and humor, and for being an excellent geneticist and co-author. See you on Teams!

Mark Kittleson for generously sharing your knowledge, and for being a (legendary!) co-author.

Erling Strandberg thank you for so kindly sharing your knowledge and expertise!

Björn Ekesten, Lena Ström and Titti Sjödahl Essén for excellent work with the "cocker eyes" and for co-authoring paper II with us.

Andrea Fascetti and Joshua Yu for valuable help with manuscripts and analyses.

Christjan Wegner for amazing help with images and illustrations in late-night sessions. I am so glad we found each other again.

Leon af Sandeberg and Carl Nilsson for excellent master's thesis and co-authoring work.

Mujde, Sandra, Lieke, Charlotte and so many more for helping me examine all my cockers.

All the **Cockers and their owners** for participating and making this possible.

Mikkel Bjaerkness for being a spark of life and making working days more fun.

Anna Selin for sharing your refreshing PhD journey with me. It sure did prepare me for what was coming (it did however not prevent deadline panic). Also thank you for being such a good friend and a sister of disaster. One of few (with Sickan) that truly gets it…

Momo and Net for being my cardio PhD colleagues. I hope we will get to work together again soon.

Malin, Ninni, Maria and Elin for being the best PhD colleagues.

My present and former cardio colleagues and friends – **Erthan, ABo, Einar, Micke, Shacko, Sara, Sjongen and Tidis** for all the fun and everything in between!

Christina, Claire, and Sofia for making everything more fun, even when it really was not, and for always having each other's backs.

Mats Wänlund for being the best big boss ever.

Emma Lindell for getting dumped the same summer, which somehow turned out to be a win.

Cozy for being where it all started.

FU for making the first years as a vet both fun and unforgettable!

Erica for always being there and not giving up on me, despite my poor phone-answering skills. Change will come!

Tilde and Åsa for so much fun and friendship.

Marie and Tuomo for kindness and support over the years.

And finally to my family ♥ – thank you:

Mamma and Göran for your ENDLESS love and support over all these years. Taking care of children, taking care of dogs, building up and repairing whatever I tear down (and those of you who know me can imagine that workload….), and thank you for "not" calling me while I wrote my thesis. ♥

Pappa and Gabriella for always being there in good and bad – some with more words than others ☺. I look forward to many days in the garden and even more evenings together on the porch in Ockelbo this summer and many more to come! ♥

Anna, Anders, Meja and Melvin for making life so much better when you are around. You are the best! Forever! ♥

Isak and Emy for everything ♥

I

Journal of Veterinary Cardiology (2023) **45**, 59—70

ELSEVIER

Journal of Veterinary Cardiology ESVC

www.elsevier.com/locate/jvc

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

K. Kriström, DVM[a,b,*], J. Häggström, DVM, PhD[a], A. Tidholm, DVM, PhD[a,b], J.Z. Yu, PhD[c], A.J. Fascetti, VMD, PhD[c], I. Ljungvall, DVM, PhD[a]

[a] *Department of Clinical Sciences, Swedish University of Agricultural Sciences, SE-750 07 Uppsala, Sweden*
[b] *Anicura Albano Small Animal Hospital, Rinkebyvägen 21A, SE-182 36 Danderyd, Sweden*
[c] *Department of Molecular Biosciences, School of Veterinary Medicine, University of California-Davis, Davis, CA 95616, USA*

Received 23 January 2022; received in revised form 16 December 2022; accepted 26 December 2022

Preliminary results of the study were presented at the European College of Veterinary Internal Medicine — Companion Animals online congress 2020.

KEYWORDS
Amino acids;
Biomarker;
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,

* Corresponding author.
E-mail address: karin.kristrom@slu.se (K. Kriström).

https://doi.org/10.1016/j.jvc.2022.12.003

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/

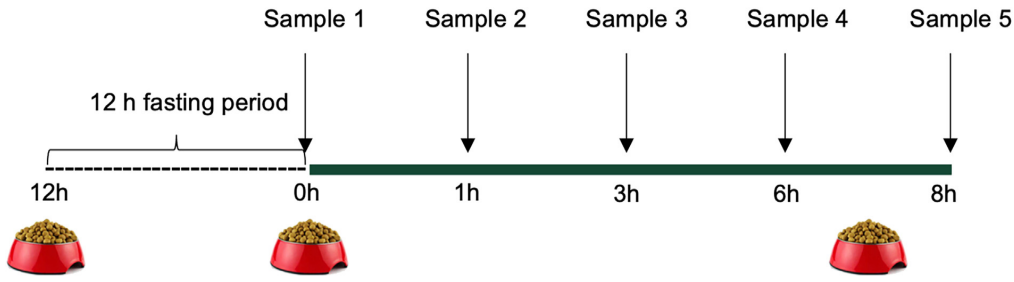


Fig. 1 Blood was collected at a total of five sampling time points over eight h. Food was offered immediately after the first sampling time point, and one h after the fourth sampling time point. The first sample was collected at arrival after approximately 12 h of fasting; the second sample was collected 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

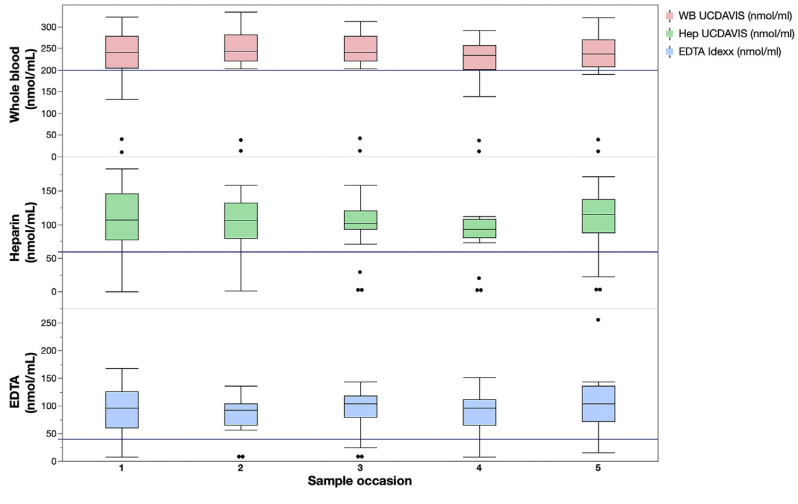


Fig. 2 Box and whiskers plots demonstrating median taurine concentrations for each sampling time point (n = 5) measured in whole blood, EDTA plasma, and heparinized plasma. The boxes (top, bottom, and central line) correspond to the 75th percentile, the 25th percentile, and 50th percentile (median), 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.

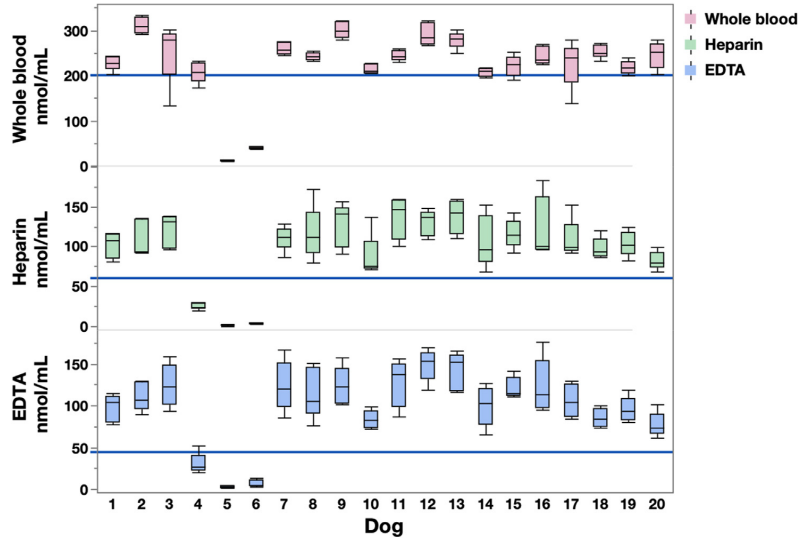


Fig. 3 Box and whiskers plots demonstrating intraindividual variation in taurine concentrations measured in whole blood, EDTA plasma and heparinized plasma at five sampling time points during an eight hour-period. Dark blue horizontal lines show normal lower reference values for each additive. Of the 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

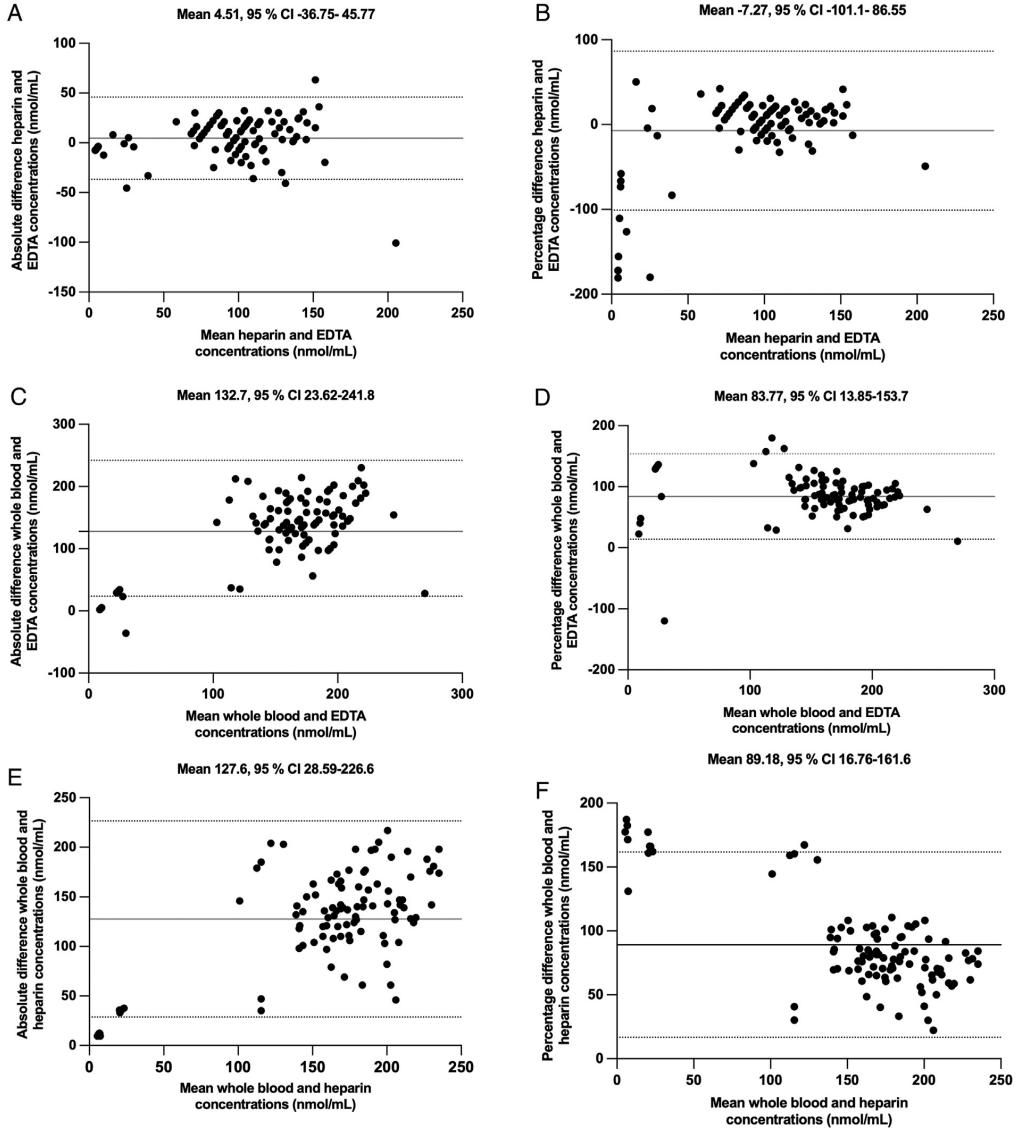


Fig. 4 Bland Altman plots showing mean and 95% confidence intervals of absolute differences (A, C, and E), and differences expressed as percentage of the mean concentrations (B, D, and F) in 100 samples obtained at five occasions from 20 dogs. A systematic absolute (and percentage) difference of 55.7 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.

Acknowledgments

This study received funding from The Greater Stockholm Veterinary Hospital Foundation, Sweden; Agria and SKK Research Fund, Sweden; Svelands Research Foundation, Sweden; Anicura Research Foundation, the Amino Acid Laboratory and the Sommer Endowment, School of Veterinary Medicine, University of California, Davis. Ulf Olsson is gratefully acknowledged for important help with statistical analyses, and Müjde Ovabasli and Johanna Holmberg for indispensable help during examination of dogs and collection of data.

References

[1] Huxtable R. Physiological actions of taurine. Physiol Rev 1992;72:101—63.
[2] Hayes K, Sturman J. Taurine in metabolism. Annu Rev Nutr 1981;1:401—25.

[3] Knopf K, Sturman JA, Armstrong M, Hayes KC. Taurine: an essential nutrient for the cat. J Nutr 1978 May;108:773—8.
[4] Pion PD, Kittleson MD, Rogers QR, Morris JG. Myocardial failure in cats associated with low plasma taurine: a reversible cardiomyopathy. Science 1987;237:764—8.
[5] Moise NS, Pacioretty LM, Kallfelz FA, Stipanuk MH, King JM, Gilmour Jr RF. Dietary taurine deficiency and dilated cardiomyopathy in the fox. Am Heart J 1991 Feb;121:541—7.
[6] Kittleson MD, Keene B, Pion PD, Loyer CG. Results of the multicenter spaniel trial (MUST): taurine- and carnitine-responsive dilated cardiomyopathy in American cocker spaniels with decreased plasma taurine concentration. J Vet Intern Med 1997;11:204—11.
[7] Miyazaki T, Sasaki SI, Toyoda A, Wei FY, Shirai M, Morishita Y, Ikegami T, Tomizawa K, Honda A. Impaired bile acid metabolism with defectives of mitochondrial tRNA taurine modification and bile acid taurine conjugation in the taurine depleted cats. Sci Rep 2020 Mar 18; 10:4915.
[8] Schaffer S, Takahashi K, Azuma J. Role of osmoregulation in the actions of taurine. AminoAcids 2000;19:527—46.
[9] Bélanger MC, Ouellet M, Queney G, Moreau M. Taurine-deficient dilated cardiomyopathy in a family of golden retrievers. J Am Anim Hosp Assoc 2005 Sep—Oct;41: 284—91.
[10] Freeman LM, Michel KE, Brown DJ, Kaplan PM, Stamoulis ME, Rosenthal SL, Keene BW, Rush JE. Idiopathic dilated cardiomyopathy in Dalmatians: nine cases (1990—1995). J Am Vet Med Assoc 1996 Nov 1;209:1592—6.
[11] Kramer GA, Kittleson MD, Fox PR, Lewis J, Pion PD. Plasma taurine concentrations in normal dogs and in dogs with heart disease. J Vet Intern Med 1995;9:253—8.
[12] Fascetti AJ, Reed JR, Rogers QR, Backus RC. Taurine deficiency in dogs with dilated cardiomyopathy: 12 cases (1997-2001). J Am Vet Med Assoc 2003 Oct 15;223:1137—41.
[13] Alroy J, Rush JE, Freeman L, Amarendhra Kumar MS, Karuri A, Chase K, Sarkar S. Inherited infantile dilated cardiomyopathy in dogs: genetic, clinical, biochemical, and morphologic findings. Am J Med Genet 2000 Nov 6;95: 57—66.
[14] Backus RC, Cohen G, Pion PD, Good KL, Rogers QR, Fascetti AJ. Taurine Deficiency in Newfoundlands fed commercially available complete and balanced diets. J Am Vet Med Assoc 2003 Oct 15;223:1130—6.
[15] Tidholm A, Häggström J, Borgarelli M, Tarducci A. Canine idiopathic dilated cardiomyopathy. Part I: aetiology, clinical characteristics, epidemiology and pathology. Vet J 2001 Sep;162:92—107.
[16] Basili M, Pedro B, Hodgkiss-Geere H, Navarro-Cubas X, Graef N, Dukes McEwan J. Low plasma taurine levels in English cocker spaniels diagnosed with dilated cardiomyopathy. J Small Anim Pract 2021 Jul;62:570—9.
[17] FDA investigating potential connections between diet and cases of canine heart disease. Administration, U.S.F.a.D; 2018. https://www.fda.gov/animal-veterinary/outbreaks-and-advisories/fda-investigation-potential-link-between-certain-diets-and-canine-dilatedcardiomyopathy.
[18] FDA. Investigation into potential link between certain diets and canine dilated cardiomyopathy. Administration, U.S.F.a.D. 2019. https://www.fda.gov/animal-veterinary/outbreaks-and-advisories/fda-investigation-potential-link-between-certain-diets-and-canine-dilated-cardiomyopathy.
[19] Smith CE, Parnell LD, Lai CQ, Rush JE, Freeman LM. Investigation of diets associated with dilated cardiomyopathy in dogs using foodomics analysis. Sci Rep 2021 Aug 5;11:15881.
[20] Freeman LM, Stern JA, Fries R, Adin DB, Rush JE. Diet-associated dilated cardiomyopathy I dogs: what do we know? J Am Vet Med Assoc 2018 Dec 1;253:1390—4.
[21] Trautwein EA, Hayes KC. Plasma and whole blood taurine concentrations respond differently to taurine supplementation (humans) and depletion (cats). Z Ernahrungswiss 1995 Jun;34:137—42.
[22] Trautwein EA, Hayes KC. Taurine concentrations in plasma and whole blood in humans: estimation of error from intra- and interindividual variation and sampling technique. Am J Clin Nutr 1990 Oct;52:758—64.
[23] Trautwein EA, Hayes KC. Evaluating taurine status: determination of plasma and whole blood taurine concentration. J Nutr Biochem 1991;2:571—6.
[24] Pacioretty L, Hickman MA, Morris JG, Rogers QR. Kinetics of taurine depletion and repletion in plasma, serum, whole blood, and skeletal muscle in cats. Amino Acids 2001 Dec; 21:417—27.
[25] Mou S, Ding X, Liu Y. Separation methods for taurine analysis in biological samples. J Chromatogr B Analyt Technol Biomed Life Sci 2002 Dec 5;781:251—67.
[26] Tôrres CL, Biourge VC, Backus RC. Plasma and whole blood taurine concentrations in dogs may not be sensitive indicators of taurine deficiency when dietary sulfur amino acid content is reduced. Front Vet Sci 2022:573.
[27] Kim SW, Morris JG, Rogers QR. Dietary soybean protein decreases plasma taurine in cats. J Nutr 1995 Nov;125: 2831—7.
[28] Tôrres CL, Backus RC, Fascetti AJ, Rogers QR. Taurine status in normal dogs fed a commercial diet associated with taurine deficiency and dilated cardiomyopathy. J Anim Physiol Anim Nutr 2003 Oct;87:359—72.
[29] Kaplan JL, Stern JA, Fascetti AJ, Larsen JA, Skolnik H, Peddle GD, Kienle RD, Waxman A, Cocchiaro M, Gunther-Harrington CT, Klose T, LaFauci K, Lefbom B, Machen Lamy M, Malakoff R, Nishimura S, Oldach M, Rosenthal S, Stauthammer C, O'Sullivan L, Visser LC, Williams R, Ontiveros E. Taurine deficiency and dilated cardiomyopathy in golden retrievers fed commercial diets. PLoS One 2018 Dec 13:13.
[30] Gray K, Alexander LG, Staunton R, Colyer A, Watson A, Fascetti AJ. The effect of 48-hour fasting on taurine status in healthy adult dogs. J Anim Physiol Anim Nutr 2016 Jun; 100:532—6.
[31] Reinero C, Visser LC, Kellihan HB, Masseau I, Rozanski E, Clercx C, Williams K, Abbot J, Borgarelli M, Scansen BA. ACVIM consensus statement guidelines for the diagnosis, classification, treatment, and monitoring of pulmonary hypertension in dogs. J Vet Intern Med 2020;34:549—73.
[32] Hansson K, Häggström J, Kvart C, Lord P. Left atrial to aortic root indices using two-dimensional and M-mode echocardiography in cavalier King Charles spaniels with and without left atrial enlargement. Vet Radiol Ultrasound 2002 Nov—Dec;43:568—75.
[33] Dukes-McEwan J, Borgarelli M, Tidholm A, Vollmar AC, Häggström J, ESVC Taskforce for Canine Dilated Cardiomyopathy. Proposed guidelines for the diagnosis of canine idiopathic dilated cardiomyopathy. J Vet Cardiol 2003 Nov;5:7—19.
[34] Wess G, Domenech O, Dukes-McEwan J, Häggström J, Gordon S. European society of veterinary cardiology screening guidelines for dilated cardiomyopathy in Doberman Pinschers. J Vet Cardiol 2017 Oct;19:405—15.
[35] Cornell CC, Kittleson MD, Della Torre P, Häggström J, Lombard CW, Pedersen HD, Vollmar A, Wey A. Allometric

scaling of M-mode cardiac measurements in normal adult dogs. J Vet Intern Med 2004;18:311–21.
[36] Idexx laboratories Germany. https://www.idexx.de/de/veterinary/reference-laboratories/tests-and-services/#menu; 2022.
[37] University of California Davis amino acid laboratory. https://www.vetmed.ucdavis.edu/labs/amino-acid-laboratory; 2022.
[38] Sharer JD, De Biase I, Matern D, Young S, Bennett MJ, Tolun AA, ACMG laboratory quality Assurance Committee. Laboratory analysis of amino acids, 2018 revision: a technical standard of the American College of Medical Genetics and Genomics (ACMG). Genet Med 2018 Dec;20:1499–507.
[39] Perry TL, Hansen S. Technical pitfalls leading to errors in the quantitation of plasma amino acids. Clin Chim Acta 1969;25:53–8.
[40] Donadelli RA, Pezzali JG, Oba PM, Swanson KS, Coon C, Varney J, Pendlebury C, Shoveller AK. A commercial grain-free diet does not decrease plasma amino acids and taurine status but increases bile acid excretion when fed to Labrador Retrievers. Transl Anim Sci 2020;4(3).
[41] Stipanuk MH. Role of the liver in regulation of body cysteine and taurine levels: a brief review. Neurochem Res 2004;29:105–10.
[42] Bagley PJ, Stipanuk MH. Rats fed a low protein diet supplemented with sulfur amino acids have increased cysteine dioxygenase activity and increased taurine production in hepatocytes. J Nutr 1995;125:933–40.
[43] Han X, Patters AB, Jones DP, Zelikovic I, Chesney RW. The taurine transporter: mechanisms of regulation. Acta Physiol 2006;187:61–73.
[44] Laidlaw SA, Sturman JA, Kopple JD. Effect of dietary taurine on plasma and blood cell taurine concentrations in cats. J Nutr 1987 Nov;117:1945–9.
[45] Backus RC, Ko KS, Fascetti AJ, Kittleson MD, Macdonald KA, Maggs DJ, Berg JR, Rogers QR. Low plasma taurine concentration in newfoundland dogs is associated with low plasma methionine and cyst(e)ine concentrations and low taurine synthesis. J Nutr 2006 Oct;136:2525–33.
[46] Longenecker JB, Hause NL. Relationship between plasma amino acids and composition of the ingested protein. II. A shortened procedure to determine plasma amino acid (PAA) ratios. Am J Clin Nutr 1961 May–Jun;9:356–62.
[47] Banton S, Pezzali JG, Verbrugghe A, Bakovic M, Wood KM, Shoveller AK. Addition of dietary methionine but not dietary taurine or methyl donors/receivers to a grain-free diet increases postprandial homocysteine concentrations in adult dogs. J Anim Sci 2021 Sep 1;99:223.
[48] Delaney SJ, Kass PH, Rogers QR, Fascetti AJ. Plasma and whole blood taurine in normal dogs of varying size fed commercially prepared food. J Anim Physiol Anim Nutr 2003;87:236–44.
[49] Chiang CF, Larsen JA, Sahtout M, Horoschak RE, Yu ZS, Fascetti AJ. Impact of storage temperature, storage duration, and deproteinization on plasma amino acid concentrations in dogs. Res Vet Sci 2021 May;136:416–21.

II

Received: 8 December 2023 | Accepted: 16 July 2024

DOI: 10.1111/jvim.17150

STANDARD ARTICLE

Journal of Veterinary Internal Medicine Open Access

ACVIM American College of Veterinary Internal Medicine

The association between taurine concentrations and dog characteristics, clinical variables, and diet in English cocker spaniels: The Canine taURinE (CURE) project

Karin Kriström[1,2] | Jens Häggström[1] | Andrea J. Fascetti[3] | Lena Ström[1] | Mark Dirven[2] | Joshua Yu[3] | Titti Sjödal Essén[4] | Anna Tidholm[1,4] | Paul D. Pion[5] | Ingrid Ljungvall[1]

[1]Department of Clinical Sciences, Swedish University of Agricultural Sciences, Uppsala, Sweden

[2]Evidensia Södra Animal Hospital, Huddinge, Sweden

[3]Department of Molecular Biosciences, School of Veterinary Medicine University of California Davis, Davis, California, USA

[4]Anicura Albano Animal Hospital, Danderyd, Sweden

[5]Veterinary Information Network, Davis, California, USA

Correspondence
Karin Kriström, Department of Clinical Sciences, Swedish University of Agricultural Sciences, Box 7054, 75007 Uppsala, Sweden.
Email: karin.kristrom@slu.se

Funding information
The Greater Stockholm Veterinary Hospital Foundation; Agria Djurförsäkringar Research Fund; Thure F och Karin Forsbergs Stiftelse; Amino Acid Laboratory and the Sommer Endowment, School of Veterinary Medicine, University of California, Davis

Abstract

Background: Occurrence of low blood taurine concentrations (B-TauC) and predisposing factors to taurine deficiency in English Cocker Spaniels (ECS) are incompletely understood.

Objectives: Investigate the occurrence of low B-TauC in a Swedish population of ECS and evaluate the association between B-TauC and dog characteristics, clinical variables, and diet composition.

Animals: One-hundred eighty privately owned ECS.

Methods: Dogs were prospectively recruited and underwent physical examination, blood analyses, and echocardiographic and ophthalmic examinations. Dogs with clinical signs of congestive heart failure (CHF) also underwent thoracic radiography. Taurine concentrations were analyzed in plasma (EDTA and heparin) and whole blood. Diets consumed by the dogs at the time of the examination were analyzed for dietary taurine- (D-TauC), cysteine- (D-CysC), and methionine concentrations (D-MetC).

Results: Fifty-three of 180 dogs (29%) had low B-TauC, of which 13 (25%) dogs had clinical and radiographic signs of CHF, increased echocardiographic left ventricular (LV) dimensions and volumes, and impaired LV systolic function. Five (9%) dogs with low B-TauC had retinal abnormalities. Dietary MetC, dietary animal protein source (red/white meat), and age were associated with B-TauC in the final multivariable regression model ($P < .001$, $R^2_{adj} = .39$).

Abbreviations: <B-TauC:CHF+, low taurine concentrations in one or more blood tube additive and clinical and radiographic signs of congestive heart failure; <B-TauC:CHF−, low taurine concentrations one or more blood tube additive and normal cardiac morphology; ACS, American Cocker Spaniel; ACVIM, American College of Veterinary Internal Medicine; AO, aorta; B-TauC, blood (plasma and/or whole blood) taurine concentrations; BW, body weight; CHF, congestive heart failure; CM, cardiomyopathy; CS, Cocker Spaniel; D-CysC, dietary cysteine concentrations; D-MetC, dietary methionine concentrations; D-TauC, dietary taurine concentrations; DCM, dilated cardiomyopathy; ECS, English Cocker Spaniel; EDTA-TauC, EDTA taurine concentrations; EDV, end diastolic volume; EF, ejection fraction; EPSS, E point to septal separation; ESV, end-systolic volume; FS, fractional shortening; Hep-TauC, heparinized plasma taurine concentrations; IQR, interquartile range; LA, left atrium; LA/AO, left atrium to aortic ratio; LV, left ventricle; LVIDd, left ventricular internal diameter in diastole; LVIDdn, left ventricular internal diameter in diastole normalized to body weight; LVIDs, left ventricular internal diameter in systole; LVIDsn, left ventricular internal diameter in systole normalized to body weight; nB-TauC, normal blood taurine concentrations; SLU, Swedish University of Agricultural Sciences; TauC, taurine concentrations; UC Davis AAL, University of California Amino Acid Laboratory; WB, whole blood; WB-TauC, whole blood taurine concentrations.

Conclusions and Clinical Importance: Low B-TauC suggests that taurine deficiency may play a role in the development of myocardial failure and CHF in ECS. Low D-MetC and diets with red meat as the animal protein source were associated with low B-TauC. Dogs with B-TauC below the normal reference range were older than dogs with normal concentrations.

KEYWORDS
amino acids, diet-associated DCM, dogs, heart disease, retinal degeneration, taurine deficiency

1 | INTRODUCTION

The amino acid taurine plays an essential role in various physiological processes.[1-3] Taurine synthesis depends on sufficient enzymatic activity and the availability of precursors methionine and cysteine, with synthesis capacity varying among species.[1,2]

Taurine is considered a dietary, nonessential amino acid in dogs receiving a balanced and bioavailable diet.[4-7] Nevertheless, taurine-responsive cardiomyopathy (CM) has been reported in certain breeds and individuals.[8-12] Insufficient dietary intake of methionine and cysteine has been associated with low blood taurine concentrations (<B-TauC) in dogs.[13] Potential associations between dietary factors and taurine deficiency have been investigated by several research groups, and although various dietary compositions have been proposed to cause taurine deficiency in dogs, no definitive conclusions have been reached.[7,14-18]

The first reports of taurine-responsive CM in dogs were published in the 1990s.[8,12] A prospective placebo-controlled study involving 11 American Cocker Spaniels with a dilated CM (DCM) phenotype and <B-TauC identified improved echocardiographic variables after taurine normalization.[8] Although these early reports led to a recommendation to supplement dogs with DCM phenotype and <B-TauC, they did not result in any general recommendations for taurine content in commercial dog foods.

The English Cocker Spaniel (ECS) traditionally has been considered predisposed to primary DCM rather than taurine-responsive CM.[19,20] However, a retrospective study reported that 13 of 16 ECS with a DCM phenotype and congestive heart failure (CHF) had <B-TauC.[11] Taurine deficiency also has been associated with retinal degeneration in dogs, although only a few reports have been published in the area.[15,21]

The Canine taURinE (CURE) project is a research project with an overall aim to investigate the occurrence of <B-TauC in selected dog populations and identify potential underlying causative factors and clinical consequences of taurine deficiency in dogs. The ultimate goal of the project is to increase knowledge about taurine deficiency in dogs and thereby decrease morbidity and mortality in the dog population. Our aims were to investigate the occurrence of <B-TauC in a Swedish population of ECS and evaluate a possible association between B-TauC and dog characteristics, clinical variables, and diet composition.

2 | MATERIALS AND METHODS

The study was approved by the Ethical Committee for Animal Welfare in Stockholm, Sweden (5.8.18-01548/2017, 5.8.18-21508/2021, and 5.8.18-04682/2020).

Client-owned dogs were prospectively recruited to the cardiology units at the University Animal Hospital of the Swedish University of Agricultural Sciences (SLU), Uppsala, Sweden, and at Anicura Albano Animal Hospital in Danderyd, Sweden, between September 2018 and April 2022. All owners received verbal and written information about the study and gave their consent before inclusion.

2.1 | Inclusion and exclusion criteria

English Cocker Spaniels were eligible for inclusion from 6 months of age, with no specified upper age limit. At the time of enrollment, dogs could either be clinically healthy, as assessed by their owners, or exhibit clinical signs indicative of cardiac disease, such as increased respiratory rate, increased respiratory effort, syncopal events, and exercise intolerance. Presumed healthy dogs were recruited via breeders, advertisements in breed magazines, and social media. Dogs with clinical signs of cardiac disease were recruited from the emergency clinics at the respective hospital and underwent study examinations after clinical signs related to cardiac disease had resolved. Exclusion criteria were clinically relevant noncardiac, systemic, or organ-related disease based on history, physical examinations, and results from blood analyses (CBC and serum biochemistry), or echocardiographic findings indicating congenital or acquired non-DCM phenotype-related cardiac disease. Congestive heart failure treatment and taurine supplementation were initiated in dogs diagnosed with CHF at enrollment. Dogs receiving cardiac medical treatment or taurine supplementation before enrollment were excluded.

2.2 | Procedures

All dogs underwent systemic blood pressure measurement, complete physical examination including assessment of body condition score on a 9-point scale, ECG registrations, echocardiographic and ophthalmologic examinations, and blood sample collection

(CBC, serum biochemistry, and B-TauC). Fasting was not required before blood sampling because fasting status does not appear to impact taurine concentration in dogs.[22,23]

Dogs with CHF initially were managed by the emergency clinic at either hospital, and received standard CHF treatment at the discretion of the attending veterinarian. The diagnosis of CHF was based on case history, physical examination findings, and results from radiographic and echocardiographic examinations. All dogs with CHF received taurine supplementation (250 mg PO q12h) after blood samples for taurine analyses had been collected, without waiting for the results of the blood taurine analyses. Dogs presenting to the emergency clinic with clinical signs of CHF were included even if only EDTA samples had been collected before initiation of taurine supplementation.

2.3 | Questionnaire

Owners completed a questionnaire regarding the dog's health status (previous diseases, medications, and surgical interventions), reproductive history (neutered yes/no, number of litters, potential reproductive problems, congenital diseases in litters), and diet (current diet, amount and type of treats, access to other animals' food or leftovers).

2.4 | Blood pressure measurements

Measurements were made using high-definition oscillometry (Vet HDO Monitor S+B medVET GmbH, Babenhausen, Germany) after a standardized protocol according to published guidelines.[24]

2.5 | Echocardiography

All echocardiographic examinations were performed and assessed by a board-certified specialist in cardiology (ILJ, JH, AT, and MD) or a resident in veterinary cardiology under supervision (KK). Dogs were unsedated and gently restrained in right and left lateral recumbency. Transthoracic echocardiographic examinations were performed during simultaneous ECG monitoring with an ultrasound unit using 5.0 to 9.2 MHz phased-array transducers (EPIQ 7G; Philips Ultrasound, Bothell, WA, USA). The echocardiographic examinations included the use of color flow Doppler, M-mode, and 2-dimensional echocardiographic modalities and were performed as previously described.[23] Left ventricular internal dimensions at end-diastole (LVIDd) and end-systole (LVIDs) were normalized for body weight (LVIDDn and LVIDSn) using the formulas by Cornell et al.[25] Measurements of LV fractional shortening (LV FS%) were made on right parasternal short axis view. E-point to septal separation (EPSS) measurements were made on right parasternal long-axis views. Left ventricular end-diastolic volume (LVEDV), end-systolic volume (LVESV), and ejection fraction (EF) were calculated using Simpson's modified method of disks. All echocardiographic measurements were made on 3 consecutive cardiac cycles, and the mean value was used for statistical analyses.

2.6 | ECG

A 3-minute standard 6-lead ECG was registered with the dog gently restrained in right lateral recumbency. Registrations were evaluated by a board-certified cardiology specialist (ILJ, JH, AT, and MD) or a cardiology resident under supervision (KK).

2.7 | Ophthalmic examinations

Each dog underwent a complete ophthalmic examination including neuro-ophthalmic testing (menace response, dazzle reflex, pupillary light reflexes, and palpebral reflexes), slit-lamp biomicroscopy of the adnexa and anterior segment, rebound tonometry and, after pharmacologic mydriasis (Tropikamid, eye drops, 0.5%, Bausch&Lomb, Stockholm, Sweden), indirect ophthalmoscopy. Fundus photography was performed in dogs with fundic lesions (Optibrand ClearView 2 Retinal Camera, Eickemeyer, Germany) and all images were reviewed by a board-certified ophthalmology specialist.

2.8 | Blood sampling

Blood samples were collected by jugular venipuncture using a butterfly needle with a Luer adapter (21G; BD Vacutainer, Eysins, Switzerland) collecting blood directly into serum, heparin, and EDTA vacutainer tubes (Greiner Bio-One GmbH, Kremsmünster, Austria).

2.9 | Hematology and biochemistry analyses

One milliliter of blood was collected in EDTA tubes for CBC and hematology. Three milliliters of blood was collected in serum tubes for routine biochemistry analyses (creatinine, blood urea nitrogen, phosphate, alanine aminotransferase, alkaline phosphatase, bile acids, potassium, sodium, calcium, albumin, total protein, c-reactive protein, thyroid stimulating hormone, and thyroxine). All samples were analyzed at the accredited commercial veterinary laboratory at the SLU or the in-house laboratory at Albano animal hospital on the same day as the samples were collected.

2.10 | Blood taurine analyses

Six milliliters of blood was collected in 2 separate heparinized tubes for whole blood (WB) samples (2 mL) and heparinized (Hep) plasma samples (4 mL), and 4 mL of blood was collected in EDTA tubes for EDTA plasma samples. Plasma samples (Hep and EDTA) were centrifuged at 3000 rpm for 5 minutes within 30 minutes of collection. EDTA plasma stored at −20°C at the in-house laboratory samples (0.5 mL) were transferred into microcentrifuge tubes (VWR International AB) until being transported frozen to IDEXX Laboratories in Germany within 5 days after collection of blood samples. Analyses of

EDTA plasma were made using liquid chromatography with mass spectrometry.[26]

Heparinized plasma samples (0.2 mL) were transferred into Eppendorf tubes and mixed with sulfosalicylic acid (0.2 mL) for deproteinization according to published recommendations.[27,28] Heparinized WB samples (2 mL) were transferred into Eppendorf tubes (1 mL). Heparinized plasma and WB samples were stored at −80°C at SLU, and at −70°C at Albano and transported frozen as a batch to the University of California Davis Amino Acid Laboratory (UC Davis AAL) within 9 months of collection. Heparinized plasma samples and WB samples were analyzed using ion exchange chromatography.[28] All blood samples were treated according to recommendations established by the respective laboratory.[26,28] The majority (94%) of samples were handled by 1 person (KK) to minimize differences in handling procedures. Plasma samples were centrifuged and separated within 30 minutes after collection and only the top layer of the plasma was transferred from each sample to avoid contamination of taurine-rich cells from the buffy coat. Hemolyzed plasma samples were not analyzed. Results are reported in nmol/mL.

Reference ranges for WB taurine concentrations (WB-TauC), EDTA plasma taurine concentrations (EDTA-TauC), and heparinized plasma taurine concentrations (Hep-TauC) in dogs were provided by the analyzing laboratory.[26,28] Thresholds established by UC Davis AAL for low blood taurine concentrations are <60 nmol/mL for heparinized plasma and <200 nmol/mL for WB. Heparinized plasma-TauC <40 nmol/mL and WB-TauC <150 nmol/mL are considered critically low, indicating a risk for taurine deficiency and potential secondary disorders.[28] Detailed thresholds have, to our knowledge, not been established for EDTA-TauC, and the single cutoff provided by IDEXX laboratories (<44 nmol/mL) was used in our study.[26] Lowest limits of quantification for B-TauC, communicated from respective laboratories, were 0.4 nmol/mL for WB-TauC and Hep-TauC, and 7.99 nmol/mL for EDTA-TauC.

2.11 | Diets

Information regarding the diet each dog consumed at the time of the examination and 3 months preceding was recorded upon enrollment. Samples consisting of 100 g each were collected from identical formulas of the various dry diets that the dogs included in the study had been consuming to analyze dietary concentrations of taurine (D-TauC), cysteine (D-CysC), and methionine (D-MetC). Samples from raw food diets were not collected for analysis because of the inconsistent content between batches in such diets. All collected samples were securely stored in sealed plastic bags at room temperature and protected from direct sunlight until shipped to an accredited external laboratory (Food&Feed Testing Sweden AB, Lidköping, Sweden) for analyses. The maximum storage time before shipping was 4 months.

Protein and carbohydrate sources were extracted from the diet ingredient list provided by the manufacturer. Dietary protein sources were categorized into 4 groups: red meat (lamb, beef, pork, reindeer, and venison), white meat (poultry and fish), mixed red/white meat, and other protein sources (soy, vegetables, and insects). A diet was classified as based on red or white meat only if all protein sources could be categorized as either red or white meat. Fish oil or animal fat were not taken into consideration in the categorization. Diets were categorized as grain-inclusive or grain-free based on whether they contained grains or grain-derived ingredients. Diets were categorized as potatoes or pulses inclusive, or both, if they contained potato, sweet potato or pulses (legumes, soybeans, peas, and lentils) or some combination of these as 1 of the first 10 ingredients on the ingredient list. Dogs that were subjected to diet changes <3 months before enrollment were excluded from analyses regarding B-TauC relation to diets.

2.12 | Grouping

Dogs were categorized based on B-TauC as normal B-TauC (nB-TauC) or B-TauC below the normal reference range (<B-TauC; EDTA-TauC <44 nmol/mL; Hep-TauC <60 nmol/mL; WB-TauC <200 nmol/mL). All dogs with clinical and radiographic signs of CHF had low B-TauC and dogs were accordingly further subdivided into <B-TauC:CHF+ or <B-TauC:CHF−.

2.13 | Statistics

Statistical analyses were performed using a commercially available software program (JMP Pro v. 16.0.0, Cary, NC, USA). Data were analyzed using descriptive as well as inferential statistics. The level of statistical significance was set at $P < .05$, if not otherwise indicated. Continuous variables were presented as medians and interquartile range (IQR).

Nonparametric Wilcoxon signed-rank test was used to analyze continuous data: age, weight, systolic blood pressure, and echocardiographic variables (LV dimension, volume, and functional variables) among groups (nB-TauC, <B-TauC:CHF−, and <B-TauC:CHF+). Chi-square and Fisher's exact tests were used to test for differences in proportions comparing categorical data (sex and neuter status).

Uni- and multivariable analyses were used to investigate the effect of dog characteristics (age, weight, sex, and neuter status), dietary factors (D-TauC, D-CysC, and D-MetC; protein source: red/white meat, grain-free y/n, and potatoes or pulses or both y/n) on B-TauC. Variables that reached a significance of $P < .2$ in the univariable regression analyses subsequently were included in the multivariable analyses. Furthermore, D-TauC, D-CysC, and D-MetC were compared between diets with red and white protein sources, and diets categorized as grain-free or grain-inclusive.

3 | RESULTS

A total of 180 ECS, 109 (60.5%) females (101 intact and 8 neutered) and 71 (39.5%) males (58 intact and 13 neutered) were included. A

total of 167 dogs were assessed as healthy, and 13 dogs presented with clinical and radiographic signs of CHF. Dog characteristics and clinical variables are presented in Table 1.

3.1 | Questionnaire

All owners filled out the questionnaire. Twenty-seven female dogs had ≥1 litter, of which 5 had ≥1 stillborn puppies in ≥1 litters. Three dogs had given birth to puppies with congenital diseases (patent ductus arteriosus n = 1 and cleft palate n = 2). All dogs experiencing reproductive problems had nB-TauC. Twenty-six dogs had a history of recurrent periods of mild self-limiting diarrhea or vomiting. Twenty of these had nB-TauC and 6 had <B-TauC.

3.2 | Blood taurine concentrations

Taurine concentrations were analyzed in EDTA plasma (n = 180), heparinized WB (n = 175), and heparinized plasma (n = 172) and results are presented in Table 1.

Fifty-three dogs (29%) had B-TauC below the normal reference range, of which 38 (21%) had B-TauC considered critically low. All 13 dogs presenting with clinical and radiographic signs of CHF had critically low B-TauC (Figure 1). A total of 42/172 (24%) dogs had <Hep-TauC, of which 26 (62%) had critically low Hep-TauC, 35/180 (19%) dogs had <EDTA-TauC, all with critically low EDTA-TauC, and 27/175 (15%) dogs had <WB-TauC, of which 15 (56%) had critically low WB-TauC. Analyses for WB-TauC and Hep-TauC were not performed in 5 and 8 dogs, respectively, because some of the dogs presenting with signs of CHF initially were managed out of hours at the emergency clinic where blood sampling for EDTA-TauC analyses was prioritized (as European commercial veterinary laboratories at that time only offered EDTA plasma-based TauC analyses) before taurine supplementation. EDTA-TauC was analyzed in all 180 dogs and used for subsequent comparisons and statistical analyses.

3.3 | Echocardiography

All 13 dogs presenting with CHF had increased LV dimensions (LVIDDn, LVIDSn) and volume variables (LVEDV and LVESV), as well as increased EPSS. The measured values of FS were within or slightly below the reported normal reference range[29-31] in 11 of the 13 < B-TauC:CHF+ dogs.

A total of 167 dogs had echocardiographic dimensional, volume, and functional variables within normal reference ranges. Forty of these dogs had <B-TauC and 127 had nB-TauC. Summary

TABLE 1 Summary of dog characteristics, taurine concentrations in EDTA-plasma, heparin plasma, and whole blood, and echocardiographic data in 180 English Cocker Spaniels.

	All dogs n = 180	nB-TauC n = 127	<B-TauC:CHF− n = 40	<B-TauC:CHF+ n = 13
Age (years)	3.6 (1.7-6.3)	3.5 (1.6-5.9)[a]	3.6 (1.4-7.0)[ab]	8.2 (3.8-10.0)[b]
BW (kg)	12.8 (11.3-14.5)	12.9 (11.4-14.5)[a]	12.3 (10.9-14.5)[a]	12.8 (11.7-15.6)[a]
BCS (U/N/O)	(0/141/39)	(0/102/25)[a]	(0/33/7)[a]	(0/6/7)[a]
Sex (f/m)	109/70	79/48[a]	23/16[a]	7/5[a]
Neutered (y/n)	21/158	15/112[a]	4/35[a]	1/11[a]
Taurine EDTA nmol/mL	71.91 (47.94-103.87)	87.89 (71.91-111.86)[a]	39.95 (31.96-55.93)[b]	7.99 (7.99-7.99)[c]
Taurine Heparin nmol/mL	92 (59-111)	103 (86-119)[a]	40 (23-54)[b]	5.5 (3.25-82.25)[b]
Taurine WB nmol/mL	261 (231.5-294)	279.5 (252.3-303.5)[a]	204 (173-245)[b]	35 (27.3-49.8)[c]
LVIDDn	1.59 (1.51-1.7)	1.57 (1.5-1.68)[a]	1.57 (1.53-1.65)[a]	2.36 (2.19-2.49)[b]
LVIDSn	1.08 (.98-1.2)	1.07 (.97-1.19)[a]	1.08 (0.96-1.13)[a]	1.84 (1.66-1.93)[b]
LVEDV (mL)	30.3 (26.3-36)	29.8 (26-34.1)[a]	30.1 (4.9-35.1)[a]	66.2 (52.6-73.05)[b]
LVESV (mL)	12.7 (10.2-15.6)	12.3 (9.7-14.3)[a]	12.7 (.5-16.3)[a]	43.6 (33.2-47.75)[b]
EPSS (cm)	.4 (.33-.5)	.4 (.3-.49)[a]	.37 (.33-.43)[a]	1.05 (.98-1.13)[b]
FS (%)	28 (24.42-32.2)	28.79 (24.73-32.71)[a]	27.81 (25.07-32.54)[a]	18.89 (16.3-20.78)[b]
EF (%)	58.50 (52.51-63.91)	60 (53.58-64.6)[a]	56.52 (53.31-62.68)[a]	32.91 (31.54-38.86)[b]

Note: Values are reported as median and interquartile ranges (IQR). Body condition score was based on a 9-point scale and divided into underweight (BCS 1-3), normal weight (BCS 4-5), and overweight (BCS 6-9). Within each row, values with the same superscript letter did not differ significantly ($P > .017$).
Abbreviations: <B-TauC:CHF−, dogs with low taurine concentrations one or more blood tube additive and normal cardiac morphology; <B-TauC:CHF+, dogs with low taurine concentrations in one or more blood tube additive and clinical and radiographic signs of CHF; BCS, body condition score; BW, body weight; CHF, congestive heart failure; DCM, dilated cardiomyopathy; LVEDV, left ventricular end-diastolic volume; EF, ejection fraction; EPSS, E point to septal separation; LVESV, left ventricular end-systolic volume; FS, fractional shortening; LVIDDn, left ventricular inner diameter in diastole normalized to body weight; LVIDSn, left ventricular inner diameter in systole normalized to body weight; nB-TauC, dogs with normal taurine concentrations and normal cardiac morphology; U/N/O, underweight/normal weight/overweight; WB, whole blood.

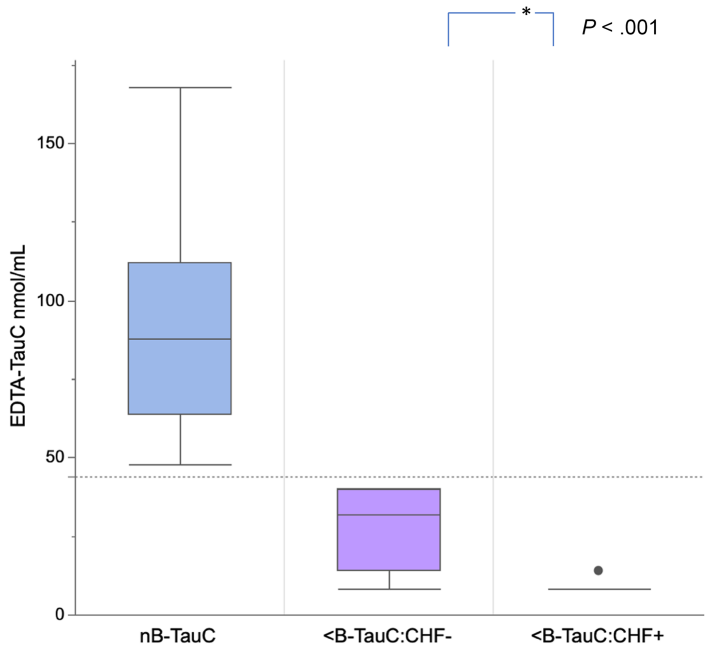


FIGURE 1 Box and whiskers plots demonstrating EDTA-plasma taurine concentrations (EDTA-TauC) in dogs with normal taurine concentrations (nB-TauC) n = 127, dogs with low taurine concentrations and normal echocardiograms (<B-Tau:CHF−) n = 40, and dogs low taurine concentrations and CHF (<B-Tau:CHF+) n = 13. The horizontal line corresponds to the normal lower reference value for EDTA plasma provided by the analyzing laboratory. Asterisk (*) indicates significant differences. Solid circle corresponds to outliers. 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, respectively. CHF, congestive heart failure.

statistics of echocardiographic variables in the different groups (nB-TauC, <B-TauC:CHF−, and <B-TauC:CHF+) are presented in Table 1.

All <B-TauC:CHF+ dogs had mild to moderate mitral regurgitation assessed as secondary to ventricular dilatation in the absence of mitral valve pathology. Trivial mitral regurgitation also was found in dogs with normal cardiac morphology and was assessed as nonpathologic in all cases.

3.4 | Electrocardiography

One dog with CHF and <B-TauC presented with atrial fibrillation at enrollment. All other dogs presented with sinus rhythm.

3.5 | Ophthalmic examinations

Five dogs with <B-TauC had bilateral and symmetrical elliptical hyperreflective lesions in the area centralis, dorso-temporal to the optic disk (Figure 2A-C). Similar changes were seen in 1 dog with nB-TauC. Also, in 2 <B-TauC dogs, bilateral symmetric tapetal hyperreflectivity and mild to moderate vessel attenuation were observed. Additional abnormal ophthalmic findings observed in a population of dogs that all had nB-TauC included multifocal retinal dysplasia (3 dogs), focal, inactive postinflammatory chorioretinal scars (7 dogs), and an optic nerve coloboma (1 dog).

3.6 | Diets

Each dog's main diet was registered. The distribution of dietary protein sources is presented in Figure 3. A total of 173 dogs (96%) were fed a commercial complete dry food, and 7 dogs (4%) were fed commercially prepared raw food. All but 5 (97%) of the included dogs had access to treats, leftovers, or other animals' food in addition to their regular diet.

Sixty-eight different diets (60 dry foods and 8 raw foods) from 29 different food manufacturers were identified. Thirty diets contained red meat (lamb, beef, pork, reindeer, and venison) as protein source, 23 diets contained white meat (poultry and fish) as protein source, 3 diets contained a mix of red and white protein sources, and 4 diets contained other protein sources (soy, vegetables, and insects). Twenty-eight (41%) diets were categorized as grain-free, and 23 (34%) diets were categorized as "potatoes with or without pulses inclusive". Fifteen (54%) grain-free diets were categorized as potatoes with or without pulses inclusive.

Sixty dry food samples were analyzed for D-TauC, D-CysC, and D-MetC (g/100 g), and results are presented in Tables S1 and S2. The diet analyses were performed "as fed" and the results were converted into dry matter basis by using a presumed moisture content of 10%.[32] Forty-two dogs (79%) with <B-TauC consumed diets with D-TauC below median concentrations of the 60 analyzed diets, 9 <B-TauC dogs (17%) consumed diets above median concentrations of the 60 analyzed diets and 2 <B-TauC dogs (4%) consumed raw food. All <B-TauC:CHF+ dogs consumed diets with D-TauC below the median concentration of the

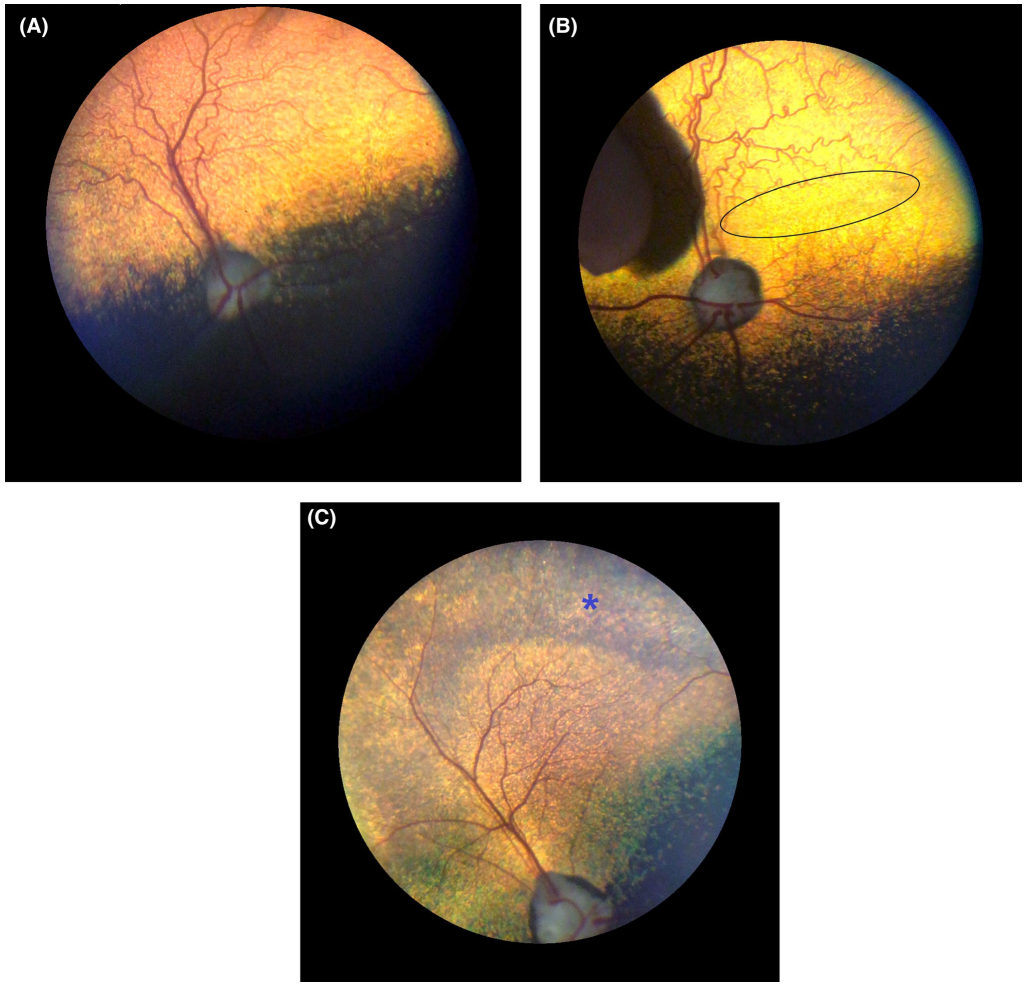


FIGURE 2 (A) Fundoscopy of a dog with normal fundus. (B) Fundoscopy in a 5-year-old female English Cocker Spaniel with abnormal EDTA-plasma taurine concentration (<7.99 nmol/mL) showing retinal lesions similar to those reported in taurine-depleted cats, including an elliptical hyperreflective lesion in the area centralis and visual streak (encircled area). Lesions were bilaterally symmetrical. (C) Fundoscopy in an 8-year-old female English Cocker Spaniel with abnormal EDTA-plasma taurine concentration (<7.99 nmol/mL) showing retinal lesions including tapetal hyperreflectivity and vessel attenuation in the periphery (*). Lesions were bilaterally symmetrical. The dog was severely visually impaired.

60 analyzed diets. Five (8%) and 3 (5%) diets had D-MetC and D-MetC +D-CysC below or just meeting the recommended daily requirements stated by The American National Research Council.[33] Four diets with <D-MetC contained red meat as protein source, and 1 diet contained mixed protein (red and white meat) as protein source. All 3 diets with <D-MetC +D-CysC contained red meat as protein source. All diets with D-MetC and D-MetC+D-CysC below recommended daily requirements were associated with <BTauC and <D-TauC. The concentrations of the 3 amino acids in the food were covariate; D-MetC and D-TauC ($P < .001$), and D-MetC and D-CysC ($P = .02$).

3.7 | Univariable analyses

Diets with red meat as the animal protein source contained lower D-TauC ($P < .001$, $R^2_{adj} = .18$), D-CysC ($P < .001$, $R^2_{adj} = .07$), and D-MetC ($P < .001$, $R^2_{adj} = .1$) compared with diets with white meat as the animal protein source. EDTA-TauC were associated with D-TauC ($P < .001$, $R^2_{adj} = .2$), and D-MetC ($P < .001$, $R^2_{adj} = .23$). Diets with red meat as the animal protein source were associated with lower EDTA-TauC when compared with diets with white meat as the animal protein source ($P < .001$, $R^2_{adj} = .21$). Diets categorized as grain-free, and diets containing potatoes

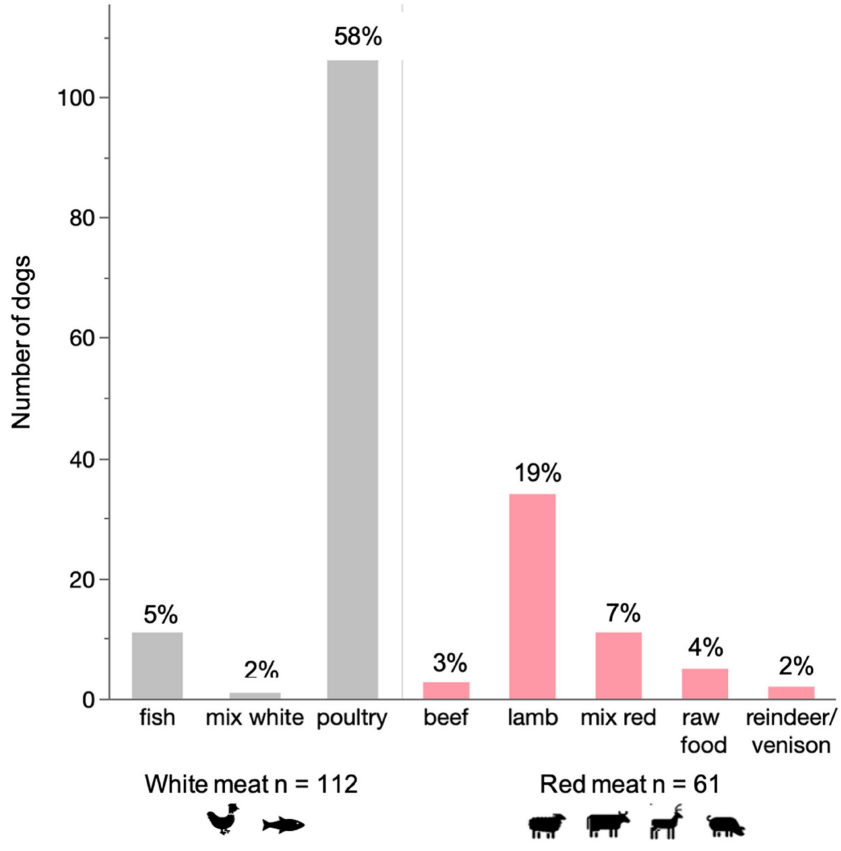


FIGURE 3 Staple diagram demonstrating the distribution of protein sources in the various diets consumed by the included dogs. Diets with several different red or white meat components in the same diet were categorized as "mix red" and "mix white". Diets based on a mixture of red and white meat in the same diet, and diets based on soy, vegetables, and insects and were excluded in this analysis. Percentages indicate the proportion of the 173 dogs that consumed the different diets containing either red or white meat as protein source.

or pulses, or both, as 1 of the top 10 ingredients were associated with lower EDTA-TauC compared with grain-inclusive diets ($P = .05$, $R^2_{adj} = .02$). Diets containing potatoes or pulses, or both, as one of the top 10 ingredients were associated with lower EDTA-TauC compared with diets that did not contain potatoes or pulses, or both ($P = .01$, $R^2_{adj} = .03$). The association between EDTA-TauC and diets with red meat as the animal protein source remained, regardless of whether or not the diets were grain-free or included potatoes or pulses, or both. Additionally, B-TauC decreased with increasing age in the dogs included in the study ($P = .02$, $R^2_{adj} = .02$).

3.8 | Multivariable analyses

Dietary MetC, protein source: red or white meat, and age remained associated with EDTA-TauC in the final multivariable regression model ($P < .001$, $R^2_{adj} = .39$; Figure 4A-C).

4 | DISCUSSION

In our study, 29% of the included dogs had B-TauC below the normal reference range, and 21% had critically low concentrations. Of dogs with low B-TauC, 25% presented with signs of CHF and 9% with retinal abnormalities. Low D-MetC, and red meat-based diets were associated with <B-TauC, and dogs with <B-TauC were older than dogs with normal concentrations.

To the best of our knowledge, ours is the first prospective exploratory study investigating the occurrence of low B-TauC in a relatively large group of ECS, including both healthy dogs and dogs presenting with CHF. One-hundred eighty ECS were examined prospectively, and 25% of the 53 dogs presenting with B-TauC below the normal reference range had signs of increased LV dimensions, systolic dysfunction, and CHF. A similar occurrence was found in a previous study including 115 Irish wolfhounds, where 53% of the included dogs had

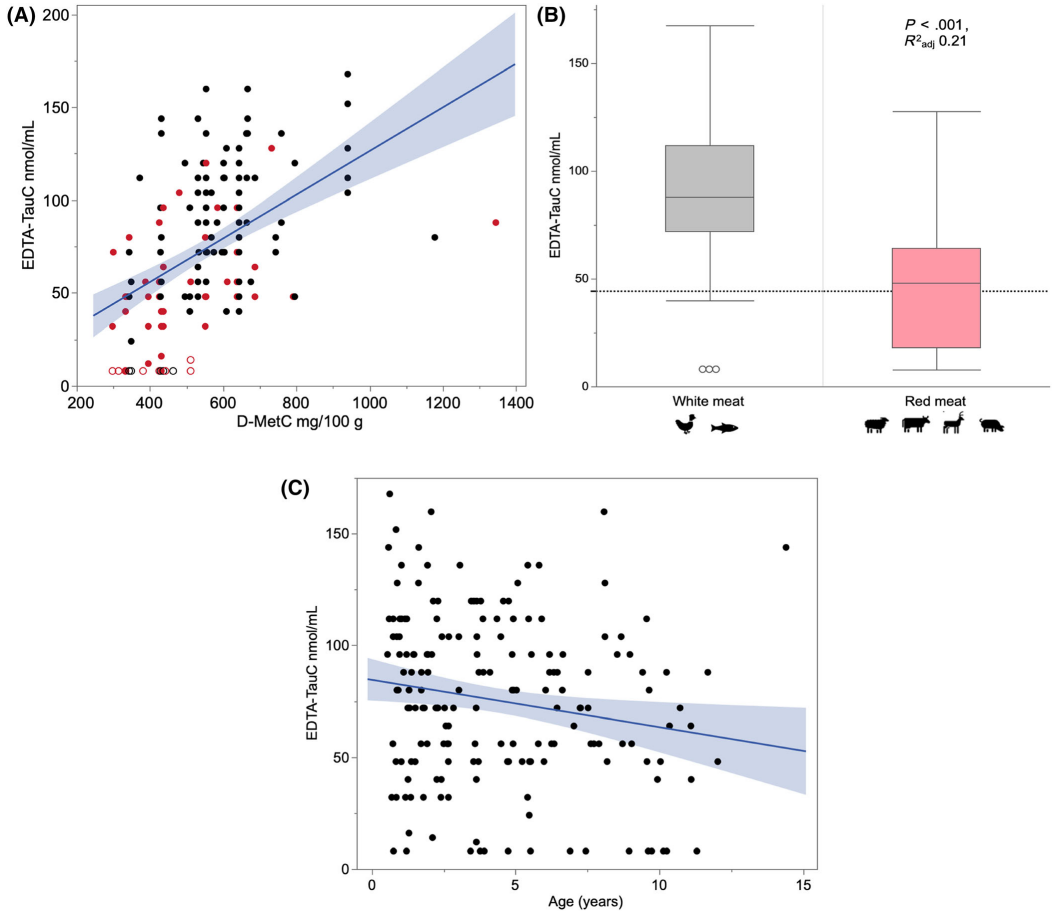


FIGURE 4 (A) Scatterplots demonstrating EDTA-taurine concentrations by dietary methionine concentrations and regression line with shaded 95% confidence interval (CI) in 167 English Cocker Spaniels consuming dry diets. Red-filled small dots represent dogs consuming diets based on red meat and black-filled small dots represent dogs consuming diets based on white meat. Red and black circles represent dogs diagnosed with congestive heart failure and low taurine concentrations that had consumed diets based on red (red circles) and white (black circles) meat, respectively. Low dietary methionine concentrations were associated with low EDTA-taurine concentrations ($P < .001$, $R^2_{adj} = .23$). D-MetC: Dietary methionine concentrations, EDTA-TauC: EDTA-taurine concentrations. (B) Box and whiskers-plot demonstrating B-TauC in 173 dogs consuming diets based on protein sources categorized as red meat (lamb, beef, venison, and pork; n = 61) and protein sources categorized as white meat (fish, poultry; N = 112). Seven dogs consumed diets based on a mixture of red and white meat or diets based on soy, vegetables, and insects, and these dogs were excluded in this analysis. Taurine concentrations were significantly lower in dogs fed a diet based on red meat then dogs fed a diet based on white meat ($P < .001$, $R^2_{adj} = .21$). The horizontal line corresponds to the normal lower reference value for EDTA-plasma communicated by IDEXX laboratories, Germany. TauC: Taurine concentrations. (C) EDTA-taurine concentrations by age and regression line with shaded 95% confidence interval (CI) in 180 ECS. Taurine concentrations decreased with increasing age ($P < .02$, $R^2_{adj} = .02$).

WB-TauC below the normal reference range, and 41% of dogs with <WB-TauC were diagnosed with a DCM phenotype.[34] A comparable number of dogs with nB-TauC also were identified with a DCM phenotype in that study, resulting in no discernible differences between the 2 groups. Taurine concentrations, however, were only analyzed in WB in that study, which leaves the possibility that dogs with low plasma-TauC might have been missed. Possibly 2 different forms of CM may coexist in the same breed.

All <B-TauC:CHF+ dogs had EDTA-TauC <15 nmol/mL, whereas many of the <B-TauC:CHF− dogs, all with normal cardiac morphology on echocardiography, had mildly decreased EDTA-TauC. This finding may suggest that severe myocardial changes primarily appear in dogs

with depleted taurine reserves. However, EDTA-TauC <15 nmol/mL also were identified in 5 dogs with echocardiographically normal hearts, indicating that the response to low B-TauC varies among individual dogs. A similar pattern has been found in studies conducted on cats fed a taurine-depleted diet over an extended time period, where only 25% to 30% of the cats developed myocardial failure although both plasma and myocardial taurine concentrations were low in all cats.[4,35] The progression from having subthreshold B-TauC to developing deficiency and secondary disorders has yet to be investigated in dogs. Also, the time required to develop clinical signs in taurine-deficient dogs remains unknown. In our study population, <B-TauC:CHF+ dogs were older than nB-TauC dogs and <B-TauC:CHF− dogs, which might indicate that disease progression is age-dependent. However, a large age range (7 months–12 years) in dogs with CHF suggests that the impact of taurine deficiency varies considerably among individuals.

The association of low B-TauC with echocardiographic signs of LV systolic dysfunction and CHF in ECS in our study corresponds with the findings in a previous retrospective study where 81% of the 16 included ECS with a DCM phenotype and CHF had low B-TauC.[11] All 13 <B-TauC:CHF+ dogs in our study population had LV dimensions, volumes, EF, and EPSS compatible with a DCM phenotype. Fractional shortening, considered indicative of LV systolic function, however, was within or just below the reported normal reference ranges in 11 of the 13 dogs.[29,30] These findings suggest that increased LV inner diameters and volumes, decreased EF, and increased EPSS may be more prominent features of taurine-responsive CM in ECS.

Six dogs had ocular changes similar to those previously reported in cats with taurine deficiency and 5 of these dogs had B-TauC below the normal reference range.[36] Retinal degeneration and subsequent blindness is a well-documented secondary consequence of taurine deficiency in cats, and retinal changes have been reported to appear after approximately 6 to 9 months of depletion.[6,37,38] Although further progression of retinal degeneration is prevented with normalized B-TauC, the retinal lesions are not reversible and typical retinal lesions in an individual with nB-TauC may indicate previous taurine deficiency.[12,39] The dog in our study population displaying retinal changes but nB-TauC may, accordingly, have had a history of taurine deficiency, explaining the retinal abnormalities. Such a scenario, however, cannot be confirmed retrospectively. Retinal lesions also were found in 3 of the 11 American cocker spaniels with taurine-responsive CM investigated in a previous study of taurine deficiency in dogs.[8,35]

Heparinized plasma analyses identified more dogs with concentrations below specified reference values than EDTA-plasma analyses in our study population. On the other hand, EDTA-plasma analyses identified more dogs with plasma concentrations considered critically low (<40 nmol/mL) than heparinized plasma analyses. This discrepancy could, at least partly, be explained by the different cutoff values used by the analyzing laboratories (IDEXX EDTA-TauC <44 nmol/mL and UC Davis AAL Hep-TauC <60 nmol/mL) and emphasizes the importance of validated reference values for the method and additive used. Amino acid concentrations also have been shown to vary across analytic methods.[40] In addition, B-TauC has been observed to have significant daily intraindividual variation in both plasma and WB, further complicating the interpretation of results.[27,28]

Seventy-nine percent of the dogs with low B-TauC and all <TauC:CHF+ dogs consumed diets with D-TauC below the median value of the 60 diets analyzed. Furthermore, diets composed of red meats were associated with lower EDTA-TauC and lower D-TauC, D-CysC, and D-MetC compared with diets composed of white meats. The protein source is essential in a diet's amino acid content, and different protein sources contain different amounts of taurine. Shellfish, fish, and chicken generally contain more taurine than lamb, beef, and pork,[41-43] but D-TauC also differs depending on which part of the animal is used in the diet. Chicken legs contain, for example, almost twice as much taurine as chicken breast.[41,42] Plant-based proteins such as soy and vegetables do not naturally contain taurine, whereas insect-based protein may be comparable with animal protein sources if the most taurine-rich insect species are used.[34] There are no official recommendations for minimum taurine content in dog food, and it is currently unknown how preparation and heat processing may affect the taurine bioavailability of the end product, further complicating the assessment of adequate supplementation requirements.[42,44]

Although the association between red meat and low TauC was observed in both blood and diets, it cannot be concluded that the protein source alone provides insufficient taurine concentrations without knowing whether the various diets are taurine supplemented by the manufacturer. Disparities between diets with the highest and lowest D-TauC exceeded 500 mg/100 g (as fed) of feed in our study, corresponding to the daily dose of taurine supplementation (250 mg q12h) given to dogs with low B-TauC in our study population. Although it is not possible to state with certainty, it is likely that the discrepancy is not solely due to the choice of protein source, and that some food manufacturers supplement their diets whereas others do not. In addition, plant-based diets analyzed in our study all had D-TauC well above median concentrations. Neither the packaging ingredient lists nor the European manufacturers' websites provided any information regarding the taurine content in the diets, whether taurine was sourced from the original ingredients, or if the diets included supplementary taurine.

It is noteworthy that dietary TauC, CysC, and MetC were covariates and that dietary MetC was the variable that remained associated with EDTA-TauC in the multivariable regression analyses. Dietary methionine and cysteine serve as precursors for taurine synthesis, and inadequate amounts have been reported as a causative factor for taurine deficiency in dogs.[13] In addition to serving as rate-limiting factors for taurine synthesis in individuals with adequate enzymatic activity, methionine and cysteine also function as precursors for glutathione, thereby playing crucial roles in the body's antioxidant defenses.[45-47]

Established recommended daily requirement guidelines for methionine and cysteine in dog diets vary with different life stages, depend on digestibility or bioavailability, and have been observed to differ among various breeds.[33,48] In addition, methionine and cysteine are interdependent, meaning that the necessary amount of 1 may change depending on the level of the other.[49] In our study, 8% and 5% of the

analyzed diets failed to meet the established requirements for D-MetC and D-MetC+D-CysC, respectively. All of these diets were associated with dogs having <B-TauC. However, >90% of the diets associated with <B-TauC had D-MetC and D-MetC+D-CysC with recommended dietary requirements or allowances, as defined by the NRC.[32] This observation suggests that adequate intake of the precursors methionine and cysteine may not be sufficient for maintaining adequate taurine concentrations in some individuals. Differences in enzymatic activity, gastrointestinal absorption, or losses, and various metabolic processes also may affect bioavailability.

Dietary grain content was weakly associated with EDTA-TauC in univariable regression analyses ($P = .05$), but the association did not remain significant in the final multivariable regression model. This finding corresponds with a previous study in ECS, where dogs fed a lamb-based diet exhibited lower B-TauC, regardless of whether the diets contained grains.[50] Grain-free diets have been suggested as a potential cause of suspected cases of nutrition-associated DCM in dogs, regardless of confirmed taurine deficiency.[17] Nevertheless, this association is debated, because several subsequent studies have failed to establish a definitive connection between grain content in the diet and the development of CM or <B-TauC.[14,50,51]

More recently, diets rich in pulses (peas, lentils, beans, and chickpeas) or various forms of potatoes, or both, have been reported as a potential causative factor in diet-associated CM in dogs of various breeds, regardless of B-TauC.[16] A weak association between potato and pulse content and B-TauC also was seen in the univariable analyses in our study. The association, however, did not remain in the multivariable analyses.

5 | LIMITATIONS

Heparin-TauC and WB-TauC were not assessed in 7 and 5 dogs, respectively, presenting with CHF out of hours at the emergency clinic of 1 of the study hospitals. Nevertheless, EDTA concentrations in all of these dogs were consistently <7.99 nmol/mL, strongly indicating that both Hep-TauC and WB-TauC would likely fall well below normal reference ranges had they been analyzed.

All of the dogs in our study were privately owned and were fed diets based on the individual decisions of their owners. This factor resulted in a varied distribution of diets among the dogs. Nearly all dogs included in the study (97%) had routine access to treats and leftovers, which adds complexity when assessing the impact of diets on our research outcomes. However, the diets registered in the study represented the primary diet for each dog and accounted for a majority of their daily dietary intake, according to their owners.

6 | CONCLUSIONS

Twenty-nine percent of the included ECS had B-TauC below the normal reference range. Signs of CHF were seen in 25% of dogs with low B-TauC and retinal abnormalities in 9% of these dogs. EDTA-TauC was associated with dietary TauC, MetC, and protein source (red/-white meat). Additionally, dogs with B-TauC below the normal reference range were on average older than dogs with normal concentrations. Low B-TauC suggests that taurine deficiency may play a role in the development of CHF in ECS. As a result, measuring and supplementing taurine in ECS with DCM phenotype seems to be a prudent and relatively low-cost strategy.

ACKNOWLEDGMENTS

Funding provided by The Greater Stockholm Veterinary Hospital Foundation, Sweden; Agria Djurförsäkringar Research Fund, Sweden; Thure F och Karin Forsbergs Stiftelse, Sweden, and the Amino Acid Laboratory and the Sommer Endowment, School of Veterinary Medicine, University of California, Davis, USA. Preliminary results were presented at the 2022 American College of Veterinary Internal Medicine (ACVIM) Forum, Austin, Texas, USA. The authors thank all participating owners for their contributions. We also thank Professor Björn Ekesten, Dr. Sara Carlsson, and Dr. Josefin Hållbus for ophthalmic examinations, and all other participating veterinary staff for their contributions.

CONFLICT OF INTEREST DECLARATION

Dr. Fascetti 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. Dr. Fascetti 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.

OFF-LABEL ANTIMICROBIAL DECLARATION

Authors declare no off-label use of antimicrobials.

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) OR OTHER APPROVAL DECLARATION

Approved by the Ethical Committee for Animal Welfare in Stockholm, Sweden (5.8.18-01548/2017, 5.8.18-21508/2021, and 5.8.18-04682/2020).

HUMAN ETHICS APPROVAL DECLARATION

Authors declare human ethics approval was not needed for this study.

ORCID

Karin Kriström https://orcid.org/0000-0003-3567-7874
Jens Häggström https://orcid.org/0000-0003-3402-023X
Andrea J. Fascetti https://orcid.org/0000-0001-9992-8148

Anna Tidholm https://orcid.org/0000-0002-6277-3936
Ingrid Ljungvall https://orcid.org/0000-0002-6617-0454

REFERENCES

1. Huxtable R. Physiological actions of taurine. *Physiol Rev*. 1992;72:101-163.
2. Hayes K, Sturman J. Taurine in metabolism. *Annu Rev Nutr*. 1981;1:401-425.
3. Huxtable RJ. From heart to hypothesis: a mechanism for the calcium modulatory actions of taurine. *The Biology of Taurine: Methods and Mechanisms*. 1987;217:371-387.
4. Pion PD, Kittleson MD, Thomas WP, Skiles ML, Rogers QR. Clinical findings in cats with dilated cardiomyopathy and relationship of findings to taurine deficiency. *J Am Vet Med Assoc*. 1992;201:267-274.
5. Lombardini JB. Taurine: retinal function. *Brain Res Rev*. 1991;16:151-169.
6. Hayes KC, Carey RE, Schmidt SY. Retinal degeneration associated with taurine deficiency in the cat. *Science*. 1975;188:949-951.
7. Kaplan JL, Stern JA, Fascetti AJ, et al. Taurine deficiency and dilated cardiomyopathy in golden retrievers fed commercial diets. *PLoS One*. 2018;13:e0209112.
8. Kittleson MD, Keene B, Pion PD, Loyer CG. Results of the multicenter spaniel trial (MUST): taurine-and carnitine-responsive dilated cardiomyopathy in American cocker spaniels with decreased plasma taurine concentration. *J Vet Intern Med*. 1997;11:204-211.
9. Fascetti AJ, Reed JR, Rogers QR, Backus RC. Taurine deficiency in dogs with dilated cardiomyopathy: 12 cases (1997–2001). *J Am Vet Med Assoc*. 2003;223:1137-1141.
10. Backus RC, Cohen G, Pion PD, Good KL, Rogers QR, Fascetti AJ. Taurine deficiency in Newfoundlands fed commercially available complete and balanced diets. *J Am Vet Med Assoc*. 2003;223:1130-1136.
11. Basili M, Pedro B, Hodgkiss-Geere H, Navarro-Cubas X, Graef N, Dukes-McEwan J. Low plasma taurine levels in English cocker spaniels diagnosed with dilated cardiomyopathy. *J Small Anim Pract*. 2021;62:570-579.
12. Kramer GA, Kittleson MD, Fox PR, Lewis J, Pion PD. Plasma taurine concentrations in normal dogs and in dogs with heart disease. *J Vet Intern Med*. 1995;9:253-258.
13. Tôrres CL, Biourge VC, Backus RC. Plasma and whole blood taurine concentrations in dogs may not be sensitive indicators of taurine deficiency when dietary sulfur amino acid content is reduced. *Front Vet Sci*. 2022;9:873460.
14. Adin D, Freeman L, Stepien R, et al. Effect of type of diet on blood and plasma taurine concentrations, cardiac biomarkers, and echocardiograms in 4 dog breeds. *J Vet Intern Med*. 2021;35:771-779.
15. Backus R, Cohen G, Pion P, et al. Taurine deficiency in Newfoundland dogs maintained on commercial diets is corrected by dietary change or methionine supplementation. *JAMA*. 2003;223:1130-1136.
16. Freeman L, Rush J, Adin D, et al. Prospective study of dilated cardiomyopathy in dogs eating nontraditional or traditional diets and in dogs with subclinical cardiac abnormalities. *J Vet Intern Med*. 2022;36:451-463.
17. FDA U. FDA investigation into potential link between certain diets and canine dilated cardiomyopathy. 2018. https://www.fda.gov/animal-veterinary/outbreaks-and-advisories/fda-investigation-potential-link-between-certain-diets-and-canine-dilated-cardiomyopathy
18. Freeman LM, Stern JA, Fries R, Adin DB, Rush JE. Diet-associated dilated cardiomyopathy in dogs: what do we know? *J Am Vet Med Assoc*. 2018;253:1390-1394.
19. Tidholm A, Häggström J, Borgarelli M, Tarducci A. Canine idiopathic dilated cardiomyopathy. Part I: aetiology, clinical characteristics, epidemiology and pathology. *Vet J*. 2001;162:92-107.
20. Martin M, Stafford Johnson M, Celona B. Canine dilated cardiomyopathy: a retrospective study of signalment, presentation and clinical findings in 369 cases. *J Small Anim Pract*. 2009;50:23-29.
21. Pion PD, Sanderson SL, Kittelson MD. The effectiveness of taurine and levocarnitine in dogs with heart disease. *Vet Clin North Am Small Anim Pract*. 1998;28:1495-1514.
22. Gray K, Alexander L, Staunton R, et al. The effect of 48-hour fasting on taurine status in healthy adult dogs. *J Anim Physiol Anim Nutr*. 2016;100:532-536.
23. Kriström K, Häggström J, Tidholm A, Yu JZ, Fascetti AJ, Ljungvall I. Impact of blood tube additives and timing of sampling on blood taurine concentrations in clinically healthy dogs. *J Vet Cardiol*. 2023;45:59-70.
24. Acierno MJ, Brown S, Coleman AE, et al. ACVIM consensus statement: guidelines for the identification, evaluation, and management of systemic hypertension in dogs and cats. *J Vet Intern Med*. 2018;32:1803-1822.
25. Cornell CC, Kittleson MD, Torre PD, et al. Allometric scaling of M-mode cardiac measurements in normal adult dogs. *J Vet Intern Med*. 2004;18:311-321.
26. https://www.idexx.de/de/veterinary/reference-laboratories/tests-and-services/#menu.llGAf.
27. Tôrres CL, Backus RC, Fascetti AJ, Rogers QR. Taurine status in normal dogs fed a commercial diet associated with taurine deficiency and dilated cardiomyopathy. *J Anim Physiol Anim Nutr*. 2003;87:359-372.
28. UDAAL. UC Davis Veterinary medicine Amieno Acid Lab, Davis. 2023. https://www.vetmed.ucdavis.edu/labs/amino-acid-laboratory
29. Wess G. Screening for dilated cardiomyopathy in dogs. *J Vet Cardiol*. 2022;40:51-68.
30. Esser LC, Borkovec M, Bauer A, Häggström J, Wess G. Left ventricular M-mode prediction intervals in 7651 dogs: population-wide and selected breed-specific values. *J Vet Intern Med*. 2020;34:2242-2252.
31. Kittleson MD, Kienle RD. Primary myocardial disease leading to chronic myocardial failure—part 3. In: Kittleson MD, ed. *Small Animal Cardiovascular Medicine*. 2nd ed. Mosby; 2005.
32. Officials AoAFC. *AAFCO Methods for Substantiating Nutritional Adequacy of Dog and Cat Foods*. Champaign, IL: AAFCO; 2014.
33. National Research Council. *Nutrient Requirements of Dogs and Cats*. National Academies Press; 2006.
34. Kouřimská L, Kvasnička F, Kurečka M, et al. Taurine content of insects used as feed. *J Insects Food Feed*. 2023;9:1151-1157.
35. Pion PD. Traditional and nontraditional effective and noneffective therapies for cardiac disease in dogs and cats. *Vet Clin North Am Small Anim Pract*. 2004;34:187-216.
36. Wen G, Sturman J, Wisniewski H, Lidsky AA, Cornwell AC, Hayes KC. Tapetum disorganization in taurine-depleted cats. *Invest Ophthalmol Vis Sci*. 1979;18:1200-1206.
37. Hayes K, Rabin AR, Berson EL. An ultrastructural study of nutritionally induced and reversed retinal degeneration in cats. *Am J Pathol*. 1975;78:505.
38. Pasantes-Morales H, Domínguez L, Campomanes MA, Pacheco P. Retinal degeneration induced by taurine deficiency in light-deprived cats. *Exp Eye Res*. 1986;43:55-60.
39. Hayes K, Trautwein EA. Taurine deficiency syndrome in cats. *Vet Clin North Am Small Anim Pract*. 1989;19:403-413.
40. Fürst P, Pollack L, Graser T, et al. Appraisal of four pre-column derivatization methods for the high-performance liquid chromatographic determination of free amino acids in biological materials. *J Chromatogr A*. 1990;499:557-569.
41. Pasantes-Morales H, Quesada O, Alcocer L, Olea RS. Taurine content in foods. *Nutr Rep Int*. 1989;40:793-801.
42. Spitze A, Wong D, Rogers Q, Fascetti A. Taurine concentrations in animal feed ingredients; cooking influences taurine content. *J Anim Physiol Anim Nutr*. 2003;87:251-262.

43. Stapleton P, Charles R, Redmond H, Bouchier-Hayes D. Taurine and human nutrition. *Clin Nutr*. 1997;16:103-108.
44. Johnson M, Parsons C, Fahey G Jr, et al. Effects of species raw material source, ash content, and processing temperature on amino acid digestibility of animal by-product meals by cecectomized roosters and ileally cannulated dogs. *J Anim Sci*. 1998;76:1112-1122.
45. Li P, Wu G. Amino acid nutrition and metabolism in domestic cats and dogs. *J Anim Sci Biotechnol*. 2023;14:1-21.
46. Finkelstein JD. Methionine metabolism in mammals. *J Nutr Biochem*. 1990;1:228-237.
47. Carballal S, Banerjee R. Overview of cysteine metabolism. *Redox Chemistry and Biology of Thiols*. Elsevier; 2022:423-450.
48. Mansilla WD, Templeman JR, Fortener L, Shoveller AK. Minimum dietary methionine requirements in miniature dachshund, beagle, and labrador retriever adult dogs using the indicator amino acid oxidation technique. *J Anim Sci*. 2020;98:skaa324.
49. Sanderson SL, Gross KL, Ogburn PN, et al. Effects of dietary fat and L-carnitine on plasma and whole blood taurine concentrations and cardiac function in healthy dogs fed protein-restricted diets. *Am J Vet Res*. 2001;62:1616-1623.
50. Poutiainen RH. Influence of a grain free diet on the blood plasma taurine concentration in English cocker spaniels. In: Eesti Maaülikool. 2022.
51. Pezzali JG, Acuff HL, Henry W, Alexander C, Swanson KS, Aldrich CG. Effects of different carbohydrate sources on taurine status in healthy beagle dogs. *J Anim Sci*. 2020;98(2):skaa010.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Kriström K, Häggström J, Fascetti AJ, et al. The association between taurine concentrations and dog characteristics, clinical variables, and diet in English cocker spaniels: The Canine taURinE (CURE) project. *J Vet Intern Med*. 2024;38(5):2620-2632. doi:10.1111/jvim.17150

Supplemental table 1 (paper II)

Diet number	Taurine mg/100g	Methionine mg/100g	Methionine mg/1000 kcal	Cysteine mg/100g	Cysteine mg/1000 kcal
1	99,8	553	1383	469	1172
2	399	531	1328	593	1483
3	190	664	1661	379	947
4	206	667	1667	548	1369
5	213	601	1503	441	1103
6	113	583	1458	427	1067
7	185	676	1689	703	1758
8	534	940	2350	614	1536
9	135	556	1389	636	1589
10	10.6	431	1078	311	778
11	15.3	333	833	232	581
12	93.71	643	1608	361	903
13	160	796	1989	356	889
14	127	687	1717	402	1006
15	68.9	732	1831	350	875
16	87.5	480	1200	298	744
17	205	759	1897	470	1175
18	252	796	1989	608	1519
19	17.8	349	872	361	903
20	77.7	1178	2944	518	1294
21	92	431	1078	434	1086
22	89.8	511	1278	424	1061
23	41.4	538	1344	422	1056
24	57.8	509	1272	514	1286
25	16.1	551	1378	323	808
26	48.4	663	1658	368	919
27	72.6	429	1072	336	839
28	34.9	603	1508	494	1236
29	68.6	568	1419	356	889
30	16.1	396	989	728	1819
31	19.6	314	786	444	1111
32	82.2	546	1364	334	836
33	27.2	466	1164	690	1725
34	200	1344	3361	308	769
35	58.9	574	1436	381	953
36	52.8	638	1594	337	842
37	81.5	716	1789	436	1089
38	86.7	611	1528	358	894
39	82.5	533	1333	320	800
40	103	743	1858	438	1094
41	83.5	447	1117	410	1025

42	100	687	1717	406	1014
43	34.9	437	1092	330	825
44	65.2	496	1239	347	867
45	43.9	463	1158	701	1753
46	13.1	442	1106	340	850
47	41.1	511	1278	331	828
48	42.3	381	953	318	794
49	4.99	298	744	306	734
50	5.26	300	750	270	675
51	36.2	342	858	416	1039
52	48.7	406	1014	338	844
53	15	609	1522	396	909
54	117	597	1492	360	900
55	43	372	931	332	831
56	28.2	584	1461	557	1392
57	42.1	426	1063	482	1206
58	31	333	833	504	1261
59	10.2	389	969	368	919
60	81.9	791	1978	441	1103

Supplemental table 1: Taurine, methionine, and cysteine concentrations in the evaluated diets (n = 60). Concentrations are expressed as mg/100 g and mg/1000 kcal, illustrating variation in sulfur amino acid content across diets

Supplemental table 2

	Median	IQR	Range
Taurine mg/100g	68.75	34.9-102.25	4.99-534
Taurine mg/100g - red meat	31.05	14.75-72.15	4.99-89.8
Taurine mg/100g - white meat	82.2	48.7-127	15-534
Methionine mg/100g	548	431-664	298-1344
Methionine mg/100g - red meat	439	373-517	298-791
Methionine mg/100g - white meat	584	509-687	343-1178
Cysteine mg/100g	399	337-479	232-728
Cysteine mg/100g - red meat	338	316-429	232-728
Cysteine mg/100g - white meat	422	358-514	332-701

Supplemental table 2. Median, interquartile range (IQR), and range of taurine, methionine, and cysteine concentrations (mg/100 g) across all diets and according to primary protein source (red meat vs. white meat).

Acta Universitatis Agriculturae Sueciae

Doctoral Thesis No. 2026:38

Taurine-responsive cardiomyopathy is potentially a reversible heart disease that occurs in association with a deficiency of the amino acid taurine. This thesis shows that English Cocker Spaniels are at risk of developing the disease and that affected dogs often improve with taurine supplementation in combination with standard medical treatment. Both hereditary and dietary factors likely influence the phenotype in affected dogs.

Karin Kriström underwent her postgraduate education at the Department of Clinical Sciences, Swedish University of Agricultural Sciences (SLU), Uppsala, Sweden. Her undergraduate degree was obtained at the Faculty of Veterinary Medicine, SLU, Uppsala, Sweden.

Acta Universitatis Agriculturae Sueciae presents doctoral theses from the Swedish University of Agricultural Sciences (SLU).

SLU generates knowledge for the sustainable use of biological natural resources. Research, education, extension, as well as environmental monitoring and assessment are used to achieve this goal.

ISSN 1652-6880
ISBN (print version) 978-91-8124-255-3
ISBN (electronic version) 978-91-8124-285-0