ed Lentils Recipe

ADULT 1+ YEARS

CALIFORNIA NATURAL™

GRAIN FREE

LIMITED INGREDIENT DIET

Kangaroo & Red Lentils Recipe

ADULT 1+ YEARS

WHY LESS IS MORE

The more ingredients a food has, the higher the likelihood it may include an ingredient your dog may be sensitive to. CALIFORNIA NATURAL™ grain-free dog food recipes use fewer ingredients and zero grains to help dogs avoid the dietary triggers commonly found in many other foods.

HIGH-QUALITY KANGAROO
for dogs in need of a novel protein source

FLAXSEED
for nourishing Omega-3 fatty acids and fiber

RED & GREEN LENTILS
for a unique carbohydrate alternative

SUNFLOWER OIL
for healthy skin and coat

PEAS & PEA FIBER
for a nutritious grain replacement and fiber

Kangaroo is the #1 ingredient

No white potatoes, corn, soy, dairy or eggs

INGREDIENTS:
Kangaroo, Red Lentils, Green Lentils, Peas, Sunflower Oil (preserved with mixed Tocopherols), Flaxseed, Pea Fiber, Dicalcium Phosphate, Natural Flavors, Calcium Carbonate, Salt, DL-Methionine, Minerals (Zinc Proteinate, Iron Proteinate, Copper Proteinate, Manganese Proteinate, Calcium Iodate), Vitamins (Betaine Hydrochloride, Vitamin A Supplement, Niacin Supplement, Calcium Pantothenate, Beta Carotene, Vitamin B12 Supplement, Vitamin D3 Supplement, Riboflavin Supplement, Pyridoxine Hydrochloride, Thiamine Mononitrate, Biotin, Folic Acid), Vitamin E Supplement, Rosemary Extract

GUARANTEED ANALYSIS:

Crude Protein (Min)	21%
Crude Fat (Min)	11%
Crude Fiber (Max)	6.5%
Moisture (Max)	10%
Linoleic Acid (Omega-6 Fatty Acid) (Min)	1.1%
Iron (Min)	100 mg/kg
Zinc (Min)	140 mg/kg
Vitamin E* (Min)	300 IU/kg
Omega-3 Fatty Acids* (Min)	1%

*Not recognized as an essential nutrient by the AAFCO Dog Food Nutrient Profiles

CALORIE CONTENT:
Metabolizable Energy: Calculated
3,487 kcal/kg
434 kcal/cup
1 cup = 4.38 oz. (124 g)

FEEDING GUIDELINES: Every cup of our food is nutrient dense so your dog gets the right amount of calories in a complete and nutritious meal. Adjust amount fed to obtain or maintain your dog's optimal weight.

To help maintain freshness, seal bag tightly and store in a cool, dry location.

Suggested Amounts to Feed Per Day (Cups)

WEIGHT (lbs.)	FEEDING AMOUNT (cups/day)
10	7/8
20	1 3/8
30	1 7/8
40	2 1/4
50	2 5/8
60	3
70	3 3/8
80	3 5/8
90	3 7/8
100	4 1/4
120	4 3/4
140	5 1/4

Approximate Kibble Size

CALIFORNIA NATURAL™ Limited Ingredient Diet Grain Free Kangaroo & Red Lentils Recipe Dog Food is formulated to meet the nutritional levels established by the AAFCO Dog Food Nutrient Profiles for adult maintenance.

TRANSITIONING TO OUR FOOD: If feeding CALIFORNIA NATURAL™ Dog Food for the first time or changing recipes, we suggest you blend increasing amounts of the new recipe with your old dog food for six days.

QUALITY STANDARDS AS HIGH AS YOURS

Good health starts with good food - that's why only the best ingredients go into every batch of CALIFORNIA NATURAL™ dog food and why we make every bag of kibble ourselves in our Nebraska plant. It's also why our plant conducts 600 quality checks a day based on industry-recognized quality and food safety standards.

✓600 QUALITY CHECKS PER DAY

MADE IN OUR NEBRASKA PLANT WITH THE WORLD'S FINEST INGREDIENTS

MARS petcare

1348053

PROOF OF PURCHASE
CALIFORNIA NATURAL™ Limited Ingredient Diet Grain Free Kangaroo & Red Lentils Recipe Dog Food

7 31485 39941 6

FOR PROOF OF PURCHASE CUT ALONG DASHED LINE

Manufactured by:
Natura Pet Products, Inc.
2779 Rademakers Way
Fremont, NE 68025 USA
®/™ Trademarks
© Mars, Incorporated.

Image on Front:
Rasheed, a fast, powerful and fearless Saluki courses through the desert near Joshua Tree National Park in California.

100% Satisfaction Guaranteed:
If for any reason you are dissatisfied with this product, return the unused portion along with the information contained within the dotted panel on this bag to your CALIFORNIA NATURAL™ products retailer for replacement or refund. Should you wish to contact our Consumer Care Department, please call 1-800-532-7261 or visit us at **www.californianaturalpet.com**

		800.261-sub 1 Case Sample				
		Fromm Heartland Gold Grain Free Large Breed Adult	**Assumptions: max moisture of 10% (label)**	**Product Typical Analysis (website label)**	**AAFCO Growth & Maint**	**Issues**
(b) (4)	Tau	45.5 mg/100g	0.05% DMB	n/a	0.1% in Cats	LOW
	Cystine	293 mg/100g	0.33% DMB	n/a	n/a	
	Met	358 mg/100g	0.4% DMB	n/a	0.35%	none
	Met-Cys	0.33 + 0.4	0.73% DMB	n/a	0.70%	none
MSU	Iodine	4.2 mg/kg	4.67% DMB		1 to 11 ppm	none

(b) (4)

Report Number:	**2119443-0**
Report Date:	**01-May-2018**
Report Status:	**Final**

Certificate of Analysis

Food and Drug Administration - CVM

8401 Muirkirk Rd.
Laurel Maryland 20708 United States

Sample Name:	**1-dog food**	(b) (4) **Sample:**	**7192972**
Project ID	FDA_CVM-20180413-0004	**Receipt Date**	13-Apr-2018
PO Number	HHSF223201610005I HHSF22301003T	**Receipt Condition**	Ambient temperature
Sample Serving Size		**Login Date**	13-Apr-2018
Description	800.261-sub	**Online Order**	20

Analysis	Result
Cystine and Methionine *	
Cystine	293 mg/100g
Methionine	358 mg/100g
Taurine	
Taurine	45.5 mg/100g

Method References	Testing Location
Cystine and Methionine (AAAC_S)	(b) (4)
Official Methods of Analysis of AOAC INTERNATIONAL, Method 982.30 E(a/b)	
Taurine (TAUR_LC_S)	(b) (4)

Official Methods of Analysis of AOAC INTERNATIONAL, Method 999.12, A●AC International Gaithersburg, MD, USA, (Modified)

R. Schuster, "Determination of Amino Acids in Biological, Pharmaceutical, Plant and Food Samples by Automated Precolumn Derivatization and HPLC", *Journal of Chromatography, 431*:271-284, (1988) (Modified)

Henderson, J.W., Ricker, R.D. Bidlingmeyer, B.A., Woodward, C., "Rapid, Accurate, Sensitive, and Reproducible HPLC Analysis of Amino Acids, Amino Acid Analysis Using Zorbax Eclipse-AAA columns and the Agilent 1100 HPLC," Agilent Publication, 2000 (Modified)

Henderson, J.W., Books, A., "Improved Amino Acid Methods using Agilent Zorbax Eclipse Plus C18 Columns for a Variety of Agilent LC Instrumentation and Separation Goals," Agilent Application Note 5990-4547, (2010).

Testing Location(s)	Released on Behalf of (b) (4)
(b) (4)	(b) (4)
(b) (4)	ilac-MRA A2LA ACCREDITED (b) (5).

These results apply only to the items tested. This certificate of analysis shall not be reproduced, except in its entirety, without the written approval of (b) (6)

*** This analysis is not ISO accredited.**

Zignature

KANGAROO FORMULA

LIMITED INGREDIENT FORMULA

Zignature® is formulated to serve your canine companions needs by delivering the highest quality, well balanced diets. Our limited ingredient formula uses Kangaroo Protein, No Potatoes, No Grains, No Chicken, No Eggs, No Glutens, No Tapioca, No Corn and No Soy. The health of your pet is our highest priority. By eliminating unnecessary ingredients, we are able to bring you a formula closer to how nature intended it (with added vitamins and minerals). With Zignature®, you can be assured you are giving your canine companions the best food for life.

PEAS, CHICKPEAS, FLAXSEED & ALFALFA MEAL

Zignature® uses only the highest quality nutrient dense legumes (Peas & Chickpeas), Flaxseed & Alfalfa Meal and appropriate nutrition. Along with regular exercise and proper feeding, Zignature® can help your dog reach and maintain an ideal weight.

ANIMAL PROTEIN FIRST

To give your canine companions the optimum health and best diet. Zignature® understands your canine companions are carnivores first, omnivores second and thrive with a good portion of their diet coming from animal or fish protein. At Zignature®, we ensure our first two ingredients are from Kangaroo proteins for healthy growth and maintenance to give your canine companions everything they need for a long and happy life with you.

EXCEEDING EXPECTATIONS IN PET NUTRITION

INGREDIENTS

Kangaroo, Kangaroo Meal, Peas, Chickpeas, Pea Flour, Sunflower Oil (preserved with Citric Acid), Flaxseed, Red Lentils, Green Lentils, Dehydrated Alfalfa Meal, Pea Protein, Natural Flavors, Salt, Minerals (Zinc Proteinate, Iron Proteinate, Copper Proteinate, Manganese Proteinate, Cobalt Proteinate, Selenium Yeast), Choline Chloride, Potassium Chloride, Calcium Carbonate, Vitamins (Vitamin A Acetate, Vitamin D3 Supplement, Vitamin E Supplement, Niacin, d-Calcium Pantothenate, Thiamine Mononitrate, Pyridoxine Hydrochloride, Riboflavin Supplement, Folic Acid, Biotin, Vitamin B12 Supplement), Lactic Acid, Calcium Iodate, Preserved With Mixed Tocopherols.

AAFCO NUTRITIONAL ADEQUACY STATEMENT

Zignature® Kangaroo Formula for dogs is formulated to meet the nutritional levels established by the Association of American Feed Control Officials (AAFCO) Dog Food Nutrient Profiles for all life stages including growth of large size dogs (70 lbs. or more as an adult).

GUARANTEED ANALYSIS

Crude Protein	26.0%	(min)
Crude Fat	14.0%	(min)
Crude Fiber	4.5%	(max)
Moisture	10.0%	(max)
Calcium	1.2%	(min)
Phosphorus	1.0%	(min)
Omega 6 Fatty Acids*	3.0%	(min)
Omega 3 Fatty Acids*	0.6%	(min)

*Not recognized as an essential nutrient by the AAFCO Dog Food Nutrient Profiles.

DAILY FEEDING GUIDELINES

WEIGHT OF DOG (lbs)	SERVING SIZE (Adult)*
10 or less	¼ - ¾ cup
10 - 20	¾ - 1¼ cups
20 - 30	1¼ - 1½ cups
30 - 40	1½ - 2 cups
40 - 60	2 - 2½ cups
60 - 80	2½ - 3 cups
80 - 100	3 - 3¼ cups

*d 8-fluid ounce measuring cup

Note: ...eding amounts may vary by breed, ...evel, temperament & climate.

PUPPIES Feed up to 2 times per pound of body weight over adult levels.

PREGNANT FEMALES Minimal increase needed during first 6 weeks. Weeks 7, 8 & 9 increase up to 25%

LACTATING FEMALES Immediately after whelping, nutritional demand increases by 50% compared to maintenance levels. During peak lactation (around 4 - 5 weeks), increase feeding up to 300% of regular intake. Feed in 3 equal meals per day.

When switching your dog's diet, we recommend that it should be done gradually over 5-7 days, increasing the amount each day with the present diet as sudden change in diet may result in digestive disturbances.

...r veterinarian regularly. Keep fresh water available. ...tain freshness, keep package sealed and store in a cool, dry place.

CALORIE CO...

Metabolizable Energy (calculated)
3,888 kcals per kg
425 kcals per cup

BEST USED BY

...KANGAROO FORMULA

100% SATISFACTION GUARA...D

All Zignature's products are 100% Satisfaction G...ed. If you and your pet are not completely satisfied with this or any Zignature's products, simply return the unused portion, along with the original receipt to your Zignature's retail store for a complete refund or replacement.

Zignature®
WWW.ZIGNATURE.COM

Manufactured for: PETS GLOBAL, INC.
...4 INDUSTRY DRIVE, VALENCIA, ...355, USA TEL: 1-888-897-7207

ANIMAL PROTEIN FIRST

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INGREDIENTS

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GUARANTEED

Crude Protein .....
Crude Fat .....
Crude Fiber .....
Moisture .....
Calcium .....
Phosphorus .....
Omega 6 Fatty Acids* ..
Omega 3 Fatty Acids* ..

*Not recognized as an es
the AAFCO Dog Food N

CALORIE CO

Metabolizable Energy
3,888 kcals pe
425 kcals per

BEST USED

Vet Qin Small Anim 36 (2006) 13'25 1341

VETERINARY CLINICS
SMALL ANIMAL PRACTICE

Taurine and Carnitine in Canine Cardiomyopathy

Sherry Lynn Sanderson, DVM, PhD

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Dilated cardiomyopathy (DCM) is one of the most common acquired car-diovascular diseases in dogs [1-4]. Although few studies of the preva-lence of DCM in the overall population of dogs have been reported, estimates range from 0.5% to 1.1% [5,6]. Only degenerative valvular disease and, in some regions of the world, heartworm infection are more common causes of cardiac morbidity and mortality in dogs. DCM is seen most commonly in large and giant breeds of dogs, although its frequency seems to be increasing in medium-sized breeds, such as the English and American cocker spaniels [4-8]. It has been reported rarely in small and miniature breeds of dogs [9].

DCM is particularly challenging to veterinarians because the cause is often unknown and can vary among dog breeds [10]. Because most cases of DCM in dogs are classified as idiopathic, most therapies can be classified as "Band-Aid therapies" that palliate the effects of this disease for a short duration but do little to address the primary disease process. Therefore, DCM is almost al-ways a progressive disease, and most dogs will eventually succumb to their dis-ease. Survival times in dogs with DCM are variable and can be influenced by several factors, including breed. However, the prognosis for survival of dogs with DCM remains poor, with reported survival rates of 17.5% at 1 year and 7.5% at 2 years [11-13]. Until recently, reported cases of DCM reversal in dogs were very rare.

With advancements in echocardiology, diagnostic capabilities in canine cardiology have improved dramatically over the past 2 decades. Therapeutic advances have made surprisingly little progress. Symptomatic treatment is the standard care and outcome remains poor.

Recently, more promising therapies for dogs with DCM have resulted from a clearer understanding of the importance of biochemistry and nutrition in managing this disease. Nutrition is now widely accepted as an important adjunct to medical therapy in dogs with DCM.

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The importance of nutrition in managing DCM has changed dramatically in the past 10 to 15 years. Historically, dietary sodium restriction was the most common nutritional recommendation for dogs with DCM. The impor-tance of other nutrients in the origin and management of this disease was largely unknown. More recently, widely accepted beliefs about the role nutri-ent deficiencies could play in DCM have been proven false, further enhancing the ability to direct therapy at an underlying cause rather than just the symptoms.

This article focuses on two nutrients, taurine and carnitine, that play an important role in the cause and treatment of DCM in some dogs. Known risk factors for developing deficiencies of these nutrients are discussed, along with the use of taurine and carnitine for treating DCM in dogs.

TAURINE

What is Taurine?

Taurine is a sulfur-containing amino acid. Unlike most other amino acids, taurine is not incorporated into proteins but rather is one of the most abundant free amino acids in the body. Taurine is found in highest tissue concentrations in cardiac muscle, skeletal muscle, the central nervous system, and platelets [14].

Other than conjugation of bile acids and detoxification of xenobiotics through conjugation and excretion in bile, the function of taurine in mammals is not well understood but is highly diverse [14,15]. Since the mid-1970s, taurine has been known to be essential for normal retinal function in cats [16]. In addition, clinical and experimental evidence collected in the late 1980s documented that taurine is essential for normal myocardial function [17–20].

Taurine is involved with numerous metabolic processes, including antioxidation, retinal photoreceptor activity, development of the nervous systems, stabilization of neural membranes, reduction in platelet aggregation, and reproduction [15,16,21–26]. Although the importance of taurine for normal myocardial function is also well recognized, the mechanisms underlying its effect on the heart remain unknown. Much of the available evidence supports the theory that taurine's major effect on cellular function in the heart is modulating tissue calcium concentrations and availability [14,27,28]. In addition, taurine may inactivate free radicals and protect the heart by changing cellular osmolality [29]. Taurine may also have an effect on osmoregulation in the myocar-dium. Taurine is a small but highly charged osmotically active molecule, and experts have proposed that alterations in cellular osmolality induced by changes in intracellular taurine concentration are a protective mechanism in nervous tissue and myocardium [29]. Other proposed mechanisms specifically related to myocardial function include N-methylation of cell membrane phospholipids [30], direct effects on contractile proteins [31,32], and interactions with the renin–angiotensin–aldosterone system [33]. Taurine is a natural antagonist of angiotension II.

Is Taurine an Essential Amino Acid in Dogs?

Taurine is an essential amino acid in cats, and it is well known that taurine deficiency can cause DCM, retinal degeneration, and reproductive anomalies in this species [18]. However, taurine is not considered an essential amino acid in dogs. One explanation for the differences in taurine requirements between cats and dogs is that the activity of cysteine sulfinic acid decarboxylase (the rate-limiting enzyme in the synthesis of taurine from cysteine and methionine) is higher in dogs than cats [34]. However, the difference in activity of this enzyme between dogs and cats does not fully explain the difference in require-ments. The activity of this enzyme in humans is even lower than in cats, and taurine is not considered an essential amino acid in healthy adult humans. Therefore, cats and dogs may have additional differences that may explain why taurine is an essential amino acid in cats and not in dogs.

A study in dogs conducted in the 1980s at the University of California at Davis showed that feeding taurine-free diets or diets found to be taurine-depleting in cats [35] did not result in taurine depletion when fed to a group of eight healthy beagles [36]. In addition, results of an early clinical study in dogs, also conducted at this University soon after the relationship between taurine deficiency and DCM was discovered in cats, were unrewarding. These studies showed that dogs could not become taurine-depleted from diet alone, and that taurine did not play a considerable role in the development of DCM in dogs.

Emergence of Taurine Deficiency in Dogs with Dilated Cardiomyopathy

The belief that taurine deficiency could not cause DCM in dogs was challenged in 1989 when taurine deficiency was linked to DCM in foxes [37]. This study reopened taurine's possible role in DCM in dogs, and a collaborative study between the University of California at Davis and the Animal Medical Center in New York City was initiated [38]. In this study, plasma taurine levels were eval-uated in dogs with DCM and in those with chronic degenerative mitral valve disease. Surprisingly, results of this study showed that plasma taurine concentration was low in 17% of 75 dogs with DCM, and this deficiency occurred in breeds not commonly afflicted with DCM, such as American cocker spaniels and golden retrievers. However, because the plasma taurine concentration in breeds more commonly affected with DCM were within the reference range, experts concluded that taurine deficiency was unlikely to play an important role in the etiopathogenesis or therapy of DCM in dogs.

Multicenter Spaniel Trial (MUST) Study

Anecdotal reports emerged regarding supplementing American cocker spaniels diagnosed with DCM with taurine; however, initial reports of taurine supplementation were unrewarding. When Kittelson and colleagues [8] gave taurine and L-carnitine supplements to two American cocker spaniels with DCM, both dogs experienced response. These findings initiated the Multicenter Spaniel Trial (MUST) study. In this study, baseline plasma taurine concentrations and echocardiograms were collected in 11 American cocker spaniels diagnosed

with DCM. All dogs were found to have low plasma taurine concentrations at baseline (<50 nmol/mL). After baseline information was collected, dogs were randomly assigned to receive supplementation with both taurine (500 mg by mouth every 8 hours) and *L*-carnitine (1000 mg by mouth every 8 hours) or a placebo for 4 months, and echocardiograms were reevaluated after 2 and 4 months of therapy. The group supplemented with both taurine and carnitine showed significant echocardiographic improvement, whereas dogs receiving the placebo did not.

After this initial 4-month period, dogs that had received the placebo initially received supplements of both taurine and carnitine, and subsequently showed echocardiographic improvement after 2 to 4 months of therapy. The magni-tude of echocardiographic improvement in the American cocker spaniels was not as dramatic as that seen after taurine supplementation in cats with taurine deficiency DCM. Nonetheless, after 4 months of supplementation, the im-provement in myocardial function in each dog was significant enough to allow discontinuation of cardiovascular drug therapy. Improvements were seen in not only cardiovascular function but also survival times. The mean survival time for dogs in this study was 28.3 ± 19.1 months, compared with an average life expectancy for dogs treated with conventional drug therapy of approxi-mately 6 months. Based on results from this study, the current recommenda-tion is to supplement American cocker spaniels diagnosed with DCM with both taurine and carnitine at the doses mentioned earlier.

University of Minnesota Study in Urolith-forming Dogs Diagnosed with Dilated Cardiomyopathy

Around the same time the MUST study was initiated, a separate clinical study was initiated at the University of Minnesota. The population of dogs studied consisted of those with either cystine or urate urolithiasis that developed DCM after long-term consumption of a protein-restricted diet that was being used to manage their stone disease (Sherry L. Sanderson, DVM, PhD, unpublished data, 1998). Dogs in group 1 underwent only conventional drug therapy for their heart disease, whereas those in group 2 underwent and taurine and/or carnitine supplementation in addition to conventional drug therapy as needed. Dogs in group 1 that were in Modified New York Heart Association (MNY-HA) functional class I and II heart failure received enalapril (0.25 mg/kg by mouth every 12 hours) and digoxin (0.01–0.02 mg/kg by mouth divided twice a day), and dogs in MNYHA functional class III and IV received furosemide (dose varied depending on severity of heart disease) in addition to enalapril and digoxin. The population of dogs in group 1 (N = 6) consisted of five English bulldogs (four with cystine urolithiasis, one with urate urolithiasis) and one Dalmatian with urate urolithiasis. The population of dogs in Group 2 (N = 8) consisted of five English bulldogs (three with cystine urolithiasis, two with urate urolithiasis), two Dalmatians with urate urolithiasis, and one miniature Dachshund with cystine urolithiasis. Because when this study was initiated experts believed that dogs with DCM did not have low plasma taurine

concentrations, none of the dogs in group 1 had these concentrations evaluated at baseline. Plasma taurine concentrations evaluated before supplementation in seven of eight dogs in group 2 ranged from 2 nmol/mL to 45 nmol/mL (mean, 20.9 nmol/mL). These results were below the reference range of 41 nmol/mL to 97 nmol/mL that the investigators established from healthy adult beagles. Echocardiography was performed at baseline and once every 2 months. Details from this study will be published later, but a few interesting and important results were noted:

1. The average life expectancy for dogs in group 1 was 10.5 months, and all dogs were euthanized because of progressive congestive heart failure that became refractory to therapy. The average life expectancy for dogs in group 2 was 47.1 months, and only three of eight dogs were euthanized because of progressive congestive heart failure. In addition, three of five dogs that did not succumb to their heart disease received only taurine and/or carnitine supplementation and no conventional drug therapy for the management of their heart disease.
2. DCM reversed in three of eight dogs in group 2. DCM returned in one dog after the owner discontinued taurine and carnitine supplementation on their own, and in an additional dog when the dose of carnitine was reduced be cause of diarrhea associated with carnitine supplementation.
3. Dogs consuming a protein restricted diet long term could develop taurine deficiency, in contrast to results from previous studies that concluded that a diet could not induce taurine deficiency in dogs. This finding provided an impetus for further examining the effects on plasma and whole blood taurine levels in healthy adult dogs consuming a protein restricted diet long term.

Diet-Induced Taurine Deficiency in Healthy Adult Dogs

Previous reports indicated that dogs could not develop diet-induced taurine deficiency, even when fed a diet devoid of taurine. However, based on the finding of University of Minnesota study that dogs developed low plasma taurine levels after consuming a protein-restricted diet long-term, a more controlled study was undertaken to determine the cause of this problem and evaluate the effects of long-term taurine deficiency on cardiac function in healthy adult dogs [39].

This study involved 17 healthy adult beagles. Baseline plasma and whole blood taurine levels were evaluated, and echocardiography was performed to assess cardiac function. Once baseline data was collected, dogs were fed one of three protein-restricted diets for 48 months. All three diets had similar levels of protein; one diet was also low in fat, a second was high in fat, and a third was high in fat and supplemented with *L*-carnitine at 200 mg/kg of diet. All diets contained methio-nine and cystine concentrations at or above recommended minimum require-ments established by the Association of American Feed Control Officials (AAFCO) [40]. After diet assignment, plasma taurine and whole blood taurine concentrations and echocardiography were evaluated every 6 months.

All three dietary treatments caused a significant decrease in whole blood taurine concentration compared with baseline concentrations. Dogs in the high-fat

group also experienced a significant decrease in plasma taurine concentration. This study was the first to show that diet could induce taurine deficiency in healthy adult dogs, in contrast to previous studies.

Another important observation was that one dog with taurine deficiency developed DCM, and that taurine supplementation resulted in almost complete reversal of the disease. This study was also the first to clearly document in dogs that taurine deficiency preceded DCM, and that taurine supplementation resulted in substantially improved cardiac function, similar to cats.

Why Did Dogs Develop Taurine Deficiency While Consuming a Protein-Restricted Diet?

The exact mechanism for this problem is unknown. However, this study showed that the AAFCO recommended minimum requirements for amino acids may need to be modified in dogs consuming a protein-restricted diet long-term. Many therapeutic diets for dogs are now supplemented with taurine.

Additional Examples of Diet-Induced Taurine Deficiency in Dogs

Soybean-based diets

Taurine deficiency was identified in two unrelated dogs fed a tofu-based diet [41]. Although the diet was low in protein, it met the National Research Council's published requirements for protein and other nutrients in dogs [42]. The authors attributed taurine deficiency to the fact that the primary protein source was soybean curd, which is low in sulfur-containing amino acids and devoid of taurine compared with meat proteins [43]. In addition, soybean curd has been shown to accelerate the loss of bile acids in cats [44].

Lamb meal and rice diets

Taurine deficiency was also identified in 12 Newfoundlands consuming two different commercially available lamb meal and rice diets [41]. Echocardiography was performed in six of the dogs, and none were diagnosed with DCM. The taurine deficiency was reversed when the diet was either changed or when the lamb meal and rice diets were supplemented with methionine. This study did not identify the exact mechanism for the development of taurine deficiency in the dogs consuming the lamb meal and rice diets.

In a study by Fascetti and colleagues [45], DCM and taurine deficiency were identified in 12 large and giant-breed dogs consuming commercially available diets that contained lamb meal, rice, or both as primary ingredients. All dogs received supplements of with taurine (1000–3000 mg by mouth every 24 hours), and significant echocardiographic improvement occurred in 9 of the 12 dogs that underwent an echocardiogram repeated after taurine supplemen-tation. The authors hypothesized that taurine deficiency caused DCM and was caused by inadequate or unavailable dietary sulfur amino acids, which are es-sential precursors of taurine synthesis.

In a similar report, five related golden retrievers were diagnosed with taurine deficiency and DCM [46]. Three of five dogs were consuming lamb meal and rice or lamb and rice diets. All showed significant improvement after taurine

supplementation (500 mg by mouth every 12 hours), and all five dogs survived for more than 3 years. The authors attribute the DCM to a suspected autoso-mal recessive mode of inheritance; however, the potential role diet played in the development of taurine deficiency warrants mentioning.

Potential Causes of Taurine Deficiency in Dogs Consuming Lamb Meal and Rice or Lamb and Rice Diets

Torres and colleagues [47] compared the effects of consuming a lamb meal and rice–based diet with effects of consuming a poultry by-product–based diet in 12 beagles aged 5 to 5.5 months. Although the differences in plasma and whole blood taurine concentrations did not differ among diet groups, dogs consuming the lamb meal and rice–based diet excreted less taurine in their urine than dogs consuming the poultry by-product–based diet. When the lamb meal and rice diet was supplemented with methionine, urinary taurine excretion increased by 54%. Because taurine homeostasis in dogs is achieved primarily through regulating renal taurine excretion, the amount of taurine excreted in urine is a sensitive indicator of the adequacy of either taurine synthesis or absorption of dietary precursor amino acids. The authors concluded that reduced bioavail-ability of sulfur amino acids in the lamb meal and rice diet is a likely cause of taurine deficiency. This finding is supported by the increase in urine taurine concentrations after supplementation with methionine. Johnson and colleagues [48] showed that ileal digestibility of amino acids in dogs depends on the raw material sources and the temperature used to process feeds and provides a mechanism for these specific dietary effects.

A second potential, although related, cause of taurine deficiency in dogs consuming lamb meal and rice diets was proposed [49,50]. When dietary protein is low in quality, undigested protein reaches the colon, where it serves as a substrate for bacterial growth. Some bacteria produce cholyltaurine hydrolase, an enzyme that causes release of taurine from taurocholic and other bile acids that are normally conserved in the enterohepatic circulation, resulting in increased fecal loss of taurine. Studies in dogs [49] and cats [50] have found that diets containing rice bran and whole rice products provide a source of moderately fermentable fiber and high amounts of fat. These fermentable fibers may increase the number of bacteria in the colon and result in a greater loss of taurine in the feces similar to the mechanism for undigested protein. The fat content of the diet can also affect taurine metabolism through altering intestinal bacteria and subsequent changes in the excretion of bile acids.

How Should Samples be Collected to Evaluate Plasma and Whole Blood Taurine Concentrations?

Fasting versus postprandial blood samples

Although fasting has no effect on plasma taurine concentrations in humans [51], food deprivation causes a small but significant reduction in plasma taurine concentrations in cats [52]. In a study by Torres and colleagues [47], plasma taurine concentrations were significantly reduced in food-restricted dogs compared with ad-libitum-fed dogs. Whole blood taurine concentrations were

also reduced, although the whole blood taurine results were not statistically significant between the two groups. Because of the potential for food intake to affect plasma and whole blood taurine concentrations in dogs, withholding food, but not water, is recommended for 8 hours before sampling.

Anticoagulant used for plasma sample collection

Paired analysis of samples comparing taurine concentrations in plasma collected in lithium heparin with those collected in sodium citrate showed that plasma taurine concentrations are higher when lithium heparin is used as the anticoagulant [38]. Because most studies have used heparinized plasma samples to evaluate plasma taurine levels in dogs, these are recommended rather than sodium citrate plasma samples.

Plasma taurine sample collection

Heparinized, nonhemolyzed blood samples should be obtained and stored on ice until they are processed. After centrifuging, the plasma should be separated immediately from the cellular components, and a small amount of plasma should be left above the buffy coat to prevent contamination of the plasma with cells. Hemolysis and platelet or white blood cell contamination falsely elevates plasma taurine concentrations. Samples should be frozen until analyzed for plasma taurine concentrations.

Whole blood taurine sample collection

Heparinized whole blood should be frozen until samples can be analyzed. Because the red blood cells are lysed before analysis, hemolyzed samples do not adversely affect whole blood taurine analysis.

Plasma and whole blood taurine samples can be sent to the Department of Molecular Biosciences at the School of Veterinary Medicine, University of California, Davis, for analysis.

Which is Better: Plasma Taurine Concentrations or Whole Blood Taurine Concentrations

Earlier studies evaluating the relationship between taurine deficiency and DCM in dogs relied primarily on plasma taurine concentrations to predict tis-sue taurine concentrations. Studies conducted in dogs by this author showed findings similar to those reported in cats [53]. Relying on plasma taurine con-centrations alone does not reliably assess tissue taurine concentrations in dogs.

Simultaneously evaluating plasma and whole blood taurine concentrations predicts skeletal and cardiac muscle taurine concentrations better than evaluating either test alone. Therefore, when evaluating taurine status in dogs with DCM, plasma and whole blood taurine concentrations should be assessed simultaneously.

Reference Ranges for Plasma and Whole Blood Taurine Concentrations in Dogs

The reference range used in earlier studies evaluating plasma and whole blood taurine concentrations in dogs was extrapolated from the reference range use in

cats. However, reference ranges for plasma and whole blood taurine concentrations in dogs were published recently (Table 1).

Delaney and colleagues [49] have also suggested that plasma taurine concentrations less than 40 nmol/mL are critically low, as are whole blood taurine concentrations less than 150 nmol/mL. In addition, Sanderson and colleagues [53] found that low plasma taurine concentrations can exist without the presence of DCM.

Therefore, results showed that the onset of clinical signs in dogs, just as in cats, was variable when taurine concentrations declined markedly below the normal range [18].

Which Dogs Diagnosed with Dilated Cardiomyopathy Should Receive Taurine Supplementation?

Evaluation of plasma and whole blood taurine concentrations is recommended for all dogs diagnosed with DCM. An association between taurine deficiency and DCM was found in various breeds of dogs, including American cocker spaniels, Newfoundlands, golden retrievers, Labrador retrievers, Dalmatians, English bulldogs, and Portuguese water dogs. Taurine supplementation is highly recommended in any of these breeds that develop DCM.

Not all dogs with DCM will show dramatic improvement with taurine supplementation. However, even if plasma and whole blood taurine concentrations are within the reference range, giving taurine supplements to dogs diagnosed with DCM may still have some benefits. Because taurine is extremely safe and inexpensive, the risks and costs of supplementation are minimal, even if dogs have normal levels of plasma and whole blood. Proposed mechanisms for the beneficial actions of taurine on the myocardium include modulating tissue calcium concentrations and availability in the heart; inactivating free radicals and protecting the heart through altering cellular osmolality; osmoregulating the myocardium; directly affecting contractile proteins; and serving as a natural antagonist of angiotension II. Dogs with DCM that do not have taurine deficiency may still benefit from some of these proposed mechanisms of action for taurine.

Table 1
Normal concentrations of taurine in dogs

Plasma (nmol/mL)	Whole blood (nmol/mL)
41 97[a]	155 347[a]
72.8 81.2[b]	255.8 276.2[b]

[a]Reference range established from 18 healthy adult beagles consuming a conned commercial maintenance diet. *Data from* 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;62:1616–23.
[b]Reference range established from 131 healthy adult dogs of various breeds consuming a variety of commercial adult maintenance diets. *Data from* Deloney SJ, Koss PH, Rogers QR, et al. Plasma and whole blood taurine in normal dogs of varying size fed commercially prepared food. J Anim Physiol 2003;87:236–44.

Recommended dose for taurine supplementation

This author has successfully used doses of 500 to 1000 mg of taurine administered orally two to three times per day for small dogs (<25 kg), and 1 to 2 g of taurine administered orally two to three times per day for large dogs (25–40 kg). These doses have been shown to normalize plasma and whole blood taurine levels in taurine-deficient dogs. Many other doses for taurine are reported in the literature. Whether a smaller or less frequent dose of taurine than what this author recommends can be used successfully remains to be determined. If doses are used that differ from those this author recommends, plasma and whole blood taurine concentrations must be reevaluated after taurine supplementation is initiated to determine if the dose being given is effective and appropriate. Another important point is that echocardiographic improvement in myocardial function is not usually documented before 2 months of supplemen-tation, and often no improvement is documented before 4 months of supple-mentation. However, the dogs may feel better clinically and be more active before improvement in cardiac function is documented. Owners must not with-draw taurine supplementation prematurely before deciding if their dogs benefit.

Where Can Taurine be Purchased?

Taurine can be purchased through several retail outlets. If taurine is purchased through a health food store, consumers must look for a product that contains a USP certification symbol on the label. This symbol ensures that what is listed on the label is exactly what is found in the product.

LEVOCARNITINE (*L*-CARNITINE)

What is *L*-Carnitine?

L-carnitine (b-hydroxy-c-trimethylaminobutyric acid) is a small water-soluble molecule with a molecular weight of 160. In dogs, carnitine is obtained ei-ther from dietary protein or endogenous synthesis in the liver using the essential precursor amino acids lysine and methionine. Synthesis also re-quires iron, vitamin C, and vitamin B_6 as cofactors [54]. Although carnitine is classified as an amino acid derivative, it is not an a-amino acid and the amino group is not free. Therefore carnitine is not used for protein synthesis [55].

Carnitine is found in the body either as free carnitine, short-chain acylcar-nitine, or long-chain acylcarnitine. Acylcarnitine is carnitine bound to a fatty acid. Total carnitine is the sum of all the individual carnitine fractions. The free carnitine fraction is normally higher than either the short-chain acylcarni-tine fraction or the long-chain acylcarnitine fraction.

Cardiac and skeletal muscles are significant storage sites, containing 95% to 98% of the carnitine in the body [56], and carnitine is concentrated in these tissues through an active membrane transport mechanism. The heart is unable to synthesize carnitine and depends on transport of carnitine from the circulation into cardiac muscle, which results in up to a 100 gradient between extracellular and intracellular concentrations.

Only the *L*-form of carnitine exists naturally in the body. The *D*-form competitively inhibits the actions of the *L*-form, thereby inhibiting carnitine enzyme systems. In addition, mammals are unable to convert *D*-carnitine to *L*-carnitine, and therefore this discussion focuses on *L*-carnitine.

Why is *L*-Carnitine Important for Normal Myocardial Function?

The normal heart obtains approximately 60% of its total energy production from oxidation of long-chain fatty acids [57]. Long-chain fatty acids in the cytosol of myocardial cells combine with coenzyme A (CoA) as the first step to-ward beta oxidation. However, long-chain fatty acids must be transported across the inner mitochondrial membrane to generate energy, and the inner mitochondrial membrane is normally impermeable to such bulky polar molecules. Therefore, transport is accomplished through a ''carnitine shuttle.'' In the carnitine shuttle, the activated fatty acid in the cytosol reacts with carnitine to form a more permeable molecule. This reaction occurs on the outer surface of the inner mitochondrial membrane and is catalyzed by the enzyme carnitine acyltransferase I. The newly formed long-chain acylcarnitine ester molecule is permeable to the inner mitochondrial membrane and is transported across this membrane, where the enzyme acyltransferase II converts the long-chain acylcarnitine back to free carnitine and the long-chain fatty acid. Therefore, carnitine functions as a cofactor of several important enzymes necessary for transport of long-chain fatty acids from the cytosol into the mitochondrial matrix [58,59]. Once inside the mitochondria, fatty acids undergo beta oxidation to generate energy [60].

Another important function of carnitine is its buffering capacity, which modulates the intramitochondrial acyl-CoA:CoA ratio [58]. This process is important because acyl-CoA is the activated form of fatty acids used for beta oxidation and lipid synthesis. However, buildup of acyl-CoA derivatives in the mitochondria results in decreased free CoA, which inhibits oxidative metabolism. Acyl-CoA derivatives also act as detergents at high concentrations. Carnitine also facilitates removal of accumulating short- and medium-chain organic acids from the mitochondria. Therefore carnitine also has a role in detoxification in the mitochondria.

What Causes *L*-Carnitine Deficiency?

Carnitine deficiency can be a primary or secondary disorder. Primary carnitine deficiencies may arise from genetic defects in synthesis, renal transport, intestinal absorption, transmembrane uptake mechanisms, or excessive degradation of carnitine [61]. In humans, primary carnitine deficiencies have been associated with cardiomyopathies that are usually not present at birth but take 3 to 4 years to develop. *L*-carnitine therapy can prevent and reverse cardiac dysfunction in some patients.

Secondary carnitine deficiencies are believed to be much more common in humans and can have many causes [61]. In humans, carnitine deficiency can result from inborn errors of metabolism or develop in patients undergoing long-term total parenteral nutrition, vegetarians, and infants fed formulas not

supplemented with carnitine. Carnitine deficiencies are recognized in dogs, but the incidence is not known.

What are the Consequences of *L*-Carnitine Deficiency?

Carnitine deficiency has been shown to cause or be associated with DCM in humans [62–64], hamsters [65,66], and dogs [36,67–69]. More widespread studies have not been undertaken in dogs because carnitine status is difficult to thoroughly assess.

What Types of Carnitine Deficiency Exist in Dogs?

Carnitine deficiency in dogs is classified as either (1) plasma carnitine deficiency, characterized by low concentrations of free plasma carnitine; (2) systemic carnitine deficiency, characterized by low concentrations of free plasma and tissue carnitine; or (3) myopathic carnitine deficiency, characterized by low free myocardial carnitine concentrations in the presence of normal and sometimes elevated plasma carnitine concentrations. Plasma carnitine deficiency alone is not a well-documented state and is included to account for the fact that plasma carnitine, but not tissue carnitine sampling, is often pursued in veterinary medicine.

For example, if plasma carnitine concentration is used to assess carnitine status of a dog, it can help diagnose carnitine deficiency when it is low. However, if plasma carnitine concentration is normal, it does not rule out the possibility of the myopathic form of carnitine deficiency, and the myopathic form of car-nitine deficiency is estimated to occur in 17% to 60% of dogs with DCM. Evaluating cardiac muscle carnitine concentrations requires a fluoroscopy-guided endomyocardial biopsy, which is not practical to perform in most private practice situations and is not without risk. Therefore, diagnosing and determining the incidence of myopathic carnitine deficiency in dogs with cardiac disease remains elusive, but may be an underdiagnosed cause of DCM in dogs.

L-Carnitine Deficiency and Associated Myocardial Disease States in Dogs

Carnitine deficiency was associated with DCM in dogs in a limited number of clinical reports [8,9,68–70] . The first reported case of carnitine deficiency was in a family of boxers [69]. The sire, dam, and two littermates were diagnosed with DCM. One offspring had a low plasma carnitine concentration and low myocardial carnitine concentration at DCM diagnosis. After undergoing treatment with high-dose *L*-carnitine (220 mg/kg/d orally), this dog's fractional shortening (FS) increased from 18% to 28%. This dog's littermate had low myocardial and normal plasma carnitine concentrations and responded similarly to high-dose *L*-carnitine supplementation, with its FS increasing from 2% to 24%. The latter dog experienced a decline in myocardial function after *L*-carnitine therapy was withdrawn. Both parents of these littermates had normal plasma and low myocardial carnitine concentrations. Unfortunately, both parents died soon after beginning *L*-carnitine supplementation.

Costa and Labuc [70] presented another case report of two boxers with DCM. One was treated with 250 mg/kg/d of *L*-carnitine orally, and the other was not treated. The myocardial concentration of carnitine was found to be low in the dog that did not receive supplementation and elevated in the dog that did.

Concurrent supplementation with carnitine and taurine has shown benefit in American cocker spaniels with DCM [8]. An unpublished study by this author in 1998 showed beneficial effects from carnitine supplementation in urolith-forming dogs diagnosed with DCM while consuming a protein-restricted diet (Sherry Lynn Sanderson, DVM, PhD, unpublished material). Both studies showed dramatic improvement in myocardial function and survival times in dogs that received supplementation.

Which Came First: Carnitine Deficiency or Dilated Cardiomyopathy?

A common argument made against the role of carnitine deficiency in dogs diagnosed with DCM is that if carnitine deficiency is diagnosed after the onset of DCM, whether carnitine deficiency caused the DCM or DCM caused the carnitine deficiency is unclear. When myocardial cells are damaged, as may occur with DCM, carnitine can leak out of the cells, resulting in low myocardial carnitine levels. In this situation, the DCM caused the carnitine deficiency. Most published studies linking carnitine deficiency to DCM in dogs have shown this scenario when carnitine deficiency was diagnosed after the onset of DCM.

In an unpublished study conducted at the University of Minnesota, this author documented carnitine deficiency before the onset of DCM in three dogs (Sherry Lynn Sanderson, DVM, PhD, unpublished material, 1998). Therefore, the association of carnitine deficiency with DCM at diagnosis may not always imply a cause-and-effect relationship. However, this study indicates that carnitine deficiency can cause DCM in dogs.

Which Dogs with Dilated Cardiomyopathy Should Receive Carnitine Supplementation?

The importance of carnitine supplementation in the treatment and survival times of some dogs with DCM should not be overlooked. In the first reported study linking carnitine deficiency to DCM in boxers, two of four dogs experienced good response to carnitine supplementation [69]. Considering the generally poor prognosis of this disease in boxers, carnitine supplementation provides owners one additional option for treating this disease, and has made a dramatic difference in the survival times and quality of life of some dogs.

The importance of carnitine supplementation in American cocker spaniels with DCM and urolith-forming dogs with DCM should also not be overlooked. Although a few anecdotal reports exist in which American cocker spaniels with DCM experienced good response to taurine supplementation alone, most cases have shown response to combined supplementation with taurine and carnitine. In the above study by this author, a miniature Dachshund diagnosed with carnitine deficiency before the onset of DCM underwent treatment

only with carnitine supplementation, and its heart disease reversed. Although DCM in many dogs is not associated with carnitine deficiency, carnitine and taurine supplementation offer the most promising hope for improved quality of life and survival times in dogs that experience response.

How is Carnitine Deficiency Diagnosed?

Because performing endomyocardial biopsies is impractical for most clinicians in private practice, most screening for carnitine deficiency relies solely on plasma carnitine levels. The method for plasma carnitine sample collection is almost identical to that used for plasma taurine sample collection. Fasting, heparinized, nonhemolyzed blood samples should be obtained and stored on ice until they are processed. The plasma should be immediately separated from the cellular components ideally in a cold-centrifuge, and a small amount of plasma should be left above the buffy coat to prevent contamination of the plasma with cells. Samples should be frozen immediately until analyzed for plasma carnitine concentrations.

What is the Recommended Dose for Carnitine Supplementation in Dogs?

The doses of carnitine being administered may contribute to the lack of favorable results with carnitine supplementation that some investigators observed. The recommended doses for carnitine supplementation in dogs with DCM vary widely in the literature. Although most authors recommend a carnitine dose of 50 to 100 mg/kg orally every 8 hours, the effective dose may depend on the form of carnitine deficiency. In a limited number of cases studied at the University of Minnesota, where pre– and post–carnitine supplemented plasma and cardiac muscle carnitine levels were obtained, this author's clinical impression was that the effective therapeutic dose in dogs with systemic carnitine deficiency was much lower than the effective dose in dogs with myopathic carnitine deficiency.

Some experts speculate that the myopathic form of carnitine deficiency may be caused by a carnitine transport defect in the heart, and much higher plasma levels of carnitine seem to be needed to overcome this defect and achieve normal concentrations of carnitine in the heart than for the systemic form of carnitine deficiency. Based on this work, the dose of carnitine recommended by this author for systemic carnitine deficiency is 100 mg/kg orally every 8 hours. However, if the myopathic form of carnitine deficiency is present or suspected, the author recommends starting carnitine supplementation at 200 mg/kg orally every 8 hours to maximize the chances that carnitine supplementation will improve myocardial function.

Carnitine is a very safe substance. Diarrhea was the only adverse effect of high doses of carnitine, reported in approximately two thirds of dogs. If diarrhea occurs, the highest dose of carnitine that the dog will tolerate without causing diarrhea should be administered. Therefore, like taurine, L-carnitine is a safe substance to administer, and, except for the expense, few drawbacks exist to supplementing a dog with DCM with carnitine (carnitine is much more expensive than taurine. Another important point is that the time it takes for

improvement in myocardial function to occur is very similar to that for taurine supplementation. Echocardiographic improvement in myocardial function is not usually documented before 2 months of supplementation with carnitine, and often improvement is not documented for up to 4 months. However, dogs may feel better clinically and be more active before improvement in cardiac function is documented. Owners must not withdraw carnitine supplementation prematurely before determining whether their dogs benefit.

Where Can *L*-Carnitine Be Purchased?

Although *L*-carnitine can be purchased from health food stores, this source is extremely expensive. Purity of the sample is also of great importance. Therefore, only products that contain the USP certification seal should be purchased from health food stores.

L-Carnitine can also be purchased less expensively in bulk. Bulk carnitine can be purchased from Ajinamotousa, Inc (500 Frank W Burr Boulevard; Park Central West; Teaneck, New Jersey). At last check, the company required a minimum purchase of 10 kg at one time. However, the individual expense can be reduced if several owners split an order. If carnitine is purchased in bulk, owners must measure out the carnitine they are giving to their dogs. One teaspoon of carnitine is equivalent to 2 g of carnitine. Therefore, fractions of a teaspoon can be administered if necessary. Owners must be sure to purchase *L*-carnitine, not *D*- or the *DL*- isomers, because *D*-carnitine interferes with *L*-carnitine use.

Which Dogs with Dilated Cardiomyopathy Should be Supplemented With Carnitine?

Carnitine supplementation should be recommended for boxers, American cocker spaniels, and dogs with cystine or urate urolithiasis that are diagnosed with DCM. Even if carnitine deficiency did not cause DCM, supplementing dogs with carnitine does not hurt them, and supplementation may be beneficial even if carnitine deficiency is not present. The major drawback to supplementing dogs with carnitine is the expense and occasional gastrointestinal upset.

What are the Reference Ranges for Carnitine Concentrations in Dogs?

The reference ranges for carnitine concentrations in dogs are listed in Table 2 [69].

SUMMARY

Some newer more promising therapies for dogs with DCM do not involve drugs but rather nutritional supplements. Two of the more common nutritional supplements administered to dogs with DCM are taurine and carnitine. Deficiencies of these nutrients have been shown to cause DCM in dogs, and some breeds have been shown to experience dramatic improvement in myocardial function after supplementation with one or both nutrients. Although most dogs diagnosed with DCM do not have a documented taurine or carnitine deficiency, they may still benefit from supplementation. Both nutrients are very

Table 2
Normal concentrations of carnitine in dogs

Carnitine fraction	Plasma carnitine (nmol/mL)	Cardiac muscle carnitine (nmol/mg of NCP)
Free	8 36	4 11
Esterified	0 7	0 4
Total	12 38	5 13

Abbreviation: NCP, noncollagenous protein.

safe to administer to dogs. For some owners, the high cost of carnitine is the only deterrent to giving their dogs supplements of both nutrients.

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Case Report

SEVERE REVERSIBLE DILATED CARDIOMYOPATHY AND HYPERTHYROIDISM: CASE REPORT AND REVIEW OF THE LITERATURE

Cristina Boccalandro, MD,[1] Fernando Boccalandro, MD,[2] Philip Orlander, MD,[1] and Chik Fong Wei, MD[2]

ABSTRACT

Objective: To describe a case of a 46-year-old woman with Graves' disease and reversible low-output congestive heart failure and present a comparative analysis of 23 similar cases reported in the literature.

Methods: A detailed case report is presented. In addition, a review of the pertinent literature published between 1960 and 2002 was performed to identify similar cases of dilated cardiomyopathy and thyrotoxicosis and to assess the findings in these patients.

Results: A 46-year-old woman without primary heart disease was admitted to the hospital with Graves' thyrotoxicosis and severe low-output congestive heart failure. Her left ventricular ejection fraction (LVEF) at the time of initial assessment was less than 20%, and her condition was categorized as New York Heart Association (NYHA) functional class III. Nineteen months after she was treated for hyperthyroidism, her LVEF was 49% and her status was NYHA class I. A severe hypotensive episode occurred when β-adrenergic blockade therapy was initiat-ed. The group of 23 similar cases from the literature plus our currently described patient had a mean age of 45 years, a maleto-female ratio of 1:1.2, Graves' disease as the principal cause, and L VEF improvement from 29% to 58%.

Conclusion: Dilated cardiomyopathy is an unusual manifestation of hyperthyroidism with unclear cause. Clinicians should be aware of this entity because it is treatable and hypotension can occur if β-adrenergic blockade treatment is initiated. **(Endocr Pract 2003;9:140-146)**

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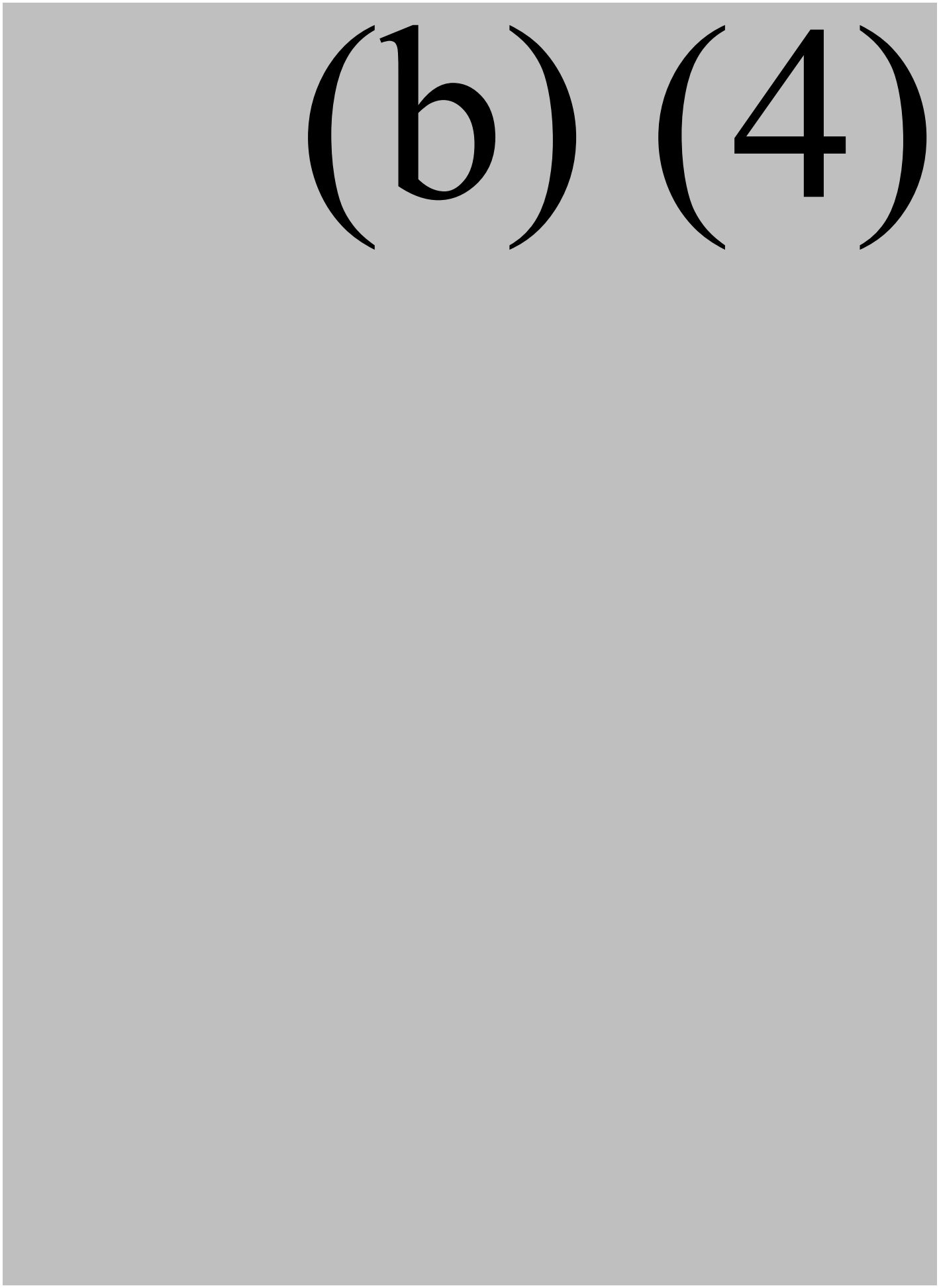
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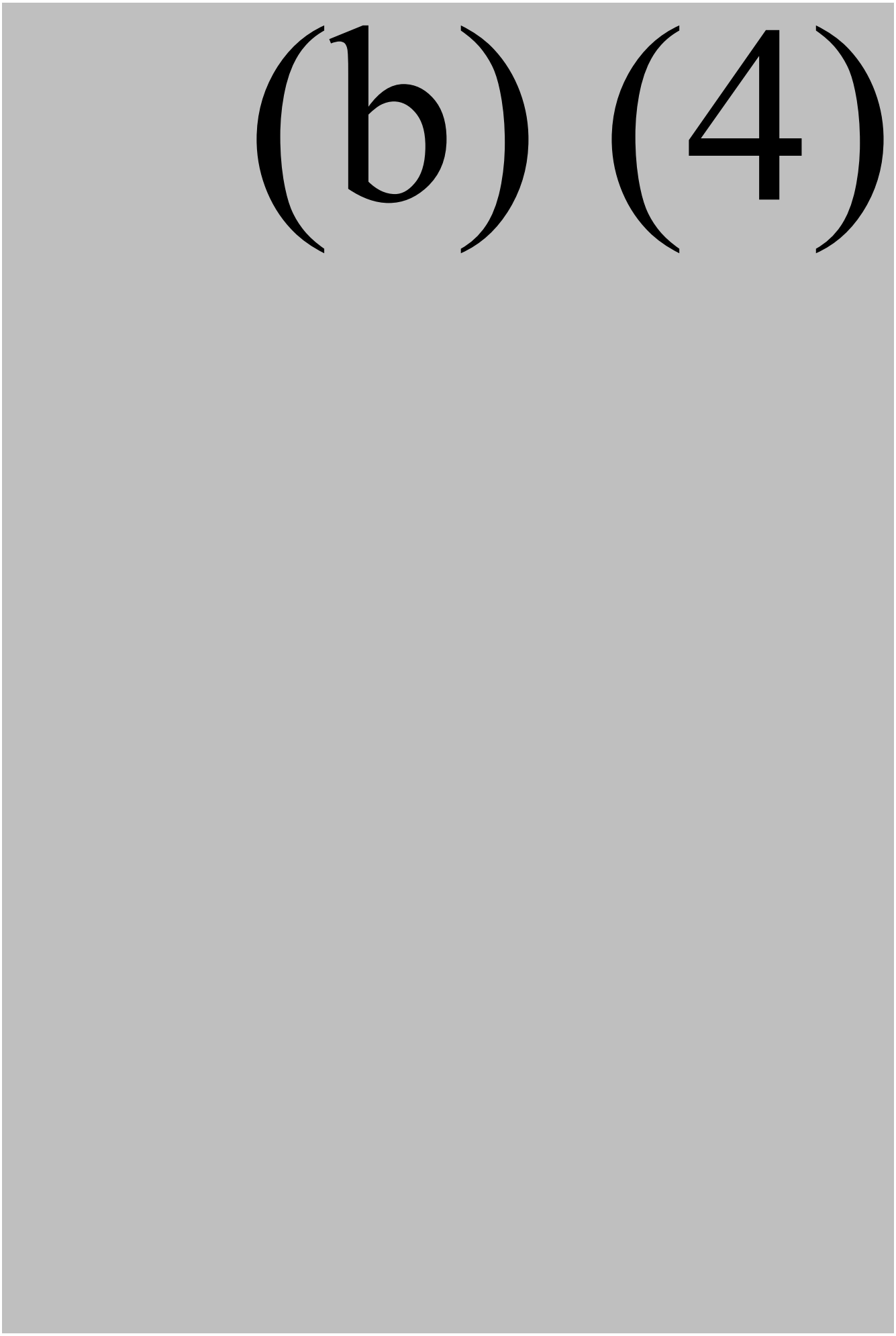
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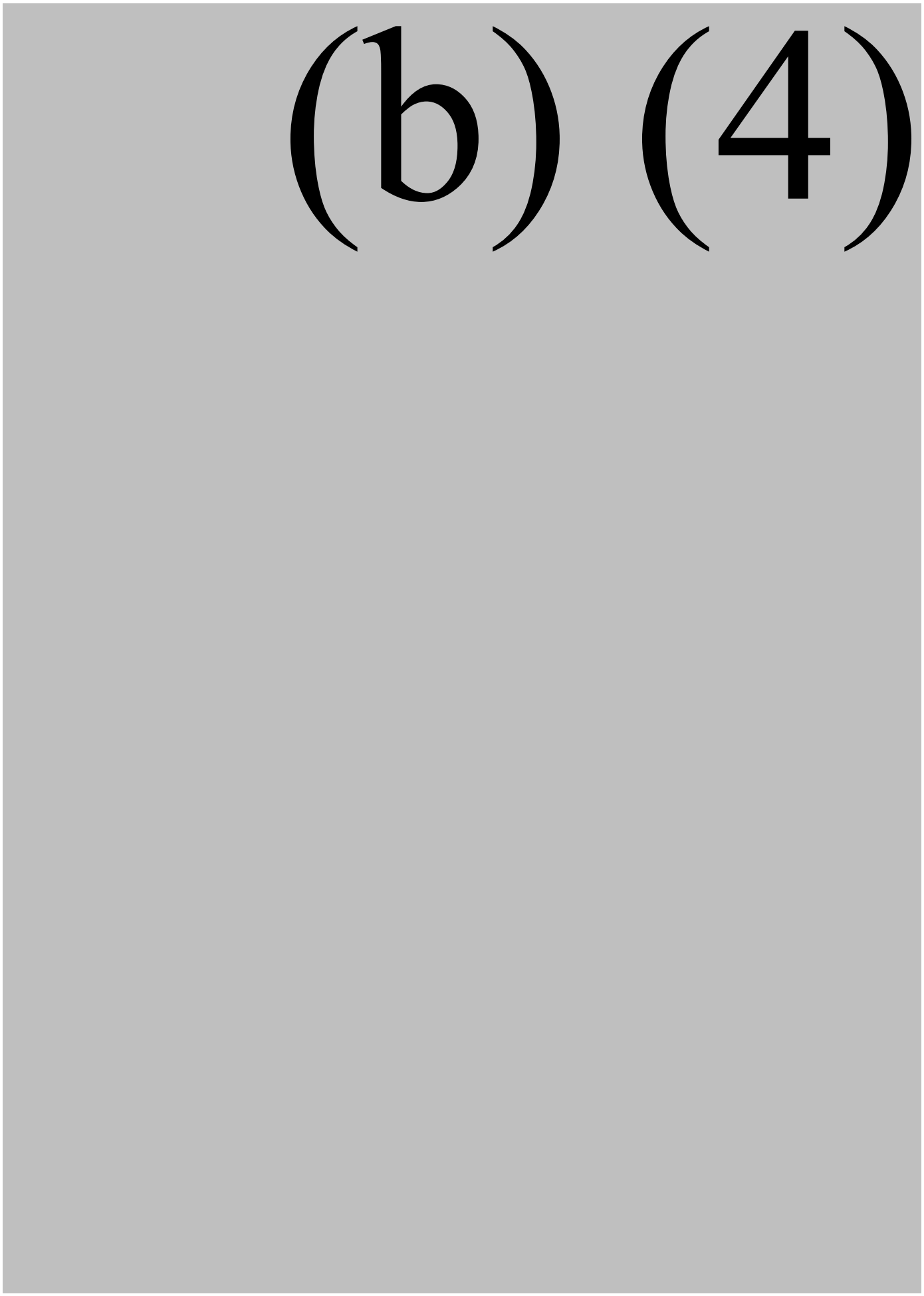
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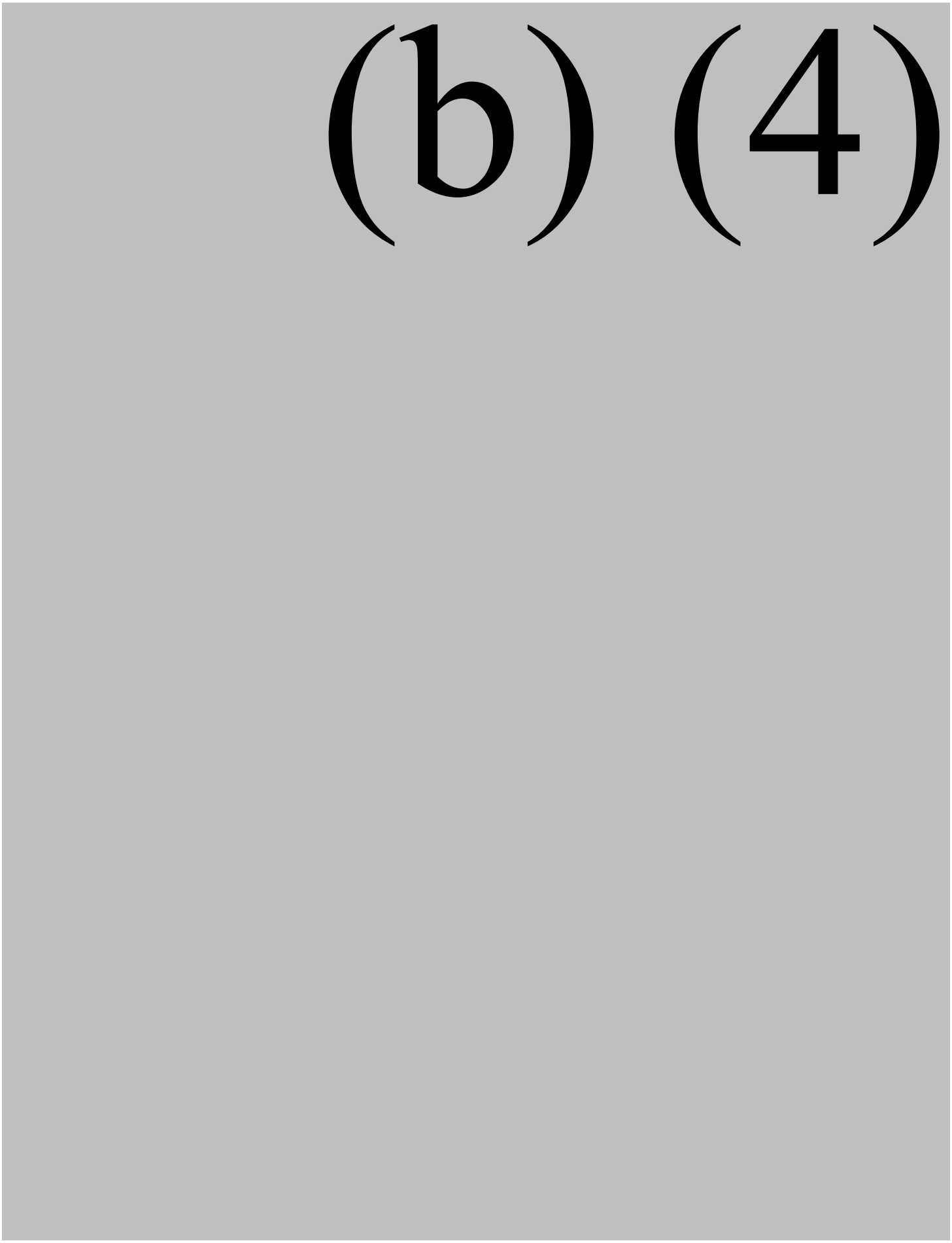
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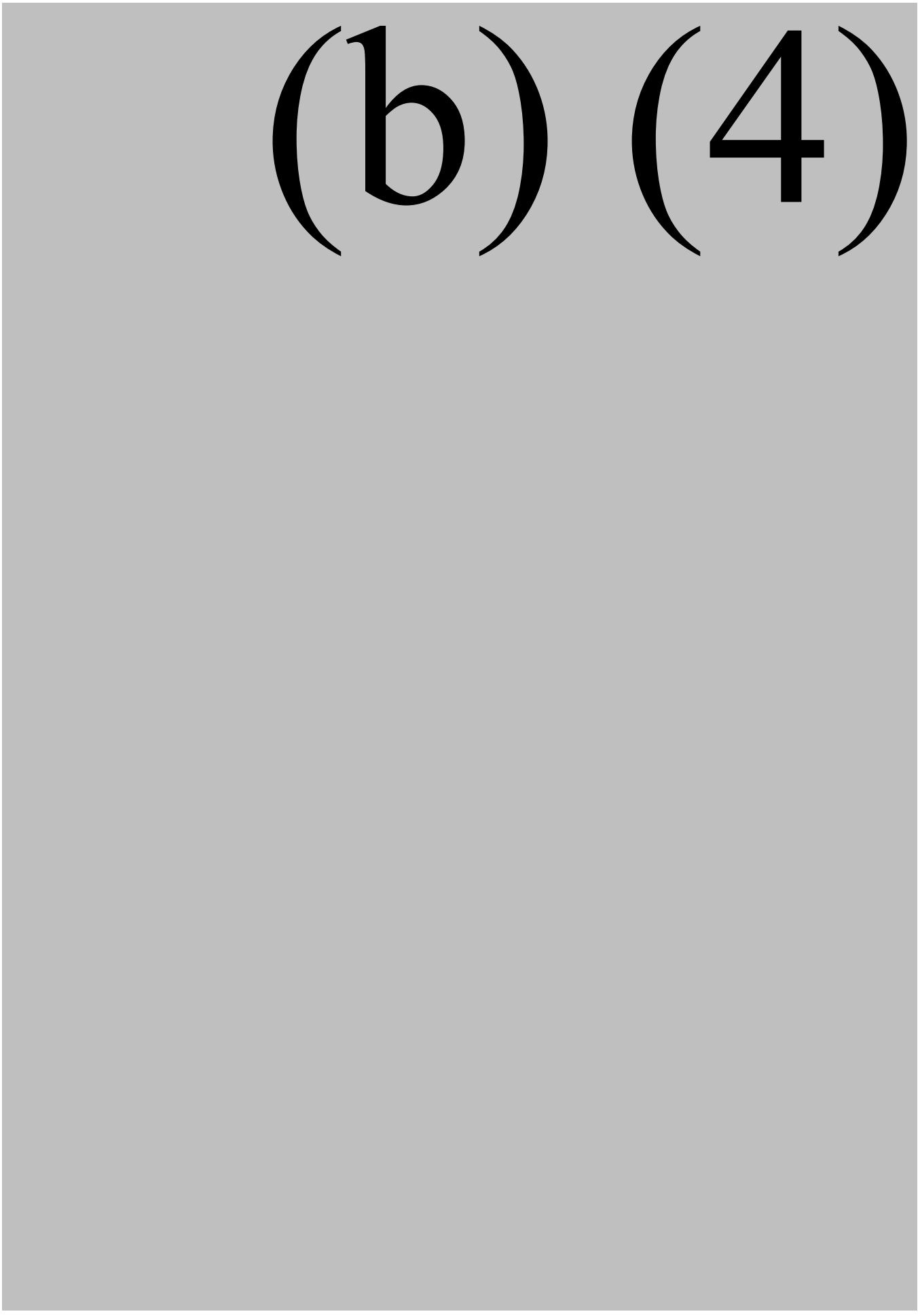
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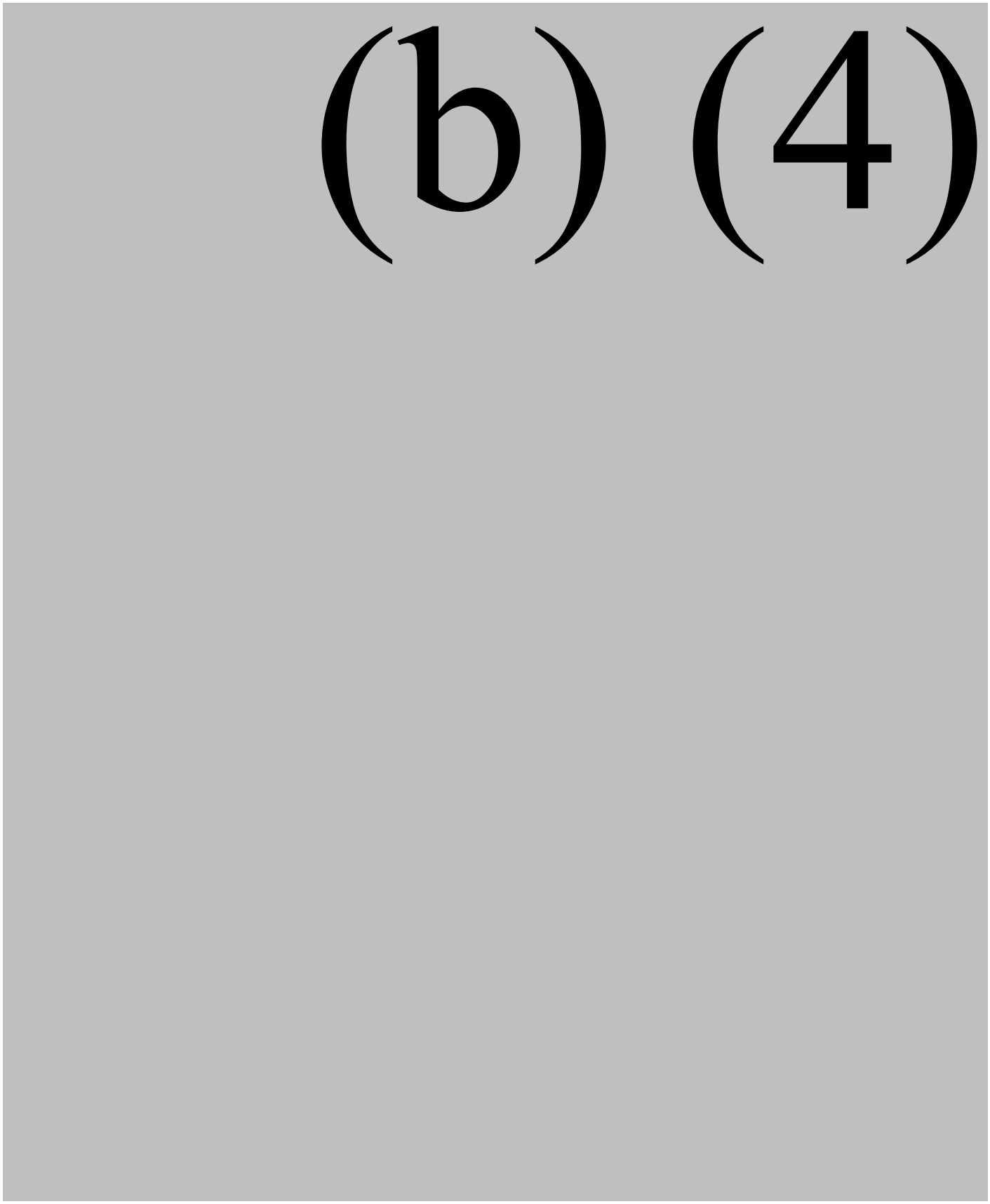
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Non-federal funds ID/Account Number to bill:_____

Amino Acid Laboratory
University of California, Davis
1020 Vet Med 3B
1089 Veterinary Medicine Drive
Davis, CA 95616
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http://www.vetmed.ucdavis.edu/vmb/labs/aal/index.cfm

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Company Name: _____
Address: _____
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Email: (b) (6)
Tel: (b) (6) **Fax:** (b) (6)

Billing Contact: Finance Dept. **TAX ID:** (b) (6)
Email: (b) (6) **Tel:** (b) (6)

Patient Name: (b)(6) (b) (6)
Species: Feline
Owner's Name: (b) (6)

Sample Type: ☐ Plasma ☒ Whole Blood ☐ Urine ☐ Food ☐ Other:_____
Test Items: ☐ Taurine ☐ Complete Amino Acid ☐ Other:_____

Taurine Results (nmol/ml)

Plasma:_____ Whole Blood: 196 Urine:_____ Food:_____

Reference Ranges (nmol/ml)

	Plasma		Whole Blood	
	Normal Range	No Known Risk for Taurine Deficiency	Normal Range	No Known Risk for Taurine Deficiency
Cat	80-120	>40	300-600	>200
Dog	60-120	>40	200-350	>150

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JOURNAL OF TRANSLATIONAL MEDICINE

REVIEW Open Access

Taurine: the appeal of a safe amino acid for skeletal muscle disorders

Annamaria De Luca*, Sabata Pierno and Diana Conte Camerino

Abstract

Taurine is a natural amino acid present as free form in many mammalian tissues and in particular in skeletal muscle. Taurine exerts many physiological functions, including membrane stabilization, osmoregulation and cytoprotective effects, antioxidant and anti-inflammatory actions as well as modulation of intracellular calcium concentration and ion channel function. In addition taurine may control muscle metabolism and gene expression, through yet unclear mechanisms. This review summarizes the effects of taurine on specific muscle targets and pathways as well as its therapeutic potential to restore skeletal muscle function and performance in various pathological conditions. Evidences support the link between alteration of intracellular taurine level in skeletal muscle and different pathophysiological conditions, such as disuse-induced muscle atrophy, muscular dystrophy and/or senescence, reinforcing the interest towards its exogenous supplementation. In addition, taurine treatment can be beneficial to reduce sarcolemmal hyperexcitability in myotonia-related syndromes. Although further studies are necessary to fill the gaps between animals and humans, the benefit of the amino acid appears to be due to its multiple actions on cellular functions while toxicity seems relatively low. Human clinical trials using taurine in various pathologies such as diabetes, cardiovascular and neurological disorders have been performed and may represent a guide-line for designing specific studies in patients of neuromuscular diseases.

Keywords: Taurine skeletal muscle, Inherited muscle disorders, Disuse muscle atrophy, Development and aging, Skeletal muscle performance

Background

Taurine (2-aminoethane-sulfonic acid) is a sulfur-containing amino acid which is not used for protein synthesis and is therefore the most abundant free amino acid in mammalian tissues, with the exception of human liver in which aspartate is the most abundant one [1, 2]. The intracellular concentration of taurine ranges between 5 and 20 μmol/g wet weight in many tissues, especially in excitable ones, such as brain, heart and skeletal muscle [1, 3, 4]. Endogenous synthesis occurs in the liver via the cysteine sulfinic acid pathway. The metabolic reaction consists in a first oxidation of the sulfhydryl group of cysteine to cysteine sulfinic acid by the enzyme cysteine dioxygenase. Cysteine sulfinic acid is then decarboxylated to hypotaurine by the cystyeine sulfinate decarboxylase. Taurine is obtained by a yet unclear spontaneous or enzymatic oxidation (by hypotaurine dehydrogenase) of hypotaurine (Fig. 1). The endogenous synthesis of taurine is highly variable between individuals also in relation to nutritional state, to the amount of protein intake and to cysteine availability [1, 5]. In turn the availability of cysteine is highly dependent on the metabolic equilibrium between homocysteine and methionine, via folic acid, vitamin B12 and the efficiency of the enzyme methyltetrahydrofolate reductase. In addition, a certain amount of taurine has to be introduced with food, mostly in carnivores and, to a minor extent, in omnivores [1]. The importance of the two sources vary quite a lot between species, with some, like felines and foxes, being highly dependent on diet acquisition of taurine, as they are unable to synthesize it. These species are also particularly susceptible to deficient states, developing severe pathophysiological conditions, such as dilated cardiomyopathy, retinal degeneration and reproduction defects

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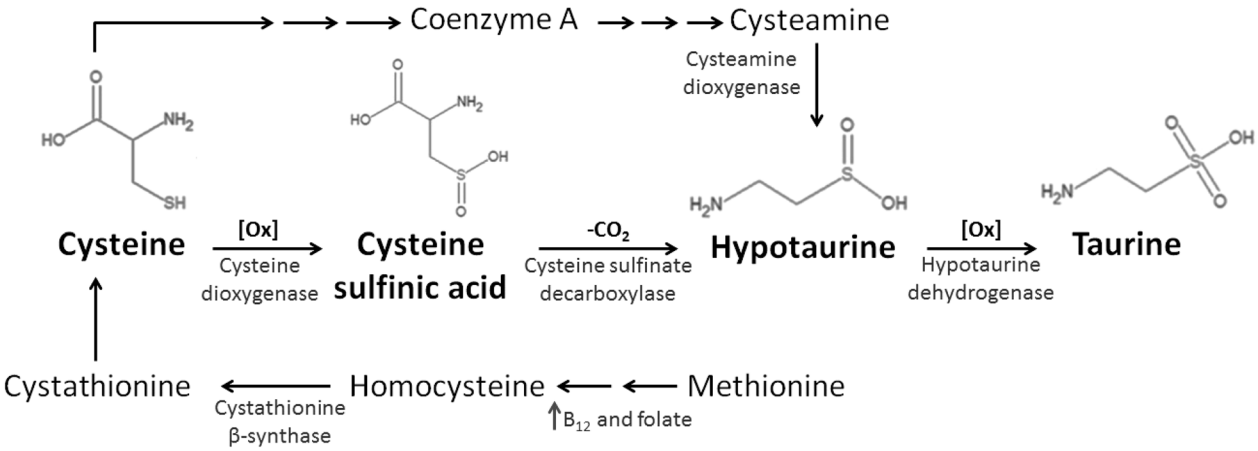

Fig. 1 Biosynthetic route of taurine from amino acids cystein and methionine. The synthesis does primarily occur in liver, although other tissues can contribute to its synthesis based on presence of key enzymes. However, the tissue synthesis is generally low, and tissues needs to uptake circulating taurine against gradients by means of the specific Na^+/Cl^- dependent transport system, TauT. Some species (i.e. felines) cannot synthesize taurine and dramatically depends on taurine intake with food. Diet is indeed an important source of the amino acid for all species, especially if reach of fish or beef meat as well as other animal-derived food (i.e. milk).

[3, 6]. These evidences first outlined the key role of taurine for mammalian tissue functions and helped to better understand the link between tissue distress in retaining proper taurine concentration and various pathophysiological conditions.

In fact, even in species able to synthesize taurine, the tissue-specific synthesis is relatively low, with liver being the main source according to the higher expression of enzymes as cysteine dioxygenase. Importantly, the activity of this latter enzyme strictly depends upon cysteine availability, so that the exact amount of taurine being endogenously synthesized is difficult to predict [7]. However, the high intracellular concentration is guaranteed by the presence of a specific active transporter that concentrates taurine inside the cells against gradients. The taurine transporter (TauT; encoded by the SLC6A6 gene) is a sodium and chloride ion-dependent transporter ubiquitously expressed in mammalian tissues. The concentration of taurine is 100-fold less in the plasma (20–100 μM) than in the tissues, suggesting that it is indeed required for modulating key cellular functions. Due to the high tissue concentration, taurine also works as an osmolyte. Its cellular efflux via volume-dependent or volume-independent pathways works to osmotically balance the excessive production of metabolic by-products. Both uptake systems and efflux pathways are tightly regulated at transcriptional and post-transcriptional level, leading to an accurate control of taurine intracellular levels [8].

Since its discovery in ox bile in 1827, several physiological functions have been described for the amino acid, ranging from the classical role of conjugating agent for bile acids, to wider actions as osmotic pressure regulator, modulator of calcium homeostasis and signaling and, more recently, as an endogenous anti-oxidant and anti-inflammatory compound in various tissues. The mechanism by which taurine exerts all these different functions is still unclear. Some of the taurine actions in central nervous system (CNS), seem to occur via specific binding sites or receptors, i.e. in thalamus taurine modulates neuronal firing via activation of extra-synaptic gamma-amino butyric acid (GABA) receptor isoforms α4β2δ with a greater affinity than GABA [9–12]. Such high affinity binding sites have not been evidenced in other tissues.

Skeletal muscle is one of the tissues able to concentrate the largest amount of body's taurine, via the TauT activity. Pioneer studies of Ryan Huxtable anticipated that the high taurine level is needed to maintain an appropriate calcium homeostasis, likely by ensuring a correct calcium re-uptake by the sarcoplasmic reticulum [13]. Similar actions were also described in heart, with taurine exerting complex modulation of calcium homeostasis in relation to external concentration of the cation with beneficial effects in contrasting arrhythmias or heart failure [1, 3, 4].

Transgenic mice lacking TauT gene have been generated by two separate groups [6, 14–16]. In line with a key role of taurine for maintaining proper physiological functions, the drastic reduction in content consequent to TauT deletion is associated to a variety of disorders in various tissues, such as eye, kidney, heart, nociceptive system and skeletal muscle [14–17]. These conditions resemble those occurring when taurine tissue content is

altered by pathophysiological states or by inhibitors of the taurine transporter. In spite the pre-clinical research has disclosed many conditions in which taurine supplementation may be beneficial, the therapeutic use of taurine is very limited. Taurine is commonly known for its claimed effects as energizer and anti-fatigue compound and it is present in many energy soft drinks as well as in supplement cocktails for athletes. The toxicity of taurine in this context is considered relatively low with respect to other active ingredients; actually it may also be protective against cardiovascular action of caffeine [18]. Such a protection may again result from multiple taurine actions, i.e. an antihypertensive effect via vasodilatation (by reducing adrenergic and angiotensin II actions as well as calcium-induced vasospasm) along with a reduced risk of cardiac arrhythmias via modulation of ion channels and ionic homeostasis [18]. However a certain caution is important especially when taurine is used in children and/or in association with drugs, alchool or other food supplements [19–23]. Apart for its nutraceutical role, taurine may exert clear pharmacological actions by modulating signaling pathways and targets or via restoration of its altered tissue levels. No systematic toxicity studies have been performed to assess the toxicological parameters for taurine; however human trials have used taurine up to 10 g/daily without overt signs of toxicity. This may also depend on the direct relationship between taurine plasma level and its excretion rate by the kidney [19].

An extensive revision of all the actions of taurine in various tissues and the wide potential usefulness of its supplementation is out of the scope of this review. However, a general overview is provided in Fig. 2. As far as inherited or acquired pathophysiological conditions of skeletal muscle are concerned, the pre-clinical findings allow to distinguish effects related to exogenous pharmacological action of taurine on rather specific targets, such as in myotonic syndromes, to conditions that may be accompanied by changes in intercellular taurine content or change in calcium homeostasis, in which a taurine supplementation may be helpful to restore altered levels.

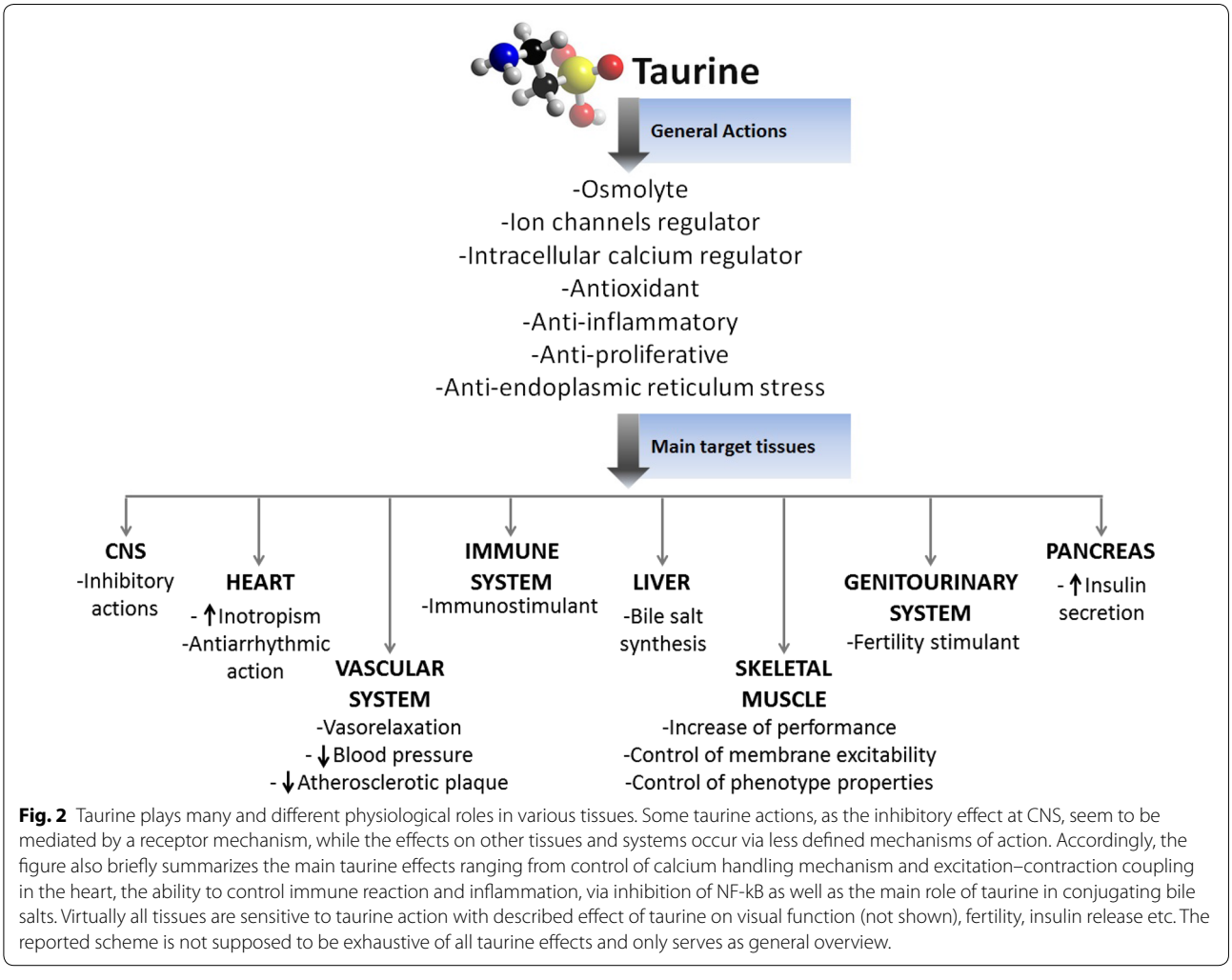

Fig. 2 Taurine plays many and different physiological roles in various tissues. Some taurine actions, as the inhibitory effect at CNS, seem to be mediated by a receptor mechanism, while the effects on other tissues and systems occur via less defined mechanisms of action. Accordingly, the figure also briefly summarizes the main taurine effects ranging from control of calcium handling mechanism and excitation–contraction coupling in the heart, the ability to control immune reaction and inflammation, via inhibition of NF-kB as well as the main role of taurine in conjugating bile salts. Virtually all tissues are sensitive to taurine action with described effect of taurine on visual function (not shown), fertility, insulin release etc. The reported scheme is not supposed to be exhaustive of all taurine effects and only serves as general overview.

The present review is aimed at providing the state-of-art of taurine research in skeletal muscle, with particular attention to its potential therapeutic application as orphan drug in inherited rare muscle disorders, as well as in pathophysiological conditions such as aging, malnutrition and/or muscle disuse.

Skeletal muscle ion channels as specific targets of taurine: the potential action of taurine as anti-myotonic drug

Taurine and skeletal muscle chloride channels ClC-1

In CNS, taurine has been long claimed to act as an "inhibitory" amino acid and neurotransmitter [1]. Neuronal synthesis of taurine and metabotropic taurine receptors have been described in specific areas of CNS, where taurine acts in a glycine or GABA-like manner, by enhancing hyperpolarizing chloride-mediated conductance in nervous cells [9, 11, 12]. Pre-clinical evidences were provided of a beneficial effect of taurine in controlling/preventing seizure discharges and neurotoxicity [1, 12, 24]. The ability of taurine to act as inhibitory amino acid raised attention to its possible effect as potential membrane stabilizer in skeletal muscle. We investigated about the actions of the amino acid on voltage-gated chloride channels CLC-1 that account for the macroscopic chloride conductance (gCl) of skeletal muscle. Resting gCl accounts for about 70–90% to the total membrane conductance of sarcolemma and plays a pivotal role in maintaining the sarcolemmal electrical stability by shunting the depolarization-driven potassium accumulation in transverse tubules. Thus the large gCl allows repolarization and muscle relaxation.

Loss-of-function mutations of CLC-1 are responsible of myotonic syndromes with either autosomal dominant (Thomsen disease) or recessive pattern of inheritance (Becker's Myotonia Congenita). The resulting decrease of gCl is responsible for the pathological hyperexcitability and for the delayed relaxation, spasms and stiffness typical of the disease in both patients and myotonic animals [25–27].

Our research has shown that taurine, acutely applied in vitro, exerts a concentration-dependent increase of gCl in rat extensor digitorum longus (EDL) myofibers, and in parallel reduces membrane excitability [28, 29]. The effective concentrations are in the millimolar range, likely in relation to the high intracellular level of the amino acid [28, 29]. A pre-clinical evaluation of the potential anti-myotonic activity of taurine has been performed. We found that taurine does not antagonize the myotonic discharges in rats made myotonic by administration of anthracene-9-carboxylic acid, a direct chloride channel blocker, nor does it restore gCl lowered in vitro by the same agent. However, when rats are made myotonic by a chronic exposure to 20,25 diazacholesterol, which reduces gCl indirectly by modifying lipid membrane composition, taurine antagonizes the electromyographic signs of myotonia if administered in vivo, while its acute in vitro application contrasts both the reduced gCl and the high frequency firing of single myofibers [30]. These results suggested that taurine can contrast myotonia if chloride channels are available for a direct modulation, implying its direct action at channel level or on a site nearby. A series of taurine analogues were tested on gCl of rat EDL myofibers to investigate the structure–activity relationship (SAR) between taurine and chloride channels. The results provided a pharmacological evidence of the presence of a specific low-affinity taurine binding site able to modulate chloride channel function and/or kinetic [31]. In particular, an increased distance between the two charged heads of taurine and/or a more distributed positive charge for the replacement of the amino group with aza-cyclo moieties lead to a decreased potency in enhancing gCl [31]. The direct action of taurine on skeletal muscle chloride channel was further confirmed by two microelectrode voltage-clamp recordings of chloride currents sustained by human ClC-1 channel heterologously expressed in Xenopous oocytes. In these conditions, the in vitro application of 20 mM taurine enhanced by 100% the chloride currents and shifted channel activation toward more negative potentials, an effect that likely accounts for the increase in resting gCl observed in native fibers [32–34]. This direct modulation adds to other possible homeostatic and modulatory roles that the high intracellular taurine has on chloride channels. However, as anticipated, the acute modulation of gCl may require fully or partly functional chloride channels, questioning about the real efficacy of taurine in ClC-1 related myotonic syndromes, especially for those mutations that seriously affect channel expression and protein level. Taurine has been tested in patients with myotonic dystrophy with encouraging results. In particular acute parenteral administrations of taurine allowed to reduce membrane excitability evaluated in relation to potassium plasma concentration after potassium-enriched infusion, suggesting again an action on membrane ionic conductance. Accordingly, a double-blind oral administration of taurine led to a long-term control of myotonic symptoms estimated as reduction of electromyographic (EMG) discharges and potassium induced-hyperexcitability [35–37]. Even taking into account the possible bias deriving from these small sized trials, the effects of taurine in myotonic dystrophy patients suggest alternative modality for decreasing membrane excitability. In fact, myotonic dystrophy type 1 (DM1) or Steinardt syndrome, is caused by expansion of a CTG trinucleotide repeat in the non-coding region of DM protein kinase with abnormalities

in mRNA metabolism and alternative splicing of certain genes. In DM1 patients, the abnormal inclusion of alternative exons 6B and/or 7A and retention of intron 2 of CLC-1 channel gene (*CLCN1)* gene have been observed. These aberrant-splicing, which may also occur in myotonic dystrophy type 2 (DM2) patients, leads to premature termination codons, with a consistent decrease of the mRNA of *CLCN1*, of ClC-1 protein and consequently of gCl [38, 39]. Therefore, the possible modulatory action of taurine on other skeletal muscle ion channels has to be taken into account.

Taurine and Nav1.4 voltage gated sodium channels

It is feasible to hypothesize a modulation by taurine of the skeletal muscle isoform of voltage-gated sodium channel (Nav1.4), involved in the generation and propagation of action potential and main target of symptomatic antimyotonic drugs [37, 40]. The effect of taurine on sodium channels of native muscle fibers has been investigated in our laboratories by cell-attached patch clamp recordings. Taurine has a dual effect. In particular taurine enhances the sodium transients elicited by depolarizing test pulses close to the threshold for channel activation (test pulse to −70/−50 mV), an effect that is likely related to the observed shift of the activation curve towards more negative potentials. However, taurine reduces sodium currents at more depolarized test pulse potentials, with a 50% inhibition of the maximal peak sodium current observed at 10 mM taurine. In parallel, a left-shift of the steady-state inactivation curve has been observed, indicating the ability of taurine to stabilize the blocked channels in the inactivated state [34, 41 Desaphy and Conte Camerino, unpublished observation]. This peculiar effect of taurine on Nav1.4 channel is similar to what has been observed on cardiac sodium currents [42, 43] and underlines a complex action of the amino acid on sodium channel gating and kinetic. Our extensive structure–activity relationship studies of inhibitors of Nav1.4 channel allow to predict that the anesthetic-like action of taurine is mediated by the amino group, a main pharmacophore moiety in sodium channel blockers [44–47]. The dual ability of taurine to open chloride channels and to block sodium channels envisages a greater therapeutic action of the amino acid in myotonic states related to gain-of-function mutations of sodium channels, such as Sodium Channel Myotonia and Paramyotonia Congenita. The verification that taurine is able to compensate mutation-related biophysical alterations of Nav1.4 channels will be helpful at this regard, and is part of future projects of our laboratory. For the moment, the action of taurine on sodium channels can account for the antimyotonic effect in conditions where chloride channels are defective or dysfunctional [35, 36]. In line with this, the mechanism of taurine action on Nav1.4 sodium channels deserves to be further investigated since it may better support its pharmacological potential and its clinical use in hyperexcitability muscle disorders (Table 1).

Role of proper taurine intramuscular level for excitation–contraction coupling and muscle performance

The ability of skeletal muscle to concentrate taurine against gradient pushed toward a better understanding of its physiological role. Adult rats were chronically treated with guanidinoethane sulfonate (GES), an inhibitor of taurine transporter (TauT) to induce a reduction of taurine content in skeletal muscle. We found that a 50% reduction of taurine in EDL muscle leads to a marked decrease in gCl, and to a parallel enhancement of sarcolemmal excitability, disclosing the ability of taurine level to exert a physiological control on chloride channel function and sarcolemmal stability [48]. The mechanism underling this effect is not clear yet, but we cannot rule out the ability of taurine to modulate ClC-1 channel function via a fine-tuning of a calcium-dependent phosphorylation-signaling pathway, as discussed below. In line with the described ability of taurine to control calcium homeostasis in both skeletal muscle and cardiac tissue [1, 4], we found a marked alteration of mechanical threshold, i.e. the voltage at which muscle fiber contracts in response to depolarizing voltage steps, in taurine-depleted EDL myofibers. Mechanical threshold depends on the kinetic of calcium release from and reuptake by sarcoplasmic reticulum, also in relation to basal cytosolic calcium concentrations. Taurine depleted EDL muscle fibers contract at more negative potentials with respect to normal ones, implying an impact of GES treatment on calcium handling [48, 49]. Both the decrease in gCl and the shift of mechanical threshold toward negative potentials were rapidly reverted by in vitro application of millimolar concentration of taurine. Actually, depleted muscles showed a higher than normal sensitivity to exogenous taurine with respect to normal ones [48], further corroborating the link between the observed alterations and the taurine level. The contractile properties and fatigability of EDL muscles depleted of taurine by a GES treatment were investigated by Bakker's group. It was found that the treatment with GES decreases muscle taurine levels to <40% of controls and decreases the peak twitch force of EDL muscles by 20%. Also, GES-treated muscles develop a lower force in force–frequency relationship and show a slower time to fatigue, likely in relation to the lower metabolic demands of the weaker muscles [50]. Primary information about the long-term effect of taurine in skeletal muscle and, consequently, of potential usefulness of its exogenous administration

Table 1 Involvement and therapeutic potential of taurine in physio-pathological conditions and diseases of skeletal muscle

Condition	Change in Taurine content / TauT	Pathogenetic mechanisms related to changes in taurine content	General symptoms	Taurine targets	Therapeutic Potential of Taurine
Post-natal development	Age-dependent increase in TauT expression and intracellular content	Delayed development and delayed acquisition of specific phenotypic properties; metabolic dysfunction	Specie-specific (due to different sensitivity to taurine deficiency)	Mitochondria; ion channels; calcium homeostasis and calcium dependent gene expression	Taurine supplementation in formula for pre-term born infants; to ensure a proper skeletal muscle phenotype differentiation
Aging	Decrease in Taurine content; no information on TauT expression	Metabolic distress; calcium dependent dysfunction; reduced regenerating ability; reduced activity of free-oxygen radicals scavengers	Sarcopenia; atrophy, weakness and fatigue degeneration, altered excitation–contraction coupling, impaired performance	Ion channels; Calcium homeostasis; oxidative stress and atrophy	To counteract the decrease in taurine content and the consequent reduction in chloride channel function and the alteration in calcium ion homeostasis; to ameliorate performance and muscle strength
Ischemia and reperfusion injury	Decrease due to a compensatory taurine efflux	Insufficient vaso-dilation in relation to muscle work; metabolic distress; oxidative stress	Hyperkaliemia, muscle dysfunction; ROS-induced inflammation and damage	Metabolic-sensitive channels; mitochondria	To counteract hyper-kaliemia by inhibiting K_{ATP} and KCa^{2+} channels; to prevent ischemia-induced taurine loss
Myotonic syndromes and periodic paralyses	Unknown	Primary inherited channelopathies due to loss-of function mutations of ClC-1 chloride channel or gain-of-function mutations of Nav1.4 sodium channel	Hyperexcitability and impaired muscle relaxation	ClC-1 chloride channel; Nav1.4 sodium channel	To reduce membrane hyper-excitability through: opening of chloride channel and increase in gCl mediated by both short and long term actions; modulation of generation and propagation of action potential, by blocking sodium channel with a local-anesthetic like mechanism
Disuse	Slow-to-fast decrease in taurine content; no change in TauT expression	Myofiber phenotype transition in postural muscle; atrophy	Atrophy, change in metabolism, slow-to-fast transition; weakness	Ion channel function and expression; calcium homeostasis	To counteract disuse-induced taurine loss; to counteract myofiber transition; potential counteraction of atrophy
Duchenne muscular dystrophy and related myopathies	Change in content related to pathology phase; possible reduction of TauT expression	Alteration of calcium homeostasis; calcium-related degeneration; oxidative stress and inflammation	Progressive muscle degeneration and weakness; muscle fiber loss and fibrosis; sarcolemmal instability; altered calcium homeostasis; inflammation and oxidative stress	Chloride channel and voltage-insensitive calcium permeable channels (Leak/TRP-like); SERCA; mitochondria	To ameliorate muscle performance; to counteract taurine loss and to modulate calcium availability for contraction; to counteract contraction-induced ischemia. To contrast degeneration-induced decrease in gCl; adjuvant therapy in combination with glucocorticoids

The table summarizes the main role of taurine in various conditions of skeletal muscle, indicating evidences in relation to changes in tissue content and potential site of taurine action. Please refer to text for more detailed information and specific references.

TauT taurine transport system, *SERCA* sarco/endoplasmatic reticulum calcium ATPasi, *gCl* macroscopic chloride conductance, *TRP* transient receptor potential channels, *ROS* reactive oxygen species, *KATP* ATP-dependent potassium channels, *KCa* calcium activated potassium channels.

derives from studies on mice in which the TauT was genetically knocked out [6, 14–16]. TauT knockout mice ($TauT^{-/-}$) show more than 90% decrease in taurine content in both muscle and heart and are characterized by a marked decrease in exercise performance in exhaustive training models. Although the force of isolated muscle has not been measured in these $TauT^{-/-}$ mice, clear abnormalities of muscle structure have been found, including signs of atrophy and muscle necrosis. Additionally, the muscles of $TauT^{-/-}$ mice have a shift of metabolism toward the glycolytic pathway, especially in condition of exercise; this has been related to a dysfunction in mitochondrial function and in fatty acid oxidative pathways [51]. In parallel, taurine deficiency leads to cardiomyopathy characterized by remodeling of ventricular cardiomyocytes, ultrastructural damages of myofilament and mitochondria, and overexpression of markers of heart failure, such as atrial natriuretic peptide, brain natriuretic peptide and beta-myosin heavy chain [15, 16].

It is therefore evident that taurine is essential to maintain muscle performance and excitation–contraction coupling; however the mechanism for these actions is still unclear. An in vitro study of Berg and Bakker clearly demonstrated the ability of taurine to increase the accumulation of calcium into sarcoplasmic reticulum (SR) in isolated skinned myofibers by 35%, an effect that accounts for the greater depolarization-induced contraction of fiber exposed to 20 mM taurine. This in spite taurine slightly reduces the sensitivity of contractile apparatus to calcium [52]. Interestingly, a recent study demonstrated that a prolonged exposure to 10–20 mM taurine increases the rate of calcium uptake in both type I and type II human myofibers; an action within the SR lumen has been proposed. An increase in contractile sensitivity to calcium was also observed but exclusively in type I fibers [53]. These results reinforce the original data of Huxtlable and Bressler about the ability of taurine to stimulate calcium uptake by vesicles of SR [13]. Recent insight into the role of taurine in skeletal muscle has been obtained by the group of Hayes, who supplemented rats with taurine and evaluated the outcome on various functional parameters [54]. Taurine supplementation significantly increases the amino acid content in skeletal muscle, without any adaptive change in TauT activity; in parallel an increase in force and a greater resistance and recovery after fatigue have been observed. These changes were paralleled by an increase in calsequestrin1, the calcium binding protein that works to maintain high amounts of calcium in the cysterna of SR. This suggests that taurine supplemented muscle can store a greater quantity of calcium with a consequent greater calcium availability for contraction. However, the involvement of sarco/endoplasmic reticulum calcium-ATPase (SERCA) remains to be better clarified. A decrease in markers of oxidative stress was also found, indicating that taurine may help to control activity-related oxidative stress [48]. In support to this view, a recent report by Silva et al. showed that a daily treatment of rats with 300 mg/kg taurine for 2 weeks protects muscles against in vivo eccentric exercise damage, such as downhill running [55]. In particular taurine reduced protein carbonylation or oxidized thiols, without increasing the expression of endogenous anti-oxidant pathways, such as superoxide dismutase or catalase [55]. Sugiura et al. similarly found that taurine administration before strenuous exercise reduces muscle DNA damage likely via down-regulation of inducible nitric oxide synthase (iNOS) and consequent reduction of nitrosative inflammation [56]. The protective effects of taurine supplementation are due to a long term modulatory effect, likely in relation to its muscle uptake and intracellular levels. In fact acute in vitro application of physiological concentrations of taurine to isolated mouse soleus muscle, does not increase muscle contractile performance in term of force, fatigue resistance and recovery and does not exert any synergistic action when associated with caffeine [57]. Despite the authors suggesting a lack of ergogenic benefit by acute taurine, it is important to underline that slow twitch soleus muscle is characterized by high intracellular taurine content [58, 59], predicting its lower dependency on extracellular concentrations. Accordingly, we have shown that a chronic treatment with taurine to dystrophic mice leads to a minor increase of its intracellular content in soleus muscle than in fast twitch muscles [59].

Although taurine supplementation enhances exercise performance, its efflux during exercise and/or ischemia, with consequent decrease in tissue concentration, can also occur [60, 61]. Whether the loss of taurine is a marker of tissue damage or rather a cytoprotective mechanism against ischemic insult, is still matter of debate [60, 62, 63]. The protective effect of taurine efflux in the above conditions can be related to the need to osmotically balance, along with water movement, the increase of by-products of metabolism in the myofibers [1, 14]. However a role in the mechanism to contrast fatigue can be envisaged. In fact, taurine exerts an inhibitory control on channels that couple the metabolic state of the myofiber with membrane excitability, such as the ATP-dependent potassium (KATP) channels and calcium-activated potassium channels [64, 65]. Taurine blocks skeletal muscle KATP channel by binding the channel complex nearby the sulphonylurea receptor [64]. During ischemia–reperfusion injury, the opening of KATP are involved in the cytoprotective effect of the preconditioning mechanisms, by preventing the influx of calcium ions and preserving the ATP

content of the muscle. The efflux of taurine during exercise and/or ischemia may be required to relief a basal inhibitory effect and to enhance the potassium efflux and membrane repolarization via the specific channels activated by ATP depletion and/or intracellular calcium accumulation. This would exert a protective action against exercise-induced fatigue or impairment in muscle performance related to ischemia–reperfusion injury [64, 65]. Accordingly, the depletion of taurine induced by GES in rat skeletal muscle significantly increases the macroscopic resting potassium conductance of about 80% [48].

Intracellular taurine can also be conjugated in mitochondria of extra-hepatic tissues to 5-taurinomethyl uridine that is present in tRNA and modulates the synthesis of mitochondrial proteins. Consequently, the fatigue and the enhanced oxidative stress observed in myopathic states by taurine depletion can also be due to respiratory chain inefficiency [4, 51, 66]. A representative scheme of the taurine actions in striated myofibers is shown in Fig. 3.

Taurine as potential therapeutic muscular agent from birth to elderly

The role of taurine for post-natal development of various organs depends upon the species-specific ability to endogenously synthesize the amino acid. Cats, that critically depend on exogenous taurine intake, develop serious impairments during post-natal development if not fed with taurine. Although less compelling for humans, prematurely born infants are believed to lack the enzymes that convert cystathionine to cysteine, and may, therefore, become taurine-deficient if not breast-fed. In fact taurine is present in mother's milk and evidences are available about potential usefulness of taurine addition in the formula especially for pre-term births [67, 68]. The actual necessity or benefit of this practice has never been rigorously studied, and as such, taurine has yet to be proven to be important during fetal development, perhaps via epigenetic and/or organogenesis related mechanisms. Recent focus has been addressed to the potential benefit of taurine supplementation in mice during gestational period, especially when mothers are exposed to

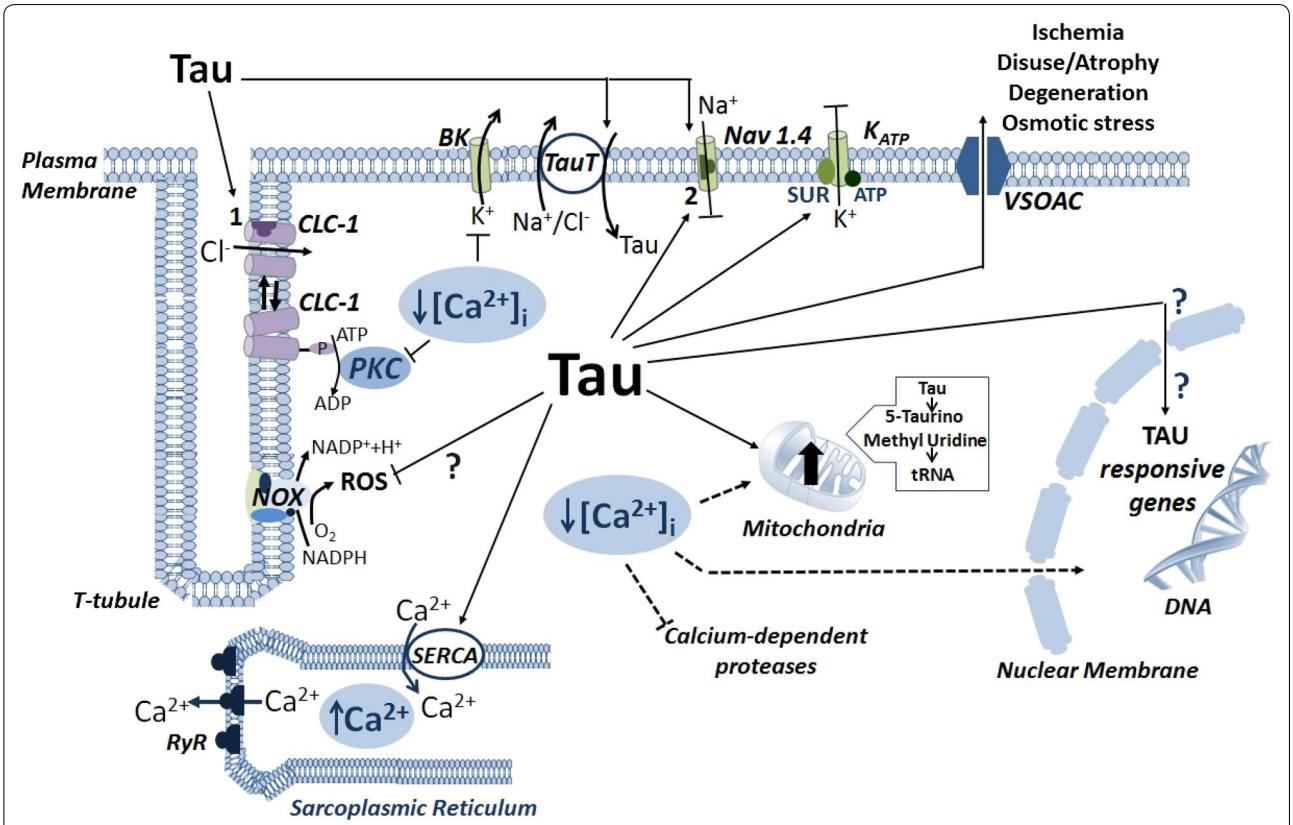

Fig. 3 Representative scheme of the action of taurine in skeletal muscle fiber. It is shown the TauT carrier which works to maintain high intracellular taurine level, along with the actions that taurine exerts on membrane channels, sarcoplasmic reticulum, mitocondria and possibly gene expression. Putative binding sites for taurine are shown (1) on ClC-1 channel and (2) as local anesthetic drug binding site. *Arrows* indicate a general stimulating action while *dotted lines* are for inhibitory effects or yet undefined pathways. A pathway for taurine efflux under stress conditions (ischemia, osmotic stress, etc.) likely via the volume-sensitive organic anion channel (VSOAC) is also shown.

low-protein diet, a condition mimicking the low weight at birth and related to the risk of developing dysmetabolic states later on [69]. In these conditions taurine protects pancreas by decreasing islet sensitivity to cytokines and shows to have an impact on gene expression and "reprogramming" in various tissues, including skeletal muscle [70–72].

In support of the pivotal role of adequate taurine level for skeletal muscle development, we demonstrated that taurine muscle level increases during the first month of rat post-natal life [73]. This increase matches the acquisition of phenotype-specific contractile properties. In particular in rat fast-twitch EDL muscle it occurs in parallel with the post-natal increase in muscle gCl and of ClC-1 channels expression; i.e. during the acquisition of the mature profile [39, 73–75]. Adult levels are likely to be attained later, since a proton nuclear magnetic resonance (H-NMR) study showed an increase in taurine in different rat skeletal muscles from 6 to 18 weeks of age [76]. Accordingly, an age dependent increase of taurine as well as of other amino acids, has been found in muscle of metabolically healthy children (age range 1–15) with respect to adults [77].

In agreement with an active role of taurine for muscle phenotype acquisition, supplementation of mothers during pregnancy and lactation as well as of new-born rats results in a higher content of the amino acid in skeletal muscle, accompanied by a more rapid development of gCl [73]. Whether such an increase is due to a modulatory action of taurine on ClC-1 channel or to an effect on its gene expression is not known yet. Importantly, a profound alteration in gene expression has been described in liver and skeletal muscle of pups that were exposed prenatally to low protein diet, while the addition of taurine to mothers via drinking water during gestation leads to a marked protection [71, 72]. Focusing on skeletal muscle, the rescuing effect of taurine did occur for genes involved in oxidative phosphorylation and in the tricarboxylic acid cycle that were markedly down-regulated in skeletal muscle by the low protein diet. Importantly, plasma taurine concentration has been suggested to be a marker of fetal well-being and a prerequisite for normal fetal development [78]. In line with the important role of taurine for skeletal muscle development, the TauT expression increases during myogenesis and its gene has consensus site for myocyte enhancing factor 2 (MEF2), being therefore under strict control of myogenic program [79]. Also, taurine has been shown to stimulate myofiber differentiation in vitro [80]. Although the mechanism through which taurine may control gene expression during development is not clear yet, it appears to be a necessary factor in myogenesis, and perhaps in mitochondrial biogenesis, with key role for tissue development (Table 1).

Another condition that may benefit from taurine supplementation is aging. Age-related sarcopenia is accompanied by profound changes in hormonal and metabolic profile of skeletal muscle. An important alteration in the content of various amino acids occurs in human muscle specimen with age, as a result of age-related increase in proteolysis; in parallel a marked decrease in taurine content has been observed [81].

Besides sarcopenia, skeletal muscle of aged rats develops features that are overlapping those observed in taurine depleted muscles, i.e. a marked decrease in gCl and a change in calcium homeostasis with a shift of mechanical threshold towards more negative potentials [82, 83]. We found by high-performance liquid chromatography (HPLC) determination that muscle taurine concentration is in fact significantly decreased in muscle of aged rats; however the levels can be restored to adult values upon the exogenous administration of taurine for 3 months (1 g/kg in drinking water) [84]. Importantly, the taurine administration counteracts the decrease in gCl and the alteration in excitation–contraction coupling of aged rat EDL muscle, supporting the key role of the amino acid in the alterations observed and the potential beneficial role of its supplementation in elderly subjects (Table 1). In the EDL muscle of aged rats supplemented with taurine an almost complete recovery of the pharmacological sensitivity of gCl to either direct and indirect channel modulators, such as the enantiomers of p-chloro-phenoxy propionic acid and the phorbol esters, respectively, was observed. The effect of these latter, along with the amelioration of mechanical threshold observed, discloses the ability of taurine to modulate gCl by reducing the phosphorylation state of the chloride channel brought about by calcium and phospholipid-dependent protein kinase C [83, 84]. This offers a unifying mechanism for physiological taurine action via calcium homeostasis and modulation of calcium-dependent signaling pathways.

In line with the above observations, TauT$^{-/-}$ mice show accelerated senescence, with greater muscular damage and endoplasmic reticulum stress due to accumulation of misfolded proteins. A central role of calcium mishandling has been proposed, along with the interest in maintaining adequate taurine level for contrasting aging-related muscle impairments [85].

Taurine and muscular dystrophy

The alteration of calcium homeostasis is a hallmark of muscles affected by inherited muscular dystrophy, such as in mice with X chromosome-linked muscular dystrophy (mdx), the most widely used model for Duchenne muscular dystrophy (DMD). It is believed that the absence of dystrophin, a protein with a key role for sarcolemmal integrity and mechano-transduction, leads to

sarcolemmal tears and to overactivity of voltage-insensitive cationic channels which enhance passive calcium entry, especially during work load [86–88]. This in turn leads to both the alteration of excitation–contraction coupling and to the activation of degenerative pathways [88, 89]. We have found that the EDL muscles of dystrophic mdx animals undergoing chronic exercise protocols, have features resembling taurine depleted ones, i.e. a reduction of gCl and a negative rheobase voltage for mechanical activation [89, 90]. Dystrophic muscle may have a reduced ability in retaining intracellular taurine; in fact we observed a trend of a lower than normal taurine muscle concentration in parallel with markedly high levels in plasma [89]. Accordingly, other authors found that taurine levels fluctuate in mdx muscles in relation to the disease phase, with compensatory increases being observed after acute degenerative period and glucocorticoid treatment [91, 92]. In this frame, taurine seems to be a useful marker of the dystrophic state of mdx mice when monitored by H1-magnetic resonance spectroscopy both in vivo and ex vivo, although technical problems may still limit the accurate peak resolution for quantitative evaluation [91–95]. In our experiments, the in vitro application of millimolar taurine concentrations fully restored the alteration of mechanical threshold observed in these animals [89]. Interestingly, similar results have been obtained upon chronic taurine treatment in exercised mdx mice. The in vivo treatment also significantly contrasted the decrease in gCl and lead to a significant increase of mouse strength in vivo, due to an interesting anabolic action of the amino acid in the dystrophic animals [90]. As previously mentioned, TauT$^{-/-}$ mice are characterized by a marked 80% decrease in exercise performance and increased fatigability, a feature that is classically observed in the mdx phenotype [6, 14, 90, 96]. The role of taurine in muscular dystrophy is also under study in Hayes' laboratory, where a lower expression of TauT in mdx mouse muscle has been demonstrated, which is not influenced by exogenous taurine administration [97], supporting the difficulty of dystrophic muscle to retain taurine. Exercise protocols may differently modulate intramuscular taurine concentration, ranging from no change to phenotype-dependent decrease, likely in relation to the exercise type; however taurine supplementation can enhance exercise performance [60, 61]. Due to the impaired mechano-transduction of dystrophic myofibers, it would be of interest to evaluate whether the exercise protocol in mdx mice can lead to a further distress in taurine concentration and in TauT expression; this is currently ongoing in our laboratory.

Based on first encouraging results, we tested the possible advantage to combine taurine with α-methylprednisolone, a glucocorticoids currently in use in dystrophic patients [58]. A synergistic action of the two drugs in enhancing mouse strength and in restoring calcium homeostasis was observed, with a normalization of mechanical threshold and a reduction of the overactivity of the cation channels likely involved in abnormal calcium entry [58, 86, 98]. The treatment was also associated with a significant increase in taurine content in fast-twitch limb muscles, suggesting that dystrophic muscle maintains the ability to uptake taurine if adequately supplemented [58]. The synergistic action observed corroborates a potential interest of taurine as adjuvant therapy in steroid-treated patients. This is also supported by the evidence that glucocorticoids exert an inhibitory action of renal taurine re-uptake, then leading to hypotaurinemia, which in turn may have long-term negative effects on cardiovascular function [5].

Importantly, the taurine treatment to mdx mice significantly reduces the high plasma level of lactate dehydrogenase, an index of metabolic distress, and it is worth to underline that a marked increase in plasma lactate actually occurs in TauT$^{-/-}$ mice [6]. Therefore taurine can also play a role in metabolism in dystrophic muscle, similarly to what observed in exercise-challenged TauT$^{-/-}$ mice [51].

Increasing evidences suggest a link between calcium homeostasis, oxidative stress and mitochondrial distress in muscular dystrophy, leading to reconcile all these taurine actions under few main mechanisms, although not fully clear yet [99, 100]. As already mentioned, taurine supplementation contrasts the exercise-induced increase in oxidative markers, without enhancing the level of endogenous anti-oxidant [55]. Other evidences support that the sulfonic amino acid is actually incapable of scavenging the common oxidants, namely, superoxide, hydrogen peroxide and hydroxyl radical, which instead are the main products of enhanced NADPH oxidase activity in dystrophic muscle [99–101]. However, the amino group of taurine can neutralize hypochlorous acid, one of the reactive species generated by myeloperoxidase-halide system in neutrophils [102]. In that reaction, taurine is converted to taurine chloramine, which is less toxic than hypochlorous acid and actually serves as a modulator of the immune system also by interfering with the production of several pro-inflammatory mediators and activation of the transcription factor nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) [102]. In addition, taurine has been proposed to directly activate peroxisome proliferator-activated receptor γ (PPARγ) in epithelial cells, a mechanism that may account for its protective action against inflammation-related diabetic retinopathy progression [103]. In consideration of the involvement of chronic inflammation and NF-kB derived mediators in dystrophic muscle [87,

104, 105], the above immunomodulatory actions of taurine are of value. However, whether the anti-inflammatory and anti-oxidant action contributes to the beneficial effect observed in dystrophic animals is not known yet and the evaluation of biomarkers in samples of taurine treated mdx mice will be useful at this regard. Our preliminary results favor a decrease in superoxide anion formation, measured by dihydroethidium staining, in tibialis anterior muscles of exercised mdx mice treated with taurine (De Luca, personal unpublished observations). An attractive hypothesis, currently under study in our laboratory, is that taurine may contrast the impaired SERCA activity in dystrophic muscle either directly or by reducing the damaging effect brought about by oxidation and/or nitrosylation [13, 54, 106]. Interesting recent results of Terrill et al. have shown that a chronic administration of the cysteine precursor 2-oxothiazolidine-4 carboxylate (OTC) markedly decreases the level of thiol oxidation in muscles of mdx mice; in parallel an amelioration of force and muscle morphology has been observed. Importantly the administration was not paralleled by an increase in cysteine or glutathione but rather by an increase in taurine level. The authors underlined that the decrease in taurine content may have a direct causative role in enhanced susceptibility to oxidative stress, disclosing a novel mechanism for beneficial effect of the classical anti-oxidant *N*-acetylcysteine [107].

Considering the mitochondrial sufferance occurring in dystrophic muscle [93], the previously described role of taurine for preserving mitochondrial function has to be taken into account for further studies. Similarly, the potential role of taurine and its chemical chaperone conjugate tauroursodeoxycholic acid in contrasting endoplasmic reticulum stress in various conditions should be considered for the acute and chronic ability of taurine to modulate signaling pathways [108, 109]. In addition, taurine may improve muscle metabolism by contrasting functional ischemia, based on the described vasodilating properties [110]. The clarification of the mechanism of action and the evaluation of long term safety and efficacy also at heart level can add important pre-clinical data to plan clinical trials in DMD patients (Table 1).

Taurine and disuse-related muscle atrophy

Muscle disuse is a general term which describes a condition of inactivity occurring after prolonged bed rest, spaceflight and/or aging. The slow-twitch muscles, devoted to postural maintenance, are the most affected ones, showing a slow-to-fast phenotype transition and severe atrophy, both leading to impaired muscle function. The adaptation of skeletal muscle to different activity includes changes in the expression of structural, metabolic and contractile proteins that fine-tune the characteristics of this tissue. The hindlimb unloaded (HU) model of disuse in rodents is a widely accepted ground-based model that mimics microgravity condition and is used to study the mechanisms responsible for the disuse-induced modification of skeletal muscle function. The soleus muscle of HU rats and mice becomes atrophic and experiences a slow-to-fast phenotype transition, characterized by an increased expression of the fast myosin heavy chain (MHC) isoform [111, 112]. Along the years, the studies on the HU model have shown that various proteins involved in the control of sarcolemma excitability, calcium ion homeostasis, energy metabolism, and contractile machinery undergo changes in the expression, turnover, and activity in accord with the entering of the slow muscle into a fast program [111, 113–117]. In particular, ClC-1 chloride and Nav1.4 sodium channels are differently expressed in fast-twitch and slow-twitch skeletal muscles, the expression of both being higher in the former. Accordingly with the change of phenotype, ClC-1 channel activity and expression as well as the intracellular resting calcium level in slow-twitch soleus muscle are significantly shifted by HU process toward the values of a fast muscle, even before the modification of MHC expression [111]. Similarly, HU increased sodium current density and sodium channel mRNA level in soleus muscle fibers [113]. All these changes alter the resistance to fatigue of antigravity muscle fibers, an effect that may contribute to the impairment of muscle function, in terms of excitability and contraction. A full understanding of the mechanisms of disuse-induced muscle alterations in humans is still incomplete and few molecules have been proposed for therapy [118, 119]. However, supplementation with essential amino acids and carbohydrates in combination with exercise attenuates muscle protein loss in humans exposed to prolonged inactivity [120, 121]. Based on these considerations and on our previous findings about the action of taurine in the modulation of calcium homeostasis and ion channel function [34, 41, 49], we focused on taurine as a potential candidate to counteract the HU-induced phenotype transition and skeletal muscle function impairment [1, 34].

In agreement with a critical role of taurine in phenotype-specific cellular function, the concentration of the amino acid is twofold higher in soleus compared to EDL muscle. The physiological relevance for this phenotypic difference is still unknown but various hypothesis can be raised based on the essential role of taurine in skeletal muscle and its actions in metabolism and phenotype-dependent properties. Interestingly, our recent findings [59] showed for the first time a marked reduction of taurine content in the soleus muscle of HU rat. This muscle loss would be consistent with an original report of National Aeronautics and Space Administration (NASA)

describing a large excretion of taurine in the urine of the astronauts of the APOLLO mission [122]. In spite of the reduction of taurine in soleus muscle of HU rats, the expression of TauT was unchanged. Indeed, TauT expression was found to be higher in slow-twitch soleus muscle with respect to the fast EDL, and was not reduced during HU, suggesting that the intracellular reduction of taurine is not associated with the change of phenotype. In addition, our data suggest that TauT activity is efficiently maintained during HU, since taurine oral supplementation fully prevents the loss of taurine content in HU-soleus muscle. Thus, we hypothesize that the reduction of intracellular taurine content during HU is likely due to increased taurine efflux. A possible explanation might be that taurine leakage compensates for intracellular osmolarity changes, which likely occurs due to muscle protein degradation and increased catabolism. Accordingly, the production of intracellular osmolytes during muscle disuse atrophy has been described, which may justify taurine escape in this condition [123–125]. Importantly in rats fed with taurine, TauT expression was reduced in soleus muscle, suggesting a negative feed-back regulation as a mechanism to control taurine intracellular level. As anticipated the TauT expression is under control of MEF2, a determinant of slow-fiber phenotype [79], thus it is tempting to speculate that TauT expression after taurine supplementation can be reduced by a mechanism involving a complex cross-talk between taurine and ClC-1 modulation during the phenotype transition.

Our findings also highlighted that taurine supplementation in HU rats has preserved resting gCl and resting cytosolic calcium level together with the slow MHC phenotype in the soleus muscle.

However, taurine had little effect on muscle atrophy, which is a severe condition occurring during HU as well as in various muscle diseases [126]. Indeed, it did not prevent the reduction of muscle-to-body weight ratio and of the fiber cross sectional area (CSA), while it partially contrasted the expression of atrogin-1 and mostly of muscle RING-finger protein-1 (MURF-1), two ubiquitin–proteasome pathway enzymes, that are strongly up-regulated as a result of HU-induced atrophy [127]. Such an effect suggests that a longer treatment or a different therapeutic schedule of taurine might have protective effect against muscle atrophy and might be useful to reach a complete muscular recovery. However complex mechanisms control the relative expression of atrogin and MURF-1 in skeletal muscle under various insults [79, 128] and further experiments are needed (Table 1).

Taurine and human skeletal muscle

Taurine has limited use in clinical settings although human use has been considered for specific diseases such as non-insulin dependent diabetes and related disorders, to treat alcohol withdrawal, congestive heart failure and arrhythmias, rheumatoid arthritis and other chronic inflammatory states, seizure disorders, and liver related disorders [19, 102, 129]. In Table 2 is a brief report of some clinical studies related to taurine supplementation, with relative dosages and outcomes. Most of them focused on diabetes mellitus, insulin resistance and diabetic complications, based on the rationale that plasma taurine concentration is reduced in patients with insulin-dependent diabetes mellitus (IDDM) [129–136]. Taurine was indicated in addition to specific drugs. Other clinical studies tested taurine in congestive heart failure, hypertension, inherited succinic semialdehyde dehydrogenase deficiency, obesity or its supplementation in aged individuals [137–143].

A part for the use in myotonic dystrophy patients [35–37], the potential therapeutic role of taurine for skeletal muscle disorders has yet to be verified in clinical settings. In fact, most of the studies about the role of taurine for skeletal muscle physiology and its potential in pathological conditions have been carried out in animal models. In these conditions taurine depletion or supplementation are directly correlated with changes in the amino acid content in skeletal muscle, which facilitate the drawing of conclusion about amino acid action and potential. However, few studies have been conducted in humans, and some contradictory reports are available, questioning about the actual usefulness of taurine supplementation or on its mechanism of action. Apart for the age-related changes reported in the previous paragraphs, one of the main issue concerns the modulation of taurine concentration in adult skeletal muscle under conditions of exercise and/or metabolic distress. Galloway et al. [144] demonstrated that taurine supplementation to exercised healthy adults leads to a marked increase in the amino acid plasma level that however is not paralleled, after 7 days of supplementation, by an increase in skeletal muscle. They proposed that intramuscular taurine concentration is tightly regulated and that high plasma level may actually work to reduce TauT activity in order to maintain constant the amino acid level. Therefore, even chronic oral taurine supplementation may cause less increase in human muscles than in rodent ones, and the observed muscle effects could be due to extracellular taurine actions. In addition, plasma levels are also tightly regulated via overexpression of TauT in kidney, which may also show specie-specific regulatory pathways [145, 146].

The dose is another important issue. In fact murine pre-clinical studies often require about tenfold higher concentration that in human trials; by the way this has to match the endogenous high level of taurine in target

Table 2 Clinical use of taurine in different pathophysiological conditions

References	Patients	Dose (g/day or mg/kg)	Duration	Result
Franconi et al. [130]	IDDM (Diabetes mellitus type 1)	1.5 g	90 days	No effect
Elizarova and Nedosugova [131]	IDDM	1 g	30 days	Glucose metabolism and trygliceride level improved
Chauncey et al. [133]	NIDDM (DM type 2)	3 g	4 months	Plasma taurine level increased
Brøns et al. [134]	Overweight non-diabetic	1.5 g	8 weeks	No effect
Xiao et al. [136]	Overweight non-diabetic	3 g	2 weeks	Insulin sensitivity improved
Nakamura et al. [132]	NIDDM with microalbuminemia	3 g	12 months	No effect
Moloney et al. [135]	IDDM	1.5 g	2 weeks	Endotelium-dependent reaction improved
Gonzales-Contreras et al. [142]	Cholestasis by parenteral nutrition	~25 mg/kg/day	~50 days	Hepatoprotection with reduction of AST, ALT and GGT
Rosa et al. [143]	Obesity	3 g/day	8 weeks	Increase in plasma levels of taurine and adiponectin; reduction of inflammatory markers
Pearl et al. [141]	Succinic semialdehyde dehydrogenase deficiency (efficacy, safety and tolerability)	50–200 mg/kg/d (age range 12 years)	13 months (mean time from 3 to 50)	No significant effects Tolerability issues at highest doses
Fujita et al. [139]	Hypertension	6 g	7 days	Systolic and diastolic pressure improved
Azuma et al. [138]	Congestive heart failure	6 g	4 weeks	Heart parameters improved
Bergamini et al. [137]	Epilepsy	200 mg–21 g	Various	Seizure frequency reduction
Durelli et al. [36]	Dystrophic myotonia	6–10 g	6 months	Myotonic symptoms improvement
Dunn-Lewis et al. [140]	Elderly	500 mg in multinurtient supplement	4 weeks	Physical function improved

organs. In addition, an accurate muscle exposure to taurine after oral ingestion requires a careful assessment of the pharmacokinetic profile that has not been extensively evaluated in humans. In line with Galloway et al. [144], a single oral dose of 4 g in healthy volunteers allows to get a maximal plasma peak in about 1.5 h and showed an half-life of 1 h with a first-order kinetic clearance; this is in line with kidney being the main organ regulating taurine level [147]. Generally the daily dose of taurine ranges between 3 and 6 g; consequently its fast kinetic can account for some of the puzzling data obtained, suggesting the need of a more careful determination of the optimum dose. It is important to underline that most of the available evidences focus on the usefulness of taurine supplementation in sustaining muscle function in trained individuals. Balshaw et al. have recently evaluated the outcome of 1 g taurine ingestion, evaluated in blind against placebo, on running performance of trained middle-distance runners. They described a modest, although significant, increase in performance in the taurine-treated group, without any change in metabolism parameters [148]. The authors claimed that a similar improvement of performance after taurine ingestion, without changes in oxygen uptake or plasma lactate, has been found in other studies [144]. Taurine muscle levels were not assessed, thus the correlation between taurine effect and a specific muscle action is rather indirect. Accordingly, they speculated about alternative potential mechanisms, such as the action of taurine at muscle membrane level, in preventing taurine drop during exercise or rather an effect on neuronal function.

In another study, a combination of taurine (2 g) and branched-chain amino acids three times a days for 2 weeks before eccentric exercise, plus 4 days after, has been tested in healthy untreated volunteers. The eccentric exercise protocol consisted of repeated sets elbow flexion at 90° to an extended position, finally leading to uncontrolled damaging stretch. The combination exerted a greater protection against muscle damage and delayed-onset muscle soreness than single administrations, although no detailed investigation has been done to clarify the mechanism of action and/or the amino acid level into the muscle [149]. Similarly, da Silva et al. have recently described the ability of 14 days taurine

administration to increase strength of the elbow flexor subjected to eccentric exercises in young adult males; in parallel, markers of oxidative stress were reduced, without increase in endogenous anti-oxidant expression nor changes in inflammatory markers. Again muscle taurine level were not determined [150]. Therefore the available evidences do not allow to conclude about the ability of supplemented taurine to actually increase its muscle level in adult healthy and trained individuals, suggesting alternative modality of action, i.e. at neuromuscular system. However, it cannot be ruled out that taurine supplementation may effectively enhances muscle taurine levels in conditions characterized by more dramatic fluctuation of its content. This applies to postnatal development and aging, and mostly to pathological conditions such as muscular dystrophy and disuse-related muscle dysfunction (Table 1) [151]. More direct evidences in humans and patients will be helpful, in order to better correlate the effect of exogenous administration of taurine with the ability of residual muscle tissue to uptake the right amount, or rather to disclosure taurine actions independent on its intracellular levels [145]. In addition, an inter-individual variation in plasma increase of taurine after supplementation may occur in relation to both nutritional state, age, drug interaction, while gene polymorphism in taurine transporter or modulation of its function and/or expression by cell metabolic state or activation of transcription factors may affect the actual level of taurine being transported into the myofibers [134, 146, 152–154]. Hence caution should be taken when concluding about lack of taurine usefulness for human muscular system without an adequate control of all variables.

Conclusion

We herein summarized the results obtained in about 30 years of research on taurine and skeletal muscle by us and other research groups. Taurine is far from themes of fashion science or from immediate interest in innovative drug development by Pharma Companies. Nevertheless the reason for such a long interest is that taurine acquired over the years a special appeal for its puzzling and multiple effects. We underlined the ability of taurine to control the function of ion channels and consequently membrane excitability as well as calcium homeostasis and excitation–contraction coupling. It has been highlighted that novel evidences are emerging regarding taurine mechanism of action, ranging from modulation of muscle metabolism to control of gene transcription, as well as in the specie-specific mechanisms underlying its intracellular levels in both chronic and acute conditions. These make the research on the topic "taurine and skeletal muscle" a continuous source of novel and exciting results allowing to renew the enthusiasm and novel working hypotheses. The wide and interconnected effects observed support a key role of the amino acid to ensure a proper muscle function and reinforce its interest as therapeutic agent in various inherited and acquired muscular disorders. The available evidences favor a greater effect of taurine in diseased condition accompanied by alterations in taurine concentration in muscle; similar benefit can occur in conditions where fluctuation in taurine level take place such as exercise, protein content in diet or post-natal development. Both acute and chronic effects of taurine supplementation are feasible, and likely occur with different time-scale although similarly interesting and important. Although a careful distinction has not been made, it is predictable that acute effects of taurine are better appreciable in situations of rapid fluctuations such as exercise, or when involving direct modulation of ion channel, or on muscles that are more dependable of external taurine such as fast-twitch ones. In parallel, chronic taurine effects, likely accompanied by changes in intracellular content, could be of value for long term control of neuromuscular function in progressive conditions, such as muscular dystrophy and disuse or aging-related dysfunction. At this regard more evidences are necessary to better understand the interest of taurine for ensuring a proper muscle function in human other than in animals. Consequently, a more clinically-oriented research will help to support the interest of taurine as novel and safer therapeutic approach of rare inherited muscle diseases and other myopathic states.

Authors' contributions

ADL: have made a substantial contribution in designing and writing the review, updating current literature and in interpretation of available data in the field; SP: was significantly involved in writing, in figures and table organizations, literature search and interpretation of available information; DCC: critically revised the manuscript and its organization and gave a substantial support to the finalization of the work. All authors have read and approved the final manuscript.

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Compliance with ethical guidelines

Competing interests

The authors declare that they have no competing interests.

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From:	Rotstein, David
To:	Jones, Jennifer L
Subject:	EON-266814; EON-266821 and EON-266827-Merrick-FW: Moisture Content: RE: Quick Review/Screening: ORA ORS Cat Food Results--Assessment of Taurine Results for Possible Product Action
Date:	Wednesday, August 24, 2016 6:35:16 AM

Here you go Jen.

David Rotstein, DVM, MPVM, Dipl. ACVP
CVM Vet-LIRN Liaison
CVM OSC/DC/ICERT
7519 Standish Place, RM 120
240-402-5613 (Office) (NEW NUMBER)
(b) (6) (BB)

From: Burkholder, William
Sent: Wednesday, July 27, 2016 4:30 PM
To: Rotstein, David; Benjamin, Linda
Cc: Krieger, Darlene; Queen, Jackie L; Hodges, April; Conway, Charlotte
Subject: RE: Moisture Content: RE: Quick Review/Screening: ORA ORS Cat Food Results--Assessment of Taurine Results for Possible Product Action

OK Everyone. The product appears to be a dry extruded product, for which the AAFCO Cat Food Nutrient Profiles content for taurine is 0.10% on a dry matter basis. Clearly all three samples were analyzed to contain more than that amount of taurine. On a dry matter basis the concentration of taurine in the samples was analyzed to be:

FACTS #	Amount Taurine Found	%Moisture	%Dry Matter	Amount Taurine on a Dry Matter Basis
958500	0.183g/100g ≈ 0.18%	2 20%	100 – 2.20 = 97.80%	0.183/0.9780 = 0 187%
958501	0.153g/100g ≈ 0.15%	1 99%	100 – 1.99 = 98.01%	0.153/0.9801 = 0 156%
958504	0.171g/100g ≈ 0.17%	2 79%	100 – 2.79 = 97.21%	0.171/0.9721 = 0 176%

All of the Dry Matter Taurine percentages are above 0.10%. IF any of the samples were canned cat food, they would not be in compliance with the AAFCO Cat Food Nutrient Profiles for the recommended minimum taurine content and IF the label indicated the product was formulated to meet the AAFCO Cat Food Nutrient Profiles the product would be misbranded.

The answer to the question of consequence/causation of the taurine content in the product from which these three samples originated to the cats in the consumer complaint is that this(ese) lot(s) of product are not indicated to be causative. However, dilated cardiomyopathy from taurine deficiency

occurs over a long period of exposure to a deficient diet (months to a year or more), so, if these cats were eating the Merrick Purrfect Bistro Grain Free Real Chicken Recipe feline dry for the 3 years indicated in the complaint, it is possible that the product was deficient for some long interval of time during that three year period and that a return to "normal" taurine levels in the diet were insufficient to correct the problem in the three cats that developed low blood taurine and the two with dilated cardiomyopathy. Treatment for dilated cardiomyopathy caused by taurine deficiency takes higher daily doses of taurine for several months than normal dietary amounts and is not completely curative.

Recommendations for regulatory steps to consider: (b) (5)

Consider recommending the owner have an ophthalmic exam performed on the cat being treated for low blood taurine to see if there are signs of retinal degeneration due to taurine deficiency.

William J. Burkholder, DVM, PhD, DACVN
Leader, Nutrition and Labeling Team I, HFV-228
Division of Animal Feeds
Center for Veterinary Medicine
United States Food and Drug Administration
7519 Standish Place
Rockville, Maryland 20855
Phone: 240-402-5900
Fax: 240-453-6882
E-mail: william.burkholder@fda.hhs.gov

From: Rotstein, David
Sent: Wednesday, July 27, 2016 2:23 PM
To: Benjamin, Linda; Burkholder, William
Cc: Krieger, Darlene; Queen, Jackie L; Hodges, April
Subject: Moisture Content: RE: Quick Review/Screening: ORA ORS Cat Food Results--Assessment of Taurine Results for Possible Product Action

Please see the moisture content below:

The moisture content for the samples are as follows:

FACTS #	Amount Taurine Found	%Moisture
958500	0.183g/100g ≈ 0.18%	2 20%
958501	0.153g/100g ≈ 0.15%	1 99%
958504	0.171g/100g ≈ 0.17%	2 79%

David Rotstein, DVM, MPVM, Dipl. ACVP
CVM Vet-LIRN Liaison
CVM OSC/DC/ICERT
7519 Standish Place, RM 120
240-402-5613 (Office) (NEW NUMBER)
(b) (6) (BB)

From: Benjamin, Linda
Sent: Wednesday, July 27, 2016 7:54 AM
To: Burkholder, William
Cc: Rotstein, David; Krieger, Darlene; Queen, Jackie L; Hodges, April
Subject: FW: Quick Review/Screening: ORA ORS Cat Food Results--Assessment of Taurine Results for Possible Product Action

Hi Bill – Could you please respond to Dave Rotstein.

Dave – As ORS's numbers are very close to the 0.2% guarantee, it might be helpful to know the AV, CV, and/or 95% confidence limit for the analytical method. Additionally, do you know if the numbers below are being reported on a dry matter basis? FYI, the sample description on the collection reports (first 3 attachments) has either "*One unopened bag of Merrick Purrfect Bistro Grain Free Real Chicken Recipe weighing 5.4kg*" or "*Opened bag of Merrick Purrfect Bistro Grain Free Real Chicken Recipe that only had 0.15kg of product. This sample was used by the consumer*" but below [my green highlight] you referenced taurine # for canned products.

Sorry Bill – I just want to make sure you have everything you need.

Thanks for the opportunity to comment,
Linda

From: Rotstein, David
Sent: Wednesday, July 27, 2016 7:22 AM
To: Benjamin, Linda
Cc: Krieger, Darlene; Queen, Jackie L; Hodges, April
Subject: Quick Review/Screening: ORA ORS Cat Food Results--Assessment of Taurine Results for Possible Product Action

Linda,

We received an email from ORS with results for taurine for a cat food. Testing was based on a consumer complaint for 3 cats with cardiac disease and low taurine.

ORS has not finalized the results, but sent on the findings for the DRY cat food and asked whether CVM considers the results to be low based on the AAFCO requirements for wet cat food.

REQUEST: To answer the following questions:

1) Is the taurine low for a dry cat food based on AAFCO nutrient profiles?
2) If the taurine is low, would it be biologically significant for cats that ate this as their sole/primary diet?

The responses will (b) (5).

REVIEWERS: Bill Burkholder, Krisztina Atkinson, Randall Lovell.

Date Needed: (b) (5)

Email from the ORS Lab:

David I hope you can help us this these findings.

We received three consumer complaint Dry Cat Food products for Amino Acid analysis. We assayed the products for the Amino Acid profile and found only Taurine low.

FACTS #	***Amount Taurine Found***
958500	***0.183g/100g ≈ 0.18%***
958501	***0.153g/100g ≈ 0.15%***
958504	***0.171g/100g ≈ 0.17%***

The label for all of the samples are the same and Taurine is declared 0.20% minimum. The AAFCO Nutrient Profile from August 2015 states that the minimum limits for Taurine is 0.20% in canned products. Do you consider these product violated?

Attachments:

Collection Reports
Pet Food Report
Vet-LIRN Summary

Medical records were collected and evaluated by Vet-LIRN. These can be provided by request.

Thank you

David Rotstein, DVM, MPVM, Dipl. ACVP
CVM Vet-LIRN Liaison
CVM OSC/DC/ICERT
7519 Standish Place, RM 120
240-402-5613 (Office) (NEW NUMBER)
(b) (6) (BB)

Food and Drug Administration Office of Regulatory Affairs

Collection Report

For Sample Number: 958500

This is an accurate reproduction of the original electronic record as of 07/27/2016

Flag | **Flag Remarks**

Episode Number	Origin	Basis	Sample Type	FIS Smpl Num	Status
	Domestic	Surveillance	Official	16260261	Completed

FEI	Date Collected	Product Code	Responsible Firm	PAC	Hours
3004211953	06/23/2016	72AYT02	Manufacturer	71R801	11

Compliance Num | **Country of Origin**: United States

Related Smpl Num	Position Class	Sampling District	NDC Number	Permit Number	Storage Rqrmnt.
	INV	NWJ-DO			Ambient

Dealer is Consumer	Crx/DEA Schedule	Recall Num	Consumer Compl. Num	Brand Name
No			146048	Merrick

Product Description
See Remarks Section.

Product Label
See continuation.

Reason for Collection
Sample collected per FACTS Assignment ID #11650647 and OP ID # 8660426 referencing Consumer Complaint #146048 reporting the illness of multiple cats from the same household. Sample testing request: Taurine.

MFG Codes
"16025DL1 38310 14131"

Expiration Date
07/26/17

Firm Legal Name	Address	Type of Firm	Firm FEI	FCE
Merrick Pet Care, Inc.	3275 Tierra Blanca Rd Hereford, TX 79045-7823 US	Manufacturer	3004211953	02944
(b) (6)	(b) (6) US	Dealer	(b) (6)	

Size of Lot	Est. Value	Rcpt Type	Carrier Name	Date Shipped
One paper bag weighing 5.4kg	$ .00	FDA484		

Description of Sample
One unopened bag of Merrick Purrfect Bistro Grain Free Real Chicken Recipe weighing 5.4kg

Method of Collection
See continuation.

How Prepared
See continuation.

Collector's Identification on Package and/or Label
"958500 06/23/2016 EB"

Collector's Identification on Seal
"958500 06/23/2016 Esteban Beltran"

Sample Delivered To
SRL-ACNA

Date Delivered	Orig C/R & Records To
06/28/2016	NWJ-DO

Lab w/Split Sample	Lab
0	SRL

Document Number	Document Date	Document Type	Document Remarks
1	06/23/2016	Other	FDA 484, Receipt for Sample, 1 page
2	06/23/2016	Other	FDA 484, Receipt for Sample Amend, 1 page
3	06/23/2016	Other	Photos of Product Labeling, 3 pages

Food and Drug Administration Office of Regulatory Affairs

Collection Report

For Sample Number: 958500

This is an accurate reproduction of the original electronic record as of 07/27/2016

Remarks
See continuation.

Payment Amount	Payment Method	704(d) Sample	702(b) Portion	Collector's Name
$27.99	Cash	No	No	Esteban Beltran

Name of Signer	Date & Time of Signature			Meaning
Esteban Beltran	07/07/2016	08:54 AM	ET	Collector

Food and Drug Administration Office of Regulatory Affairs

Collection Report

For Sample Number: 958500

This is an accurate reproduction of the original electronic record as of 07/27/2016

Continuation:

Product Label
Finished Product: Label on bag read in parts: "***Lot #: 16025DL1 38310 14131 *** Merrick Whole Health Made Right Purrfect Bistro Grain Free *** REAL CHICKEN RECIPE FISH-FREE *** Net Wt 12Lb (5.4kg) *** WHOLE HEALTH MADE RIGHT! *** SQF INSTITUE CERTIFIED *** MERRICK PET CARE, INC. P.O. BOX 9800 AMARILLO, TX 79105 USA WWW.MERRICKCARE.COM *** Best By: July 26, 2017***"

Method of Collection
On 06/23/2016, I collected a sample from the storage area of a retail store. The sample was placed in a clear plastic bag. I officially sealed the clear plastic bag containing the sample with a FDA415a on site. The sample was transported via GOV to the NBRP sample prep room.

How Prepared
On 06/28/16, I attached an FDA 525 envelope to the sample and I placed the sample into a shipping box with bubble wrap. I secured the shipping box for shipment to SRL-ACNA via UPS (Tracking #: 1Z A47 51E 01 9888 4498). I delivered the sample inside to the NBRP reception area for UPS pickup.

Remarks
Product Description: Poultry based dry cat food packed in a dark orange paper bag with brown letters and an image of a cat on the front.

An amendment to the original FDA 484 was done in order to further describe what each sample number consists of and to identify what lot number of the product pertains to the sample number. CSO Gobiga Vanniyasingam assisted during the assignment. Related samples include 958501, 958502, 958503, 958504.

Food and Drug Administration Office of Regulatory Affairs

Collection Report

For Sample Number: 958504

This is an accurate reproduction of the original electronic record as of 07/27/2016

Flag

Flag Remarks

Episode Number	Origin	Basis	Sample Type	FIS Smpl Num	Status
	Domestic	Surveillance	Official	16260366	Completed

FEI	Date Collected	Product Code	Responsible Firm	PAC	Hours
3004211953	06/28/2016	72AYT02	Manufacturer	71R801	11

Compliance Num	Country of Origin
	United States

Related Smpl Num	Position Class	Sampling District	NDC Number	Permit Number	Storage Rqrmnt.
958500	INV	NWJ-DO			Ambient

Dealer is Consumer	Crx/DEA Schedule	Recall Num	Consumer Compl. Num	Brand Name
No			146048	Merrick

Product Description
See Remarks Section.

Product Label
See continuation.

Reason for Collection
Sample collected per FACTS Assignment ID #11650647 and OP ID # 8660426 referencing Consumer Complaint #146048 reporting the illness of multiple cats from the same household. Sample testing request: Taurine.

MFG Codes
"16025DL1 38310 14131"

Expiration Date
07/26/17

Firm Legal Name	Address	Type of Firm	Firm FEI	FCE
Merrick Pet Care, Inc.	3275 Tierra Blanca Rd Hereford, TX 79045-7823 US	Manufacturer	3004211953	02944
(b) (6)	(b) (6) - (b) (6)	Dealer	(b) (6)	

Size of Lot	Est. Value	Rcpt Type	Carrier Name	Date Shipped
One paper bag weighing 0.15kg	$ .00	FDA484		

Description of Sample
Opened bag of Merrick Purrfect Bistro Grain Free Real Chicken Recipe that only had 0.15kg of product. This sample was used by the consumer.

Method of Collection
See continuation.

How Prepared
See continuation.

Collector's Identification on Package and/or Label
"958504 06/28/2016 EB"

Collector's Identification on Seal
"958504 06/28/2016 Esteban Beltran"

Sample Delivered To
SRL-ACNA

Date Delivered	Orig C/R & Records To
06/28/2016	NWJ-DO

Lab w/Split Sample	Lab
0	SRL

Document Number	Document Date	Document Type	Document Remarks
1	06/28/2016	Other	FDA 484, Receipt for Sample, 1pg.
2	06/28/2016	Other	Photos of Product Labeling, 2pgs.

Food and Drug Administration Office of Regulatory Affairs

Collection Report

For Sample Number: 958504

This is an accurate reproduction of the original electronic record as of 07/27/2016

Remarks
See continuation.

Payment Amount	Payment Method	704(d) Sample	702(b) Portion	Collector's Name
$0.00	No Charge	No	No	Esteban Beltran

Name of Signer	Date & Time of Signature			Meaning
Esteban Beltran	07/07/2016	08:57 AM	ET	Collector

Food and Drug Administration Office of Regulatory Affairs

Collection Report

For Sample Number: 958504

This is an accurate reproduction of the original electronic record as of 07/27/2016

Continuation:

Product Label
Finished Product. Label on bag read in parts: "***Lot #:16025DL3 38310 14131***Merrick Whole Health Made Right Purrfect Bistro Grain Free *** REAL CHICKEN RECIPE FISH-FREE *** Net Wt 12Lb (5.4kg) *** WHOLE HEALTH MADE RIGHT! *** SQF INSTITUE CERTIFIED *** MERRICK PET CARE, INC. P.O. BOX 9800 AMARILLO, TX 79105 USA WWW.MERRICKCARE.COM *** Best By: July 26, 2017***"

Method of Collection
On 06/28/16, I collected the used, opened bag which had been provided by the consumer to the (b) (6) (b) (6) The sample was placed in a clear plastic bag. I officially sealed the clear plastic bag containing the sample with a FDA 415a on site. The sample wastransported via GOV to the NBRP sample prep room.

How Prepared
On 06/28/16, I attached an FDA 525 envelope to the sample and I placed the sample into a shipping box with bubble wrap. I secured the shipping box for shipment to SRL-ACNA via UPS (Tracking #: 1Z A47 51E 01 9888 4498). I delivered the sample inside to the NBRP reception area for UPS pickup.

Remarks
Product Description: Poultry based dry cat food packed in a dark orange paper bag with brown letters and an image of a cat on the front.

CSO Gobiga Vanniyasingam assisted during the assignment. Related samples include 958500, 958501, 958502, 958503.

Patient History Report

Client: (b) (6)
Phone: (b) (6)
Address: (b) (6)
(b) (6)

Patient: (b) (6)
Species: Feline
Age: 12 Yrs. 5 Mos.
Color: Black

Breed: Shorthair, Domestic
Sex: Spayed Female

Date	Type	Staff	History
6/6/2016	C	(b) (6)	RECEPTION ACTIONS NOTE Sympathy card sent- (b) (6)
6/6/2016	C	(b) (6)	MEDICAL COMMENTS 6/6/2016 11:15 FDA complaint submitted: Pet Food Safety Report, ID 53897, was successfully submitted on 6/6/2016 11:15:17 AM EST to the FDA, and it was issued an Individual Case Safety Report Number (ICSR) of 1053335.
(b) (6)	C	(b) (6)	MEDICAL COMMENTS 16:26 (b) (6) AllForwardActions To: (b) (6) Sent ItemsThursday, (b) (6) 4:24 PM Hi (b) (6) Sorry for the delay in getting back with you, I needed to get permission from the owner's before providing you with their contact information. Below is the their information as well as the names of the individual cats. The cat, (b) (6) , with dilated cardiomyopathy was euthanized yesterday. Owners: (b) (6) Cats: (b) (6) : 5/9/2016 Plasma Taurine 24nmol/ml (normal 60-120, critical level <40) - test performed at University of Wisconsin, results were received on 5/15/2016, euthanized on 5/21/2016 - Whole Blood Taurine submitted at the University of California Davis on remaining 4 cats consuming this food (normal 300-600 nmol/ml, no known risk for deficiency >200), results were received on 5/27/2016 - (b) (6) : 9yr male neutered domestic long hair: 196 nmol/ml -(b) (6): 8y female spayed domestic short hair: 368 nmol/ml (b) (6) : 9yr male neutered domestic long hair: 124 nmol/ml - (b) (6) : 9yr male neutered domestic long hair: 536 nmol/ml Please let me know if you have any other questions.

B:Billing, C:Med note, CB:Call back, CK:Check-in, CM:Communications, D:Diagnosis, DH:Declined to history, E:Examination, ES:Estimates, I:Departing instr, L:Lab result, M:Image cases, P:Prescription, PA:PVL Accepted, PB:problems, PP:PVL Performed, PR:PVL Recommended, R:Correspondence, T:Images, TC:Tentative medl note, V:Vital signs

Patient History Report

Client: (b) (6)
Phone: (b) (6)
Address: (b) (6)
(b) (6)

Patient: (b) (6)
Species: Feline
Age: 12 Yrs. 5 Mos.
Color: Black

Breed: Shorthair, Domestic
Sex: Spayed Female

Date	Type	Staff	History
			Sincerely, (b) (6) (b) (6) (b) (6) Reply All Wednesday, (b) (6) 12:56 PM Thank You for providing me this information (b) (6). Could you provide us the pet parents information as well. We would like to reach out to the pet parent as well and speak with her. Thanks.
(b) (6)	C	(b) (6)	COMMUNICATIONS WITH CLIENT (b) (6) 15:55 (b) (6) - expressed my condolences. asked for permission to provide contact info to company and the FDA - owner consented. Discussed what to expect when talking to company. Owner thankful for call.
(b) (6)	R	(b) (6)	Euthanasia Notice - FINAL (b) (6) - Euthanasia Notice

(b) (6)

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Patient History Report

Client: (b) (6)
Phone: (b) (6)
Address: (b) (6)
(b) (6)

Patient: (b) (6)
Species: Feline
Age: 12 Yrs. 5 Mos.
Color: Black

Breed: Shorthair, Domestic
Sex: Spayed Female

Date	Type	Staff	History

TO: (b) (6)

FAX #: (b) (6)
FROM: (b) (6)
DATE: (b) (6)

RE:
Client: (b) (6)
Patient: (b) (6)
Breed: Shorthair, Domestic
Age: 12 Yrs. 5 Mos. Sex: Spayed Female

~ EUTHANASIA NOTIFICATION ~

Dear Dr. (b) (6):

This letter is to inform you that your patient, (b) (6) was visited by (b) (6) *At Home* house call service today for end-of-life care.

If you have any questions, please feel free to contact me at the location noted above.

Thank you,

(b) (6)

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Patient History Report

Client: (b) (6)
Phone: (b) (6)
Address: (b) (6)
(b) (6)

Patient: (b) (6)
Species: Feline
Age: 12 Yrs. 5 Mos.
Color: Black

Breed: Shorthair, Domestic
Sex: Spayed Female

Date	Type	Staff	History
(b) (6)	C	(b) (6)	MEDICAL COMMENTS - Closed Jun 04/2016 (b) (6) 18:03 Seen on emergency today, embolic event secondary to DCM. Discussed necropsy and advised that nutritionist and cardiologist agreed that prior test results were sufficient; necropsy would not reveal anything not already documented. Owner had already admin 0.2ml buprenex sublingually, requested I admin the remaining 0.3ml dispensed today which I did. They then spent time privately with the patient prior to euthanasia. Flushed cephalic catheter in right front leg; patent. Admin 20mg (2ml) expired propofol IV, apneic and unresponsive Admin 975mg (2.5ml) beuthanasia IV, 3 exhalation spasms followed Confirmed deceased by prolonged thoracic auscultation Removed IVC, placed (b) (6) in coffin, nested in owner's blanket
(b) (6)	D	(b) (6)	Pleural Effusion Final
(b) (6)	D	(b) (6)	Feline Arterial Thromboembolic Disease Final
(b) (6)	R	(b) (6)	Referral Letter - Cardio Resident Eval and labs - FINAL (b) (6) - REF fxd

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Patient History Report

Client: (b) (6)
Phone: (b) (6)
Address: (b) (6)
(b) (6)

Patient: (b) (6)
Species: Feline
Age: 12 Yrs. 5 Mos.
Color: Black

Breed: Shorthair, Domestic
Sex: Spayed Female

Date	Type	Staff	History

(b) (6)

TO: (b) (6)

FAX #: (b) (6)
FROM: (b) (6)
DATE: Wednesday, (b) (6)
RE:
Client: (b) (6)
Patient: (b) (6)
Breed: Shorthair, Domestic
Age: 12 Yrs. 5 Mos. **Sex:** Spayed Female
Current Weight: 5.3 kilograms as of 5/25/2016

Thank you for referring (b) (6). The following is a case summary.

Date of evaluation: Wednesday, (b) (6)

Date of previous cardiac evaluation: Wednesday, (b) (6)

CHIEF COMPLAINT: heavy breathing, dragging RH limb

HISTORY: (b) (6) previously doing well. Appetite had improved, eating 2/3 can max cal per day. Normal breathing. Owner noted acute onset of dragging RH limb this morning and heavy breathing. No interest in food this morning. Brought in to ER immediately. Received 9mg lasix IV total and 0.075mg buprenohpine IV on presentation.
Previous hx: Diagnosed with DCM (b) (6). Initially presented to ER for lethargy and ADR. Pleural effusion present. A renal panel performed overnight revealed azotemia (BUN 67, Creat 5.3). Prior to discharge BUN was 74, creatinine was 1.4.
Taurine level confirmed taurine deficiency

PHYSICAL EXAM: The patient was quiet, alert and responsive. No murmur on auscultation. The patient was tachypnic with moderate increased effort, RR 48. Normal BV sounds, no crackles on auscultation. Unable to bear weight on RH limb, dragging. Femoral pulses were fair in left hind, absent in left hind limb. Right paw pads cold to the touch. Heart rate was 160 bpm, regular rhythm. PCS 0/4. BCS 8/9.

(b) (6)

RADIOGRAPHS (DV, both laterals) 5/8/16: Diffusely increased opacity obscuring the cardiac silhouette. Area of moderate increased opacity in the region of the caudal segment of the left cranial lobe. Pulmonary vasculature appears wnl.

Patient History Report

Client:	(b) (6)	**Patient:**	(b) (6)		
Phone:	(b) (6)	**Species:**	Feline	**Breed:**	Shorthair, Domestic
Address:	(b) (6)	**Age:**	12 Yrs. 5 Mos.	**Sex:**	Spayed Female
	(b) (6)	**Color:**	Black		

Date	Type	Staff	History

Cursory Ultrasound: small volume pleural effusion. No pericardial effusion. Large thrombus in LV.
Brief Echo 5/25/16: moderate volume pleural effusion. No pericardial effusion. Large mass noted in left ventricle unchanged from previous.
Brief Echo 5/15/16: small volume pleural effusion. No pericardial effusion. Large mass noted in left ventricle.
ECHOCARDIOGRAM (b) (6) 2016:
IVSd: 0.37 cm LVIDd: 1.94 cm LVPWd: 0.48 cm
IVSs: 0.35 cm LVDs: 1.86 cm LVPWs: 0.48 cm %FS: 4 %
Ao: 0.8 cm LAD: 1.6 cm LA:Ao ratio 2 LA max: 1.5 cm LLAD: 1.57 cm
Comments: The left atrium is moderately enlarged. The left ventricle is enlarged in systole and diastole with poor left ventricular function. Mild right atrial and ventricular enlargement. Trivial MR and TR noted. No evidence for systolic anterior motion of the mitral valve (SAM).
Aortic and pulmonic flows were reduced. Moderate volume pleural effusion, no pericardial effusion. ECG on echo: HR 160, sinus rhythm.

DIAGNOSIS/PROBLEM LIST: Dilated Cardiomyopathy (DCM), taurine deficiency, moderate left atrial enlargement, pleural effusion, hx azotemia, LV thrombus, FATE (partial)

THORACOCENTESIS 4/10/16: a total of 180ml of yellow fluid was removed with an 18 gauge needle from the right side of the chest in right lateral recumbency. No sedation. A post-tap echo showed scant effusion remained in the patient.
THORACOCENTESIS (b) (6): a total of 120 cc of yellow tinged fluid was removed with an 18 gauge needle from the right side of the chest in right lateral recumbency. No sedation. A post-tap echo showed mild effusion remained in the patient.
ER thoracocentesis 5/8/16: 25ml yellow tinged fluid from the right side.

SUMMARY: (b) (6) clinical signs are due to a partial aortic thromboembolus and recurrent left sided congestive heart failure. Thrombus previously noted in LV is still present. There is a very guarded prognosis of recovery with ATE. Discussed with owners, if they wants to go forward with treatment recommend hospitalization to manage CHF and pain. Discussed risk of reperfusion injury, main concern hyperkalemia which can be fatal. At home care will consist primarily of pain medication and nursing care. She may or may not gain function back to hind limb, and this could take up to 1-2 months to see improvement if any. Owner elected to take home to spend a few hours to spend time and have (b) (6) come for euthanasia this evening. IVC left in place (right cephalic). Sent home with additional dose of buprenorphine to keep her comfortable until euthanasia. Owners understand she may pass on her own. Gave owners my condolences.

MEDICATIONS:
Buprenorphine 0.3mg/ml- give entire contents of syringe (0.5ml) at 3pm today for pain control

Thank you for the courtesy of this interesting referral. Please feel free to contact me

with any questions or comments.

Sincerely,

(b) (6)

(b) (6)
Sent electronically - no signature required

(b) (6)

Client ID: (b) (6) Patient ID: (b) (6) Patient Name: (b) (6)

Pain Condition Scores
Have you noticed the "PCS" aka Pain Condition Score in our medical records? As part of our AAHA specialty accreditation, we evaluate the level of pain or discomfort for each and every patient we see. You will see this reflected in the physical exam recordings as a "PCS". We follow the guidelines created and published by Colorado State University. If you have questions about interpreting pain or treatment options, or you need to schedule a pain management consult, please speak with our Anesthesiology & Pain Management Department by calling (b) (6).

Clinical Studies

Patient History Report

Client: (b) (6)
Phone: (b) (6)
Address: (b) (6)
(b) (6)

Patient: (b) (6)
Species: Feline
Age: 12 Yrs. 5 Mos.
Color: Black

Breed: Shorthair, Domestic
Sex: Spayed Female

Date	Type	Staff	History

We are recruiting for a variety of clinical studies across multiple specialties and are looking for eligible patients. For a complete list, please visit our website at and click on Veterinary Professionals and then Current Clinical Studies.

Avian & Exotics

(b) (6) is happy to re-introduce our Avian & Exotics Department and welcome (b) (6) DVM, ABVP (Avian Practice) to our team! We offer advanced diagnostics and treatment methods to complement the care provided by referring veterinarians. In addition to referral services, we provide primary care and medical boarding. Our facility maintains a dedicated exotics ward and a 24-hour monitored critical care unit. The Avian & Exotics Department accepts appointments 5 days a week (Tuesday-Saturday) and our emergency service receives patients, including exotic species, 24 hours a day. You can contact (b) (6)

(b) (6) C (b)(6) EMERGENCY PHYSICAL EXAM - Closed (b) (6)
(b) (6)

Chief Complaint: Respiratory distress

History: (b) (6) presented for STAT evaluation of respiratory distress. Owner noticed progressive tachypnea this AM and difficulty using right hindlimb. She did not want to eat this AM so she did not receive her AM medication. She is currently under the care of our Cardiology Service for Dilated Cardiomyopathy (DCM) (suspect secondary to taurine deficiency), moderate left atrial enlargement, pleural effusion, azotemia, and LV thrombus.

Other Medical Problems: None

Medications/Supplements: Pimobendan, Lasix, taurine supplementation, appetite stimulant

Environment: indoors only, several other cats

Vaccination Status: UTD

Current Diet (Type): Tempting to eat
- Frequency:

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Patient History Report

Client: (b) (6)
Phone: (b) (6)
Address: (b) (6)
(b) (6)

Patient: (b) (6)
Species: Feline
Age: 12 Yrs. 5 Mos.
Color: Black

Breed: Shorthair, Domestic
Sex: Spayed Female

Date	Type	Staff	History

- Amount:

Physical Examination:

S(ubjective): BAR/distressed, hydration WNL, BCS 7/9, pain score: 1/4

O(bjective):
Weight: 5.3 kilograms
TPR: T: 94.8 HR: 188, RR/RE: 60/rapid/shallow
EENT: clear AU/OU, no nasal discharge, normal cervical palpation, mm pink, moist/CRT<2 sec
INTEG: Hair coat ok
PLN: WNL
CV: NSR, no murmur ausculted, left femoral pulse moderate/synchronous, right very difficult to feel to absent
RESP: tachypneic, sl. dull ventrally, no crackles/wheezes
GI: soft, nonpainful, no masses
UG: FS, NSF
M/S: laterally recumbent, a
Neuro: alert/appropriate, cranial nerves intact, no placing deficits or spinal/neck pain

Problems/Differential Diagnoses:
Respiratory distress, decreased motor/absent femoral pulse RHL

Diagnostics:
None performed

Assessment:
12yo FS DSH
- absent to faint femoral pulse RHL, decreased motor, hypothermia, hx: DCM with LV thrombus- r/o saddle thrombus vs. other
- respiratory distress, mild amount pleural effusion on TFAST- r/o secondary to CHF secondary to DCM (suspect taurine deficiency)
- hx: Dilated Cardiomyopathy (DCM) (suspect secondary to taurine deficiency), moderate left atrial enlargement, pleural effusion, azotemia, and LV thrombus.

Treatment:
Placed in oxygen. IVC placed. 4mg Lasix IV, followed by additional 5mg IV. 0.015mg/kg Buprenorphine IV. Improved rr/re with above.

Plan/Recommendations:
Discussed PE at length with owner. Concerned for partial vs. full saddle thrombus RHL secondary to LV thrombus we know she has. Discussed options- point to

Patient History Report

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(b) (6)

Patient: (b) (6)
Species: Feline
Age: 12 Yrs. 5 Mos.
Color: Black

Breed: Shorthair, Domestic
Sex: Spayed Female

Date	Type	Staff	History
			severity of underlying disease- ATH, repeat Echo, supportive care, vs. euthanasia. Owner elected to continue supportive care until they could speak with (b) (6), considering euthanasia. Elected RED code, transferred to cardiology.
(b) (6)	P	(b) (6)	0.50 mg of Buprenex (Buprenorphine) 0.3mg/mL MG C3 (MOBHL0) Rx #: 2579780 0 Of 0 Refills Give the entire contents of the syringe (0.5ml) under the tongue at 3pm.
(b) (6)	C	(b)	CARDIAC EVALUTION - CLOSED 06/04/2016 - Cardiac Evaluation

Date of evaluation: Wednesday, (b) (6)

Date of previous cardiac evaluation: Wednesday, May 25, 2016

CHIEF COMPLAINT: heavy breathing, dragging RH limb

HISTORY: (b) (6) previously doing well. Appetite had improved, eating 2/3 can max cal per day. Normal breathing. Owner noted acute onset of dragging RH limb this morning and heavy breathing. No interest in food this morning. Brought in to ER immediately. Received 9mg lasix IV total and 0.075mg buprenohpine IV on presentation.
Previous hx: Diagnosed with DCM 5/9/16. Initially presented to ER for lethargy and ADR. Pleural effusion present. A renal panel performed overnight revealed azotemia (BUN 67, Creat 5.3). Prior to discharge BUN was 74, creatinine was 1.4.
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Patient History Report

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(b) (6)

Patient: (b) (6)
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Age: 12 Yrs. 5 Mos.
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Breed: Shorthair, Domestic
Sex: Spayed Female

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MEDICATIONS:
Buprenorphine 0.3mg/ml- give entire contents of syringe (0.5ml) at 3pm today for pain control

(b) (6) CK (b)(6)
STAT
Reason for Visit: Emergency
Date Patient Checked Out: (b) (6) Practice TF

(b) (6) TC (b) (6)
MEDICAL COMMENTS - TENTATIVE
(b) (6) 10:40
SW (b) (6) at Merrick - updated company that we have documented taurine deficiency in 2 other cats in house hold. The quality assurance team indicated that the level of taurine in the lot # I gave them was sufficient - discussed that this likely takes 3-6 month to develop and likely to be related to earlier lot and they need to

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Patient History Report

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(b) (6)

Patient: (b) (6)
Species: Feline
Age: 12 Yrs. 5 Mos.
Color: Black

Breed: Shorthair, Domestic
Sex: Spayed Female

Date	Type	Staff	History

investigate further. Asked if the level they gave me was from them retesting the food after I called or from an earlier test - not sure. Asked for information from the taurine tests - told I can send summary of lab results. Also indicated that I am reporting this to the FDA. She give new info to her manager and quality assurance. Told them I expect them to follow up with me. Below email sent to Merrick:

Taurine Levels
(b) (6)
To:
(b) (6)@merrickpetcare.com
Hi (b) (6)

Thank you for your help with these cases. Here is the summary of the lab results:

12yr female spayed domestic short hair diagnosed and clinical for dilated cardiomyopathy
-5/9/2016 Plasma Taurine 24nmol/ml (normal 60-120, critical level <40) - test performed at University of Wisconsin, results were received on 5/15/2016

5/21/2016 - Whole Blood Taurine submitted at the University of California Davis on remaining 4 cats consuming this food (normal 300-600 nmol/ml, no known risk for deficiency >200), results were received on 5/27/2016
-9yr male neutered domestic long hair: 196 nmol/ml
-8y female spayed domestic short hair: 368 nmol/ml
-9yr male neutered domestic long hair: 124 nmol/ml
-9yr male neutered domestic long hair: 536 nmol/ml

Please let me know if you have any other questions.

Sincerely,

(b) (6)

(b) (6)
Clinical Nutrition Department

(b) (6)

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Patient History Report

Client: (b) (6)
Phone: (b) (6)
Address: (b) (6)
(b) (6)

Patient: (b) (6)
Species: Feline
Age: 12 Yrs. 5 Mos.
Color: Black

Breed: Shorthair, Domestic
Sex: Spayed Female

Date	Type	Staff	History
(b) (6)	TC	(b)(6)	COMMUNICATIONS WITH CLIENT - TENTATIVE (b) (6) 08:42 O lmom- was previously doing well. Eating ~2/3 can of max cal per day, sRR 6-7breaths/15sec. Now, this morning, dragging RH limb and breathing heavier. SWO- recommended to come in as soon as possible since (b) (6) breathing heavy. Unfortunately, cardiology will be in surgery this morning. Should go through emergency and I will consult.
(b) (6)	B	(b)(6)	.08 mg of Buprenex (Buprenorphine) 0.3mg/mL MG C3 (MOBHL0) by (b)(6)
(b) (6)	B	(b) (6)	1.00 Specialty/Referral Exam Level 3 (REF03) by (b)(6)
(b) (6)	B	(b) (6)	Echo Guided Thoracocentesis Group (EGT) by (b)(6)
(b) (6)	B	(b) (6)	1.00 EGT Procedure (USSC50) by (b) (6)
(b) (6)	B	(b) (6)	1.00 Equipment Service & Preparation (USEQPT) by (b)(6)
(b) (6)	B	(b)(6)	1.00 Cared for by (b) (6)
(b) (6)	B	(b) (6)	.50 mg of Buprenex (Buprenorphine) 0.3mg/mL MG C3 (MOBHL0) by (b)(6)
(b) (6)	B	(b) (6)	At Home Euthanasia Group (HCEUTH) by (b) (6)
(b) (6)	B	(b) (6)	1.00 At Home Euthanasia Service (HC08) by (b)(6)
(b) (6)	B	(b) (6)	1.00 At Home Burial (HC10) by (b)(6)
(b) (6)	B	(b) (6)	100.00 mg of Telazol 100mg/mL inj per mg (C3-N) 2103 (MLTZL1) by (b)(6)
(b) (6)	B	(b) (6)	10.00 mg of Acepromazine 10mg/mL Inj per mg (MLA2L1) by (b)(6)
(b) (6)	B	(b) (6)	1.00 mg of Butorphanol 10mg/mL Inj per mg (C4) (MOB2L10) by (b)(6)
(b) (6)	B	(b) (6)	100.00 mg of Beuthanasia Soln 390 mg/mL (C-3N) / MG (MOB2L1) by (b)(6)
(b) (6)	B	(b)(6)	1.00 IV Catheter Placement (CATH) by (b)(6)
(b) (6)	B	(b)(6)	1.00 each of Tx Catheter IV 22g x 1" Surflo (BLUE) (H113) by (b)(6)
(b) (6)	B	(b)(6)	1.00 each of Tx IV Ext T Set Hospira 1265028 (H027) by (b)(6)
(b) (6)	B	(b) (6)	At Home Euthanasia Group (HCEUTH) by (b) (6)
(b) (6)	B	(b) (6)	1.00 At Home Euthanasia Service (HC08) by (b) (6)
(b) (6)	B	(b) (6)	1.00 At Home Burial (HC10) by (b) (6)
(b) (6)	B	(b) (6)	-100.00 mg of Telazol 100mg/mL inj per mg (C3-N) 2103 (MLTZL1) by (b) (6)
(b) (6)	B	(b) (6)	-10.00 mg of Acepromazine 10mg/mL Inj per mg (MLA2L1) by (b) (6)
(b) (6)	B	(b) (6)	-1.00 mg of Butorphanol 10mg/mL Inj per mg (C4) (MOB2L10) by (b) (6)
(b) (6)	B	(b) (6)	875.00 mg of Beuthanasia Soln 390 mg/mL (C-3N) / MG (MOB2L1) by (b) (6)
(b) (6)	B	(b) (6)	1.00 Cared for by (b) (6) ((b) (6)) by (b) (6)
(b) (6)	B	(b)(6)	1.00 each of Tx Injection Cap/Plug Termo 007110 (H118) by (b)(6)
(b) (6)	B	(b)(6)	Oxygen Therapy (Caged)/Hour (Group) (O2CAGE) by (b)(6)
(b) (6)	B	(b)(6)	1.00 O2 Therapy Per Hour (T044) by (b)(6)
(b) (6)	B	(b)(6)	1.00 Oxygen-related Patient Care / Hour (O2CARE) by (b)(6)
(b) (6)	B	(b)(6)	1.00 Equipment Service & Preparation (USEQPT) by (b)(6)
(b) (6)	B	(b)(6)	4.00 mg of Furosemide (Lasix) 5% Injection per mg (MMF2L5) by (b)(6)
(b) (6)	B	(b)(6)	5.00 mg of Furosemide (Lasix) 5% Injection per mg (MMF2L5) by (b)(6)

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Patient History Report

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Phone: (b) (6)
Address: (b) (6)
(b) (6)

Patient:
Species: Feline
Age: 12 Yrs. 5 Mos.
Color: Black
Breed: Shorthair, Domestic
Sex: Spayed Female

Date	Type	Staff	History
(b) (6)	B	(b)(6)	1.00 Cared for by (b) (6) (EGB) by (b) (6)
(b) (6)	B	(b)(6)	1.00 Emergency Exam Level 4 (EE04) by (b)(6)
(b) (6)	P	(b) (6)	7.00 can of Iams K9/Fel Max-Cal Plus 6oz cn 041484 (FI791) Rx #: 2578487 0 Of 6 Refills Feed up to 1 can daily.
(b) (6)	TC	(b)(6)	COMMUNICATIONS WITH CLIENT - TENTATIVE (b) (6) 13:52 (b) (6)- (b) (6) doing ok. sRR7breaths/15 sec. Ate ~3/4 can of the Iams max cal last night. Had normal BM yesterday. Hind limbs are very weak, one is worse than the other, but able to take a few steps on it before needing a rest. Does not see painful or distressed. Rec continue lasix 1/4 tab SID for now until appetite is consistent, then may consider incresasing. Continue pimo and taurine. Will put refill through for max cal. Emailed Client: I put through a prescription for 7 cans of food for (b) (6). She needs just under 1 can per day (although if she eats a whole can per day that is fine). There are also refills on the prescription if you need more. I will call to check in on her in a few days. Please call me with any concerns.
(b) (6)	B	(b) (6)	7.00 can of Iams K9/Fel Max-Cal Plus 6oz cn 041484 (FI791) by (b) (6)
(b) (6)	C	(b) (6)	PHARMACY NOTE Returned O call, left voice message that medication is ready for pick up
(b) (6)	P	(b) (6)	21.00 tablet of Pimobendan 1.5mg Tab (Cpd) (M00472) Rx #: 2569385 1 Of 12 Refills Filled by: (b) (6) Give 1 tablet by mouth twice daily with food.
(b) (6)	B	(b) (6)	21.00 tablet of Pimobendan 1.5mg Tab (Cpd) (M00472) by (b) (6)
(b) (6)	TC	(b)(6)	COMMUNICATIONS WITH CLIENT - TENTATIVE (b) (6) 10:12 (b) (6)- (b) (6) back to licking gravy, not eating a lot of solid food. Hind legs are weak. Owner not able to get sRR yet but seems comfortable. A/o to continue with current meds. If stops eating, then stop lasix. Otherwise will touch base in a few days. Gave owner my cell phone number if they need anything.

B:Billing, C:Med note, CB:Call back, CK:Check-in, CM:Communications, D:Diagnosis, DH:Declined to history, E:Examination, ES:Estimates, I:Departing instr, L:Lab result, M:Image cases, P:Prescription, PA:PVL Accepted, PB:problems, PP:PVL Performed, PR:PVL Recommended, R:Correspondence, T:Images, TC:Tentative medl note, V:Vital signs

Patient History Report

Client: (b) (6)
Phone: (b) (6)
Address: (b) (6)
(b) (6)

Patient: (b) (6)
Species: Feline
Age: 12 Yrs. 5 Mos.
Color: Black

Breed: Shorthair, Domestic
Sex: Spayed Female

Date	Type	Staff	History
5/25/2016	TC	(b)(6)	COMMUNICATIONS WITH CLIENT - TENTATIVE 5/25/2016 18:38 (b) (6)- BW wnl, kidney values have decresed to normal. A/o to give lasix 12.5mg tabs- 1/4 tab SID. Will adjust based on appetite and breathing. Continue with other meds (pimo, taurine and app stimulant). Owner thankful.
5/25/2016	D	(b) (6)	Pleural Effusion Final
5/25/2016	C	(b)(6)	CARDIAC EVALUTION - CLOSED 05/28/2016 - Cardiac Evaluation

Date of evaluation: Wednesday, May 25, 2016

Date of previous evaluation: Sunday, May 15, 2016

CHIEF COMPLAINT: heavy breathing

HISTORY: Owners noted heavy breathing yesterday. Decreased appetite yesterday and today. Prior to that her appetite was improving. Owners transitioned her to royal canin and she started eating small amounts of solid food, previously only licking gravy.
Previous hx: Diagnosed with DCM 5/9/16. Initially presented to ER for lethargy and ADR. Pleural effusion present. A renal panel performed overnight revealed azotemia (BUN 67, Creat 5.3). Prior to discharge BUN was 74, creatinine was 1.4.
Taurine level confirmed taurine deficiency

PHYSICAL EXAM: The patient was bright, alert and responsive. No murmur on auscultation. The patient was tachypnic with mild increased effort, RR 48. Normal BV sounds, no crackles on auscultation. Faint referred upper airway noise. Femoral pulses were fair and synchronous. Heart rate was 180 bpm, regular rhythm. PCS 0/4. BCS 8/9.

RADIOGRAPHS (DV, both laterals) 5/8/16: Diffusely increased opacity obscuring the cardiac silhouette. Area of moderate increased opacity in the region of the caudal segment of the left cranial lobe. Pulmonary vasculature appears wnl.

Brief Echo 5/25/16: moderate volume pleural effusion. No pericardial effusion. Large mass noted in left ventricle unchanged from previous.
Brief Echo 5/15/16: small volume pleural effusion. No pericardial effusion. Large mass noted in left ventricle.
ECHOCARDIOGRAM 5/9/2016:
IVSd: 0.37 cm LVIDd: 1.94 cm LVPWd: 0.48 cm
IVSs: 0.35 cm LVDs: 1.86 cm LVPWs: 0.48 cm %FS: 4 %
Ao: 0.8 cm LAD: 1.6 cm LA:Ao ratio 2 LA max: 1.5 cm LLAD: 1.57 cm

B:Billing, C:Med note, CB:Call back, CK:Check-in, CM:Communications, D:Diagnosis, DH:Declined to history, E:Examination, ES:Estimates, I:Departing instr, L:Lab result, M:Image cases, P:Prescription, PA:PVL Accepted, PB:problems, PP:PVL Performed, PR:PVL Recommended, R:Correspondence, T:Images, TC:Tentative medl note, V:Vital signs

Patient History Report

Client: (b) (6)
Phone: (b) (6)
Address: (b) (6)
(b) (6)

Patient: (b) (6)
Species: Feline
Age: 12 Yrs. 5 Mos.
Color: Black

Breed: Shorthair, Domestic
Sex: Spayed Female

Date	Type	Staff	History

Comments: The left atrium is moderately enlarged. The left ventricle is enlarged in systole and diastole with poor left ventricular function. Mild right atrial and ventricular enlargement. Trivial MR and TR noted. No evidence for systolic anterior motion of the mitral valve (SAM).
Aortic and pulmonic flows were reduced. Moderate volume pleural effusion, no pericardial effusion. ECG on echo: HR 160, sinus rhythm.

DIAGNOSIS/PROBLEM LIST: Dilated Cardiomyopathy (DCM), moderate left atrial enlargement, pleural effusion, azotemia, LV thrombus

THORACOCENTESIS 4/10/16: a total of 180ml of yellow fluid was removed with an 18 gauge needle from the right side of the chest in right lateral recumbency. No sedation. A post-tap echo showed scant effusion remained in the patient.
THORACOCENTESIS 5/9/16: a total of 120 cc of yellow tinged fluid was removed with an 18 gauge needle from the right side of the chest in right lateral recumbency. No sedation. A post-tap echo showed mild effusion remained in the patient.
ER thoracocentesis 5/8/16: 25ml yellow tinged fluid from the right side.

SUMMARY: (b) (6) had 180ml fluid removed from her chest today. A renal panel showed normal renal values (BUN 31, creatinine 1.3)- previous azotemia. Will start with very low dose of lasix since decreased appetite right now (decreased appetite seemed to correlate with onset of heavy breathing). If appetite improves, can consider increasing lasix dose. Continue other medications as below. Recheck in 2 weeks, sooner if concerns.

MEDICATIONS:
START:
Lasix 12.5mg tablets- give ¼ tablet by mouth once daily

CONTINUE:
Taurine 250mg by mouth twice daily
Mirtazepine 15mg tablets: Give ¼ tablet by mouth every 3 days as needed.
Pimobendan 1.5mg tiny tabs: Give 1 tablet by mouth two times a day WITH FOOD.

5/25/2016	I	(b) (6)	Cardiology Discharge Instructions (b) (6) (b) (6) (b) (6) (b) (6) (b) (6) had 160ml of fluid removed from her chest tonight. The clot in her heart appears similar to previous. I will call you with the bloodwork results and we can determine what to do with her lasix dose.

B:Billing, C:Med note, CB:Call back, CK:Check-in, CM:Communications, D:Diagnosis, DH:Declined to history, E:Examination, ES:Estimates, I:Departing instr, L:Lab result, M:Image cases, P:Prescription, PA:PVL Accepted, PB:problems, PP:PVL Performed, PR:PVL Recommended, R:Correspondence, T:Images, TC:Tentative medl note, V:Vital signs

Patient History Report

Client: (b) (6)
Phone: (b) (6)
Address: (b) (6)
(b) (6)

Patient: (b) (6)
Species: Feline
Age: 12 Yrs. 5 Mos.
Color: Black

Breed: Shorthair, Domestic
Sex: Spayed Female

Date	Type	Staff	History

Recheck in 2 weeks, sooner if concerns.

MEDICATIONS:
CONTINUE:
Taurine 250mg by mouth twice daily
Mirtazepine 15mg tablets: Give ¼ tablet by mouth every 3 days as needed.
Pimobendan 1.5mg tiny tabs: Give 1 tablet by mouth two times a day WITH FOOD.

WAIT TO BE INSTRUCTED FURTHER ON LASIX DOSE

Watch (b) (6) for the following clinical signs and call a veterinarian if you see any of these:
Initiation of or increase in cough
Excessive panting or wheezing
Restlessness, unable to get comfortable
Decreased appetite
Lethargy/weakness
Collapse or fainting

It has been a pleasure caring for (b) (6). Thank you for entrusting us with his care. If you have any further questions or problems, please don't hesitate to call.

5/25/2016 L (b) (6)

(b) (6), (b) (6)
Cardiac Panel #10 results from (b) (6) Requisition ID: 0 Posted Final

Test	Result	Reference Range
HCT =	34 %	
NA+ =	147.3 mmol/L	146.2 – 156.2
K+ =	6.65 mmol/L H	3.41 – 4.71
CL– =	115.1 mmol/L L	117.0 – 125.3
BUN =	31 mg/dL	22 – 33
CREA =	1.3 mg/dL	0.07 – 1.9

Manually entered.
PCV = 32% TS = 6.0g/dL

5/25/2016 P (b) (6)

30.00 ml of DNULsix 10mg/ml/ML (M0568)
Rx #: 2576809 0 Of 12 Refills
Give 0.5ml by mouth once daily or as directed by your veterinarian.

5/25/2016 V (b)(6)

May 25, 2016 04:26 PM Staff: (b)(6)

Weight : 5.30 kilograms
Rm. 14

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Patient History Report

Client: (b) (6)
Phone: (b) (6)
Address: (b) (6)
(b) (6)

Patient: (b) (6)
Species: Feline
Age: 12 Yrs. 5 Mos.
Color: Black

Breed: Shorthair, Domestic
Sex: Spayed Female

Date	Type	Staff	History
5/25/2016	CK	(b) (6)	breathing heavy Reason for Visit: Recheck Date Patient Checked Out: 05/25/16 Practice(b)(6)
5/25/2016	TC	(b)(6)	COMMUNICATIONS WITH CLIENT - TENTATIVE 5/25/2016 14:40 (b) (6)- (b) (6) breathing heavy today. No interest in food yesterday or today. Owner to bring in this afternoon.
5/25/2016	B	(b) (6)	1.00 Specialty/Referral Exam Level 2 (REF02) by (b) (6)
5/25/2016	B	(b) (6)	Echo Guided Thoracocentesis Group (EGT) by (b) (6)
5/25/2016	B	(b) (6)	1.00 EGT Procedure (USSC50) by (b) (6)
5/25/2016	B	(b) (6)	1.00 Equipment Service & Preparation (USEQPT) by (b) (6)
5/25/2016	B	(b) (6)	1.00 Thoracocentesis Therapeutic (R33) by (b) (6)
5/25/2016	B	(b) (6)	Laboratory Request / Sample Handling (LABS) by (b) (6)
5/25/2016	B	(b) (6)	1.00 In-house lab (XNBALIX) by (b) (6)
5/25/2016	B	(b) (6)	1.00 Sample Handling & Disposal (LFEE) by (b) (6)
5/25/2016	B	(b) (6)	1.00 Lab Sample Label (TL) by (b) (6)
5/25/2016	B	(b) (6)	1.00 Cardiac (b) (6) Panel #10 ((b) (6)) by (b) (6)
5/25/2016	B	(b) (6)	1.00 Cared for by (b) (6) (b) (6) by (b) (6)
5/25/2016	B	(b) (6)	30.00 ml of DNULsix 10mg/ml/ML (M0568) by (b) (6)
5/25/2016	B	(b) (6)	-30.00 ml of DNULsix 10mg/ml/ML (M0568) by (b)(6)
5/22/2016	TC	(b)(6)	COMMUNICATIONS WITH CLIENT - TENTATIVE 5/22/2016 16:23 (b) (6)- Appetite is still the same, but now (b) (6) will go to the food on her own instead of owners bringing it to her. Still only eating gravy, no solid food yet. Owner has not tried Max Cal, recommended trying that. Cats who go prolonged period without eating at risk for hepatic lipidosis. Personatlity wise, she is much improved, almost back to normal self. Ambulating around the house as before. Very social. Owner bought Royal Canin as new diet. Gets taurine and pimo BID now. Told owner to continue appetite stimulant for now (had stopped this). Urinating and defecating outside of the litter box, not a SE of meds, likley behavioral. Soft stools. A/o let me know if soft stools continue.
5/19/2016	C	(b) (6)	COMMUNICATIONS WITH CLIENT 5/19/2016 16:18

B:Billing, C:Med note, CB:Call back, CK:Check-in, CM:Communications, D:Diagnosis, DH:Declined to history, E:Examination, ES:Estimates, I:Departing instr, L:Lab result, M:Image cases, P:Prescription, PA:PVL Accepted, PB:problems, PP:PVL Performed, PR:PVL Recommended, R:Correspondence, T:Images, TC:Tentative medl note, V:Vital signs

Patient History Report

Client: (b) (6)
Phone: (b) (6)
Address: (b) (6)
(b) (6)

Patient: (b) (6)
Species: Feline
Age: 12 Yrs. 5 Mos.
Color: Black

Breed: Shorthair, Domestic
Sex: Spayed Female

Date	Type	Staff	History
			(b) (6) - updating that I did talk with Merrick customer service today to take my complaint and are filing it with their quality assurance. I am not sure when they will get back with me, but I will let them know as soon as I hear anything. Owner thankful for call.
5/19/2016	C	(b) (6)	MEDICAL COMMENTS ***ADDENDUM 5/19/2016 5/19/2016 11:49 Called Merrick at 1(800)664-7387 to report taurine deficiency possibly related to consumption of their product, Merrick Purrfect Bistro Grain Free Real Chicken Recipe feline dry (best by 7/26/2017, lot #16025 DL1 38310 14131 - lost # difficult to read), USB# 22808 38310). Owner has been feeding this food for approximately 3 years, 5 cats total in household, product has been purchased from the (b) (6) . Requesting that the company investigate this possible deficiency, also discussed that I would like for the other cats in the household to be tested. (b) (6) @ Merrick - said I could expect call back in 2 weeks, let her know I would like to know when to expect a call. She will submit complaint and let me know. ADDENDUM on 5/19/2016 at 15:28:19 from (b) (6) DVM, DACVN Merrick called back - additional questions of how long the cat has been sick - presented to ER on 5/8 and sick day before; also wanted to know if bag was new - yes bag was purchased about 2 weeks prior per owner. My concern however is that it takes several months for this to develop and I do not believe this is a single bag/lot issue.
5/19/2016	C	(b) (6)	COMMUNICATIONS WITH CLIENT 5/19/2016 10:10 (b) (6) - introduce3d myself, asked owner about diet history, has been feeding Merrick Purrfect Bistro Grain Free Real Chicken Recipe for approximately 3 years and purchasing from the (b) (6) Prior to this feeding Dick Van Pattons Indoor Formula Dry, chicken and salmon flavor. Discussed with owner that I will contact the company and also report to the FDA. Will let owner know of communication. In my experience sometimes the company will also want to reach out to the client. Owner thankful for call.
5/18/2016	TC	(b)(6)	COMMUNICATIONS WITH CLIENT - TENTATIVE 5/18/2016 18:16 (b) (6) - (b) (6) still not eating, had a little gravy this morning. Drank a lot of water today. Breathing is normal. Owner dropping food off tonight.

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Patient History Report

Client: (b) (6)
Phone: (b) (6)
Address: (b) (6)
(b) (6)

Patient: (b) (6)
Species: Feline
Age: 12 Yrs. 5 Mos.
Color: Black

Breed: Shorthair, Domestic
Sex: Spayed Female

Date	Type	Staff	History
5/18/2016	TC	(b)(6)	COMMUNICATIONS WITH DOCTOR - TENTATIVE 5/18/2016 18:15 lmom for (b) (6) regarding appt on Satruday with other cats in house. Would like whole blood taurine levels sent to the UC Davis amino acid lab. Call me or speak with nutrition regarding any questions.
5/17/2016	P	(b) (6)	3.00 can of Iams K9/Fel Max-Cal Plus 6oz cn 041484 (FI791) Rx #: 2573240 0 Of 3 Refills Feed as directed
5/17/2016	TC	(b) (6)	COMMUNICATIONS WITH CLIENT - TENTATIVE 5/17/2016 15:58 (b) (6)- did not eat much this morning. O gave appetite stimulant this morning and then left her alone with some food. Has not checked on her yet. Normal BM last night. sRR6brs/15sec last night. Vet coming for house call Saturday morning to take taurine sample for other cats. A/o to transition after that- recommended Hills Science Diet, Purina, Royal Canin. Will also rx Iams max cal for her to pick up here and offer (b) (6).
5/17/2016	B	(b) (6)	3.00 can of Iams K9/Fel Max-Cal Plus 6oz cn 041484 (FI791) by (b)(6)
5/16/2016	TC	(b)(6)	COMMUNICATIONS WITH CLIENT - TENTATIVE 5/16/2016 18:04 (b) (6)- (b) (6) seemed to be doing better last night. A little brighter when they got home. Started taurine supplementation last night. Not eating solid foods yet, but licking gravy- had the gravy from almost 3 cans last night. Owner put solid food in blender, but (b) (6) not interested (may have been too thick still). Drinking water. No BM, not a concern becuase she is not eating. Asked owner to bring in the food in original package as soon as possible, owner was planning on dropping off tomorrow. Also discussed to get other 4 cats tested for taurine levels tis week, as since we are changing their diet we would like to know levels on current diet. Owner will call to either have mobile vet come to house or schedule here with GP this week. Told owner I will talk to nutrition about recommended diets.
5/15/2016	R	(b) (6)	Referral Letter - Cardio Resident Eval and labs - FINAL 05/15/2016 - (b)(6)

B:Billing, C:Med note, CB:Call back, CK:Check-in, CM:Communications, D:Diagnosis, DH:Declined to history, E:Examination, ES:Estimates, I:Departing instr, L:Lab result, M:Image cases, P:Prescription, PA:PVL Accepted, PB:problems, PP:PVL Performed, PR:PVL Recommended, R:Correspondence, T:Images, TC:Tentative medl note, V:Vital signs

Patient History Report

Client: (b) (6)
Phone: (b) (6)
Address: (b) (6)
(b) (6)

Patient: (b) (6)
Species: Feline
Age: 12 Yrs. 5 Mos.
Color: Black

Breed: Shorthair, Domestic
Sex: Spayed Female

Date	Type	Staff	History

(b) (6)

TO: (b) (6)

FAX #: (b) (6)
FROM: (b) (6)
DATE: Sunday, May 15, 2016
RE:
Client: (b) (6)
Patient: (b) (6)
Breed: Shorthair, Domestic
Age: 12 Yrs. 4 Mos. **Sex:** Spayed Female
Current Weight: 5.2 kilograms as of 5/15/2016

Thank you for referring (b) (6) The following is a case summary.

Date of evaluation: Sunday, May 15, 2016

Date of previous cardiac evaluation: Monday, (b) (6)

CHIEF COMPLAINT: Recheck, not eating

HISTORY: (b) (6) has not eaten since discharge on (b) (6). Owner gave mirtazapine yesterday. Today licking some of the liquid off the food and very polydyspic, but no interest in eating any solid food. Will take a few steps and then lay down, very weak. Owner not able to get sRR, awake RR 6breaths/15sec. No heavy breathing noted.
Previous hx: Diagnosed with DCM (b) (6). Initially presented to ER for lethargy and ADR. Pleural effusion present. A renal panel performed overnight revealed azotemia (BUN 67, Creat 5.3). Prior to discharge BUN was 74, creatinine was 1.4.

PHYSICAL EXAM: The patient was quiet, alert and responsive. No murmur on auscultation. The patient was eupnic, RR 32. Normal BV sounds, no crackles on auscultation. Femoral pulses were fair and synchronous. Heart rate was 180 bpm, regular rhythm. PCS 0/4. BCS 8/9.

RADIOGRAPHS (DV, both laterals) 5/8/16: Diffusely increased opacity obscuring the cardiac silhouette. Area of moderate increased opacity in the region of the caudal segment of the left cranial lobe. Pulmonary vasculature appears wnl.

(b) (6)

Brief Echo 5/15/16: small volume pleural effusion. No pericardial effusion. Large mass noted

in left ventricle.
ECHOCARDIOGRAM (b) (6):
IVSd: 0.37 cm LVIDd: 1.94 cm LVPWd: 0.48 cm
IVSs: 0.35 cm LVDs: 1.86 cm LVPWs: 0.48 cm %FS: 4 %
Ao: 0.8 cm LAD: 1.6 cm LA:Ao ratio 2 LA max: 1.5 cm LLAD: 1.57 cm
Comments: The left atrium is moderately enlarged. The left ventricle is enlarged in systole and diastole with poor left ventricular function. Mild right atrial and ventricular enlargement. Trivial MR and TR noted. No evidence for systolic anterior motion of the mitral valve (SAM).
Aortic and pulmonic flows were reduced. Moderate volume pleural effusion, no pericardial effusion. ECG on echo: HR 160, sinus rhythm.

DIAGNOSIS/PROBLEM LIST: Dilated Cardiomyopathy (DCM), moderate left atrial enlargement, pleural effusion, azotemia, LV thrombus

THORACOCENTESIS 5/9/16: a total of 120 cc of yellow tinged fluid was removed with an 18 gauge needle from the right side of the chest in right lateral recumbency. No sedation. A post-tap echo showed mild effusion remained in the patient.
ER thoracocentesis 5/8/16: 25ml yellow tinged fluid from the right side.

SUMMARY: The small amount of pleural effusion was not enough to warrant thoracocentesis, especially since patient eupnic. (b) (6) taurine level came back low (24nmol/ml). Recommended supplementing taurine at below dose. Discussed with owner it can take up to 2-3 weeks to see an effect of this and even longer (3 months) to see changes on echocardiogram. There is a chance (b) (6) will succumb to congestive heart failure before we see the positive effects of the taurine. A renal panel revealed azotemia. Discussed with owner the challenge going forward of managing the heart failure with azotemia. If (b) (6) develops trouble breathing, may not have options to treat. Euthanasia may be the most humane option for (b) (6) at that time.
The echocardiogram showed a large mass in the left ventricle, consistent with a thrombus. There is a risk that this clot, or a piece of it, leaves the heart and causes a thromboembolic event. The most common place for thromboembolic disease in cats is the aortic bifurcation, but can occur anywhere including the lungs which can be fatal. Advised owners, if FATE suspected at home, bring (b) (6) to closest emergency facility. At that time, euthanasia is recommended. We discussed holding off on an asprin or Plavix for now, as it will not affect the current clot, and (b) (6) is not yet eating.
Owners willing to start taurine supplementation and take it day by day. Owners understand the possible outcomes we discussed today. I will call to check in tomorrow.

MEDICATIONS:
START:
Taurine 250mg by mouth twice daily

CONTINUE:
Mirtazepine 15mg tablets: Give ¼ tablet by mouth every 3 days as needed.

HOLD FOR NOW:
Furosemide (Salix, Lasix) 12.5 mg tablets: Give 1/4 tablet once daily.
Pimobendan 1.5mg tiny tabs: Give 1 tablet by mouth two times a day WITH FOOD.

Thank you for the courtesy of this interesting referral. Please feel free to contact me with any questions or comments.

Sincerely,

(b) (6) (Cardiology Resident)

(b) (6), BVSc, MRCVS, ACVIM (Cardiology)
Sent electronically - no signature required

(b) (6)

Client ID: (b) (6) Patient ID: (b) (6) Patient Name (b) (6)

DATE/TIME	TEST	RESULT	REFERENCE RANGE
5/15/2016	BUN	= 61 mg/dL (H)	22 - 33
5/15/2016	CL-	= 104.5 mmol/L (L)	117.0 - 125.3
5/15/2016	CREA	= 3.1 mg/dL (H)	0.07 - 1.9
5/15/2016	HCT	= 43 %	
5/15/2016	K+	= 3.33 mmol/L (L)	3.41 - 4.71
5/15/2016	NA+	= 145.3 mmol/L (L)	146.2 - 156.2

Patient History Report

Client:	(b) (6)	**Patient:**	(b) (6)		
Phone:	(b) (6)	**Species:**	Feline	**Breed:**	Shorthair, Domestic
Address:	(b) (6)	**Age:**	12 Yrs. 5 Mos.	**Sex:**	Spayed Female
	(b) (6)	**Color:**	Black		

Date	Type	Staff	History

Lab Comments: Manually entered.

Additional Comments: BUN/Cre=19.7mg/mg PCV=39% TS=7.8g/dL Normal

Pain Condition Scores

Have you noticed the "PCS" aka Pain Condition Score in our medical records? As part of our AAHA specialty accreditation, we evaluate the level of pain or discomfort for each and every patient we see. You will see this reflected in the physical exam recordings as a "PCS". We follow the guidelines created and published by Colorado State University. If you have questions about interpreting pain or treatment options, or you need to schedule a pain management consult, please speak with our Anesthesiology & Pain Management Department by calling (b) (6).

Clinical Studies

We are recruiting for a variety of clinical studies across multiple specialties and are looking for eligible patients. For a complete list, please visit our website at and click on Veterinary Professionals and then Current Clinical Studies.

Avian & Exotics

(b) (6) is happy to re-introduce our Avian & Exotics Department and welcome (b) (6), ABVP (Avian Practice) to our team! We offer advanced diagnostics and treatment methods to complement the care provided by referring veterinarians. In addition to referral services, we provide primary care and medical boarding. Our facility maintains a dedicated exotics ward and a 24-hour monitored critical care unit. The Avian & Exotics Department accepts appointments 5 days a week (Tuesday-Saturday) and our emergency service receives patients, including exotic species, 24 hours a day. You can contact (b) (6)

5/15/2016	TC	(b)(6)	COMMUNICATIONS WITH CLIENT - TENTATIVE 5/15/2016 16:34 (b) (6)- discussed azotemia. Since (b) (6) is eupnic, would hold off on lasix for now. Hope would be that she may be able to breathe comfortably without lasix for enough time that taurine may start to help. Otherwise may give low dose of lasix, but going to be a big challange with azotemia. Owners are to start taurine tonight. Discussed case with nutrition. Will file a complaint about the food. Will have more

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Patient History Report

Client: (b) (6)
Phone: (b) (6)
Address: (b) (6)
(b) (6)

Patient: (b) (6)
Species: Feline
Age: 12 Yrs. 5 Mos.
Color: Black

Breed: Shorthair, Domestic
Sex: Spayed Female

Date	Type	Staff	History
			information on this tomorrow. Will call to check in tomorrow.
5/15/2016	D	(b)(6)	Taurine Deficiency Final
5/15/2016	D	(b)(6)	Azotemia Tentative
5/15/2016	D	(b) (6)	Pleural Effusion Final
5/15/2016	C	(b)(6)	CARDIAC EVALUTION - CLOSED 05/18/2016 - Cardiac Evaluation

Date of evaluation: Sunday, May 15, 2016

Date of previous cardiac evaluation: Monday, (b) (6)

CHIEF COMPLAINT: Recheck, not eating

HISTORY: (b) (6) has not eaten since discharge on (b) (6) Owner gave mirtazapine yesterday. Today licking some of the liquid off the food and very polydyspic, but no interest in eating any solid food. Will take a few steps and then lay down, very weak. Owner not able to get sRR, awake RR 6breaths/15sec. No heavy breathing noted.
Previous hx: Diagnosed with DCM (b) (6). Initially presented to ER for lethargy and ADR. Pleural effusion present. A renal panel performed overnight revealed azotemia (BUN 67, Creat 5.3). Prior to discharge BUN was 74, creatinine was 1.4.

PHYSICAL EXAM: The patient was quiet, alert and responsive. No murmur on auscultation. The patient was eupnic, RR 32. Normal BV sounds, no crackles on auscultation. Femoral pulses were fair and synchronous. Heart rate was 180 bpm, regular rhythm. PCS 0/4. BCS 8/9.

RADIOGRAPHS (DV, both laterals) 5/8/16: Diffusely increased opacity obscuring the cardiac silhouette. Area of moderate increased opacity in the region of the caudal segment of the left cranial lobe. Pulmonary vasculature appears wnl.

Brief Echo 5/15/16: small volume pleural effusion. No pericardial effusion. Large mass noted in left ventricle.
ECHOCARDIOGRAM 5/9/2016:
IVSd: 0.37 cm LVIDd: 1.94 cm LVPWd: 0.48 cm
IVSs: 0.35 cm LVDs: 1.86 cm LVPWs: 0.48 cm %FS: 4 %
Ao: 0.8 cm LAD: 1.6 cm LA:Ao ratio 2 LA max: 1.5 cm LLAD: 1.57 cm
Comments: The left atrium is moderately enlarged. The left ventricle is enlarged in systole and diastole with poor left ventricular function. Mild right atrial and ventricular enlargement. Trivial MR and TR noted. No evidence for systolic anterior motion of the mitral valve ((b)(6)).
Aortic and pulmonic flows were reduced. Moderate volume pleural effusion, no pericardial effusion. ECG on echo: HR 160, sinus rhythm.

Patient History Report

Client:	(b) (6)	**Patient:**	(b) (6)		
Phone:	(b) (6)	**Species:**	Feline	**Breed:**	Shorthair, Domestic
Address:	(b) (6)	**Age:**	12 Yrs. 5 Mos.	**Sex:**	Spayed Female
	(b) (6)	**Color:**	Black		

Date	Type	Staff	History

DIAGNOSIS/PROBLEM LIST: Dilated Cardiomyopathy (DCM), moderate left atrial enlargement, pleural effusion, azotemia, LV thrombus

THORACOCENTESIS 5/9/16: a total of 120 cc of yellow tinged fluid was removed with an 18 gauge needle from the right side of the chest in right lateral recumbency. No sedation. A post-tap echo showed mild effusion remained in the patient.
ER thoracocentesis 5/8/16: 25ml yellow tinged fluid from the right side.

SUMMARY: The small amount of pleural effusion was not enough to warrant thoracocentesis, especially since patient eupnic. (b) (6) taurine level came back low (24nmol/ml). Recommended supplementing taurine at below dose. Discussed with owner it can take up to 2-3 weeks to see an effect of this and even longer (3 months) to see changes on echocardiogram. There is a chance (b) (6) will succumb to congestive heart failure before we see the positive effects of the taurine. A renal panel revealed azotemia. Discussed with owner the challenge going forward of managing the heart failure with azotemia. If (b) (6) develops trouble breathing, may not have options to treat. Euthanasia may be the most humane option for (b) (6) at that time.
The echocardiogram showed a large mass in the left ventricle, consistent with a thrombus. There is a risk that this clot, or a piece of it, leaves the heart and causes a thromboembolic event. The most common place for thromboembolic disease in cats is the aortic bifurcation, but can occur anywhere including the lungs which can be fatal. Advised owners, if FATE suspected at home, bring (b) (6) to closest emergency facility. At that time, euthanasia is recommended. We discussed holding off on an asprin or Plavix for now, as it will not affect the current clot, and (b) (6) is not yet eating.
Owners willing to start taurine supplementation and take it day by day. Owners understand the possible outcomes we discussed today. I will call to check in tomorrow.

MEDICATIONS:
START:
Taurine 250mg by mouth twice daily

CONTINUE:
Mirtazepine 15mg tablets: Give ¼ tablet by mouth every 3 days as needed.

HOLD FOR NOW:
Furosemide (Salix, Lasix) 12.5 mg tablets: Give 1/4 tablet once daily.
Pimobendan 1.5mg tiny tabs: Give 1 tablet by mouth two times a day WITH FOOD.

5/15/2016 I (b) (6) Cardiology Discharge Instructions
(b) (6)
(b) (6)
(b) (6)
(b) (6)

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Patient History Report

Client: (b) (6)
Phone: (b) (6)
Address: (b) (6)
(b) (6)

Patient: (b) (6)
Species: Feline
Age: 12 Yrs. 5 Mos.
Color: Black

Breed: Shorthair, Domestic
Sex: Spayed Female

Date	Type	Staff	History

(b) (6) has a small amount of fluid in her chest today. It was not enough to warrant draining today.

Her taurine levels came back low. Please start supplementing taurine as below. It can take up to 2-3 weeks to see an effect of this.

The echocardiogram showed a large mass in one of the chambers of her heart (the left ventricle). There is a risk that this clot, or a piece of it, leaves the heart. If that happens, it can travel to any part of the body (lungs, hind legs, etc) and this can be fatal. We discussed holding off on an asprin or Plavix medication for now, as it will not do anything for the current clot, and (b) (6) is not yet eating. I will call you with her bloodwork results this afternoon.

MEDICATIONS:
START:
Taurine 250mg by mouth twice daily

Watch (b) (6) for the following clinical signs and call a veterinarian if you see any of these:
Initiation of or increase in cough
Excessive panting or wheezing
Restlessness, unable to get comfortable
Decreased appetite
Lethargy/weakness
Collapse or fainting

It has been a pleasure caring for (b) (6). Thank you for entrusting us with his care. If you have any further questions or problems, please don't hesitate to call.

5/15/2016 L (b) (6)

(b) (6), (b) (6)

Cardiac Panel #10 results from (b) (6) In-Clinic Requisition ID: 0 Posted Final

Test	Result	Reference Range
HCT =	43 %	
NA+ =	145.3 mmol/L L	146.2 - 156.2
K+ =	3.33 mmol/L L	3.41 - 4.71
CL- =	104.5 mmol/L L	117.0 - 125.3
BUN =	61 mg/dL H	22 - 33
CREA =	3.1 mg/dL H	0.07 - 1.9

Manually entered.
BUN/Cre=19.7mg/mg PCV=39% TS=7.8g/dL Normal

5/15/2016 V (b)(6) May 15, 2016 03:24 PM Staff: (b)(6)

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Patient History Report

Client: (b) (6)
Phone: (b) (6)
Address: (b) (6)
(b) (6)

Patient: (b) (6)
Species: Feline
Age: 12 Yrs. 5 Mos.
Color: Black

Breed: Shorthair, Domestic
Sex: Spayed Female

Date	Type	Staff	History
			------------------------------ Weight : 5.20 kilograms cardio baby scale
5/15/2016	CK	(b)(6)	Reason for Visit: Recheck Date Patient Checked Out: (b) (6) Practice (b)(6)
5/15/2016	TC	(b)(6)	COMMUNICATIONS WITH CLIENT - TENTATIVE (b) (6) 13:29 (b) (6) (mrs)- owner gave mirtazapine, no improvement in appetite. Drinking excesivly. Having a hard time walking, very weak. Owner not able to get sRR, awake breathing 6breaths/15sec. Offered to see (b) (6) today. Made appt for 3pm
5/15/2016	B	(b) (6)	1.00 Specialty/Referral Exam Level 2 (REF02) by (b) (6)
5/15/2016	B	(b) (6)	Laboratory Request / Sample Handling (LABS) by (b) (6)
5/15/2016	B	(b) (6)	1.00 In-house lab (XNBALIX) by (b) (6)
5/15/2016	B	(b) (6)	1.00 Sample Handling & Disposal (LFEE) by (b) (6)
5/15/2016	B	(b) (6)	1.00 Lab Sample Label (TL) by (b) (6)
5/15/2016	B	(b) (6)	1.00 Cardiac (b) (6) Panel #10 ((b) (6)) by (b) (6)
5/15/2016	B	(b) (6)	Echo Guided Thoracocentesis Group (EGT) by (b) (6)
5/15/2016	B	(b) (6)	1.00 EGT Procedure (USSC50) by (b) (6)
5/15/2016	B	(b) (6)	1.00 Equipment Service & Preparation (USEQPT) by (b) (6)
5/15/2016	B	(b) (6)	1.00 Cared for by (b) (6) by (b) (6)
5/14/2016	TC	(b) (6)	COMMUNICATIONS WITH CLIENT - TENTATIVE 5/14/2016 17:42 Mrs called and lmovm that (b) (6) hasn't been eating well. I called back and sw Mr. He said she is eating only very tiny amounts and not improving, wanted to know if I had suggestions. Owners feel breathing is still ok, 6 breaths/15 seconds but coughed a little today. I told Mr she could have poor app due to fluid reforming or azotemia or her heart disease in general. She may need to be rechecked sooner than later to evaluate this and r/o fluid and worsening azo. Owners plan to discuss w/ (b)(6) but wanted to know if there is something they could give her before morning. I offered to prescribe appetite stimulant, explained that this may not work b/c it doesn't override what is causing the inappetance in the 1st place but it's fine to try. Mr was thankful, said he may or may not pick it up tonight but is glad to have the option.
5/14/2016	P	(b) (6)	2.00 tablet of Mirtazapine 15mg Tablet (M1052)

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Patient History Report

Client: (b) (6)
Phone: (b) (6)
Address: (b) (6)
(b) (6)

Patient: (b) (6)
Species: Feline
Age: 12 Yrs. 5 Mos.
Color: Black

Breed: Shorthair, Domestic
Sex: Spayed Female

Date	Type	Staff	History
5/14/2016	B	(b) (6)	Rx #: 2571986 0 Of 3 Refills Give 1/4 tablet once every 3 days as needed to stimulate appetite. 2.00 tablet of Mirtazapine 15mg Tablet (M1052) by (b) (6)
5/12/2016	C	(b)(6)	TRIAGE CALL 5/12/2016 21:23 Per owner, (b) (6) appetite has been decreasing over the last couple days. Yesterday only ate about 2 tablespoons, today less. Let owner know that if the appetite has been decreasing recommend a recheck. Owner wants to talk to cardio first to see about an appetite stimulant. She will call tomorrow to speak with cardio department.
5/11/2016	TC	(b)(6)	COMMUNICATIONS WITH CLIENT - TENTATIVE 5/11/2016 13:16 (b) (6) (mrs)- still weak and unstable, but up and walking around short distances. sRR was 5breaths/15sec this morning. Ate 2 teaspoons canned food last night, so owner gave 1/4 tab lasix. Has not eaten yet this morning. Advised owner since sRR wnl, hold off on lasix for now. Will restart when either (b) (6) has a good appetite or if sRR >8br/15esc. Owner understands. Also hold off on pimo and taurine supplement. Should have taurine level back by recheck in 2 weeks. Owner asked about starting asprin. Can consider asprin/plavix at rehceck if appetite is good. Discussed that they may lower risk, but do not prevent risk of clot formation. Will call to check on appetite in a few days. Owner to call sooner with concerns.
5/10/2016	TC	(b)(6)	COMMUNICATIONS WITH CLIENT - TENTATIVE 5/10/2016 10:13 (b) (6) (mrs)- (b) (6) was drinking a lot last night. Has not eaten anything yet. sRR 7breaths/15sec. Has gotten up and walked around, otherwise sleeping. Advised owner to hold off on meds today, would like her eating before restarting them. Will call tomorrow to check on appetite and advised what to do with lasix.
5/10/2016	L	(b) (6)	**Miscellaneous results from (b) (6)** **(East) Requisition ID: 189206 Posted Final** **Ascn: (b) (6) Profile: Taurine** **RE: 16758 Sample: PLASMA,HEPARIN** **RE: 16759 Taurine 24 NMOL/ML nmol/ml** **Feline taurine ranges: normal plasma 60-120 nmol/mL critical level <40** **nmol/mL; whole blood normal 300-600 nmol/mL**

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Patient History Report

Client: (b) (6)
Phone: (b) (6)
Address: (b) (6)
(b) (6)

Patient: (b) (6)
Species: Feline
Age: 12 Yrs. 5 Mos.
Color: Black

Breed: Shorthair, Domestic
Sex: Spayed Female

Date	Type	Staff	History
			critical level <200 nmol/mL. **TEST PERFORMED AT THE UNIVERSITY OF WISCONSIN** **FELINE PLASMA NORMALS: 60–120 nmol/ml WHOLE BLOOD NORMALS: 300–600 nmol/ml** **PLASMA CRITICAL: LESS THAN 40 nmol/ml WHOLE BLOOD CRITICAL: LESS THAN 200** **nmol/ml** **K9 PLASMA NORMALS: 60–120 nmol/ml WHOLE BLOOD NORMALS: 200–350 nmol/ml PLASMA** **CRITICAL: LESS THAN 40 nmol/ml WHOLE BLOOD CRITICAL: LESS THAN 150 nmol/ml**
(b) (6)	R	(b)(6)	Referral Letter - Cardio Resident Eval and labs - FINAL (b) (6) - (b)(6)

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Patient History Report

Client: (b) (6)
Phone: (b) (6)
Address: (b) (6)
(b) (6)

Patient: (b) (6)
Species: Feline
Age: 12 Yrs. 5 Mos.
Color: Black

Breed: Shorthair, Domestic
Sex: Spayed Female

Date	Type	Staff	History

(b) (6)

TO: (b) (6)

FAX #: (b) (6)
FROM: (b) (6)
DATE: Monday, May 09, 2016
RE:
Client: (b) (6)
Patient: (b) (6)
Breed: Shorthair, Domestic
Age: 12 Yrs. 4 Mos. **Sex:** Spayed Female
Current Weight: 15.6 pounds as of 12/28/2009

Thank you for referring (b) (6). The following is a case summary.

Date of evaluation: Monday, (b) (6)

CHIEF COMPLAINT: pleural effusion

HISTORY: Presented to ER last night for lethargy and ADR. Cursory ultrasound revealed pleural effusion. Thoracocentesis yielded 25ml yellow tinged fluid from the right side. Patient received 12mg lasix IV and was placed in oxygen overnight. Her RR was wnl, with slight effort noted overnight. She ate a small amount. A renal panel performed overnight revealed azotemia (BUN 67, Creat 5.3).

PHYSICAL EXAM: The patient was bright, alert and responsive. No murmur on auscultation, but heart sound slightly muffled. The patient was eupnic, RR 30 with slight effort. Normal BV sounds, no crackles on auscultation. Pulses were fair and synchronous. Heart rate was 170 bpm, regular rhythm. PCS 0/4. BCS 8/9.

RADIOGRAPHS (DV, both laterals) 5/8/16: Diffusely increased opacity obscuring the cardiac silhouette. Area of moderate increased opacity in the region of the caudal segment of the left cranial lobe. Pulmonary vasculature appears wnl.

Comments: The left atrium is moderately enlarged. The left ventricle is enlarged in systole and diastole with poor left ventricular function. Mild right atrial and ventricular enlargement. Trivial MR and TR noted. No evidence for systolic anterior motion of the mitral valve (SAM). Aortic and pulmonic flows were reduced. Moderate volume pleural effusion, no pericardial

(b) (6)

effusion. ECG on echo: HR 160, sinus rhythm.

DIAGNOSIS/PROBLEM LIST: Dilated Cardiomyopathy (DCM), moderate left atrial enlargement, pleural effusion, azotemia

THORACOCENTESIS 5/9/16: a total of 120 cc of yellow tinged fluid was removed with an 18 gauge needle from the right side of the chest in right lateral recumbency. No sedation. A post-tap echo showed mild effusion remained in the patient.

SUMMARY: (b) (6) has dilated cardiomyopathy (DCM) and has developed pleural effusion due to congestive heart failure. The pleural effusion was removed today and we are starting the medications as described below. Although taurine deficiency is a rare cause for dilated cardiomyopathy in cats, we submitted a taurine level to the lab today to look for this. The test results can take up to 2 weeks; we will fax results when they are available. Unfortunately the prognosis for DCM (with no taurine deficiency) in cats is poor, with survival time 3-6 weeks or in rare cases 4-6 months. A renal panel was recheck today and showed elevated BUN (74) and normal creatinine (1.4). Discussed with owner the azotemia may make treating the congestive heart failure challenging.
We discussed that with heart enlargement there is a risk for developing a blood clot, or stroke. Although aspirin +/- plavix can be given in hopes of reducing clot formation, they have not been definitively proven to prevent blood clot formation in cats. If the owners elect to start this medication I would recommend waiting until we know she is eating and feeling well on the below medications as aspirin/plavix can cause GI side effects (vomiting, inappetance) in some cats. A recheck with cardiology is recommended in 2 weeks, or sooner if clinical signs.
Advised owner congestive heart failure likely contributing to lethargy, but concern that weakness may be secondary to the DCM and low cardiac output. If that is the case, may not improve once we get her out of failure. Owner comfortable taking (b) (6) home to see how she does. (b) (6) was discharged on the below medications. If she does not improve at home over the next few days, owner to consider euthanasia.

MEDICATIONS:
START: Furosemide (Salix, Lasix) 12.5 mg tablets: Give 1/4 tablet once daily.

START in 3 DAYS IF EATING
Pimobendan 1.5mg tiny tabs: Give 1 tablet by mouth two times a day WITH FOOD.
If eating and easy to medicate, start: Taurine 250 mg by mouth twice a day with food.

Thank you for the courtesy of this interesting referral. Please feel free to contact me

with any questions or comments.

Sincerely,

(b) (6)

Client ID: (b) (6) Patient ID: (b) (6) Patient Name: (b) (6)

DATE/TIME	TEST	RESULT	REFERENCE RANGE
(b) (6)	**CREA**	**= 1.4 mg/dL**	**0.8 - 2.4**

Lab Comments: CREA: Test results for the latest analyzer run have been multiplied by the dilution factor for a dilution of 1 in 4 total.

DATE/TIME	TEST	RESULT	REFERENCE RANGE
(b) (6)	**ALB**	**= 2.8 g/dL**	**2.3 - 3.9**
(b) (6)	**ALB/GLOB**	**= 0.8**	
(b) (6)	**ALKP**	**= 11 U/L (L)**	**14 - 111**
(b) (6)	**ALT**	**= 140 U/L (H)**	**12 - 130**
(b) (6)	**BUN/UREA**	**= 74 mg/dL (H)**	**16 - 36**
(b) (6)	**Chloride**	**= 100 mmol/L (L)**	**112 - 129**
(b) (6)	**CREA**	**- --.-- mg/dL**	**0.8 - 2.4**
(b) (6)	**GLOB**	**= 3.3 g/dL**	**2.8 - 5.1**
(b) (6)	**GLU**	**= 105 mg/dL**	**71 - 159**
(b) (6)	**Na/K**	**= 29**	
(b) (6)	**OSM calc**	**= 298 mmol/kg**	
(b) (6)	**PHOS**	**= 7.0 mg/dL**	**3.1 - 7.5**
(b) (6)	**Potassium**	**= 4.7 mmol/L**	**3.5 - 5.8**
(b) (6)	**Sodium**	**= 138 mmol/L (L)**	**150 - 165**

Patient History Report

Client: (b) (6)
Phone: (b) (6)
Address: (b) (6)
(b) (6)

Patient: (b) (6)
Species: Feline
Age: 12 Yrs. 5 Mos.
Color: Black

Breed: Shorthair, Domestic
Sex: Spayed Female

Date	Type	Staff	History

DATE/TIME	TEST	RESULT	REFERENCE RANGE
(b) (6)	TP	= 6.1 g/dL	5.7 - 8.9

Pain Condition Scores
Have you noticed the "PCS" aka Pain Condition Score in our medical records? As part of our AAHA specialty accreditation, we evaluate the level of pain or discomfort for each and every patient we see. You will see this reflected in the physical exam recordings as a "PCS". We follow the guidelines created and published by Colorado State University. If you have questions about interpreting pain or treatment options, or you need to schedule a pain management consult, please speak with our Anesthesiology & Pain Management Department by calling (b) (6)

Clinical Studies
We are recruiting for a variety of clinical studies across multiple specialties and are looking for eligible patients. For a complete list, please visit our website at and click on Veterinary Professionals and then Current Clinical Studies.

Avian & Exotics
(b) (6) is happy to re-introduce our Avian & Exotics Department and welcome (b) (6), DVM, ABVP (Avian Practice) to our team! We offer advanced diagnostics and treatment methods to complement the care provided by referring veterinarians. In addition to referral services, we provide primary care and medical boarding. Our facility maintains a dedicated exotics ward and a 24-hour monitored critical care unit. The Avian & Exotics Department accepts appointments 5 days a week (Tuesday-Saturday) and our emergency service receives patients, including exotic species, 24 hours a day. You can contact (b) (6)

(b) (6) I (b) (6) Cardiology Discharge Instructions
(b) (6)

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(b) (6)

Patient: (b) (6)
Species: Feline
Age: 12 Yrs. 5 Mos.
Color: Black

Breed: Shorthair, Domestic
Sex: Spayed Female

Date	Type	Staff	History

A cardiologist has evaluated (b) (6) and has diagnosed her with Dilated Cardiomyopathy (DCM). DCM means she has poor muscle contraction of the heart and she has developed significant heart enlargement over time. Her clinical signs were due to congestive heart failure (fluid buildup around the lungs called pleural effusion), which developed secondary to the enlarged heart. We removed all the pleural effusion today. The fluid will reform but how fast this occurs is unpredictable. Please start the medications as below to help clear fluid and slow the fluid formation.

Although taurine deficiency is a rare cause for cardiomyopathy in cats, we submitted a taurine level to the lab today to look for this. The test results can take up to 2 weeks; we will call you when the results are available.

As we discussed, (b) (6) has elevations in her kidney values. This can make treating her heart disease challenging because she may not tolerate the lasix. If her kidney values become elevated to a certain degree, it will make her feel sick and she will likely have a decreased appetite or stop eating. We will monitor her kidney values with bloodwork over time.

Cats with heart enlargement are at risk for developing a blood clot, or stroke. Although aspirin and/or plavix can be given in hopes of reducing clot formation, they have not been proven to prevent blood clot formation in cats. If you elect to start this medication I would recommend waiting until she is eating and feeling well at home as these medications can cause GI side effects (vomiting, inappetance) in some cats.

Please periodically take a sleeping respiratory rate (sRR) at home. WHILE (b) (6) IS SLEEPING, count the number of times she breathes in over 15 seconds. She should breathe 8 or fewer breaths in 15 seconds.

A recheck with cardiology is recommended in 2 weeks, or sooner if you see any of the below signs.

MEDICATIONS:
START TODAY: Furosemide (Salix, Lasix) 12.5 mg tablets: Give 1/4 tablet by mouth once a day
Furosemide: Also called Salix or Lasix. This is a diuretic and will help clear the fluid from your pet's lungs. Side effects include electrolyte abnormalities (if they stop eating), dehydration and kidney enzyme elevations. Blood work can be done to monitor these. This medication will be probably given for the life of your pet.

START IN 3 DAYS IF EATING:
Pimobendan 1.5 mg tiny tabs: Give 1 tablet by mouth two times a day WITH

Patient History Report

Client: (b) (6)
Phone: (b) (6)
Address: (b) (6)
(b) (6)

Patient: (b) (6)
Species: Feline
Age: 12 Yrs. 5 Mos.
Color: Black

Breed: Shorthair, Domestic
Sex: Spayed Female

Date	Type	Staff	History
			FOOD. Pimobendan is a phosphodiesterase inhibitor that gives increased contractility and arterial vasodilation. This will help the heart function better, allow your cat to feel better and live longer. Any medication can upset the stomach. This drug does not typically cause this, but if you see any changes, please stop the drug till you talk to a doctor here at (b) (6) Please give this with (b) (6) meals. Giving on empty stomach is more likely to make her nauseous. We have called this medication into (b) (6). Please call them to order it and they will mail it to you. If eating, start: Taurine 250 mg by mouth twice a day with food. I have submitted blood for a taurine level. The result may not return for 2 weeks. In the meantime, please start Taurine at home, 250 mg two times a day with food. This can be purchased at any health food store. If she is not eating well or if it is difficult to give her this medication, you can skip this until we get the taurine result from the blood work. Watch (b) (6) for the following clinical signs and call a veterinarian if you see any of these: Initiation of or increase in cough Excessive panting or wheezing Restlessness, unable to get comfortable Decreased appetite Lethargy/weakness Collapse or fainting It has been a pleasure caring for (b) (6). Thank you for entrusting us with his care. If you have any further questions or problems, please don't hesitate to call.
(b) (6)	L		(b) (6) **Chemistry results from (b) (6) In-clinic** **Laboratory Requisition ID: 197 Posted Final** **Test Result Reference Range** **CREA = 1.4 mg/dL 0.8 - 2.4** **CREA: Test results for the latest analyzer run have been multiplied by the dilution factor for a dilution of 1 in 4 total.**
(b) (6)	C	(b) (6)	PHARMACY NOTE Called (b) (6) and spoke to (b) (6). Ordered Pimobendan

B:Billing, C:Med note, CB:Call back, CK:Check-in, CM:Communications, D:Diagnosis, DH:Declined to history, E:Examination, ES:Estimates, I:Departing instr, L:Lab result, M:Image cases, P:Prescription, PA:PVL Accepted, PB:problems, PP:PVL Performed, PR:PVL Recommended, R:Correspondence, T:Images, TC:Tentative medl note, V:Vital signs

Patient History Report

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Address: (b) (6)
(b) (6)

Patient: (b) (6)
Species: Feline
Age: 12 Yrs. 5 Mos.
Color: Black

Breed: Shorthair, Domestic
Sex: Spayed Female

Date	Type	Staff	History

1.5mg tiny tabs. Give 1 tablet by mouth twice daily with food. #100, 12 refills

---

(b) (6) L

Chemistry results from (b) (6) **In-clinic**
Laboratory Requisition (b)(6) **Posted** **Final**

Test	Result	Reference Range
ALB =	2.8 g/dL	2.3 – 3.9
ALKP =	11 U/L L	14 – 111
ALT =	140 U/L H	12 – 130
BUN/UREA =	74 mg/dL H	16 – 36
Chloride =	100 mmol/L L	112 – 129
CREA =	--.-- mg/dL	0.8 – 2.4
GLU =	105 mg/dL	71 – 159
PHOS =	7.0 mg/dL	3.1 – 7.5
Potassium =	4.7 mmol/L	3.5 – 5.8
Sodium =	138 mmol/L L	150 – 165
TP =	6.1 g/dL	5.7 – 8.9
GLOB =	3.3 g/dL	2.8 – 5.1
ALB/GLOB =	0.8	
Na/K =	29	
OSM calc =	298 mmol/kg	

(b) (6) TC (b)(6)

COMMUNICATIONS WITH CLIENT - TENTATIVE
(b) (6) 12:35
10am- (b) (6) (mrs)- Discussed echo confirmed heart disease, DCM. Reviewed causes of DCM (unlikley taurine def, but will submit for levels) and prognosis with owner. Risk of future episodes of CHF, when is unpredictable. Oenwer consented to thoracocentesis. If continues to breath comfortably out of oxygen can go home this afternoon.

12:30pm- (b) (6)- (b) (6) breathing is stable out of oxygen. Very weak and lethargic. Ate a small amount of food this morning. Discussed since breathing is comfortable, can try at home. If energy level does not improve at home over the next few days, may consider euthanasia. Discussed elevated kidney values and how that is giong to make treating CHF with lasix challenging. Owner is comfortable with trying (b) (6) at home to see how she does. Will have husband call back to set up a time.

(b) (6) P (b) (6)

21.00 tablet of Pimobendan 1.5mg Tab (Cpd) (M00472)
Rx #: 2569385 0 Of 12 Refills
Give 1 tablet by mouth twice daily with food.

(b) (6) P (b) (6)

60.00 tablet of Lasix (Salix / Furosemide) 12.5mg Tablet (M568)

B:Billing, C:Med note, CB:Call back, CK:Check-in, CM:Communications, D:Diagnosis, DH:Declined to history, E:Examination, ES:Estimates, I:Departing instr, L:Lab result, M:Image cases, P:Prescription, PA:PVL Accepted, PB:problems, PP:PVL Performed, PR:PVL Recommended, R:Correspondence, T:Images, TC:Tentative medl note, V:Vital signs

Patient History Report

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Address: (b) (6)
(b) (6)

Patient: (b) (6)
Species: Feline
Age: 12 Yrs. 5 Mos.
Color: Black

Breed: Shorthair, Domestic
Sex: Spayed Female

Date	Type	Staff	History
			Rx #: 2569382 0 Of 0 Refills Give 1/2 tablet by mouth twice daily.
(b) (6)	D	(b) (6)	Pleural Effusion Final
(b) (6)	D		Left Atrial Enlargement Final
(b) (6)	D		Dilated Cardiomyopathy Final

(b) (6) C (b) (6) RADIOLOGY REVIEW - FINAL (b) (6)

The DV view of the thorax obtained on (b) (6) has been reviewed and there is a moderate to large amount of pleural effusion that obscures visualization of the cardiac silhouette. There is also an area of increased opacity in the left hemithorax in the region of the caudal segment of left cranial lung lobe. The remaining lung parenchyma appears to be within normal limits and the pulmonary vessels appear normal. This combination of findings may be the result of heart failure or neoplasia and a cardiac consult is recommended for further evaluation.

This review was written by: (b) (6), DVM, DACVR, DACVS

(b) (6) C (b)(6) CARDIAC EVALUTION - CLOSED (b) (6) - Cardiac Evaluation

Date of evaluation: Monday, (b) (6)

CHIEF COMPLAINT: pleural effusion

HISTORY: Presented to ER last night for lethargy and ADR. Cursory ultrasound revealed pleural effusion. Thoracocentesis yielded 25ml yellow tinged fluid from the right side. Patient received 12mg lasix IV and was placed in oxygen overnight. Her RR was wnl, with slight effort noted overnight. She ate a small amount. A renal panel performed overnight revealed azotemia (BUN 67, Creat 5.3).

PHYSICAL EXAM: The patient was bright, alert and responsive. No murmur on auscultation, but heart sound slightly muffled. The patient was eupnic, RR 30 with slight effort. Normal BV sounds, no crackles on auscultation. Pulses were fair and synchronous. Heart rate was 170 bpm, regular rhythm. PCS 0/4. BCS 8/9.

RADIOGRAPHS (DV, both laterals) (b) (6)**:** Diffusely increased opacity obscuring the cardiac silhouette. Area of moderate increased opacity in the region of the caudal segment of the left cranial lobe. Pulmonary vasculature appears wnl.

ECHOCARDIOGRAM (b) (6):
IVSd: 0.37 cm LVIDd: 1.94 cm LVPWd: 0.48 cm

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Patient History Report

Client:	(b) (6)	**Patient:**	(b) (6)		
Phone:	(b) (6)	**Species:**	Feline	**Breed:**	Shorthair, Domestic
Address:	(b) (6)	**Age:**	12 Yrs. 5 Mos.	**Sex:**	Spayed Female
	(b) (6)	**Color:**	Black		

Date	Type	Staff	History

IVSs: 0.35 cm LVDs: 1.86 cm LVPWs: 0.48 cm %FS: 4 %
Ao: 0.8 cm LAD: 1.6 cm LA:Ao ratio 2 LA max: 1.5 cm LLAD: 1.57 cm
Comments: The left atrium is moderately enlarged. The left ventricle is enlarged in systole and diastole with poor left ventricular function. Mild right atrial and ventricular enlargement. Trivial MR and TR noted. No evidence for systolic anterior motion of the mitral valve (SAM).
Aortic and pulmonic flows were reduced. Moderate volume pleural effusion, no pericardial effusion. ECG on echo: HR 160, sinus rhythm.

DIAGNOSIS/PROBLEM LIST: Dilated Cardiomyopathy (DCM), moderate left atrial enlargement, pleural effusion, azotemia

THORACOCENTESIS (b) (6): a total of 120 cc of yellow tinged fluid was removed with an 18 gauge needle from the right side of the chest in right lateral recumbency. No sedation. A post-tap echo showed mild effusion remained in the patient.

SUMMARY: (b) (6) has dilated cardiomyopathy (DCM) and has developed pleural effusion due to congestive heart failure. The pleural effusion was removed today and we are starting the medications as described below. Although taurine deficiency is a rare cause for dilated cardiomyopathy in cats, we submitted a taurine level to the lab today to look for this. The test results can take up to 2 weeks; we will fax results when they are available. Unfortunately the prognosis for DCM (with no taurine deficiency) in cats is poor, with survival time 3-6 weeks or in rare cases 4-6 months. A renal panel was recheck today and showed elevated BUN (74) and normal creatinine (1.4). Discussed with owner the azotemia may make treating the congestive heart failure challenging.
We discussed that with heart enlargement there is a risk for developing a blood clot, or stroke. Although aspirin +/- plavix can be given in hopes of reducing clot formation, they have not been definitively proven to prevent blood clot formation in cats. If the owners elect to start this medication I would recommend waiting until we know she is eating and feeling well on the below medications as aspirin/plavix can cause GI side effects (vomiting, inappetance) in some cats. A recheck with cardiology is recommended in 2 weeks, or sooner if clinical signs.
Advised owner congestive heart failure likely contributing to lethargy, but concern that weakness may be secondary to the DCM and low cardiac output. If that is the case, may not improve once we get her out of failure. Owner comfortable taking (b) (6) home to see how she does. (b) (6) was discharged on the below medications. If she does not improve at home over the next few days, owner to consider euthanasia.

MEDICATIONS:
START: Furosemide (Salix, Lasix) 12.5 mg tablets: Give 1/4 tablet once daily.

START in 3 DAYS IF EATING
Pimobendan 1.5mg tiny tabs: Give 1 tablet by mouth two times a day WITH FOOD.

Patient History Report

Client: (b) (6)
Phone: (b) (6)
Address: (b) (6)
(b) (6)

Patient: (b) (6)
Species: Feline
Age: 12 Yrs. 5 Mos.
Color: Black

Breed: Shorthair, Domestic
Sex: Spayed Female

Date	Type	Staff	History

If eating and easy to medicate, start: Taurine 250 mg by mouth twice a day with food.

(b) (6) L (b) (6)

Basic Metabolic Profile, (b) (6) results from (b) (6)
In-Clinic Requisition ID: 0 Posted Final

Test	Result	Reference Range
HCT =	40 %	12 - 70
HB =	13.1 g/dL	9.9 - 14.9
NA+ =	146.3 mmol/L	146.2 - 156.2
K+ =	4.99 mmol/L H	3.41 - 4.71
CL+ =	107.8 mmol/L L	117.0 - 125.3
CA++ =	1.17 mmol/L	1.16 - 1.35
MG++ =	1.08 mmol/L H	0.33 - 0.49
GLU =	156 mg/dL H	72 - 132
LAC =	9.7 mg/dL H	0.7 - 1.9
BUN =	67 mg/dL H	22 - 33
CREAT =	5.3 mmo/L H	1.1 - 3.5
O2CAP =	18.2 mL/dL	
TCO2 =	19.9 mmol/L	
GAP =	20.3 mmol/L	
CA/MG =	1.1 mol/mol	
OSM =	313.5 mOsm/kg	
BUN/CREA =	12.7 mg/mg	

Manually entered.
PCV: 43% T.S: 6.6mg/dl

(b) (6) TC (b) (6) LAB RESULTS - NOTES - TENTATIVE
(b) (6) 00:00
Lab Results: PCV: 42% TS g/dl: 6.8 Serum: Normal
Original Lab Date:

(b) (6) B (b) (6) Laboratory Request / Sample Handling (LABS) by (b) (6)
(b) (6) B (b) (6) 1.00 Sample Handling & Disposal (LFEE) by (b) (6)
(b) (6) B (b) (6) 1.00 Basic Metabolic (b) (6) Panel # 2 ((b) (6)) by (b) (6)
(b) (6) B (b) (6) 1.00 Specialty/Referral Exam Level 3 (REF03) by (b) (6)
(b) (6) B (b) (6) Echocardiogram Level 3 Group (USSC19) by (b) (6)
(b) (6) B (b) (6) 1.00 VRC04 Procedure (VRC04) by (b) (6)
(b) (6) B (b) (6) 1.00 Equipment Service & Preparation (USEQPT) by (b) (6)
(b) (6) B (b) (6) 1.00 Thoracocentesis Therapeutic (R33) by (b) (6)

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Patient History Report

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(b) (6)

Patient: (b) (6)
Species: Feline
Age: 12 Yrs. 5 Mos.
Color: Black

Breed: Shorthair, Domestic
Sex: Spayed Female

Date	Type	Staff	History
	B	(b) (6)	60.00 tablet of Lasix (Salix / Furosemide) 12.5mg Tablet (M568) by (b) (6)
	B	(b) (6)	21.00 tablet of Pimobendan 1.5mg Tab (Cpd) (M00472) by (b) (6)
	B		Oxygen Therapy (Caged)/Hour (Group) (O2CAGE) by (b) (6)
	B		7.00 O2 Therapy Per Hour (T044) by (b) (6)
	B		7.00 Oxygen-related Patient Care / Hour (O2CARE) by (b) (6)
	B		1.00 Equipment Service & Preparation (USEQPT) by (b) (6)
	B	(b) (6)	Hospitalization Hours Smart Group (HOSPIT) by (b) (6)
	B	(b) (6)	17.00 Hospitalization Hours- Feline (H01) by (b) (6)
	B	(b) (6)	1.00 In-House Nutrition Assessment Level 1 (NTR012) by (b) (6)
	B	(b) (6)	17.00 Critical Care Level 2- Hours (CCU2) by (b) (6)
	B	(b) (6)	.20 ml of DNULsix 50mg/ml/ML (T106) by (b) (6)
	B	(b)	1.00 Cared for by (b) (6) ((b) (6) by (b) (6)
	B	(b) (6)	1.00 Cared by (b) (6) - Cardiology ((b) (6)) by (b) (6)
(b) (6)	B	(b) (6)	Laboratory Request / Sample Handling (LABS) by (b) (6)
(b) (6)	B	(b) (6)	1.00 Taurine U of Wisc (via (b) (6) S16755) (L0245) by (b) (6)
(b) (6)	B	(b) (6)	1.00 Taurine U of Wisc (via (b) (6) S16755) (L0245) by (b) (6)
(b) (6)	B	(b) (6)	Laboratory Request / Sample Handling (LABS) by (b) (6)
(b) (6)	B	(b) (6)	1.00 Chemistry IV Renal Panel (b) (6) (CH25) by (b) (6)
(b) (6)	B	(b) (6)	Laboratory Request / Sample Handling (LABS) by (b) (6)
(b) (6)	B	(b) (6)	1.00 Dilution Verification Catalyst CREA (CH11DV) by (b) (6)

(b) (6) C (b) (6) EMERGENCY PHYSICAL EXAM - Closed May
(b) (6)

Chief Complaint: Lethargic

History: Starting yesterday patient was noted to be lethargic and not herself. 4 other cats so difficult to say if she was eating but they think she was. Not sure about U/BM. Indoor only. Did not notice she was having issues breathing.

Other Medical Problems: None

Medications/Supplements: None

Environment: Indoor only

Vaccination Status:

Current Diet (Type):
- Frequency:
- Amount:

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Patient History Report

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(b) (6)

Patient: (b) (6)
Species: Feline
Age: 12 Yrs. 5 Mos.
Color: Black

Breed: Shorthair, Domestic
Sex: Spayed Female

Date	Type	Staff	History

Physical Examination:

S(ubjective): QAR, hydration WNL, BCS 7/9, pain score: 0/4

O(bjective):
Weight: 15.6 pounds
TPR: [temp - 93]F, [HR - 150] bpm, [RR - 60] bpm
EENT: clear AU/OU, no nasal discharge, normal cervical palpation, mm pink, moist/CRT<2 sec
INTEG: Hair coat ok
PLN: WNL
CV: Heart sounds muffled
RESP: Increased RE, dull lung sounds
GI: soft, nonpainful, no masses
UG: SF, WNL
M/S: amb x 4
Neuro: alert/appropriate, cranial nerves intact

Problems/Differential Diagnoses:
Dyspnea
Lethargy

Diagnostics:
Cursory ultrasound - mild to moderate amount of pleural effusion R>L
DV thoracic radiograph - cardiac silhouette difficult to visualize, pleural effusion
(b) (6) 2

Assessment:
12 yr SF DSH
1. Pleural effusion, dyspnea - r/o cardiac (HCM) vs neoplasia (lymphoma vs other)

Treatment:
12 mg Lasix IM at 10 PM
Place in O2 cage
Thoracocentesis - 25 mL clear to yellow fluid removed from the right side
Place IVC, 12 mg Lasix IV at 2 AM

Plan/Recommendations:
Discussed differentials for pleural effusion - cardiac vs neoplasia. Due to pleural effusion cannot tell on radiographs if this is cardiac over neoplastic. Rec thoracocentesis to make (b) (6) breath more comfortably - o consents. Rec echocardiogram in the morning to see if this is heart disease. If this is CHF spoke about disease process and prognosis. If this is neoplasia owner's may decide to

Patient History Report

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Phone: (b) **Species:** Feline **Breed:** Shorthair, Domestic
Address: (b) **Age:** 12 Yrs. 5 Mos. **Sex:** Spayed Female
(b) **Color:** Black

Date	Type	Staff	History

stop. Discussed possibility of taking repeat radiographs tomorrow if needed.

(b) (6) R (b) (6) Tx Template (blank)- Old WTS - TENTATIVE

WARD TREATMENT SHEET

DATE:	WARD: **ccu**
LAST NAME:	CLINICIAN:
PATIENT NAME:	TRANSFER DOCTOR:
BREED: **Shorthair, Domestic** COLOR:**Black**	**LEGEND:**
AGE: **12 Yrs. 4 Mos.** SEX: **Spayed Female**	**O** = scheduled **X** = performed **D/C** = discontinued
WEIGHT: 15.6 pounds as of: 12/28/2009	
PROBLEM/WORKING DIAGNOSIS:	**inc** = increase **dec** = decrease ⊖ = not given
Pleural effusion	SURGERY:
ALERT: **increased RR**	IV CATHETER: CODE: **RED**

			Technician STAFF ID ==>																								
DR Staff ID	TREATMENTS	Call Parameters	8	9	10	11	N	1	2	3	4	5	6	7	8	9	10	11	M	1	2	3	4	5	6	7
	Temperature	<99, >102.5																								
	HR/MM/CRT	<140, >240																								
	RR/RE	>40, increased RE	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O
	Pulse Quality	wk																								
	Assess Pain & Note Score	2																								
	WEIGH																									
	RER: 262 kcal/day Diet: 115 g EN BID - any -				O										O											
	Quantify Food Intake						O										O									
	Lasix 12 mg IV - ask		O																		O					
	O2 cage																									

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(b)

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Date	Type	Staff	History

Primary Infusion	Additives	ml/hr	
		Daily Cumulative	
OBSERVATION COMMENTS ON REVERSE		ADDITIONAL BAGS	

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Breed: Shorthair, Domestic
Sex: Spayed Female

Date	Type	Staff	History

	AM SHIFT
	MID SHIFT
	OVER

TIME	Init. TECH	Temp	HR	Pulse qual.	RR/ effort	MM color	CRT	PCV/ ts	BG	Turn	Blood Pressure	Pain Score	BM	Urine	Vomit	Grams Consumed	Init. AA	
8 a																		
9 a																		
10 a																		
11a																		
N																		
1 p																		
2 p																		
3 p																		
4 p																		
5 p																		
6 p																		
7 p																		
8 p																		
9 p																		
10 p																		
11 p																		
M																		
1 a																		
2 a																		
3 a																		
4 a																		
5 a																		
6 a																		
7 a																		

TIME	ADDITIONAL COMMENTS

B:Billing, C:Med note, CB:Call back, CK:Check-in, CM:Communications, D:Diagnosis, DH:Declined to history, E:Examination, ES:Estimates, I:Departing instr, L:Lab result, M:Image cases, P:Prescription, PA:PVL Accepted, PB:problems, PP:PVL Performed, PR:PVL Recommended, R:Correspondence, T:Images, TC:Tentative medl note, V:Vital signs

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Breed: Shorthair, Domestic
Sex: Spayed Female

Date	Type	Staff	History

(b) (6)	T	(b) (6)	Image: Thorax Received via DICOM C-STORE on Sun (b) (6) EDT (b) (6) Client First Name: (b) (6) Client Last Name: (b) (6) Patient Id: (b) (6) Patient Name: (b) (6) l' Patient DOB: 'Thu (b) (6) Patient Sex: 'Female'
(b) (6)	CK	(b) (6)	lack of energy Reason for Visit: Emergency Date Patient Checked Out: (b) (6) Practice b) (6)
(b) (6)	B	(b) (6)	Hospitalization Hours Smart Group (HOSPIT) by (b) (6)
(b) (6)	B	(b) (6)	0.00 Admission time was 10:10 PM (ADMTIME) by b) (6)
(b) (6)	B	(b) (6)	3.00 Hospitalization Hours- Feline (H01) by b) (6)
(b) (6)	B	(b) (6)	3.00 Critical Care Level 2- Hours (CCU2) by b) (6)
(b) (6)	B	(b) (6)	1.00 Emergency Exam Level 4 (EE04) by (b) (6)
(b) (6)	B	(b) (6)	.24 ml of DNULsix 50mg/ml/ML (T106) by (b) (6)
(b) (6)	B	(b) (6)	1.00 Thoracocentesis Therapeutic (R33) by b) (6)
(b) (6)	B	(b) (6)	Oxygen Therapy (Caged)/Hour (Group) (O2CAGE) by (b) (6)

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(b) ((b) (6)

Patient History Report

Client: (b) (6)
Phone: (b) (6)
Address: (b) (6)
(b) (6)

Patient: (b) (6)
Species: Feline
Age: 12 Yrs. 5 Mos.
Color: Black

Breed: Shorthair, Domestic
Sex: Spayed Female

Date	Type	Staff	History
(b) (6)	B	(b) (6)	3.00 O2 Therapy Per Hour (T044) by (b) (6)
(b) (6)	B	(b) (6)	3.00 Oxygen-related Patient Care / Hour (O2CARE) by (b) (6)
(b) (6)	B	(b) (6)	1.00 Equipment Service & Preparation (USEQPT) by (b) (6)
(b) (6)	B	(b) (6)	IV Catheter with Injection Cap (IVCATCP) by (b) (6)
(b) (6)	B	(b) (6)	1.00 IV Catheter Placement (CATH) by (b) (6)
(b) (6)	B	(b) (6)	1.00 each of Tx Catheter IV 20g x 2" Surflo (PINK) (H0112) by (b) (6)
(b) (6)	B	(b) (6)	1.00 each of Tx IV Ext T Set Hospira 1265028 (H027) by (b) (6)
(b) (6)	B	(b) (6)	1.00 each of Tx Injection Cap/Plug Termo 007110 (H118) by (b) (6)
(b) (6)	B	(b) (6)	1.00 Cared for by (b) (6) (b) (6)) by (b) (6)
(b) (6)	B	(b) (6)	Thorax Radiographic Study Group (RADTH) by (b) (6)
(b) (6)	B	(b) (6)	1.00 Radiograph Preparation (XFEE) by (b) (6)
(b) (6)	B	(b) (6)	1.00 One view rdgh stdy (RAD1V) by (b) (6)
(b) (6)	B	(b) (6)	1.00 Radiologist Review Fee (RADGN) by (b) (6)

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Patient History Report

Client: (b) (6)) **Patient:** (b) (6)
Phone: (b) (6) **Species:** Feline **Breed:** Longhair, Domestic
Address: (b) (6) **Age:** 9 Yrs. 10 Mos. **Sex:** Neutered Male
(b) (6) **Color:** Calico

Date	Type	Staff	History
6/7/2016	TC	(b) (6)	COMMUNICATIONS WITH CLIENT - TENTATIVE 6/7/2016 12:33 On the phone with client discussing (b) (6) also discussed (b) (6) T4 and liver values need to be rechecked 6 weeks after his meds began to test for any needed dose adjustments. We can come to the home or he can schedule with a (b) (6) in the hospital. We discussed that he's enjoyed working with (b) (6) before.
6/6/2016	C	(b) (6)	MEDICAL COMMENTS 6/6/2016 11:47 FDA complaint submitted: Pet Food Safety Report, ID 54405, was successfully submitted on 6/6/2016 11:44:41 AM EST to the FDA, and it was issued an Individual Case Safety Report Number (ICSR) of 1053339
6/1/2016	TC	(b) (6)	MEDICAL COMMENTS - TENTATIVE 6/1/2016 10:46 SW (b) (6) at Merrick - updated company that we have documented taurine deficiency in 2 other cats in house hold. The quality assurance team indicated that the level of taurine in the lot # I gave them was sufficient - discussed that this likely takes 3-6 month to develop and likely to be related to earlier lot and they need to investigate further. Asked if the level they gave me was from them retesting the food after I called or from an earlier test - not sure. Asked for information from the taurine tests - told I can send summary of lab results. Also indicated that I am reporting this to the FDA. She give new info to her manager and quality assurance. Told them I expect them to follow up with me. Below email sent to Merrick: Taurine Levels (b) (6) To: (b) (6)@merrickpetcare.com Hi (b) (6) Thank you for your help with these cases. Here is the summary of the lab results: 12yr female spayed domestic short hair diagnosed and clinical for dilated cardiomyopathy -5/9/2016 Plasma Taurine 24nmol/ml (normal 60-120, critical level <40) - test performed at University of Wisconsin, results were received on 5/15/2016 5/21/2016 - Whole Blood Taurine submitted at the University of California Davis on

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Patient History Report

Client: [redacted])
Phone: [redacted]
Address: [redacted]

Patient: [redacted]
Species: Feline
Age: 9 Yrs. 10 Mos.
Color: Calico
Breed: Longhair, Domestic
Sex: Neutered Male

Date	Type	Staff	History

remaining 4 cats consuming this food (normal 300-600 nmol/ml, no known risk for deficiency >200), results were received on 5/27/2016
-9yr male neutered domestic long hair: 196 nmol/ml
-8y female spayed domestic short hair: 368 nmol/ml
-9yr male neutered domestic long hair: 124 nmol/ml
-9yr male neutered domestic long hair: 536 nmol/ml

Please let me know if you have any other questions.

Sincerely,

[redacted]

[redacted], DVM Diplomate ACVN
Clinical Nutrition Department

[redacted]

5/31/2016 C [redacted]

COMMUNICATIONS WITH CLIENT - Closed Jun 02/2016
5/31/2016 16:14
Spoke with husband, he confirmed email was received and [redacted] and [redacted] have begun taurine supplementation. Discussed results are a different normal range for the whole blood testing I did vs the plasma testing the cardiologist did on [redacted]. He notes [redacted] had been getting some Fancy Feast so that likely explains why his values are top of the normal range. Discussed [redacted] elevated globulins, need protein electrophoresis to better define the issue, can be chronic inflammatory response or a few types of cancer. If cancer, because they are [redacted] clients they can consult with oncology at no charge to hear what the treatment options would be. They can bring [redacted] into the office with a [redacted] doctor for the bloodwork or I can do the bloodwork at the house. If I am doing it, I would not charge any recheck exam, just the travel and diagnostic test costs. If a [redacted] in the hospital, they shouldn't have to charge for an exam either because she was just checked in late May. Owner likes [redacted]; advised he could schedule that with her. Discussed rechecking [redacted] T4 and liver values 6 weeks after starting meds to check if dose needs adjusting; can be done at the house or in the office as well. Advised Nutrition is contacting Merrick about the taurine results and she feels the owner shouldn't have to pay for the taurine testing; she wants Merrick to have to pay for it directly. So I

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Patient History Report

Client: (b) (6)) **Patient:** (b) (6)
Phone: (b) (6) **Species:** Feline **Breed:** Longhair, Domestic
Address: (b) (6) **Age:** 9 Yrs. 10 Mos. **Sex:** Neutered Male
(b) (6) **Color:** Calico

Date	Type	Staff	History
			told the owner that first and foremost, they are responsible for payment of the testing to (b) (6) and once we advise them of a charge, they would be required to pay it. If the Nutritionist is able to circumvent that by having Merrick pay us directly, that would be a nice advantage for the client. He understands.
5/31/2016	C	(b) (6)	COMMUNICATIONS WITH CLIENT - Closed Jun 02/2016 5/31/2016 15:39 LMOM on husband cell making sure they received my treatment advice in the email from over the weekend. Please call back or reply to email so I can be certain the treatment guidelines were received.
5/28/2016	C	(b) (6)	COMMUNICATIONS WITH CLIENT - Closed May 30/2016 5/28/2016 15:46 Email to client: Hi Mr and Mrs (b) (6) – I left a (long-winded) message on (b) (6) voicemail earlier today. The nutritionist has since been in contact with me and advised that both (b) (6) and (b) (6) should be started on taurine supplementation. She recommends 250mg taurine twice daily for 2-3 weeks. Because you've already switched them to another diet, after 2-3 weeks, supplementation can be discontinued. (b) (6) and (b) (6) tested safely within the normal range for taurine, so they do not require any supplementation. I presume since you have already been treating (b) (6), you likely have a supply of taurine supplement. If not, feel free to contact me (b) (6) or the nutritionist or cardiologist to get a larger supply in order to treat the brothers. The nutritionist also advised she'll be contacting Merrick again now that the data has been received. Once she has heard more from them, she'll be in contact with you, as well. I also mentioned in the voicemail that (b) (6) blood test was repeated and verified that she does have elevated globulins. The most harmless reason would be chronic inflammation, but since she's been otherwise healthy, it is valuable to pursue further diagnostic inquiry. Unfortunately, elevated globulins can also indicate cancer, so we want to determine precisely what is happening with her. We can collect another blood sample from her at any time in order to perform a test called protein electrophoresis which further defines which specific immunoglobulins are elevated. You may choose to bring her into the office or have us out to the home again. (b) (6)will need repeat bloodwork after he's been on his thyroid supplement for 6 weeks, we could collect her second sample at that time as well, if

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Patient History Report

Client: (b) (6)) **Patient:** (b) (6)
Phone: (b) (6) **Species:** Feline **Breed:** Longhair, Domestic
Address: (b) (6) **Age:** 9 Yrs. 10 Mos. **Sex:** Neutered Male
(b) (6) **Color:** Calico

Date	Type	Staff	History

you choose.

Feel free to contact me with any questions. I will next be in the office on Tuesday May 31st.
Happy Memorial Day weekend –
(b) (6)

5/28/2016 C (b) (6)

COMMUNICATIONS WITH DOCTOR - Closed May 30/2016
(b) (6) 15:45
Email response from (b) (6) to (b) (6):
I'm also going to talk with (b) (6) this week about the cost of the taurine test. In my opinion this should be paid for by the company. I don't want the owner to pay the cost yet until I talk with (b) (6) and the company again.

(b) (6)

_____
From: (b) (6)
Sent: Saturday, May 28, 2016 2:16 PM
To: (b) (6)
Subject: RE: price for taurine test
There is a risk of deficiency with anything <200, so that's why I would go ahead and supplement both cats...and it's harmless:-)

_____
From: (b) (6)
Sent: Saturday, May 28, 2016 2:13 PM
To: (b) (6)
Subject: RE: price for taurine test
It is really interesting...probably the same reason some puppies raised on an unbalanced home cooked diet never have issues and other do.

Great the diet has been changed. We should get the cats that tested low on some supplementation for 2-3 weeks just to cover our bases. 250mg taurine PO BID...if she needs to use a powder form and mix with the cats food that's fine

Let the owner know I will touch base with the company after Memorial day...I have not heard back from them yet. This will also give me much more to go on when reporting to the FDA...who know this might turn into a pet food recall (it should turn into a recall)!

Thank you so much for the update!

(b) (6)

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Patient History Report

Client: (b) (6))
Phone: (b) (6)
Address: (b) (6)
(b) (6)

Patient: (b) (6)
Species: Feline
Age: 9 Yrs. 10 Mos.
Color: Calico

Breed: Longhair, Domestic
Sex: Neutered Male

Date	Type	Staff	History
			From: (b) (6) Sent: Saturday, May 28, 2016 1:55 PM To: (b) (6) Subject: RE: price for taurine test Hi (b) (6) – Thanks for providing this justification for the lab decision; I really appreciate it! I left you a voicemail earlier today – the results are in. 2 cats tested within the normal range [(b) (6) 368, (b) (6) 536 (300-600)]. (b) (6) was 196 and (b) (6) was way down at 124. All 4 cats were switched to Royal Canin food about 7 days ago. I left a voicemail for the client advising of the results, but told him I wanted your input before devising a treatment strategy. I would think of these 4, only (b) (6) would benefit substantially from taurine supplementation. I presume (b) (6) levels are sufficient now that he's been put on a properly formulated diet. Do you agree? This case is so interesting… how the cats fall all along the clinical spectrum, including some that have sufficient taurine, despite all eating the same presumably flawed diet. Thanks for your input, (b) (6)
5/28/2016	C	(b) (6)	COMMUNICATIONS WITH CLIENT - Closed May 30/2016 5/28/2016 13:44 LMOM - advised client taurine results have been received, I have notified nutrition dept who will weigh in on treatment decision-making. Normal range is 300-600 and (b) (6) and (b) (6) tested within that range. Clinical signs are unlikely above 200, (b) (6) was 196, so it is likely he wouldn't show any issues. b) (6) tested at 124 so he might be the one to benefit from additional supplementation, aside from just the diet change to the Royal Canin food. We will wait to initiate any therapy until the nutritionist has a chance to comment; we are working as a team on this. Since we have results, we likely have an invoice from the lab as well, so we should be able to advise of the cost of this testing in the short-term. I had spoken with his wife about (b) (6) having elevated globulins and on the re-test that status persists, was verified. Recommend additional blood testing for further work-up, could be collected when we visit (b) (6) for bloodwork 6 weeks after starting his thyroid meds. Please call back to discuss these results.
5/27/2016	TC	(b) (6)	LAB RESULTS - NOTES - TENTATIVE 5/27/2016 15:32 Lab Results: Fax from U of Cal Davis. Original Lab Date: - Attachment(s)

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Patient History Report

Client: (b) (6)) **Patient:** (b) (6)
Phone: (b) (6) **Species:** Feline **Breed:** Longhair, Domestic
Address: (b) (6) **Age:** 9 Yrs. 10 Mos. **Sex:** Neutered Male
(b) (6) **Color:** Calico

Date	Type	Staff	History
5/24/2016	P	(b) (6)	100.00 tablet of Tapazole (Methimazole) 5mg Tablet (M066) Rx #: 2576298 0 Of 0 Refills Give 1 tablet by mouth twice daily. Check bloodwork for dose adjustment 6 weeks after starting medication.
5/24/2016	C	(b) (6)	COMMUNICATIONS WITH CLIENT - Closed May 26/2016 5/24/2016 16:41 Spoke with Mrs; advised hyperthyroid with some liver elevations. Reviewed life-long treatment, bloodwork 6 weeks after med started and then twice yearly if stable. If dose is changed after first bloodwork, we repeat bloodwork again 6 weeks later until properly regulated. Med can be tablet, liquid or transdermal. Owner wants to crush tablet into canned food; advised this is fine as long as we're certain he's the only one who might consume the medicated food within their group-housing situation. Owner feels she can guarantee that. Meds will be at 197 pharmacy. Taurine pending, will call. Advised final pricing on taurine at CA lab not yet determined, will be in touch with that info as soon as finalized. Owner asked why use a diff lab; advised nutritionists recommended this lab, specialized testing at university, two labs finding low levels strengthens case against food company.
5/24/2016	B	(b) (6)	100.00 tablet of Tapazole (Methimazole) 5mg Tablet (M066) by (b) (6)
5/24/2016	B	(b) (6)	1.00 Cared for by (b) (6) (b) (6) by (b) (6)
5/21/2016	C	(b) (6)	GP PHYSICAL EXAM - Closed May 26/2016 Date Presented: 5/21/2016 Chief Complaint: Weight loss/Taurine check History: Owner notes chronic weight loss across the recent months. Was losing hair for over a year, but was told it was related to anxiety. Eats with voracious appetite. Normal activity level. Normal ur/def. No known V/D/C/S. 5 cat, 2 dog household; he is one of 4 cats that live together in a room above the garage. Indoor only. (b) (6) has DCM from taurine deficiency, checking status of other cats in household. Diet (type, freq, amt): Merrick Purrfect Bistro Grain Free chicken dry food Meds: none S: BAR, PCS 0/4 - slightly feisty O: MM / ORPH: Pink, moist, crt <2 sec, mild tartar E/E: mild black debris in outer cartilages of left ear, deep canal WNL, right ear WNL. ophtho WNL.

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Patient History Report

Client: (b) (6)) **Patient:** (b) (6)
Phone: (b) (6) **Species:** Feline **Breed:** Longhair, Domestic
Address: (b) (6) **Age:** 9 Yrs. 10 Mos. **Sex:** Neutered Male
(b) (6) **Color:** Calico

Date	Type	Staff	History
			INT: alopecia caudal dorsum, ventrum, lateral thighs. no ectoparasites observed. PLN: WNL CV / Resp: Reg rhythm, no murmur, SSP, clear and eupneic GI/UG: Compliant, no masses MS/NS: Normal amb x4 BCS: 1.5-2/5 4.4kg A: 9yr9mo MN DLH 1) weight loss - r/o hyperthyroid, diabetes mellitus, organ dz (kidney, liver), other endocrinopathy, neoplasia, nutritional problem 2) alopecia - r/o FAD, other derm issue, psychogenic (stress, pain-related) 3) dental disease 4) otic debris - r/o infection vs inadequate grooming P: PE Taurine level CBC/Superchem/T4 Advised client of marked weight loss from last documented weight. Systemic bloodwork may illuminate the reason; will call with results next week. Taurine level will take 7-10 days. Advised client we are sending taurine test to a different lab than the one that tested (b) (6) sample, at the advice of the nutrition service. We do not have a price in our computer system for this test through this lab, so the client will be invoiced for the taurine level (for all 4 cats) once that is established. Client paid today's services during the visit and is aware of the pending charge; advised the (b) (6) charge was $214 and the charge at the other lab will likely be within $50 under/over that fee. He commits to paying taurine test fees once advised of final fee. Stated we want to submit samples for testing ASAP and he understands fee structure will not be set until after tests are underway.
5/21/2016	C	(b) (6)	COMMUNICATIONS WITH CLIENT - Closed May 23/2016 5/21/2016 16:25 mailed welcome card, magnet, Rabies certificates ((b) (6), (b) (6)) and feedback postcard
5/21/2016	V	(b)	May 21, 2016 11:21 AM Staff: (b) (6) ---------- Weight : 4.40 kilograms HC-RS scale
5/21/2016	L		**Hematology results from (b) (6) (East) Requisition ID: 209396 Posted Final**

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Patient History Report

Client: (b) (6)) **Patient:** (b) (6)
Phone: (b) (6) **Species:** Feline **Breed:** Longhair, Domestic
Address: (b) (6) **Age:** 9 Yrs. 10 Mos. **Sex:** Neutered Male
(b) (6) **Color:** Calico

Date	Type	Staff	History

Test	Result	Reference Range
HCT	42 %	29 – 48
HGB	15.0 g/dL	9.3 – 15.9
MCHC	35.7 g/dL	30 – 38
WBC	14.2 10^3/uL	3.5 – 16.0
Bands	0 %	0 – 3
RBC	9.5 10^6/uL	5.92 – 9.93
MCV	44 fL	37 – 61
MCH	15.8 pg	11 – 21
ABS BASO	0 /uL	0 – 150
ABS NEUTB	0 /uL	0 – 150
Platelet C	254 10^3/uL	200 – 500
Platelet E	ADEQUATE	ADEQUATE –
Neutrophil	53 %	35 – 75
Lymphocyte	41 %	20 – 45
Monocytes	2 %	1 – 4
Eosinophil	4 %	2 – 12
Basophils	0 %	0 – 1
Absolute N	7526 /uL	2500 – 8500
Absolute L	5822 /uL	1200 – 8000
Absolute M	284 /uL	0 – 600
Absolute E	568 /uL	0 – 1000

Ascn: (b) (6) Profile: Complete Blood Count

5/21/2016 L

Chemistry results from (b) (6) (East) Requisition
ID: 209396 Posted Final

Test	Result	Reference Range
ALB	3.4 g/dL	2.5 – 3.9
ALKP	174 U/L H	6 – 102
ALT	243 U/L H	10 – 100
AMYL	882 U/L	100 – 1200
AST	46 U/L	10 – 100
BUN/UREA	17 mg/dL	14 – 36
Ca	9.2 mg/dL	8.2 – 10.8
Chloride	111 mEq/L	104 – 128
CHOL	181 mg/dL	75 – 220
CK	124 U/L	56 – 529
CREA	0.6 mg/dL	0.6 – 2.4
GGT	3 U/L	1 – 10
GLU	80 mg/dL	64 – 170
Mg	1.7 mEq/L	1.5 – 2.5
PHOS	5.9 mg/dL	2.4 – 8.2
Potassium	4.5 mEq/L	3.4 – 5.6
Sodium	150 mEq/L	145 – 158
TBIL	0.1 mg/dL	0.1 – 0.4
TP	5.9 g/dL	5.2 – 8.8
TRIG	58 mg/dL	25 – 160
GLOB	2.5 g/dL	2.3 – 5.3

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Patient History Report

Client: (b) (6)) **Patient:** (b) (6)
Phone: (b) (6) **Species:** Feline **Breed:** Longhair, Domestic
Address: (b) (6) **Age:** 9 Yrs. 10 Mos. **Sex:** Neutered Male
(b) (6) **Color:** Calico

Date	Type	Staff	History
			A/G Ratio 1.4 Ratio 0.35 - 1.5 B/C Ratio 28 Ratio 4 - 33 Na/K Ratio 33
5/21/2016	L		Endocrinology results from (b) (6) (East) Requisition ID: 209396 Posted Final Test Result Reference Range T4 20.2 ug/dL H 0.8 - 4.0 Ascn: (b) (6) Profile: Total T4 Result verified.
5/21/2016	L		Miscellaneous results from (b) (6)ics (East) Requisition ID: 209396 Posted Final Ascn: (b) (6) Profile: Superchem RE: 1045 PrecisionP 28 U/L 8 - 26 PresisionPSL elevations correlate closely with abnormal PLI concentrations. In cats with appropriate clinical signs, this PrecisionPSL is supportive of, but not definitive, for a diagnosis of pancreatitis. In cats without clinical signs of pancreatitis, a mild elevation is an insignificant finding. RE: 11067 Comment Hemolysis 1+ No significant interference.
5/21/2016	B	(b) (6)	1.00 Superchem Cbc T4 (b) (6) Sa120 (L85) by (b) (6)
5/21/2016	B	(b) (6)	1.00 House Call Travel Level 2 (HC06) by (b) (6)
5/21/2016	B	(b) (6)	1.00 At Home Appointment (HC04) by (b) (6)
5/21/2016	B	(b) (6)	Laboratory Request / Sample Handling (LABS) by (b) (6)
5/21/2016	B	(b) (6)	1.00 Outside Lab (XTBALUO) by (b) (6)
5/21/2016	B	(b) (6)	1.00 Sample Handling & Disposal (LFEE) by (b) (6)
5/21/2016	B	(b) (6)	1.00 Lab Sample Label (TL) by (b) (6)
5/21/2016	B	(b) (6)	1.00 Cared for by (b) (6) ((b) (6)) by (b) (6)
5/20/2016	C	(b) (6)	COMMUNICATIONS WITH CLIENT - Closed May 21/2016 5/20/2016 15:00 Called to confirm tomorrow's appointment fro (b) (6), (b) (6) (b) (6) and (b) (6) at 9 am. I also mentioned in my message that we should use the address (b) (6) (b) (6) in the GPS. If any questions please call (b) (6)

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Patient History Report

Client: (b) (6)
Phone: (b) (6)
Address: (b) (6)
(b) (6)

Patient: (b) (6)
Species: Feline
Age: 9 Yrs. 10 Mos.
Color: Calico

Breed: Longhair, Domestic
Sex: Neutered Male

Date	Type	Staff	History
5/17/2016	C	(b) (6)	COMMUNICATIONS WITH CLIENT - Closed May 19/2016 5/17/2016 12:29 Responding to owner's message, booked (b) (6), (b) (6), (b) (6), (b) (6) for House Call on Saturday (b)(6). (b) (6) was seen on emergency and diagnosed with low taurine, so all cats need to be screened. Had been eating Merrick dry food. Cats are kept in a finished room above the garage; he thinks they won't need to be confined/isolated more than that in order to work on them. Discussed senior bloodwork as well. He notes this emergency with (b) (6) was a wake-up call and he'd like to thoroughly have everyone checked out. (b) (6) is losing weight. (b) (6) and (b) (6) have been to another vet and have a current Rabies; (b) (6) and (b) (6) haven't been to the vet in a long time. Discussed PureVax 1yr vs 3yr vs standard RabVac, vaccine-associated sarcoma issue - owner wants the purified vaccine, prefers the one year since they should be examined annually anyway. Advised if (b) (6) status progresses and we need to be checking her as well, please call to inform us in case we need special items/supplies for her care. Owner notes we should use (b) (6) with the GPS; his home address was renamed/renumbered a few years ago, but GPS cannot often find (b) (6)

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Patient History Report

Client: (b) (6)
Phone: (b) (6)
Address: (b) (6)
(b) (6)

Patient: (b) (6)
Species: Feline
Age: 8 Yrs. 0 Mos.
Color: brown tabby

Breed: Shorthair, Domestic
Sex: Spayed Female

Date	Type	Staff	History
(b) (6)	TC	(b) (6)	COMMUNICATIONS WITH CLIENT - TENTATIVE (b) (6) 12:25 Spoke with owner, advised of specialist's comments about protein electrophoresis. Can conduct further testing through our Internal Medicine service of ultrasounds of chest and abdomen, essentially searching for origin of chronic inflammation. It is possible that testing will come back normal, despite the bloodwork indicating the elevated globulins. Another option is to track her body weight at home once monthly and report any weight loss promptly; if none noted, recheck bloodwork in 3 months to see if globulins are resolved. Specific cancers cause specific spikes and her results do not show those elevations, so that is good news. But we don't have a definite reason for her chronic inflammation. Owner understands and will relate to wife.
(b) (6)6	TC	(b) (6)	COMMUNICATIONS WITH DOCTOR - TENTATIVE (b) (6) 12:11 Spoke with (b) (6) at (b) (6); she notes monoclonal globulin spikes are most concerning because they define lymphoma, myeloma. Polyclonal spikes can be FIP, but also any cause of chronic inflammation. There is a chance chronic inflammation can be associated with neoplastic process though. She notes the degree of elevation is mild. If the owners want to work this up aggressively, full body imaging, best with ultrasound, to search for cancer. If they would like to monitor, recheck chemistry panel in 3 months and assess globulin count at that time. Only repeat electrophoresis if significantly higher elevation. If pet is losing weight, neoplasia moves up the list of differentials. Advised chronic otitis externa in this otherwise healthy patient; doctor concurs that wouldn't be sufficient to cause systemic response.
(b) (6)	L		**Chemistry results from (b) (6) (East) Requisition ID: 209396 Posted Final** **Test — Result — Reference Range** **ALB — 2.9 g/dL — 2.5 – 3.9** **TP — 8.2 g/dL — 5.2 – 8.8** **GLOB — 5.3 g/dL — 2.3 – 5.3** **ALPHA 1 — 0.3 g/dL — 0.2 – 1.1** **ALPHA 2 — 0.7 g/dL — 0.4 – 0.9** **BETA — 0.6 g/dL — 0.3 – 0.9** **GAMMA — 3.6 g/dL H — 0.3 – 2.5**
6/2/2016	L		**Miscellaneous results from (b) (6)**

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Patient History Report

Client: (b) (6)
Phone: (b) (6)
Address: (b) (6)
(b) (6)

Patient: (b) (6)
Species: Feline
Age: 8 Yrs. 0 Mos.
Color: brown tabby

Breed: Shorthair, Domestic
Sex: Spayed Female

Date	Type	Staff	History
			(East) Requisition ID: (b) (6) Posted Final **Ascn: (b) (6) Profile: Protein Electrophoresis,Serum** **RE: 1140 Interpreta** **The gamma globulin fraction is elevated, characterized by a broad** **polyclonal band, resulting from a mixture of increased immunoglobulins** **associated with an immune response. Potential causes include suppurative disease, chronic infectious disease (bacterial; protozoal;** **viral; rickettsial; fungal), connective tissue disease, chronic** **granulomatous disease, etc. Correlate with clinical findings.** **PATHOLOGIST:** **(b) (6), BVSc (Hons 1), DACVP** **(b) (6)** **(b) (6)** **Due to difference in method of analysis, there may be slight differences in the** **quantitative albumin and calculated globulin results between serum** **electrophoresis results compared to a generalchemistry panel.**
(b) (6)	C	(b) (6)	MEDICAL COMMENTS - Closed (b) (6) (b) (6) 18:35 Drew sample for protein electrophoresis while at the home for EOL care for (b) (6).
(b) (6)	B	(b) (6)	1.00 House Call Travel Level 2 (HC06) by (b) (6)
(b) (6)	B	(b) (6)	Laboratory Request / Sample Handling (LABS) by (b) (6)
(b) (6)	B	(b) (6)	1.00 Outside Lab (XTBALUO) by (b) (6)
(b) (6)	B	(b) (6)	1.00 Protein Electrophor. Serum (b) (6)T240 (L018) by (b) (6)
(b) (6)	B	(b) (6)	1.00 Sample Handling & Disposal (LFEE) by (b) (6)
(b) (6)	B	(b) (6)	1.00 Lab Sample Label (TL) by (b) (6)
(b) (6)	B	(b) (6)	1.00 Cared for by (b) (6) ((b) (6)) by (b) (6)
(b) (6)	C	(b) (6)	COMMUNICATIONS WITH CLIENT - Closed (b) (6) (b) (6) 16:21 (See full phone call under (b) (6) record)

B:Billing, C:Med note, CB:Call back, CK:Check-in, CM:Communications, D:Diagnosis, DH:Declined to history, E:Examination, ES:Estimates, I:Departing instr, L:Lab result, M:Image cases, P:Prescription, PA:PVL Accepted, PB:problems, PP:PVL Performed, PR:PVL Recommended, R:Correspondence, T:Images, TC:Tentative medl note, V:Vital signs

Patient History Report

Client: (b) (6)
Phone: (b) (6)
Address: (b) (6)
(b) (6)

Patient: (b) (6)
Species: Feline
Age: 8 Yrs. 0 Mos.
Color: brown tabby

Breed: Shorthair, Domestic
Sex: Spayed Female

Date	Type	Staff	History
			Discussed (b) (6) elevated globulins, need protein electrophoresis to better define the issue, can be chronic inflammatory response or a few types of cancer. If cancer, because they are (b) (6) clients they can consult with oncology at no charge to hear what the treatment options would be. They can bring (b) (6) into the office with a (b) (6) doctor for the bloodwork or I can do the bloodwork at the house. If I am doing it, I would not charge any recheck exam, just the travel and diagnostic test costs. If a (b) (6) in the hospital, they shouldn't have to charge for an exam either because she was just checked in late May. Owner likes (b) (6); advised he could schedule that with her.
(b) (6)	TC	(b) (6)	LAB RESULTS - NOTES - TENTATIVE (b) (6) 15:36 Lab Results: Fax from U of Cal Davis. Original Lab Date: - Attachment(s)
(b) (6)	C	(b) (6)	COMMUNICATIONS WITH DOCTOR - Closed (b) (6) (b) (6) 16:48 Spoke with doctor at (b) (6) consult line - she opted to rerun the full chemistry profile to validate the results since (b) (6) remaining profile is so normal. If globulins are truly elevated, protein electrophoresis is the next step. Ddx: myeloma, lymphoma, FIP, other neoplasia, chronic inflammatory condition. Asked specifically about taurine based on (b) (6) and current investigation into whole household's taurine status; not aware of any relationship between globulins and taurine.
(b) (6)	C	(b) (6)	COMMUNICATIONS WITH CLIENT - Closed (b) (6) (b) (6) 16:46 Spoke with Mrs; (b) (6) has elevated globulins which can indicate cancer or a chronic inflammatory condition. Spoke with specialist and no correlation with taurine deficiency. Lab is going to re-run her full profile to validate the results. Expect an update in 1-2 days. If verified, we may need to collect additional blood for the next level of testing which tells us which specific pattern of globulins is elevated. Taurine pending, will call.
(b) (6)	C	(b) (6)	GP PHYSICAL EXAM - Closed (b) (6) Date Presented: (b) (6) Chief Complaint: Wellness/Taurine check History: Doing well. Normal activity level. Normal ur/def. No known V/D/C/S. 5 cat, 2 dog household; she is one of 4 cats that live together in a room above the

B:Billing, C:Med note, CB:Call back, CK:Check-in, CM:Communications, D:Diagnosis, DH:Declined to history, E:Examination, ES:Estimates, I:Departing instr, L:Lab result, M:Image cases, P:Prescription, PA:PVL Accepted, PB:problems, PP:PVL Performed, PR:PVL Recommended, R:Correspondence, T:Images, TC:Tentative medl note, V:Vital signs

Patient History Report

Client: (b) (6)
Phone: (b) (6)
Address: (b) (6)
(b) (6)

Patient: (b) (6)
Species: Feline
Age: 8 Yrs. 0 Mos.
Color: brown tabby

Breed: Shorthair, Domestic
Sex: Spayed Female

Date	Type	Staff	History
			garage. Indoor only. (b) (6) has DCM from taurine deficiency, checking status of other cats in household. Diet (type, freq, amt): Merrick Purrfect Bistro Grain Free chicken dry food Meds: none S: BAR, PCS 0/4 O: MM / ORPH: Pink, moist, crt <2 sec, small suspect FORL right upper PM3, mild tartar overall. E/E: copious black debris AU, mildly pruritic while cleaning. ophtho WNL. INT: WNL; no evidence of ectoparasites observed PLN: WNL CV / Resp: Reg rhythm, no murmur, SSP, clear and eupneic GI/UG: Compliant, no masses MS/NS: Normal amb x4 BCS: 3/5 4.15kg A: 8yr FS DSH 1) otitis externa - r/o bacterial/fungal vs ear mites 2) dental disease P: PE Taurine level CBC/Vetscreen Disp Tresaderm 7.5ml - apply 2-3 drops in each ear twice daily for 7-10 days, keep in fridge ear cleaning PureVax Rabies 1yr SQ right hind (lot#17390B, exp 12/11/2016) Discussed ear infection and treatment. Will call with lab results; systemic early next week, taurine in 7-10 days.
5/21/2016	I	(b) (6)	An animal is not considered immunized for at least 28 days after the initial or primary vaccination is administered. For this reason, pets receiving their first rabies vaccine should not be left outdoors unattended.
5/21/2016	P	(b) (6)	1.00 bottle of Tresaderm 7.5ml (Merial] (M225) Rx #: 2574865 0 Of 0 Refills Apply 2-3 drops in each ear twice daily for 7-10 days.
5/21/2016	V	(b)	May 21, 2016 11:15 AM Staff: (b) ---------- Weight : 4.15 kilograms HC-RS scale
5/21/2016	L		**Hematology results from (b) (6) (East) Requisition ID: 209396 Posted Final**

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Patient History Report

Client: (b) (6)
Phone: (b) (6)
Address: (b) (6)
(b) (6)

Patient:
Species: Feline
Age: 8 Yrs. 0 Mos.
Color: brown tabby
Breed: Shorthair, Domestic
Sex: Spayed Female

Date	Type	Staff	History

Test	Result	Reference Range
HCT	31 %	29 – 48
HGB	11.0 g/dL	9.3 – 15.9
MCHC	35.5 g/dL	30 – 38
WBC	13.4 10^3/uL	3.5 – 16.0
Bands	0 %	0 – 3
RBC	6.7 10^6/uL	5.92 – 9.93
MCV	46 fL	37 – 61
MCH	16.4 pg	11 – 21
ABS BASO	0 /uL	0 – 150
ABS NEUTB	0 /uL	0 – 150
Platelet C	375 10^3/uL	200 – 500
Platelet E	ADEQUATE	ADEQUATE –
Neutrophil	55 %	35 – 75
Lymphocyte	37 %	20 – 45
Monocytes	2 %	1 – 4
Eosinophil	6 %	2 – 12
Basophils	0 %	0 – 1
Absolute N	7370 /uL	2500 – 8500
Absolute L	4958 /uL	1200 – 8000
Absolute M	268 /uL	0 – 600
Absolute E	804 /uL	0 – 1000

Ascn: (b) (6) Profile: Complete Blood Count

5/21/2016 L

Chemistry results from (b) (6) (East) Requisition
ID: 209396 Posted Final

Test	Result	Reference Range
ALB	2.6 g/dL	2.5 – 3.9
ALKP	18 U/L	6 – 102
ALT	14 U/L	10 – 100
AST	11 U/L	10 – 100
BUN/UREA	29 mg/dL	14 – 36
Ca	8.8 mg/dL	8.2 – 10.8
Chloride	113 mEq/L	104 – 128
CHOL	94 mg/dL	75 – 220
CK	62 U/L	56 – 529
CREA	1.2 mg/dL	0.6 – 2.4
GLU	79 mg/dL	64 – 170
PHOS	5.7 mg/dL	2.4 – 8.2
Potassium	4.8 mEq/L	3.4 – 5.6
Sodium	148 mEq/L	145 – 158
TBIL	0.1 mg/dL	0.1 – 0.4
TP	9.1 g/dL H	5.2 – 8.8
GLOB	6.5 g/dL H	2.3 – 5.3
A/G Ratio	0.4 Ratio	0.35 – 1.5
B/C Ratio	24 Ratio	4 – 33
Na/K Ratio	31	

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Patient History Report

Client: (b) (6)
Phone: (b) (6)
Address: (b) (6)
(b) (6)

Patient: (b) (6)
Species: Feline
Age: 8 Yrs. 0 Mos.
Color: brown tabby

Breed: Shorthair, Domestic
Sex: Spayed Female

Date	Type	Staff	History
5/21/2016	L		**Miscellaneous results from** (b) (4) **(East) Requisition ID: 209396 Posted Final** **Ascn:** (b) (6) **Profile: Vet Screen** **RE: 11067 Comment** **Hemolysis 1+ No significant interference.**
5/21/2016	B	(b) (6)	1.00 At Home Additional Pet Appointment (HC03) by b) (6)
5/21/2016	B	(b) (6)	Laboratory Request / Sample Handling (LABS) by (b) (6)
5/21/2016	B	(b) (6)	1.00 Outside Lab (XTBALUO) by (b) (6)
5/21/2016	B	(b) (6)	1.00 Vetscreen Cbc Antec SA030 (L00030) by (b) (6)
5/21/2016	B	(b) (6)	1.00 Sample Handling & Disposal (LFEE) by (b) (6)
5/21/2016	B	(b) (6)	1.00 Lab Sample Label (TL) by (b) (6)
5/21/2016	B	(b) (6)	1.00 Fel Vax Rabies 1 Year Purevax (Merial) (V21) by (b) (6)
5/21/2016	B	(b) (6)	1.00 bottle of Tresaderm 7.5ml (Merial] (M225) by (b) (6)
5/21/2016	B	(b) (6)	1.00 Cared for by (b) (6) by (b) (6)
(b) (6)	C	(b) (6)	COMMUNICATIONS WITH CLIENT - Closed (b) (6) (b) (6) 15:04 Called to confirm tomorrow's appointment fro (b) (6), (b) (6), (b) (6) and (b) (6) at 9 am. I also mentioned in my message that we should use the address (b) (6) in the GPS. If any questions please call (b) (6)

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Patient History Report

Client: (b) (6)
Phone: (b) (6)
Address: (b) (6)

Patient: (b) (6)
Species: Feline
Age: 9 Yrs. 10 Mos.
Color: Calico

Breed: Longhair, Domestic
Sex: Male

Date	Type	Staff	History
6/1/2016	TC	(b) (6)	MEDICAL COMMENTS - TENTATIVE (b) (6) 10:46 SW (b) (6) at Merrick - updated company that we have documented taurine deficiency in 2 other cats in house hold. The quality assurance team indicated that the level of taurine in the lot # I gave them was sufficient - discussed that this likely takes 3-6 month to develop and likely to be related to earlier lot and they need to investigate further. Asked if the level they gave me was from them retesting the food after I called or from an earlier test - not sure. Asked for information from the taurine tests - told I can send summary of lab results. Also indicated that I am reporting this to the FDA. She give new info to her manager and quality assurance. Told them I expect them to follow up with me. Below email sent to Merrick: Taurine Levels (b) (6) To: (b) (6)@merrickpetcare.com Hi (b) (6), Thank you for your help with these cases. Here is the summary of the lab results: 12yr female spayed domestic short hair diagnosed and clinical for dilated cardiomyopathy -5/9/2016 Plasma Taurine 24nmol/ml (normal 60-120, critical level <40) - test performed at University of Wisconsin, results were received on 5/15/2016 5/21/2016 - Whole Blood Taurine submitted at the University of California Davis on remaining 4 cats consuming this food (normal 300-600 nmol/ml, no known risk for deficiency >200), results were received on 5/27/2016 -9yr male neutered domestic long hair: 196 nmol/ml -8y female spayed domestic short hair: 368 nmol/ml -9yr male neutered domestic long hair: 124 nmol/ml -9yr male neutered domestic long hair: 536 nmol/ml Please let me know if you have any other questions. Sincerely, (b) (6) (b) (6), DVM Diplomate ACVN Clinical Nutrition Department

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Patient History Report

Client: (b) (6)
Phone: (b) (6)
Address: (b) (6)
(b) (6)

Patient: (b) (6)
Species: Feline
Age: 9 Yrs. 10 Mos.
Color: Calico

Breed: Longhair, Domestic
Sex: Male

Date	Type	Staff	History
			(b) (6)
5/27/2016	TC	(b) (6)	LAB RESULTS - NOTES - TENTATIVE 5/27/2016 15:34 Lab Results: Fax from U of Cal Davis. Original Lab Date: - Attachment(s)
5/24/2016	C	(b) (6)	COMMUNICATIONS WITH CLIENT - Closed May 26/2016 5/24/2016 16:50 Spoke with Mrs; systemic blood results WNL for (b) (6). Taurine pending.
5/21/2016	C	(b) (6)	GP PHYSICAL EXAM - Closed May 26/2016 Date Presented: 5/21/2016 Chief Complaint: Wellness/Taurine check History: Doing well. Normal activity level. Normal ur/def. No known V/D/C/S. 5 cat, 2 dog household; he is one of 4 cats that live together in a room above the garage. Indoor only (b) (6) has DCM from taurine deficiency, checking status of other cats in household. Diet (type, freq, amt): Merrick Purrfect Bistro Grain Free chicken dry food Meds: none S: BAR, PCS 0/4 O: MM / ORPH: Pink, moist, crt <2 sec, moderate tartar overall E/E: ophtho/otoscopic exams WNL INT: no evidence of ectoparasites observed. matted hair present. PLN: WNL CV / Resp: Reg rhythm, no murmur, SSP, clear and eupneic GI/UG: Compliant, no masses MS/NS: Normal amb x4 BCS: 4/5 9.5kg A: 9yr9mo MN DLH 1) overweight 2) dental disease

B:Billing, C:Med note, CB:Call back, CK:Check-in, CM:Communications, D:Diagnosis, DH:Declined to history, E:Examination, ES:Estimates, I:Departing instr, L:Lab result, M:Image cases, P:Prescription, PA:PVL Accepted, PB:problems, PP:PVL Performed, PR:PVL Recommended, R:Correspondence, T:Images, TC:Tentative medl note, V:Vital signs

Patient History Report

Client: (b) (6)
Phone: (b) (6)
Address: (b) (6)
(b) (6)

Patient: (b) (6)
Species: Feline
Age: 9 Yrs. 10 Mos.
Color: Calico

Breed: Longhair, Domestic
Sex: Male

Date	Type	Staff	History

3) matted hair
P: PE
Taurine level
CBC/Vetscreen

Recommend weight loss, frequent brushing +/- clippers to remove mats or using a groomer. Dental condition warrants treatment. Will call with systemic blood results early next week, taurine level in 7-10 days.

5/21/2016 V (b)

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May 21, 2016 11:21 AM Staff: (b)
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Weight            : 9.50 kilograms
   HC-RS scale
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5/21/2016 L (b) (6)

Hematology results from (b) (6) (East) Requisition

ID: 209396 Posted Final

Test	Result	Reference Range
HCT	40 %	29 – 48
HGB	12.3 g/dL	9.3 – 15.9
MCHC	30.8 g/dL	30 – 38
WBC	11.6 10^3/uL	3.5 – 16.0
Bands	0 %	0 – 3
RBC	7.9 10^6/uL	5.92 – 9.93
MCV	51 fL	37 – 61
MCH	15.6 pg	11 – 21
ABS BASO	0 /uL	0 – 150
ABS NEUTB	0 /uL	0 – 150
Platelet C	188 10^3/uL L	200 – 500
Platelet E	ADEQUATE	ADEQUATE –
Neutrophil	72 %	35 – 75
Lymphocyte	21 %	20 – 45
Monocytes	3 %	1 – 4
Eosinophil	4 %	2 – 12
Basophils	0 %	0 – 1
Absolute N	8352 /uL	2500 – 8500
Absolute L	2436 /uL	1200 – 8000
Absolute M	348 /uL	0 – 600
Absolute E	464 /uL	0 – 1000

Ascn: (b) (6) Profile: Complete Blood Count Ascn: (b) (6) Profile: Complete Blood Count

Platelet count reflects the minimum number due to platelet clumping.

5/21/2016 L (b) (6)

Chemistry results from (b) (6) (East) Requisition

Patient History Report

Client: (b) (6)
Phone: (b) (6)
Address: (b) (6)
(b) (6)

Patient: (b) (6)
Species: Feline
Age: 9 Yrs. 10 Mos.
Color: Calico

Breed: Longhair, Domestic
Sex: Male

Date	Type	Staff	History

ID: (b) (6) Posted Final

Test	Result	Reference Range
ALB	3.1 g/dL	2.5 - 3.9
ALKP	27 U/L	6 - 102
ALT	64 U/L	10 - 100
AST	44 U/L	10 - 100
BUN/UREA	26 mg/dL	14 - 36
Ca	9.3 mg/dL	8.2 - 10.8
Chloride	112 mEq/L	104 - 128
CHOL	98 mg/dL	75 - 220
CK	157 U/L	56 - 529
CREA	1.2 mg/dL	0.6 - 2.4
GLU	90 mg/dL	64 - 170
PHOS	5.9 mg/dL	2.4 - 8.2
Potassium	5.1 mEq/L	3.4 - 5.6
Sodium	150 mEq/L	145 - 158
TBIL	0.1 mg/dL	0.1 - 0.4
TP	8.4 g/dL	5.2 - 8.8
GLOB	5.3 g/dL	2.3 - 5.3
A/G Ratio	0.6 Ratio	0.35 - 1.5
B/C Ratio	22 Ratio	4 - 33
Na/K Ratio	29	

5/21/2016 L (b) (6)

Miscellaneous results from (b) (6)
(East) Requisition ID: 209396 Posted Final
Ascn: (b) (6) Profile: Vet Screen
RE: 11067 Comment
Hemolysis 1+ No significant interference.
Ascn: (b) (6) Profile: Vet Screen
RE: 11067 Comment
Hemolysis 1+ No significant interference.

5/21/2016 B (b) (6) 1.00 At Home Additional Pet Appointment (HC03) by (b) (6)
5/21/2016 B (b) (6) Laboratory Request / Sample Handling (LABS) by (b) (6)
5/21/2016 B (b) (6) 1.00 Outside Lab (XTBALUO) by (b) (6)
5/21/2016 B (b) (6) 1.00 Vetscreen Cbc Antec SA030 (L00030) by (b) (6)
5/21/2016 B (b) (6) 1.00 Sample Handling & Disposal (LFEE) by (b) (6)
5/21/2016 B (b) (6) 1.00 Lab Sample Label (TL) by (b) (6)
5/21/2016 B (b) (6) 1.00 Cared for by (b) (6) ((b) (6)) by (b) (6)

5/20/2016 C (b) (6) COMMUNICATIONS WITH CLIENT - Closed May 21/2016
5/20/2016 15:04
Called to confirm tomorrow's appointment fro (b) (6), (b) (6), (b) (6) and (b) (6) at 9

B:Billing, C:Med note, CB:Call back, CK:Check-in, CM:Communications, D:Diagnosis, DH:Declined to history, E:Examination, ES:Estimates, I:Departing instr, L:Lab result, M:Image cases, P:Prescription, PA:PVL Accepted, PB:problems, PP:PVL Performed, PR:PVL Recommended, R:Correspondence, T:Images, TC:Tentative medl note, V:Vital signs

Patient History Report

Client: (b) (6)
Phone: (b) (6)
Address: (b) (6)
(b) (6)

Patient: (b) (6)
Species: Feline
Age: 9 Yrs. 10 Mos.
Color: Calico

Breed: Longhair, Domestic
Sex: Male

Date	Type	Staff	History
			am. I also mentioned in my message that we should use the address (b) (6) (b) (6) in the GPS. If any questions please call (b) (6)

B:Billing, C:Med note, CB:Call back, CK:Check-in, CM:Communications, D:Diagnosis, DH:Declined to history, E:Examination, ES:Estimates, I:Departing instr, L:Lab result, M:Image cases, P:Prescription, PA:PVL Accepted, PB:problems, PP:PVL Performed, PR:PVL Recommended, R:Correspondence, T:Images, TC:Tentative medl note, V:Vital signs

Patient History Report

Client: (b) (6)
Phone: (b) (6)
Address: (b) (6)
(b) (6)

Patient: (b) (6)
Species: Feline
Age: 9 Yrs. 7 Mos.
Color:

Breed: Longhair, Domestic
Sex: Neutered Male

Date	Type	Staff	History
6/7/2016	TC	(b) (6)	COMMUNICATIONS WITH CLIENT - TENTATIVE 6/7/2016 12:32 While speaking with owner about (b) (6), discussed (b) (6) dental. Spends the day at 197, but most often home that same night after procedure. Bloodwork is good for 2 months. Can schedule with GP or dentistry according to owner's preference.
5/27/2016	TC	(b) (6)	LAB RESULTS - NOTES - TENTATIVE 5/27/2016 15:38 Lab Results: Fax from U of Cal Davis. Original Lab Date: - Attachment(s)
5/24/2016	C	(b) (6)	COMMUNICATIONS WITH CLIENT - Closed May 26/2016 5/24/2016 16:51 Spoke with Mrs; bloodwork WNL, excellent news for planning anesthesia and dental work. Important that taurine status is addressed prior to anesthesia, but dental work should be planned for the next 4-8 weeks. Taurine pending, will call.
5/21/2016	C	(b) (6)	GP PHYSICAL EXAM - Closed May 26/2016 Date Presented: 5/21/2016 Chief Complaint: Wellness/Taurine check History: Doing well. Normal activity level. Normal ur/def. No known V/D/C/S. 5 cat, 2 dog household; he is the one cat who lives in the house ((b) (6) is aggressive toward (b) (6), so he lives away from other cats). Indoor only. (b) (6) has DCM from taurine deficiency, checking status of other cats in household. Diet (type, freq, amt): Merrick Purrfect Bistro Grain Free chicken dry food Meds: none S: BAR, PCS 0/4 O: MM / ORPH: Pink, moist, crt <2 sec; right upper canine tooth loose, significant gingivitis locally. heavy tartar on PM3s bilaterally. missing incisors. E/E: brown debris in outer ear cartilages bilaterally, but canals clean/free of debris. ophtho exam WNL. INT: matted hair. no evidence of ectoparasites observed. PLN: WNL CV / Resp: Reg rhythm, no murmur, SSP, clear and eupneic GI/UG: Compliant, no masses

B:Billing, C:Med note, CB:Call back, CK:Check-in, CM:Communications, D:Diagnosis, DH:Declined to history, E:Examination, ES:Estimates, I:Departing instr, L:Lab result, M:Image cases, P:Prescription, PA:PVL Accepted, PB:problems, PP:PVL Performed, PR:PVL Recommended, R:Correspondence, T:Images, TC:Tentative medl note, V:Vital signs

Patient History Report

Client: (b) (6)
Phone: (b) (6)
Address: (b) (6)
(b) (6)

Patient: (b) (6)
Species: Feline
Age: 9 Yrs. 7 Mos.
Color:

Breed: Longhair, Domestic
Sex: Neutered Male

Date	Type	Staff	History

MS/NS: Normal amb x4
BCS: 3-3.5/5 6.7kg
A: 9yr7mo MN DLH
1) dental disease
2) matted hair
P: PE
Taurine level
CBC/Superchem
PureVax Rabies 1yr SQ right hind (lot# 17390B, exp 12/11/2016)

Advised dental status is poor and likely painful; recommend prompt dental cleaning under general anesthesia with extraction of canine +/- other teeth. Can use dental specialists or general practitioner depending on owner's preference. Recommend waiting for taurine level and any management pertaining to that issue before scheduling anesthetic procedure. Can be shaved down during anesthesia; frequent brushing +/- intermittent clipping or taking to a groomer is needed. Will call with systemic blood results early next week; taurine level will take 7-10 days.

5/21/2016 I (b) (6)

An animal is not considered immunized for at least 28 days after the initial or primary vaccination is administered. For this reason, pets receiving their first rabies vaccine should not be left outdoors unattended.

5/21/2016 V (b)

```
May 21, 2016 11:24 AM Staff: (b)
------------------------------
Weight            : 6.70 kilograms
   HC-RS scale
```

5/21/2016 L

Hematology results from (b) (6) (East) Requisition ID: 209396 Posted Final

Test	Result	Reference Range
HCT	36 %	29 - 48
HGB	11.8 g/dL	9.3 - 15.9
MCHC	32.8 g/dL	30 - 38
WBC	9.8 10^3/uL	3.5 - 16.0
Bands	0 %	0 - 3
RBC	7.9 10^6/uL	5.92 - 9.93
MCV	46 fL	37 - 61
MCH	14.9 pg	11 - 21
ABS BASO	0 /uL	0 - 150
ABS NEUTB	0 /uL	0 - 150
Platelet C	490 10^3/uL	200 - 500
Platelet E	ADEQUATE	ADEQUATE -
Neutrophil	59 %	35 - 75
Lymphocyte	33 %	20 - 45
Monocytes	2 %	1 - 4
Eosinophil	6 %	2 - 12

B:Billing, C:Med note, CB:Call back, CK:Check-in, CM:Communications, D:Diagnosis, DH:Declined to history, E:Examination, ES:Estimates, I:Departing instr, L:Lab result, M:Image cases, P:Prescription, PA:PVL Accepted, PB:problems, PP:PVL Performed, PR:PVL Recommended, R:Correspondence, T:Images, TC:Tentative medl note, V:Vital signs

Patient History Report

Client: (b) (6)
Phone: (b) (6)
Address: (b) (6)
(b) (6)

Patient: (b) (6)
Species: Feline
Age: 9 Yrs. 7 Mos.
Color:

Breed: Longhair, Domestic
Sex: Neutered Male

Date	Type	Staff	History

Test	Result	Reference Range
Basophils	0 %	0 – 1
Absolute N	5782 /uL	2500 – 8500
Absolute L	3234 /uL	1200 – 8000
Absolute M	196 /uL	0 – 600
Absolute E	588 /uL	0 – 1000

Ascn: (b) (6) Profile: Complete Blood Count

5/21/2016 L

Chemistry results from (b) (6) (East) Requisition ID: 209396 Posted Final

Test	Result	Reference Range
ALB	3.8 g/dL	2.5 – 3.9
ALKP	25 U/L	6 – 102
ALT	32 U/L	10 – 100
AMYL	1067 U/L	100 – 1200
AST	14 U/L	10 – 100
BUN/UREA	32 mg/dL	14 – 36
Ca	9.9 mg/dL	8.2 – 10.8
Chloride	112 mEq/L	104 – 128
CHOL	125 mg/dL	75 – 220
CK	76 U/L	56 – 529
CREA	1.3 mg/dL	0.6 – 2.4
GGT	1 U/L	1 – 10
GLU	99 mg/dL	64 – 170
Mg	2.2 mEq/L	1.5 – 2.5
PHOS	5.5 mg/dL	2.4 – 8.2
Potassium	5.1 mEq/L	3.4 – 5.6
Sodium	150 mEq/L	145 – 158
TBIL	0.1 mg/dL	0.1 – 0.4
TP	7.8 g/dL	5.2 – 8.8
TRIG	97 mg/dL	25 – 160
GLOB	4.0 g/dL	2.3 – 5.3
A/G Ratio	1.0 Ratio	0.35 – 1.5
B/C Ratio	25 Ratio	4 – 33
Na/K Ratio	29	

5/21/2016 L

Miscellaneous results from (b) (6) (East) Requisition ID: 209396 Posted Final
Ascn: (b) (6) Profile: Superchem
RE: 1045 PrecisionP 17 U/L 8 – 26
Acute pancreatitis is unlikely. Chronic pancreatitis is not excluded by a
normal PrecisionPSL.
RE: 11067 Comment
Hemolysis 1+ No significant interference.

B:Billing, C:Med note, CB:Call back, CK:Check-in, CM:Communications, D:Diagnosis, DH:Declined to history, E:Examination, ES:Estimates, I:Departing instr, L:Lab result, M:Image cases, P:Prescription, PA:PVL Accepted, PB:problems, PP:PVL Performed, PR:PVL Recommended, R:Correspondence, T:Images, TC:Tentative medl note, V:Vital signs

Patient History Report

Client: (b) (6)
Phone: (b) (6)
Address: (b) (6)
(b) (6)

Patient: (b) (6)
Species: Feline
Age: 9 Yrs. 7 Mos.
Color:

Breed: Longhair, Domestic
Sex: Neutered Male

Date	Type	Staff	History
5/21/2016	B	(b) (6)	1.00 At Home Additional Pet Appointment (HC03) by (b) (6)
5/21/2016	B		Laboratory Request / Sample Handling (LABS) by (b) (6)
5/21/2016	B	(b) (6)	1.00 Outside Lab (XTBALUO) by (b) (6)
5/21/2016	B	(b) (6)	1.00 Superchem Cbc (b) (6) Sa020 (L07) by (b) (6)
5/21/2016	B	(b) (6)	1.00 Sample Handling & Disposal (LFEE) by (b) (6)
5/21/2016	B	(b) (6)	1.00 Lab Sample Label (TL) by (b) (6)
5/21/2016	B		1.00 Fel Vax Rabies 1 Year Purevax (Merial) (V21) by (b) (6)
5/21/2016	B		1.00 Cared for by (b) (6) by (b) (6)
5/20/2016	C	(b) (6)	COMMUNICATIONS WITH CLIENT - Closed May 21/2016 5/20/2016 15:05 Called to confirm tomorrow's appointment fro (b) (6), (b) (6), (b) (6) and (b) (6) at 9 am. I also mentioned in my message that we should use the address (b) (6) (b) (6) in the GPS. If any questions please call (b) (6)

B:Billing, C:Med note, CB:Call back, CK:Check-in, CM:Communications, D:Diagnosis, DH:Declined to history, E:Examination, ES:Estimates, I:Departing instr, L:Lab result, M:Image cases, P:Prescription, PA:PVL Accepted, PB:problems, PP:PVL Performed, PR:PVL Recommended, R:Correspondence, T:Images, TC:Tentative medl note, V:Vital signs

Report Details - EON-266821

ICSR:	1053339	
Type Of Submission:	Initial	
Report Version:	FPSR.FDA.PETF.V.V1	
Type Of Report:	Adverse Event (a symptom, reaction or disease associated with the product)	
Reporting Type:	Voluntary	
Report Submission Date:	2016-06-06 11:44:41 EDT	
Reported Problem:	**Problem Description:**	Another household cat diagnosed with dilated cardiomyopathy and taurine deficiency - separate report filed (FDA ICSR ID 1053335). Euthanized on (b) (6) due to aortic thromboembolism. Review of the patient's diet history revealed that all 5 cats in household had been fed Merrick Purrfect Bistro Grain Free Real Chicken Recipe Feline dry for approximately 3 years. Remaining 4 cats in household tested for taurine deficiency - whole blood samples submitted to University of California Davis (normal 300-600 nmol/ml, no known risk for deficiency >200), results received on 5/27/16 - (b) (6) 196nmol/ml - started on taurine supplementation 250mg PO BID for 2-3 weeks. Diet was changed at the time of other cat's diagnosis (5/15/15). Patient also diagnosed with hyperthyroidism on same day as blood submitted for taurine testing - history of weight loss. An echo was not performed on this patient therefore it is unknown if he had evidence of DCM.
	Date Problem Started:	05/27/2016
	Concurrent Medical Problem:	No
	Outcome to Date:	Not Applicable
Product Information:	**Product Name:**	Merrick Purrfect Bistro Grain Free Real Chicken Recipe
	Product Type:	Pet Food
	Lot Number:	**Lot Number:** 16025 DL1 38310 14131 **Expiration Date:** 07/26/2017
	UPC:	2280838310
	Package Type:	BAG
	Package Size:	5.4 kilogram
	Number Purchased:	1
	Possess Opened Product:	Yes
	Storage Conditions:	stored in bag indoors
	Product Use Information:	**Description:** fed to cats in bowl **Last Exposure Date:** 05/15/2016 **Product Use Stopped After the Onset of the Adverse Event:** Yes **Perceived Relatedness to Adverse Event:** Definitely related **Other Foods or Products Given to the Animal During This Time Period:** No
	Manufacturer/Distributor Information:	**Name:** Merrick Pet Care, Inc **Type(s):** Manufacturer **Address:** P.O. Box 9800 Amarillo Texas 79105 United States

		Contact:	Phone:	18006647387
			Web Address:	www.merrickpetcare.com
		Possess One or More Labels from This Product:	Yes	
	Purchase Location Information:	Name:	(b) (6)	
		Address:	(b) (6) United States	
Animal Information:	Name:	(b) (6)		
	Type Of Species:	Cat		
	Type Of Breed:	Mixed (Cat)		
	Gender:	Male		
	Reproductive Status:	Neutered		
	Weight:	4.4 Kilogram		
	Age:	9 Years		
	Assessment of Prior Health:	Fair		
	Number of Animals Given the Product:	5		
	Number of Animals Reacted:	3		
	Owner Information:	Owner Information provided:	Yes	
		Contact:	Name:	(b) (6)
			Phone:	(b) (6)
		Address:	(b) (6) United States	
	Healthcare Professional Information:	Practice Name:	(b) (6)	
		Contact:	Name:	(b) (6)
			Phone:	(b) (6)
			Email:	(b) (6)
		Address:	(b) (6) United States	
Sender Information:	Name:	(b) (6)		
	Address:	(b) (6) United States		
	Contact:	Phone:	(b) (6)	
		Email:	(b) (6)	
	Permission To Contact Sender:	Yes		

	Preferred Method Of Contact:	Email
	Reported to Other Parties:	Manufacturer
Additional Documents:		

Report Details - EON-345822

ICSR:	2040525	
Type Of Submission:	Initial	
Report Version:	FPSR.FDA.PETF.V.V1	
Type Of Report:	Adverse Event (a symptom, reaction or disease associated with the product)	
Reporting Type:	Voluntary	
Report Submission Date:	2018-01-22 17:19:17 EST	
Reported Problem:	**Problem Description:**	(b) (6) was presented for evaluation of cough, labored breathing, multiple episodes of collapse, cardiomegaly, and suspected congestive heart failure. Congestive heart failure was confirmed with thoracic radiographs and echocardiogram revealed dilated cardiomyopathy.
	Date Problem Started:	12/30/2017
	Concurrent Medical Problem:	Yes
	Pre Existing Conditions:	Canine atopy controlled with current treatment of sublingual immunotherapy and Apoquel.
	Outcome to Date:	Better/Improved/Recovering
Product Information:	**Product Name:**	California Natural Grain-Free Kangaroo and Red Lentils Recipe
	Product Type:	Pet Food
	Lot Number:	
	Package Type:	BAG
	Purchase Date:	01/02/2018
	Possess Unopened Product:	No
	Possess Opened Product:	Yes
	Product Use Information:	**Description:** (b) (6) had been eating this dog food since she first displaced signs of pruritis as a puppy and food allergy was considered as a potential contributor.
		Last Exposure Date: 01/10/2018
		Time Interval between Product Use and Adverse Event: 6 Years
		Product Use Stopped After the Onset of the Adverse Event: Yes
		Adverse Event Abate After Product Stop: Unknown
		Product Use Started Again: No
		Perceived Relatedness to Adverse Event: Probably related
		Other Foods or Products Given to the Animal During This Time Period: Yes
	Manufacturer /Distributor Information:	
	Purchase Location Information:	

Animal Information:	**Name:**	(b) (6)
	Type Of Species:	Dog
	Type Of Breed:	Mixed (Dog)
	Gender:	Female
	Reproductive Status:	Neutered
	Weight:	25.1 Kilogram
	Age:	6 Years
	Assessment of Prior Health:	Good
	Number of Animals Given the Product:	4
	Number of Animals Reacted:	4
	Owner Information:	**Owner Information provided:** Yes
		Contact: **Name:** (b) (6)
		Phone: (b) (6)
		Email: (b) (6)
		Address: (b) (6) United States
	Healthcare Professional Information:	**Practice Name:** (b) (6)
		Contact: **Name:** (b) (6)
		Phone: (b) (6)
		Other Phone: (b) (6)
		Email: (b) (6)
		Address: (b) (6) United States
Sender Information:	**Name:**	(b) (6)
	Address:	(b) (6) United States
	Contact:	**Phone:** (b) (6)
		Other Phone: (b) (6)
		Email: (b) (6)
	Permission To Contact Sender:	Yes
	Preferred Method Of Contact:	Phone
	Reported to Other Parties:	Other
Additional Documents:		
	Attachment:	Medical record.pdf
	Description:	Medical record and echo report
	Type:	Medical Records

Attachment:	Taurine level.pdf
Description:	Taurine level
Type:	Laboratory Report

Attachment:	Listserve on kangaroo and lentil diets.pdf
Description:	Discussion amongst veterinary cardiologists of dilated cardiomyopathy in patients eating either kangaroo and lentil or vegan diets with lentils
Type:	Other

17962

Sample Submission Form

UC CUSTOMERS ONLY:
Non-federal funds ID/Account Number to bill:_____

Amino Acid Laboratory
University of California, Davis
1020 Vet Med 3B
1089 Veterinary Medicine Drive
Davis, CA 95616
Tel: (530)752-5058, Fax: (530)752-4698

http://www.vetmed.ucdavis.edu/vmb/aal/aal.html

Vet/Tech Contact: Account # (b) (6) / Contact: (b) (6) Date: 1-10-18
Company Name: (b) (6)
Address: (b) (6)
(b) (6)

Email: (b) (6)
Tel: (b) (6) Fax: (b) (6)

Billing Contact: (b) (6) TAX ID:_____
Email: (b) (6) Tel: (b) (6)

Patient Name: (b) (6)
Species: K9
Owner's Name: (b) (6)

Sample Type: ☐ Plasma ☑ Whole Blood ☐ Urine ☐ Food ☐ Other:_____
Test Items: ☑ Taurine ☐ Complete Amino Acid ☐ Other:_____

Taurine Results (nmol/ml)

Plasma:_____ Whole Blood: 292 Urine:_____ Food:_____

Reference Ranges (nmol/ml)

	Plasma		Whole Blood	
	Normal Range	No Known Risk for Taurine Deficiency	Normal Range	No Known Risk for Taurine Deficiency
Cat	80-120	>40	300-600	>200
Dog	60-120	>40	200-350	>150

From: Norris, Anne
To: Rotstein, David; Hartogensis, Martine; DeLancey, Siobhan
Subject: RE: Calls Complete
Date: Tuesday, June 25, 2019 9:13:41 AM

Thought you'd find this interesting https://www.petfoodprocessing.net/articles/13194-midwestern-manufacturer-debuts-legume-free-dog-foods

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From: Rotstein, David
Sent: Monday, June 24, 2019 12:57 PM
To: Hartogensis, Martine <Martine.Hartogensis@fda.hhs.gov>; Norris, Anne <Anne.Norris@fda.hhs.gov>; DeLancey, Siobhan <Siobhan.Delancey@fda.hhs.gov>
Subject: RE: Calls Complete

Martine deserves a lot of credit. Some of the firms were challenging.

From: Hartogensis, Martine <Martine.Hartogensis@fda.hhs.gov>
Date: June 24, 2019 at 12:22:40 PM EDT
To: Norris, Anne <Anne.Norris@fda.hhs.gov>, DeLancey, Siobhan <Siobhan.Delancey@fda.hhs.gov>
Cc: Rotstein, David <David.Rotstein@fda.hhs.gov>
Subject: RE: Calls Complete

No problem. It was an interesting morning!

Martine

From: Norris, Anne
Sent: Monday, June 24, 2019 12:15 PM
To: Hartogensis, Martine <Martine.Hartogensis@fda.hhs.gov>; DeLancey, Siobhan <Siobhan.Delancey@fda.hhs.gov>
Cc: Rotstein, David <David.Rotstein@fda.hhs.gov>
Subject: RE: Calls Complete

Understood, just confirming. Thanks for handling this, I know it was an unpleasant situation.

From: Hartogensis, Martine
Sent: Monday, June 24, 2019 12:13 PM
To: Norris, Anne <Anne.Norris@fda.hhs.gov>; DeLancey, Siobhan <Siobhan.Delancey@fda.hhs.gov>
Cc: Rotstein, David <David.Rotstein@fda.hhs.gov>
Subject: RE: Calls Complete

No – I thought the plan was (b) (5). We had several firms that were pretty upset BTW.

From: Norris, Anne
Sent: Monday, June 24, 2019 12:11 PM
To: DeLancey, Siobhan <Siobhan.Delancey@fda.hhs.gov>; Hartogensis, Martine <Martine.Hartogensis@fda.hhs.gov>
Cc: Rotstein, David <David.Rotstein@fda.hhs.gov>
Subject: RE: Calls Complete

Thanks! Did you speak to PFI?

From: DeLancey, Siobhan
Sent: Monday, June 24, 2019 12:06 PM
To: Hartogensis, Martine <Martine.Hartogensis@fda.hhs.gov>; Norris, Anne <Anne.Norris@fda.hhs.gov>
Cc: Rotstein, David <David.Rotstein@fda.hhs.gov>
Subject: RE: Calls Complete

Thanks!

From: Hartogensis, Martine
Sent: Monday, June 24, 2019 12:04 PM
To: Norris, Anne <Anne.Norris@fda.hhs.gov>; DeLancey, Siobhan <Siobhan.Delancey@fda.hhs.gov>
Cc: Rotstein, David <David.Rotstein@fda.hhs.gov>
Subject: Calls Complete

From:	Norris, Anne
To:	Hartogensis, Martine
Cc:	DeLancey, Siobhan
Subject:	RE: Clearing DCM Comms
Date:	Tuesday, June 18, 2019 9:26:34 AM
Attachments:	DCM Project Plan.docx image001.png image002.jpg image003.jpg image004.jpg image005.jpg image006.jpg

Thanks! I think these look good. I believe Tracey and Nadine started a script – see attached. I think Dave knows best about what the current marching orders are with the divisions so you may want to touch base with him directly. Let me know what I can do to help.

Anne

From: Hartogensis, Martine
Sent: Tuesday, June 18, 2019 7:59 AM
To: Norris, Anne <Anne.Norris@fda.hhs.gov>
Cc: DeLancey, Siobhan <Siobhan.Delancey@fda.hhs.gov>
Subject: RE: Clearing DCM Comms

Hi Anne!

I reviewed and these are cleared by me. I added some suggested language (3rd paragraph CVM Update and question 3 in Q&A) (b) (5). See what you think!

(b) (5)

I missed the meeting last week, so I am not sure where we are on that and the script. I am happy to write a script if there isn't one yet.

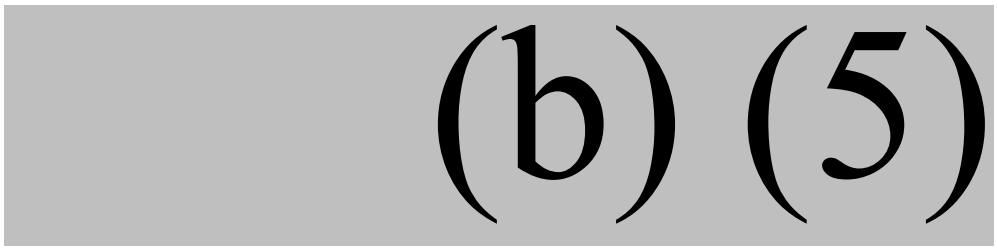
Lastly, I added in a few places ..labeled as "grain-free" so we are consistent. Not a huge deal, but it might help some of our readers recall the issue.

Thanks again!

Martine

From: Norris, Anne
Sent: Monday, June 17, 2019 4:48 PM
To: Hartogensis, Martine <Martine.Hartogensis@fda.hhs.gov>
Cc: DeLancey, Siobhan <Siobhan.Delancey@fda.hhs.gov>
Subject: Clearing DCM Comms

When you go through and clear the comms, do you want to enter in the language that you feel most comfortable with regarding (b) (5)



Is tomorrow late morning a reasonable timeline to get your clearance? Happy to discuss.

Thanks!

Anne Norris
Strategic Initiatives

Office of the Director
Center for Veterinary Medicine
U.S. Food & Drug Administration
O: 240-402-0132
M: (b) (6)
Anne.Norris@fda.hhs.gov

DCM Plan

Activity	Lead	Notes	Timing
(b) (5)			

(b) (5)

(b) (5)	

From:	Norris, Anne
To:	Palmer, Lee Anne; Carey, Lauren; Rotstein, David; Jones, Jennifer L; Peloquin, Sarah; Reimschuessel, Renate; Hartogensis, Martine; Burkholder, William; DeLancey, Siobhan
Subject:	RE: DCM paper - Darcy Adin, 2019 Vet Cardiology
Date:	Thursday, February 21, 2019 12:12:01 PM
Attachments:	sky488.pdf image001.png image002.jpg image003.jpg image004.jpg image005.jpg image006.jpg

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I've lost track of whether we circulated this paper internally, but sharing because it caught the eye of Phyllis Entis from Food Safety News. She hasn't written about it (at least not yet). One of the authors is Greg Aldrich.

From: Norris, Anne
Sent: Tuesday, February 19, 2019 9:09 AM
To: Palmer, Lee Anne <LeeAnne.Palmer@fda.hhs.gov>; Carey, Lauren <Lauren.Carey@fda.hhs.gov>; Rotstein, David <David.Rotstein@fda.hhs.gov>; Jones, Jennifer L <Jennifer.Jones@fda.hhs.gov>; Peloquin, Sarah <Sarah.Peloquin@fda.hhs.gov>; Reimschuessel, Renate <Renate.Reimschuessel@fda.hhs.gov>; Hartogensis, Martine <Martine.Hartogensis@fda.hhs.gov>; Burkholder, William <William.Burkholder@fda.hhs.gov>
Subject: RE: DCM paper - Darcy Adin, 2019 Vet Cardiology

Thanks!

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Subject: DCM paper - Darcy Adin, 2019 Vet Cardiology

Hi – please forgive me if we have this already, but I think this just came out.

I haven't read it yet.

Thanks, lee Anne

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Special topic: The association between pulse ingredients and canine dilated cardiomyopathy: addressing the knowledge gaps before establishing causation[1]

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ABSTRACT: In July 2018, the Food and Drug Administration warned about a possible relationship between dilated cardiomyopathy (**DCM**) in dogs and the consumption of dog food formulated with potatoes and pulse ingredients. This issue may impede utilization of pulse ingredients in dog food or consideration of alternative proteins. Pulse ingredients have been used in the pet food industry for over 2 decades and represent a valuable source of protein to compliment animal-based ingredients. Moreover, individual ingredients used in commercial foods do not represent the final nutrient concentration of the complete diet. Thus, nutritionists formulating dog food must balance complementary ingredients to fulfill the animal's nutrient needs in the final diet. There are multiple factors that should be considered, including differences in nutrient digestibility and overall bioavailability, the fermentability and quantity of fiber, and interactions among food constituents that can increase the risk of DCM development. Taurine is a dispensable amino acid that has been linked to DCM in dogs. As such, adequate supply of taurine and/or precursors for taurine synthesis plays an important role in preventing DCM. However, requirements of amino acids in dogs are not well investigated and are presented in total dietary content basis which does not account for bioavailability or digestibility. Similarly, any nutrient (e.g., soluble and fermentable fiber) or physiological condition (e.g., size of the dog, sex, and age) that increases the requirement for taurine will also augment the possibility for DCM development. Dog food formulators should have a deep knowledge of processing methodologies and nutrient interactions beyond meeting the Association of American Feed Control Officials nutrient profiles and should not carelessly follow unsubstantiated market trends. Vegetable ingredients, including pulses, are nutritious and can be used in combination with complementary ingredients to meet the nutritional needs of the dog.

Key words: dilated cardiomyopathy, dogs, feed formulation, grain-free, nutrition, pulse ingredients

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INTRODUCTION

In July 2018, the Food and Drug Administration (**FDA**) issued a statement relating dilated cardiomyopathy (**DCM**) in dogs to the consumption of foods that have potatoes and/or pulse ingredients, such as peas and lentils or their coproducts, as main ingredients (FDA, 2018). The FDA's statement, as well as media attention, has raised concern in some pet owners, veterinarians, nutritionists, and the pet food manufacturing and retail industry. The underlying cause for concern with pet food and DCM is that there is a link between nutrition that was previously tied to DCM and insufficient circulating taurine (Fascetti et al., 2003; Backus et al., 2006). The result was an increased need for dietary taurine or its precursor methionine due to higher fermentation of taurine and greater fecal excretion with dietary fermentable fiber (Kim et al., 1996a, 1996b). Whether this has any link to dietary pulses or the greater inclusion of pulses in grain-free dog food has yet to be directly demonstrated and mechanistic research is warranted.

Pulses are a subset of legumes, harvested as a dry crop, with low concentrations of lipid. They include peas, lentils, chickpeas, and dry beans (Marinangeli et al. 2017) which have been used as ingredients in dog food for their protein and fiber for more than 2 decades (Butterwick et al., 1994; Rice and Ihle, 1994). As a source of protein, the amino acid (AA) profile in peas, lentils, chickpeas, and beans is generally high in lysine and low in methionine (NRC, 2006) and serves as a complementary protein to both animal and plant-derived ingredients. As an example, soybean meal is derived from defatted soybeans and has an AA profile similar to pulses. In a 24-wk study that evaluated graded concentrations of soybean meal up to 17% (as-fed basis) in dog foods, soybean meal inclusion did not affect the nutrient status of dogs as indicated by serum biochemistry analysis (Menniti et al., 2014). However, Yamka et al. (2003) demonstrated that using soybean meal at more than 15% inclusion on a dry matter basis decreased crude protein digestibility. Based on the authors assessment of current formulas in the market, there is a high likelihood that legume seed use in some foods may be greater than 40%. This inclusion exceeds concentration of legumes previously investigated in dogs. When used to complement the nutritional profile of other ingredients, pulses can be used as nutrient-rich vehicles to meet the nutritional requirements of dogs and other companion animals. Given that companion animals most often consume static diets for long periods of time, overuse of any ingredient could facilitate higher risk of certain nutrient deficiencies if nutrient balance is not considered in the formulation. Thus, the formulation of static diets that use significant concentrations of a single ingredient, relative to other ingredients in the formulation, requires an in-depth knowledge of nutrient interactions, animal physiology, and effects of processing, beyond that of simply meeting minimum nutrient profiles stipulated in the Official Publication of The Association of American Feed Control Officials (AAFCO, 2018).

The present commentary discusses the following: 1) The limited data being used to support linkages between DCM and pulse ingredients; 2) The nutritional factors and physiological mechanisms that should be explored to establish causation between nutritional deficiencies and incidence of DCM; 3) The factors that nutritionists should consider when formulating complete diets destined for long-term consumption; and 4) The disadvantages of formulating protein and minimal AA recommendations rather than a balanced indispensable AA profile.

The Development of Canine DCM, Historical Linkages to Taurine Deficiency, and Pulses

Dilated cardiomyopathy is a disease of the myocardium that results in both mechanical dysfunction (enlarged heart cavities and congestion) and/or electrical dysfunction (arrhythmias and sudden death) (Sisson et al., 2000; Maron et al., 2006; Dutton and López-Alvarez, 2018). Development of DCM is slow and few clinical signs manifest over time. As DCM progresses, signs include lethargy, anorexia, shallow breathing, sudden fainting, and potential death. In some cases, animals may die from irregular heart rhythm without previous signs of the disease. In dogs, DCM can be

caused by various factors. Genetic predisposition is thought to play the most important role in the development of DCM in several dog breeds, mostly large and giant breeds. Genetic mutations associated with DCM have been discovered in American lines of Doberman and Boxer dogs (Meurs et al., 2012; Meurs et al., 2013). However, the Doberman variant's association was not upheld in a European population of Dobermans (Owczarek-Lipska et al., 2013). Similarly, a United Kingdom population of Boxers did not uphold their published DCM-associated variant (Cattanach et al., 2015). It is becoming increasingly clear that the genetic basis for DCM in dogs is not monogenic, but complex and polygenic. Breeds with the highest prevalence of DCM include Dobermans, Boxers, Great Danes, Newfoundlands, Irish Wolfhounds, English Cocker Spaniels, and Portuguese Water Dogs (Monnet et al., 1995; Borgarelli et al., 2006; Werner et al., 2008; Martin et al., 2009), and the genetic basis of DCM in each of these breeds has been investigated (Dutton and López-Alvarez, 2018). In addition, Golden Retrievers and American Cocker Spaniels appear to have breed predispositions to taurine deficiency (Kramer et al., 1995; Bélanger et al., 2005). When dogs are not genetically predisposed for developing DCM, diet and physiology are other factors that may be associated with the disease.

The first link between taurine deficiency and DCM was demonstrated in cats in 1987. Cats diagnosed with DCM recovered after taurine supplementation (Pion et al., 1987). Similarly, an inverse association between dietary taurine and the incidence of DCM in a population of foxes was documented by Moise et al. (1991) and established the importance of taurine in the family Canidae. In dogs, DCM diagnoses related to low whole blood taurine concentrations have been reported in Cocker Spaniels, Dalmatians, Boxers, Newfoundlands, Portuguese Water Dogs, English Setters, Alaskan Malamutes, and Scottish Terriers (Freeman et al., 1996; Kittleson et al., 1997; Pion et al., 1998; Alroy et al., 2000; Fascetti et al., 2003; Backus et al., 2006). In all these cases, taurine supplementation improved cardiac function. However, dogs, in contrast to cats, can endogenously synthesize taurine from methionine and cysteine (Figure 1). Therefore, the above-mentioned data do not unequivocally establish taurine intake as the underlying mechanism for the development of DCM in dogs, whether they are genetically predisposed. Dietary supply of precursor AAs necessary for taurine synthesis (i.e., methionine and cysteine), metabolic intermediates, and cofactors (such as methyl donors) cannot be ruled out as factors that contribute to the susceptibility of dogs to developing genetic and diet-related DCM. When DCM is diet-related, the formulation and the provision of all nutrients, including indispensable AAs, to facilitate optimum health and wellbeing of dogs should be considered.

Recent reports, including the statement by the FDA (2018), have implicated that lentils, peas, and other legumes seeds could be responsible for the development of DCM in dogs not genetically predisposed to this disease. Such statements and associations between pulse ingredients and incidence of DCM are, at the present time, premature. Animals, including dogs, have no minimum or maximum requirements for ingredients. Ingredients serve

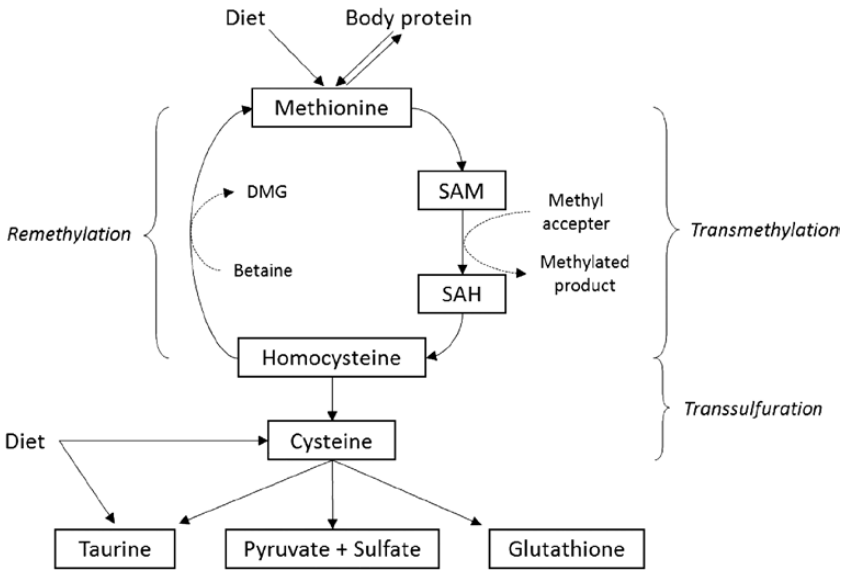

Figure 1. Metabolism of sulfur amino acids. DMG = dimethylglycine; SAH = S-denosylhomocysteine; SAM = S-adenosylmethionine.

as the vehicle to providing nutrients to animals. As such, animals have nutrient requirements, not ingredient requirements. In diets that have nutrient deficits, imbalances, or exceed maximums, the final nutrient composition of the diet, not the ingredients, should be critiqued. In addition, animal nutritionists should consider that the nutrient concentration of ingredients can vary, nutrient availability is not 100%, and diets formulated to marginally meet requirements could actually be deficient. Overall, it is the responsibility of nutritionists to use different ingredients to formulate diets that can be produced and safely meet the nutritional needs of animals.

Taurine Deficiency and the Development of Canine DCM

For dogs, taurine is a dispensable AA synthesized from methionine and cysteine primarily in the liver (Figure 1). Taurine is not incorporated into proteins. Instead, it is used as a mediator for various biological processes and is the most abundant free AA intracellularly (Huxtable, 1992). In the heart, taurine represents ~60% of the total AA free pool (Huxtable, 1992). The high concentration of taurine in cardiac cells may explain the role of a taurine deficiency in the development of DCM. It has been speculated that taurine contributes to the reabsorption of calcium by the sarcoplasmic reticulum and increases the sensitivity of the myofilaments to calcium (Bakker and Berg, 2002). Thus, low dietary taurine intake and/or reduced synthesis of taurine from methionine and cysteine can deplete calcium pools in the cardiac cells and impede proper contraction of the cardiac muscle tissue, resulting in DCM in dogs.

For diagnosing DCM in dogs and cats, among other diagnostic methods including electrocardiograms and echocardiography, it is common to measure taurine concentration in whole blood. Whole blood samples, and not plasma samples, should be used to assess circulating taurine concentrations. In plasma, free taurine concentrations are much lower compared with intracellular taurine. This suggests that the plasma pool is not representative of taurine in other pools (Schaffer et al., 2010). In platelets, taurine concentration is high and is considered a marker of taurine status. Taurine concentration in platelets is captured when whole blood is analyzed (Huxtable, 1992). However, platelet count can vary depending on the immune status of the animal and whole blood taurine concentration can be affected. In this scenario, whole blood taurine may not represent concentrations of taurine in muscle cells, including cardiac muscle. These additional variables related to the measurement of taurine status may explain why some dogs diagnosed with DCM have normal whole blood taurine concentrations.

As taurine can be synthesized endogenously in dogs, taurine is not considered an indispensable AA for the species Canidae. Thus, there are no recommendations on minimum dietary concentrations of taurine for dogs reported by the National Research Council (NRC, 2006) or AAFCO (2018). The lack of regulation on minimum taurine concentrations in commercial dog foods suggests that endogenous synthesis of taurine can meet the metabolic needs in all dogs and at all life stages. This assumption may not be accurate as studies have determined that synthesis of taurine is related to the size of dog (Ko et al., 2007), and some dietary factors can increase the physiological need for taurine (Story, 1978). Nutritional factors that increase the dietary requirement, reduce the supply, or increase the excretion of taurine in dogs are discussed in subsequent sections of this review and should be considered to avoid taurine deficiency in dogs and the risk of DCM.

Physiological factors can increase taurine utilization in dogs, and endogenous synthesis of taurine could be insufficient for meeting taurine requirements. For example, compared with smaller size dogs, synthesis of taurine in large dog breeds is up to 50% lower per unit of metabolic body weight (Ko et al., 2007). These results demonstrate that larger dogs are at higher risk for insufficient endogenous taurine synthesis, and dietary supplementation or fortification may be required, even when there is no minimum dietary taurine concentration according to current recommendations (AAFCO, 2018). Obesity and diabetes have also been related to lower concentrations of taurine in blood in humans and rats, respectively (Merheb et al., 2007; Nardelli et al., 2011; Ito et al., 2012), and may increase the requirement for sulfur AAs necessary for endogenous taurine synthesis. This is of importance given that approximately half of dogs in North America are obese (Linder and Mueller, 2014). Data from rats and cats suggest that age and sex could also affect whole body taurine status. Hepatic activity of cysteine sulfonate decarboxylase, the enzyme responsible for taurine synthesis, was shown to be 16 times higher in adult male rats vs. female rats. In the same study, the activity of cysteine sulfonate decarboxylase was higher in 5- to 6-wk-old kittens compared with 15-mo-old cats and in 8-wk-old mice compared with 16-wk-old mice; changes of

the enzyme activity in dogs have not been tested (Worden and Stipanuk, 1985). Overall, these studies suggest that, despite some capacity for endogenous synthesis, physiological need of taurine can be heavily dependent on breed, age, sex, and physiological status. These physiological factors could help us to predict the risk for developing DCM when genotypic and environmental factors, such as diet, are simultaneously considered to ensure that dogs maintain adequate concentrations of taurine and other sulfur AAs.

Given that there are no recommendations for the minimum concentration of taurine in dog food, the concentration of taurine in dog foods can vary substantially depending on the ingredients used. Taurine is very low in plant-based ingredients (Table 1) but is higher in some algae and fungi species and is ubiquitously found in animal tissues, especially in the heart, brain, and white blood cells (Huxtable, 1992). This is relevant, as many grain-free and/or high legume dog foods attempt to limit the use of animal byproducts, which can substantially decrease the levels of dietary taurine. In the context of providing adequate and preventive nutrition, dog foods should include organ meat or animal byproducts or be fortified with taurine and/or its precursors (methionine and/or cysteine) to ensure the delivery of sufficient levels of taurine.

Effect of Dietary Fiber on Taurine Status and Risk of Canine DCM

Dietary fiber has been shown to affect the taurine status in dogs. For example, commercial diets formulated with lamb meal and rice bran were shown to cause taurine deficiency in part because of low bioavailable cysteine from lamb meal and possibly more importantly due to the effects of rice bran fiber on gastrointestinal metabolism of taurine (Johnson et al., 1998; Tôrres et al., 2003). It has been hypothesized that high-fiber diets can increase susceptibility to taurine deficiency by 2 mechanisms of action linked to obligatory bile acid conjugation with taurine in dogs (O'Mádille et al., 1965) and reliance on enterohepatic circulation for the reabsorption of bile acids and taurine. First, high-fiber diets may increase fecal output and losses of taurine-conjugated bile. This would require higher synthesis rates of bile in the liver, and consequently, higher utilization of taurine

Table 1. Crude protein (CP), fiber, selected amino acids, and carnitine contents in the principal legumes, cereals, and animal-derived ingredients used in dog food formulation

				α-amino acids, mg/g protein[1]				
Ingredients		CP, %	Crude fiber,[1] %	Lys	Met	Cys	Tau, mg/kg[2]	Carnitine, mg/kg[3]
Legumes	Fava beans	27.2	8.55	23.9	7.0	12.5	–	–
	Phaseolus beans	22.9	NR	72.9	12.7	12.7	–	–
	Kidney beans	20.0	6.40	26.5	14.0	12.0	–	–
	Lentils	26.0	NR	65.8	6.9	10.4	–	–
	Lupins	32.4	14.25	48.7	6.5	14.2	–	–
	Chick peas	20.3	6.16	69.4	14.8	21.6	–	–
	Soybean meal	47.7	3.89	62.0	13.8	14.7	–	–
Grains	Barley	11.3	3.90	35.3	17.7	22.9	–	–
	Corn, yellow dent	8.2	1.98	30.3	21.8	23.1	–	–
	Oats	11.2	2.20	43.9	60.9	32.3	–	–
	Rice	7.9	0.52	44.5	31.8	22.9	–	–
	Rye	11.7	2.71	36.9	13.7	16.3	–	–
	Sorghum	9.4	2.14	21.4	17.1	19.2	–	–
	Wheat hard, red	14.5	2.57	27.0	15.2	22.8	–	–
Animal-derived ingredients	Beef, meat	15.0	–	77.3	28.7	15.3	296	150
	Chicken, meat and skin	17.6	–	81.3	26.7	13.1	159	57
	Chicken, by product	59.0	–	48.1	17.3	16.8	3049	120
	Lamb, ground	16.6	–	88.0	25.9	12.0	473	282.3
	Rendered meat	54.1	2.50	53.8	14.2	11.3	NR	NR

Cys = cysteine; Lys = lysine; Met = methionine; NR = not reported; Tau = taurine.

Values are presented on as-fed basis.

[1]NRC, 2006; NRC, 2012.

[2]Spitze et al. 2003.

[3]Arslan, 2006.

(Story, 1978). Second, high consumption of fermentable fibers may increase the abundance of microbial populations that degrade taurine in the intestinal lumen (Kim et al., 1996a, 1996b). Either alone or together, increased excretion or degradation of taurine from high-fiber diets may decrease enterohepatic circulation and recycling of taurine. Given that taurine is the only AA used for bile acid conjugation in dogs, over time, high-fiber diets could increase the risk of taurine insufficiency in dogs and lead to DCM.

This should not be interpreted as dietary fiber being deleterious to the health of dogs. However, there may be a limit to the benefit for soluble fibers. Legume seeds contain an appreciable quantity of oligosaccharides which are known to be fermentable (Tosh and Yada, 2010). Thus, by a similar mechanism as described above, high levels of legume seed oligosaccharides could ostensibly contribute to taurine depletion via excretion in the feces as bile conjugation and degradation by colonic bacteria. In addition to the physiological benefits of high-fiber diets in certain dogs, formulators should also be cognizant of possible nutritional risks associated with high concentrations of fiber in dog foods. Consequently, dog foods with high concentrations of dietary fiber should be accompanied by higher supplies of taurine or sulfur AAs for endogenous taurine synthesis. Overall, the digestibility and bioavailability of taurine in ingredients used and the effect of other nutrients in taurine metabolism should be considered to avoid taurine deficiency and the development of DCM.

Carnitine Deficiency and Risk of Canine DCM

Carnitine is not nutritionally indispensable since it is endogenously produced in the liver and kidneys from lysine and methionine; it can also be attained exogenously from animal-based products. Carnitine is highly abundant in skeletal and cardiac muscles. Together, these represent >95% of the total carnitine in the body. Carnitine is essential for metabolism of fatty acids used for energy production (Hoppel, 2003). In the heart, where 60% of the energy is derived from fatty acid oxidation, carnitine facilitates the uptake of free fatty acids into the mitochondria to produce ATP (Hoppel, 2003). Plant-based ingredients do not contain carnitine (Table 1). Therefore, in commercial dog foods with reduced inclusion of animal-based ingredients, intakes of carnitine could be decreased if diets are not fortified. Reduced dietary carnitine intake translates into increased reliance on endogenous synthesis to meet physiological requirements.

Given that carnitine is required for sufficient energy production in cardiac muscle, it is not surprising that carnitine deficiency is associated with DCM. In 1991, a family of Boxers diagnosed with DCM were also diagnosed with carnitine deficiency (Keene et al., 1991). In dogs, carnitine deficiency can occur with aberrations of carnitine regulation in disorders such as cardiomyopathy (including DCM), diabetes, sepsis, and malnutrition (Flanagan et al., 2010). However, carnitine deficiency as a causative factor in the development of DCM or a consequence of cardiac malfunction remains as a subject of debate (Freeman and Rush, 2006). Despite the interest in this metabolite, little progress has been made on determining the effect of carnitine supplementation on alleviating risk of DCM. However, both taurine and carnitine are often supplemented in supraphysiological concentrations once DCM is diagnosed. This practice is supported by positive clinical outcomes, albeit without comparison groups (Kittleson et al., 1997; Sanderson et al., 2001). Concentrations of carnitine in the plasma are relatively insensitive to dietary carnitine, and more invasive techniques (biopsies) are required to determine the concentration of carnitine in muscle tissue (Flanagan et al., 2010; Răşanu et al., 2012). The invasive nature of testing for carnitine status is likely the reason why carnitine is rarely explored when investigating possible causes of canine DCM.

Preventing Diet-Mediated DCM in Dogs by Providing Adequate Sulfur AAs and Maximizing Endogenous Taurine Synthesis

Although taurine is considered a dispensable AA in dogs, endogenous taurine synthesis requires an adequate supply of bioavailable sulfur AA precursors cysteine or methionine (Figure 1). Thus, providing marginal concentrations of these 2 sulfur AAs, or providing sources with lower bioavailability, could increase the risk of taurine deficiency and facilitate the development of DCM. Contrary to taurine, methionine cannot be synthesized endogenously in dogs (NRC, 2006). Therefore, dogs depend on the provision of dietary methionine to meet daily sulfur AA requirements, which includes production of taurine. From an ingredient perspective, methionine and lysine are usually the first or second limiting AAs in dog diets formulated with soybean meal and rendered meats (NRC, 2006). In addition, methionine is particularly susceptible to damage, and subsequent reduction in bioavailability,

secondary to heat processing (Marshall et al. 1982; Hurrell et al., 1983). This suggests that the risk of methionine deficiency is more likely than any other indispensable AA in commercial dog diets. Although the primary role for methionine is protein synthesis, in pigs at least 50% of absorbed methionine acts as a methyl donor and a precursor in the production of cysteine, taurine, sulfate, and pyruvate (Robinson et al., 2016a; Figure 1). These functions of methionine become more crucial when dietary intake of cysteine, taurine, and/or dietary methyl donors (e.g., folate, betaine, and their precursors) is limited (Robinson et al., 2016b), and they need to be considered when nutritionists set criteria for delivery of sulfur AAs in pet foods.

Methionine and cysteine both contribute to the total sulfur AA requirements for humans and animals. For adult dogs at maintenance, the latest guidelines from the NRC (2006) recommend that adult dog foods contain 0.33% (on dry matter basis) methionine when cysteine is provided in excess, and 0.65% for methionine + cysteine. These NRC (2006) recommendations are not based on dose–response studies, but on a 4-yr study where adult dogs were fed low-crude protein diets (Sanderson et al., 2001). In that study, the lowest concentration of methionine in the diet that reported no observable deficiencies was used as the recommended requirement. As companion animals are typically fed a single static diet during adulthood, and for most of their lifespan, it is necessary that AA requirements of dogs should be measured empirically (Baker, 1986). In addition to the lack of empirical data corresponding to the AA requirements of dogs, it is equally important to understand how other dietary (e.g., dietary fiber), environmental, other physiological variables, and breed/genotype may alter AA requirements. The lack of recommendations for taurine in commercial dog food puts a higher stress on accurately meeting requirements for sulfur AAs, not only for protein synthesis, but also for the endogenous synthesis of taurine, for support of optimal methyl status, and for the synthesis of secondary metabolites.

Rethinking Indispensable AA Targets in Commercial Dog Foods

Currently, the ingredients permitted in pet foods and the corresponding nutrient targets are guided by recommendations made by AAFCO (2018). These recommendations are based on the peer-reviewed scientific literature and represented in the Nutrient Requirement of Dogs and Cats (NRC, 2006). However, AA recommendations made by AAFCO correspond to total AA content within the formulation and do not consider the true ileal digestibility of ingredients. True ileal digestibility of AAs is more representative of nutrient absorption capacity and bioavailability compared with fecal digestibility or total AA content in the diet (Columbus and de Lange, 2012). To account for the reduced digestibility and bioavailability of protein-bound AAs in food ingredients, AAFCO arbitrarily increases AA recommendations relative to those from the NRC to ensure that an adequate supply of AAs is provided, regardless of the ingredients and effects of processing (Table 2). However, this increment is only applied to lysine, threonine, and tryptophan and not applied to other indispensable AAs, including methionine (AAFCO, 2018). For example, the recommended allowance for lysine reported in NRC (2006) is 0.35% for adult dogs at maintenance, whereas the minimum content of lysine to meet AAFCO (2018) recommendations is 0.63%. Nonruminant animals, including dogs, absorb AAs from the duodenum to the terminal ileum (Columbus and de Lange, 2012). Hence, feeding diets with lower ileal digestibility coefficients could decrease actual concentrations of available indispensable AAs, even when meeting AAFCO recommendations. This is of special concern for dietary taurine and other sulfur AAs, considering that there is no regulated minimum threshold for taurine in dog foods and that AAFCO (2018) recommendations for sulfur AAs are not increased compared with NRC (2006) recommendations to account for potential ileal digestibility coefficients. There is a dearth of data in this area to justify empirical adjustments based on different dietary variables. As such, future research should pursue how AA requirements change under different dietary variables that can affect small intestinal digestibility and whole body availability.

It is worthwhile to note that minimum dietary nutrient contents for dog foods, as reported in AAFCO (2018), only consider differences between growth/reproduction and adult life stages. This lack of data places the pregnant bitch in the same group as growing animals. Moreover, most studies on nutrient requirements in dogs have been established using Beagles as a proxy for all dogs. Using a single breed creates a homogenous sample and likely does not account for nutritional variability across pure and mixed breeds, or those of different sizes. Unpublished data from Shoveller et al. investigated the minimum methionine (with excess cysteine) requirements of

Table 2. Recommended allowance (RA) and minimum dietary content suggested by AAFCO for crude protein and essential amino acids in dog food, and their physiological roles and potential interactions

Nutrient	NRC RA[1], % DM	AAFCO[2], % DM	Important physiological roles and potential interactions
Crude protein	10	18	Necessary for synthesis of nonessential amino acids
Arginine	0.35	–	Competes with lysine absorption, arginine should be increased when high lysine concentrations in the diet
Histidine	0.19	–	
Lysine	0.35	0.63	Highly reactive to reducing sugars during heating (Maillard reaction), reducing bioavailability
Methionine	0.33	0.33	Requirement increases when methyl donors/acceptors and cysteine are reduced in the diet
Methionine + cystine	0.65	0.65	Requirement is increased with low supply of taurine and during immune challenge
Phenylalanine	0.45	0.45	
Phenylalanine + tyrosine	0.74	0.74	
Threonine	0.43	0.48	Abundant in mucosal proteins (mucin), requirement increases when feeding high fermentable fibers
Tryptophan	0.14	0.16	Precursor for serotonin synthesis. Ratio of Trp: LNAA should be considered; lower ratios may deprive appetite
Valine	0.49	0.49	Abnormal Increment of valine, leucine, or isoleucine (BCAA) will cause catabolism of the other BCAA in the muscle
Isoleucine	0.38	–	
Leucine	0.68	0.68	

AAFCO = The Association of American Feed Control Officials; BCAA = branched chain amino acids; DM = dry matter; NRC = National Research Council; RA = recommended allowance; Trp:LNAA = tryptophan to large neutral amino acid ratio.

[1]Recommended Allowance requirements for adult dogs at maintenance, Nutrient Requirements of Dogs and Cats (NRC, 2006).

[2]Miminum dietary content, AAFCO (2018).

Miniature Dachshunds, Beagles, and Labrador Retrievers as proxies for small, medium, and large dog breeds and found that methionine requirements may differ across breeds or size of dogs and be greater than previously estimated. Thus, given the methods of derivation, single indispensable AA requirements for all dog populations, as presented in AAFCO (2018), may not consider variable AA requirements across dog phenotypes. Moreover, it is widely assumed that endogenous synthesis of dispensable AAs, such as taurine in the dog, is sufficient for meeting metabolic demands. However, recent studies suggest that under some metabolic conditions, dispensable AAs may also be required in diets (Hou et al., 2015). Taurine, as described in this commentary, is a clear example of this paradigm shift. Dietary taurine or the capacity for its adequate endogenous synthesis, especially in circumstances where excessive losses might occur, should be considered in the final formulation of dog foods to decrease the risk of canine DCM.

Nutritionists and regulatory agencies should be aware that, in the spectrum of nutrient requirements, dog populations with higher AA requirements relative to energy intake and other factors could be at a higher risk for a taurine deficiency. More precise categorization of requirements among different canine populations would help us to optimize nutritional adequacy and decrease risk of diseases, such as DCM, that are possibly linked to nutrient deficiencies.

Effect of Processing on Antinutritional Factors in Plant-Based Ingredients

Just as understanding the inherent nutritional characteristics and the interaction between ingredients is important for preventing nutritional imbalances in pet foods, the effects of processing on these factors are equally important. Raw cereals and legumes contain antinutritional factors such as trypsin inhibitors, phytates, hematoglutinins, and polyphenols that can decrease protein digestion, nutrient absorption, and/or cause illness. Some of these antinutritional factors are thermolabile and, under the right conditions, can be effectively destroyed during the extrusion process improving the overall quality of plant-based ingredients and the final diet (Patterson et al., 2017). Recent reviews across a variety of legumes and legume-derived ingredients show that the activities of trypsin inhibitor, chymotrypsin inhibitor, and hemagglutinating activity were decreased by up to 95% across a variety of thermal treatment conditions, including extrusion (Patterson et al., 2017; Avilés-Gaxiola et al., 2018). Extrusion had modest effects on levels of phytate with reductions ranging from 7% to 26% and varied by legume and extrusion conditions (Patterson

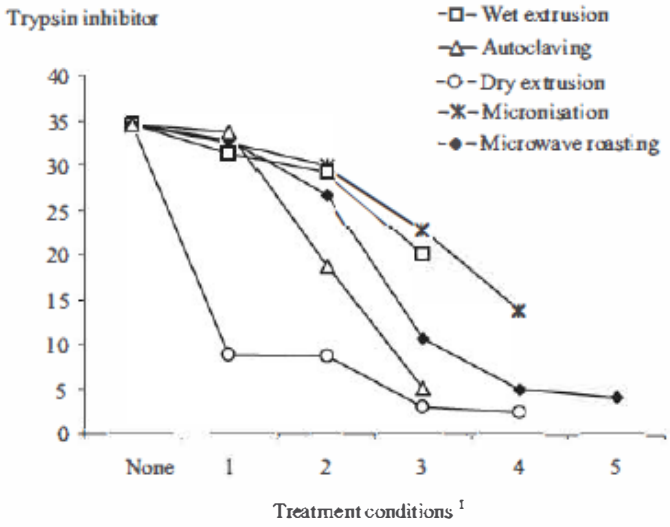

Figure 2. Effect of thermal processing methods on trypsin inhibitor levels (mg/g) soybean kernel. [1]Treatment conditions: None = no treatment; dry extrusion for 25 to 30 sec (1 = 100 °C; 2 = 125 °C; 3 = 140 °C; 4 = 150 °C); wet extrusion for 25 to 30 s with 6% to 8% added moisture (1 = 100 °C; 2 = 125 °C; 3 = 140 °C); micronization with near-infrared rays wavelength of 1.8 to 3.4 µm for 90 s (1 = 100 °C; 2 = 125 °C; 3 = 140 °C; 4 = 150 °C); microwave roasting at 800 W and 2450 MHz (1 = 1 min [kernel temp = 57 °C], 2 = 2 min [kernel temp = 88 °C], 3 = 3 min [kernel temp = 108 °C], 4 = 4 min [kernel temp =121 °C], 5 = 5 min [kernel temp = 132 °C]); Autoclaving at 120 °C and 1.2 bars (1 = 10 min, 2 = 20 min, 3 = 30 min). Reprinted with permission from Žilić et al. (2012).

et al., 2017). Figure 2 highlights the variability between processing methods and thermic conditions for decreasing antinutritional factors. For example, when soybeans were subjected to extrusion at increasing temperatures that ranged from 100 to 150 °C, trypsin inhibitor levels were incrementally decreased. At 140 °C, dry extrusion was considerably more effective at decreasing trypsin inhibitors (−91%) compared with wet extrusion (−44%). When the dry extrusion temperature was increased to 150 °C, reductions in trypsin inhibitors were further decreased by 94% (Žilić et al., 2012). Other thermal treatments, such as micronisation, microwave roasting, and autoclaving, also facilitated incremental reductions in trypsin inhibitors with increasing temperatures (Žilić et al., 2012). When formulating foods with higher concentrations of plant-based ingredients, consideration should also be given to the processing methods and the parameters used to effectively optimize the nutritional density and decrease antinutritional factors.

It is important to mention that, while temperature and pressure processing can greatly decrease antinutritional factors, they can also negatively affect bioavailability of AAs. The Maillard reaction is a well-known example of heat-damaged protein (Teodorowicz et al., 2017). In this reaction, lysine interacts with reducing sugars present in the diets forming the Maillard product. The complex formed can be digested and absorbed by the animal but cannot be utilized for metabolic processes (e.g., protein synthesis). Thus, in heat-damaged proteins, digestibility of AAs can greatly overestimate bioavailability (Moehn et al., 2005). Other products of heat damage on proteins include racemization of AAs (alteration from L to D form) and the formation of cross-linked AAs. Such components can decrease bioavailability of AAs and digestibility of proteins, and their effects on protein quality cannot usually be determined using conventional methods of AA analysis. Pet foods with higher levels of plant-based ingredients may also require optimization of processing methods to maximize their nutritional density and nutrient bioavailability.

Recommendations for Formulating Dog Food With Novel Ingredients

Considering the AA profile of dog foods. Feed formulation for agricultural and companion animals should be based on the ideal protein concept (Baker, 1991; Swanson et al., 2013). The ideal protein is defined as that in which all AAs are in perfect balance compared with the animal's AA requirements (mg/g protein). Hence, all indispensable AAs are equally limiting. However, this is impossible to achieve in practical animal feed formulation, and diets should be formulated considering the first limiting indispensable AA. The first limiting indispensable AA refers to the indispensable AA that is present in the lowest proportion compared with the animal's requirement. By meeting the first indispensable limiting AA requirement, requirements for all other indispensable AAs are also inherently satisfied. Moreover, to avoid the formulation of diets with excessive protein concentration or an excess of indispensable AAs relative to the requirements of dogs, animal nutritionists combine multiple ingredients that are complementary in their AA profiles. Commonly, dog foods are formulated with a higher proportion of animal-derived ingredients, and a lower proportion of plant-based ingredients to meet nutrient recommendations. More recently, however, cereal grains have been removed in some diet formulations or the proportion of animal-based ingredients has been reduced. The production of these types of formulations is often driven by consumer perception, rather than scientific evidence. Allowing consumers to direct the ingredient composition of dog foods, or other pet foods, could perpetuate nutrient deficits that affect the health of animals in the long term.

In the formulation of grain-free pet foods, cereal grains are replaced with alternative ingredient(s). Animal-derived ingredients are expensive relative to plant-based ingredients. Thus, pulses, a subset of legumes, are often used as the replacement. In addition to containing substantial fiber, pulses also contain significant concentrations of protein and are used to partly meet indispensable AA requirements. Of interest, soybean meal and pulses contain 48% and 25% crude protein, respectively, which is substantially greater than the average protein concentration for grains (11%; Table 1). Although the high-protein content in soybean meal and pulses is indicative of higher concentration of AAs compared with grains, it does not imply AA balance. Soybean meal and pulses are high in lysine (mg/g protein) but low in sulfur AAs (mg/g protein), whereas the reverse is true for cereals. Plant-based ingredients tend to have lower ileal digestibility coefficients for protein compared with protein from animal sources (FAO and WHO, 1991). Thus, dog foods that contain substantial amounts of pulses, lower proportions of animal-based ingredients, and do not address AA imbalances through the addition of alternate ingredients or fortification, may risk AA deficiencies. To mitigate this risk across the pet food industry and ensure the final pet diets are nutritionally adequate and balanced, it is prudent that the digestibility coefficients of all final pet food products be calculated.

Considering the addition of high-fiber ingredients to dog foods. By definition, dietary fiber is carbohydrates that are resistant to digestion by endogenous enzymes in the gastrointestinal tract (NRC, 2006). Typical fibers include arabinoxylan, raffinose, inulin, β-glucan, cellulose, and pectin (NRC, 2006). Common ingredients to increase fiber content in companion animal diets include beet pulp, corn fiber, rice bran, whole grains, and pulse fibers (de Godoy et al., 2013). Achieving an optimal fiber concentration in canine diets has diverse positive physiological effects in the gastrointestinal tract; for example, higher fermentable fiber intake has been shown to slow the transit time of digesta, increasing satiety of the animal (Haber et al., 1977). Moreover, high-fiber diets generally have lower energy density making them an important nutritional strategy for controlling body weight (Johnson et al., 2008) and reducing the incidence of diarrhea (Homann et al., 1994). Gut health is also improved with higher consumption of fiber; fermentable fiber can act as a prebiotic and increase the population of health-promoting microbiota including lactobacilli and bifidobacteria (Roberfroid, 2005). Although not required by AAFCO to fulfill the criteria of "complete and balanced," fiber is an important component of the diet, and depending on the type of fiber and the amount consumed, fiber can increase the gut health status. Adding the necessary amount and type of fiber in the diet is crucial for optimal dog nutrition.

Despite the benefits of fiber in the diet, fiber can also affect enterohepatic recycling of taurine (discussed above). In monogastric species, including humans, high dietary fermentable fiber may also decrease digestibility and availability of dietary AAs (Blackburn and Southgate, 1981; Degen et al., 2007) and, in some cases, increase the risk of DCM in dogs fed diets that marginally meet requirements for sulfur AAs. Moreover, higher concentrations of dietary fiber increase the size of the gastrointestinal tract in pigs and poultry (Nyachoti et al., 2000), increasing nutrient utilization in this organ. It has been determined in pigs that on average the gastrointestinal tract catabolizes 30% of dietary indispensable AAs during absorption, and this utilization represents ~50% for sulfur AAs (Stoll et al., 1998; Mansilla et al., 2018), further reducing precursor availability for taurine synthesis and increasing the risk for taurine deficiency. For some high-fiber diets, fortification of specific nutrients, including taurine and other sulfur AAs, might be beneficial to avoid nutrient deficiencies.

Compared with the pet food industry, in other industries where high-fiber ingredients (coproducts) are routinely used (e.g., swine industry), the effects of fiber on the absorption of nutrients have been given more attention when formulating diets (NRC, 2012). For example, highly fermentable fiber in swine diets increases the threonine requirement to compensate for the increase in mucus (mucin protein) production in the intestinal cell lining (Lien et al., 1997; Mathai et al., 2016). This has underpinned the development of "requirement models" (NRC, 2012) to tailor nutrient requirements for pigs while accounting for the different nutrient interactions. In contrast, in the pet food industry, the only concentrations of nutrients used for comparison are those recommended by AAFCO (2018). Such recommendations are static and may not encompass all the effects of the different nutrient combinations in the final diet. There is a clear need in companion animal nutrition to improve the understanding of the interactions of different ingredients and how these alter nutrient requirements for different breeds, age, and physiological status of dogs.

Other recent publications highlight the need for careful nutrient formulation. Several recent papers, both original research and reviews, likewise highlight the unknowns surrounding grain-free diets (typically legume or pulse-based, but sometimes also with "exotic" ingredients such as kangaroo, bison, or wild boar) and DCM. For example, Adin et al. (2019) examined 48 dogs of many breeds with diagnosed DCM and having a known diet history. Among grain-free diets being consumed in this study, 1 dog was particularly associated with DCM, possibly underscoring the importance of specific diet formulation. Furthermore, 2 dogs switched from that diet to other grain-free diets showed improvement in their DCM; it is unclear if those dogs were taurine deficient or if they also received taurine and/or carnitine supplementation. This suggests that grain-free composition per se may not be the root cause of DCM. Another recently published case series of 24 Golden Retrievers with DCM and known diet histories were evaluated, and an association between grain-free diets and DCM was suggested (Kaplan et al., 2018). Most dogs (15 of 24) were fed a single diet which was significantly associated with low blood taurine concentrations, again suggesting that specific diet formulation may play an important role. However, as in the previous study, soluble vs. insoluble fiber concentrations were not available for the diets, nor were taurine, methionine, or cysteine concentrations, meaning that the true nutrient profiles of the diets could not be assessed and reinforcing the point that diet formulation for nutrients—not ingredients—is essential. It also suggests that nutrient requirements may vary widely based on breed, diet, and other phenotypic data. Indeed, most of the dogs with DCM in the previously described study were consuming less energy compared with their predicted requirements (Kaplan et al., 2018). It also bears pointing out that the numbers in both studies were very low (representing less than 100 DCM-affected dogs between them), which surely represents a fraction of the dogs consuming grain-free, pulse-based diets. A recent thoughtful review supports these conclusions by reiterating the crucial need for plant-based diets for dogs to be formulated with sufficient quantities of bioavailable methionine and cysteine to support adequate taurine synthesis (Dodd et al., 2018). This can be achieved with the addition of purified AAs and other sources that are readily available (Gloaguen et al., 2014). Finally, a recent commentary carefully concludes that a true cause-and-effect relationship between grain-free diets and DCM has not been proven, and other factors may ultimately be more important (Freeman et al., 2018). Taken together, these recent publications may point to faulty nutrient formulation in some, but not all, grain-free diets.

CONCLUSIONS

Recently, it has been suggested that pulse ingredients in commercial dog foods are associated with a limited number of cases of DCM. Although pulse ingredients have been implicated for having negative effects on the taurine status in dogs (deficiency of which is a known cause of canine DCM) based on the available evidence, the relationship between pulses and canine DCM remains undefined. However, the FDA statement may harm consideration of protein alternatives, such as pulses, as quality ingredients in pet foods and undermine attempts to diversify ingredients used across the food chain as the global population continues to grow. Ingredients do not represent the nutritional composition of the diet, and therefore, nutrient deficiencies should not be attributed to individual ingredients. The authors of this commentary recognize the important role of endogenous, and perhaps exogenous, taurine in the prevention of DCM in some dogs. The assurance of appropriate concentrations of all indispensable sulfur AAs, including methionine and cysteine, is crucial for ensuring adequate endogenous synthesis of taurine and to meet the metabolic demands of dogs. Additional dietary factors, such as methyl donors required for sulfur AA metabolism, carnitine for energy production in muscle, and dietary fiber, as well as animal factors, such as breed, size, and health status, should also be investigated when nutrient deficiency-related DCM is suspected.

It is the responsibility of animal nutritionists to formulate balanced diets for dogs, and other animals, by looking beyond the goal of meeting AAFCO recommendations or satisfying unsubstantiated market trends. Pulses and other plant-based ingredients can be used to formulate nutritionally adequate dog foods, and final product formulations should be assessed for nutrient balance and bioavailability, especially when using a limited number of ingredients. Although dietary factors are important in the prevention of sulfur AA deficiency and development of DCM, empirical data and mechanistic studies are required to better understand the indispensable AA requirements of dogs and preventing DCM. In diets that contain high concentrations of dietary fiber, compensative inclusion

of dietary indispensable sulfur AAs, including exogenous taurine, might be required to offset the possibility of increased fecal excretion or microbial assimilation of taurine in the large intestine. Processing conditions may also require adjustments to ensure the presence or effects of antinutritional factors are minimized and nutrient bioavailability is not compromised. Greater awareness of AA balance is crucial for ensuring that AA requirements are met for dogs consuming static diets.

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From: Palmer, Lee Anne
To: Murphy, Jeanette; Norris, Anne; Hartogensis, Martine
Cc: Forfa, Tracey
Subject: RE: DCM roll-out timing question?
Date: Thursday, June 20, 2019 10:44:49 AM

(b) (5)

I don't have slides on research gaps, and I think the other slides I would have are going to be shared with the update. Others may have thoughts...(good luck with this).

From: Murphy, Jeanette
Sent: Thursday, June 20, 2019 10:37 AM
To: Norris, Anne <Anne.Norris@fda.hhs.gov>; Palmer, Lee Anne <LeeAnne.Palmer@fda.hhs.gov>; Hartogensis, Martine <Martine.Hartogensis@fda.hhs.gov>
Cc: Forfa, Tracey <Tracey.Forfa@fda.hhs.gov>
Subject: DCM roll-out timing question?

Greetings Ladies

I am at a training this week and am with one of the ORA division reps who got Dave Rotstein's email about contacting firms and timing.

I took from Dave's email that the roll-out (b) (5).

I am speaking at a conference next Friday (conference is Thurs/Fri) about FSMA updates. The general focus of the conference is research in rendering and pet food for food safety. I anticipate some of the firms that are listed in the complaint release will be there in addition to members of PFI's nutrition sub-committee who has been working on the DCM issue.

Do you have a slide or two I can incorporate on research gaps in DCM or about current science you want shared. And any side bar talking points you want me to have if asked questions.

(b) (6)

From:	Edwards, David
To:	Hartogensis, Martine
Cc:	Norris, Anne; Murphy, Jeanette
Subject:	RE: Invitation to participate in panel discussion on DCM investigation at Petfood Forum conference
Date:	Friday, November 30, 2018 9:12:00 AM

Bill is willing to be on the panel. He is going to help with the AAFCO labeling workshop as well.

Thanks,
Dave

From: Hartogensis, Martine
Sent: Thursday, November 29, 2018 9:14 PM
To: Edwards, David <David.Edwards@fda.hhs.gov>
Cc: Norris, Anne <Anne.Norris@fda.hhs.gov>; Murphy, Jeanette <Jenny.Murphy@fda.hhs.gov>
Subject: RE: Invitation to participate in panel discussion on DCM investigation at Petfood Forum conference

Hi Dave,

My apologies as I missed your reply message back (somehow I am not getting all my emails). Anyway, they are still interested in a CVM rep and I think Bill B would be an excellent choice.

If that works, do you want to ask him? I can ask, but let me know what works best for you.

Thanks again!

Martine

From: Edwards, David <David.Edwards@fda.hhs.gov>
Date: November 9, 2018 at 1:45:59 PM EST
To: Hartogensis, Martine <Martine.Hartogensis@fda.hhs.gov>
Subject: RE: Invitation to participate in panel discussion on DCM investigation at Petfood Forum conference

I know that AAFCO is putting on a pet food labeling workshop at the Forum as well, so there will likely be some from FDA going to participate in that (Bill, maybe). Should we try to get a 2 for 1 and have him go? Unless you are wanting to go.

Dave

From: Hartogensis, Martine
Sent: Friday, November 09, 2018 1:40 PM
To: Edwards, David <David.Edwards@fda.hhs.gov>

Subject: FW: Invitation to participate in panel discussion on DCM investigation at Petfood Forum conference

Hi Dave,

See the request below. Looks like the conference is in Kansas City, MO: https://www.petfoodforumevents.com/. I could probably attend, but others may want to do it (Ok with me).

We used to bring speaking requests to CEB, but I haven't seen any of those discussions recently. Let me know your thoughts and I am happy to send to Susan DeWitt if you like.

Martine

From: Debbie Phillips <DPhillips@wattglobal.com>
Sent: Friday, November 09, 2018 12:32 PM
To: Hartogensis, Martine <Martine.Hartogensis@fda.hhs.gov>
Cc: Norris, Anne <Anne.Norris@fda.hhs.gov>
Subject: Invitation to participate in panel discussion on DCM investigation at Petfood Forum conference

Hi Dr. Hartogensis:

Thank you again for participating in our webinar in September on the atypical cases of canine DCM and their possible link to certain pet diets. This continues to be a topic of conversation and concern in the industry, as you probably are aware, so we are planning a follow-up/update during our annual conference, Petfood Forum, in late April/early May. Could you or one of your colleagues please consider serving as a panel member for this discussion?

The session is currently scheduled for the afternoon of Wednesday, May 1. I know that seems a long way off, but we prefer to issue invitations in advance, as opposed to the hasty invitation for the webinar!

Other panel members will likely include Dr. Greg Aldrich as moderator (who also participated in the webinar, as you know), plus Dr. Jennifer Adolphe, nutrition manager for Petcurean Pet Food; Dr. Kate Shoveller, assistant professor at the University of Guelph; and Dr. Chris Marinangeli, director or nutrition, scientific and regulatory affairs for Pulse Canada.

In case you are not familiar with Petfood Forum, we just held our 26th edition this past April. It is the only event of its kind for the global pet food industry, drawing more than 3,000 people each year from pet food companies around the world, plus from retailers and related businesses, academia and regulatory organizations, such as AAFCO. In addition to education (concurrent scientific tracks plus panel discussions, general sessions, keynotes and other), it includes a trade show featuring the industry's leading suppliers of ingredients, equipment, packaging materials, testing and other services. This year's show had over 400 booths with more than 250 exhibiting companies.

We offer an honorarium to our speakers and panel members, cover their hotel costs and conference registration and reimburse all other travel expenses.

Please let me know if you have questions about Petfood Forum or this panel discussion. Thank you in advance for considering the request!

Sincerely
Debbie

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Check out these upcoming Petfood Forum conferences!
Petfood Forum and Petfood Innovation Workshop 2019: April 29-May 1
Petfood Forum Europe 2019: June 13
Petfood Forum China 2019: August 20
Petfood R&D Showcase: October 2019
Visit www.PetfoodForumEvents.com

From: Rotstein, David
To: Hartogensis, Martine
Subject: RE: logistics-DCM Firm Contact -Quick List 6-18-2019.xlsx
Date: Tuesday, June 18, 2019 2:10:52 PM
Attachments: image001.png
image002.jpg
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image004.jpg
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image006.jpg

Martine,

I'm still confused on that part—it seems like (b) (5) .

I think we can figure out the logistics for the calls. I think having a two day window—one day to hopefully finish and a snow day for firms that we can't get ahold of.

As for the Divisions, (b) (5)

I do have one Division that has not responded to me with a second email request. If I don't hear from them by tomorrow, I'll go the person above them about it.

I do have thoughts on the call scheduling including a call-in line for the whole day (but I'm worried if we go over or a firm calls in early).

Dave

David Rotstein, DVM, MPVM, Dipl. ACVP
CVM Vet-LIRN Liaison
CVM OSC/DC/CERT
7519 Standish Place
(b) (6) **(BB)**

From: Hartogensis, Martine
Sent: Tuesday, June 18, 2019 1:49 PM
To: Rotstein, David <David.Rotstein@fda.hhs.gov>
Subject: RE: logistics-DCM Firm Contact -Quick List 6-18-2019.xlsx

Thanks Dave! (b) (5)

Hopefully we can clarify today.

Thanks again!

Martine

From: Rotstein, David
Sent: Tuesday, June 18, 2019 11:39 AM
To: Hartogensis, Martine <Martine.Hartogensis@fda.hhs.gov>
Cc: Rotstein, David <David.Rotstein@fda.hhs.gov>
Subject: logistics-DCM Firm Contact -Quick List 6-18-2019.xlsx

Martine,

I'm still awaiting word from CA about two of the firms, but here is the breakdown.

As you can see HAF5E has a the bulk of firms.

Here are some thoughts for discussion:

(b) (5)

.

Dave

From: Palmer, Lee Anne
To: Murphy, Jeanette; Hartogensis, Martine
Subject: RE: Meeting with PFI - thoughts, invitees?
Date: Monday, April 08, 2019 12:42:56 PM

One more piece to this – Jen Jones is giving an FDA-wide presentation to the White Oak Veterinarians group on 4/17/19 at noon. Just a heads up, since a wider presentation sometimes grows legs.

From: Palmer, Lee Anne
Sent: Monday, April 8, 2019 11:58 AM
To: Murphy, Jeanette <Jenny.Murphy@fda.hhs.gov>; Hartogensis, Martine <Martine.Hartogensis@fda.hhs.gov>
Subject: RE: Meeting with PFI - thoughts, invitees?

Hi Jenny – I defer to Martine, (b) (5) - a collaboration would be good to pursue. I think an initial meeting to explore ways we can collaborate would be a nice way to begin. I this is going to take industry (PFI nutrition sub-committee group), FDA and our academic partners to really get to the bottom of it.

(b) (5)

It may take some discussion and planning first with them before we can provide anything. I could do either week, but hoping on right on the 22nd, will just be getting back from leave.

Thanks, Lee Anne

From: Murphy, Jeanette
Sent: Monday, April 8, 2019 11:39 AM
To: Palmer, Lee Anne <LeeAnne.Palmer@fda.hhs.gov>; Hartogensis, Martine <Martine.Hartogensis@fda.hhs.gov>
Subject: Meeting with PFI - thoughts, invitees?

LeeAnne and Martine,

My apologies for getting this to you so late.

I met with PFI 2 weeks ago to talk about the work of their nutrition sub-committee and there request to get together with our DCM team.

Their nutrition sub-committee is (b) (4), (b) (5)

(b) (4), (b) (5)

I told them that I was not sure (b) (5), (b) (4)

(b) (5), (b) (4)

I am happy to help set things up or we can potentially get Mia to help schedule/coordinate.

I don't know who all from CVM should be invited but know there are a lot of folks working on the issue.

Thoughts?
Jenny

Jenny Murphy
Office of Surveillance and Compliance
Center for Veterinary Medicine
7519 Standish Place
Rockville, MD 20855
Telephone: **240-402-6246**
Email: jenny.murphy@fda.hhs.gov

From: Conway, Charlotte
To: Murphy, Jeanette
Subject: RE: SME assistance for meeting with PFI and Tim next week
Date: Thursday, April 11, 2019 8:44:00 AM

10-4

From: Murphy, Jeanette
Sent: Wednesday, April 10, 2019 9:26 PM
To: Palmer, Lee Anne <LeeAnne.Palmer@fda.hhs.gov>; Conway, Charlotte <Charlotte.Conway@fda.hhs.gov>; Lambkin, Sonya <Sonya.Lambkin@fda.hhs.gov>
Cc: Large, Machelle <Machelle.Large@fda.hhs.gov>
Subject: SME assistance for meeting with PFI and Tim next week

Greetings LeeAnne, Charlotte, and Sonya,

PFI is coming in next week, Wed 4/17 (2-3:00 p.m.), to have their initial meeting with Tim. This will serve as both a meet and greet and a chance to discuss some issues.

PFI is only bringing in 3 people (b) (6). PFI has proposed the following agenda topics, and Tim has recommend each of you attend to help discuss topics below. While I know there are a lot of people who work on all of these, probably best to keep the invite list small to not totally outnumber the PFI staff. (FYI…I will be on leave next week and will not be in attendance)

In person attendance likely preferred. Mia will provide you with the calendar invite.

Please let me or Mia know if you have any concerns/conflicts and will not be able to make it.
Thanks,
Jenny

Agenda Items:

(b) (4), (b) (5)

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JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

High Potential for Selenium Biofortification of Lentils (*Lens culinaris* L.)

DIL THAVARAJAH, JAMIE RUSZKOWSKI, AND ALBERT VANDENBERG*

Crop Development Centre, College of Agriculture and Bioresources, University of Saskatchewan, 51 Campus Drive, Saskatoon, Saskatchewan S7N 5A8, Canada

Beneficial forms of selenium (Se) and their impact on human health are a global topic of interest in public health. We are studying the genetic potential for Se biofortification of pulse crops to improve human nutrition. Lentils (*Lens culinaris* L.) are an important protein and carbohydrate food and are a valuable source of essential dietary components and trace elements. We analyzed the total Se concentration of 19 lentil genotypes grown at eight locations for two years in Saskatchewan, Canada. We observed significant genotypic and environmental variation in total Se concentration in lentils and that total Se concentration in lentils ranged between 425 and 673 μg kg^{-1}, providing 77−122% of the recommended daily intake in 100 g of dry lentils. Over 70% of the Se was present as selenomethionine (SeMet) with a smaller fraction (<20%) as inorganic Se and very small amounts as selenocysteine (SeCys). We found that soils from the locations where the lentils were grown were rich in Se (37−301 μg kg^{-1}) and that lentils grown in Saskatchewan have the potential to provide an excellent natural source of this essential element. Our analyses gave us a preliminary understanding of the genetic basis of Se uptake in lentil and indicated that any potential strategy for micronutrient biofortification in lentil will require choice of field locations that minimize the spatial variability of soil Se content.

KEYWORDS: Selenium; selenomethionine (SeMet); selenocysteine (SeCys); lentils

INTRODUCTION

Selenium (Se) is an essential micronutrient in human nutrition and is involved in important regulatory and protective mechanisms. The nutritional benefits of Se were first published in 1957 (*1*). Se or, more specifically, selenocysteine (SeCys) is a key component of certain enzymes, for example, in the Se-dependent iodothyronine deiodinases involved in activating thyroid hormone. It also forms the integral parts of glutathione peroxidases and selenoprotein P (*2*), containing one or more atoms of Se per protein molecule. Essential Se-based roles in enzymes, antioxidants, and protective pathways have been discovered and have recently gained importance in cancer suppression, HIV treatment, free radical induced diseases, and protection from toxic heavy metals (*3*−*5*).

Selenium content in the human diet has increasing importance, as the effect of Se deficiency on human health is becoming a topic of interest in public health systems around the world. A recommended dietary allowance (RDA) of 55 μg of Se day^{-1} has been established for regular adults in the United States (*6*), and 60−75 μg of Se day^{-1} has been recommended for regular adults in the United Kingdom (*7*). This requirement is generally met by North Americans; however, large numbers of people in Europe, Asia, Australia, and parts of Africa have intakes of less than the RDA level. Selenium-enriched commercial fertilizers have been recommended in Finland since 1984 to increase Se content in their major food crops. The recommended fertilizer rate for cereals and other crops was 16 mg of Se as sodium selenate (Na_2SeO_4) per kilogram of fertilizer. Since then, the daily Se intake of the Finnish population has increased from 39 μg of Se day^{-1} in 1984 to 110 μg of Se day^{-1} in 1998 (*8*). Low intake of Se (<25 μg day^{-1}) is linked to specific diseases such as arsenicosis in Bangladesh and fatal juvenile cardiomyopathy (Keshan disease) in China. Deficiency is also linked to specific diseases such as poor skeletal muscle strength in older adults (*9*), and even a slight deficiency has now been associated with other disorders including chronic heart failure and prostate and bladder cancers (*10*−*14*). A dietary intake of 55−200 μg of Se per day is now recommended as safe and adequate to reduce the risks of several types of cancer (*15*, *16*). Recently, several clinical studies examined the relationship between serum Se levels and the prevalence of diabetes among U.S. adults and suggested that the adverse effects of a high intake of Se may increase primary or secondary diabetes (*12*, *17*, *18*).

The Se content of the soil from which foods are derived is the major influence on dietary intake of Se. Soil Se is highly variable in distribution and chemical availability. Most soils around the world contain 0.1−2 μg of Se kg^{-1} (*19*). Deficient soils in New Zealand, Australia, Denmark, central Siberia, northeast to south central China, parts of India, and Bangladesh

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Table 1. Market Class, Major Consuming Countries, Protein Content and Cotyledon Color of 19 Lentil Genotypes Grown in Saskatchewan, Canada

market class	seed wt (mg)	major consuming countries	genotype	protein[a] (%)	cotyledon color
extra small red	<30	Bangladesh, Pakistan, Egypt	CDC Robin	26	red
			CDC Rosetown	27	red
			CDC Imperial	27	red
small red	30−50	England, Middle East, Sri Lanka, India, Pakistan	CDC Blaze	27	red
			CDC Impact	27	red
			CDC Redberry	25	red
			CDC Rouleau	22	red
large red	>65	USA, Dubai, Sri Lanka	Red Chief	25	red
large green	>65	Spain, Turkey, Iran, Germany, Algeria	Laird	24	yellow
			CDC Grandora	23	yellow
			CDC Greenland	24	yellow
			CDC Plato	22	yellow
			CDC Sedley	22	yellow
			CDC Sovereign	24	yellow
medium green	50−60	Latin America, Europe	CDC Richlea	20	yellow
			CDC Meteor	23	yellow
small green	<40	Italy, Morocco, Greece, mexico	CDC Viceroy	24	yellow
			CDC Milestone	25	yellow
			Eston	25	yellow

[a] Protein (%) was calculated on the basis of the total seed nitrogen content ($n = 57$).

produce crops that are very low in Se (*20*). On the other hand, soils of Ireland, Colombia, and Venezuela and of the Great Plains of the United States and Canada are naturally rich in Se (*16*). Our initial research showed that Saskatchewan soils have abundant Se and that lentils grown in Saskatchewan may have the potential to provide a significant natural source of this essential element (*21*). However, we have limited understanding of the potential for genetic improvement of Se uptake in Saskatchewan grown lentils.

Biofortification by enrichment of the nutritional contribution of staple food crops through plant breeding is one option that is now widely discussed in the fields of nutrition, public health, and agriculture at national and international levels. Development of an effective biofortification strategy requires the application of genetics and agronomy to provide a solution to wide-scale nutrition problems (*22*). Studies have demonstrated that there is significant genetic variation for Se uptake in soybean, wheat, and *Brassica* vegetables (*23*−*25*).

Pulses combined with cereals are central to the diets of billions of people, and the potential for Se biofortification of pulses is high because of their relatively high protein content. World lentil production on an annual basis is approximately 4 million metric tonnes, and about 20−25% is grown in Saskatchewan, Canada (*26*). Saskatchewan supplies lentils to consumers in more than 100 countries with concentrated regions of consumption in Europe, the Middle East, and most notably South Asia (**Table 1**). Health problems due to Se deficiency affect over 100 million people around the world, many of them in lentil-consuming countries. Progress has been made in controlling Se deficiency through dietary supplementation, food fortification, and agronomic fertilization, but new approaches such as biofortification of basic foodstuffs are needed. Supplying essential Se through widely consumed meals such as lentils and rice could help increase the intake of dietary Se in regions where Se intake is insufficient (*7*). Research is needed to determine whether significant genetic variation exists in pulse crops for Se uptake to develop appropriate breeding strategies in the future. This also requires an understanding of the Se content of soils. We investigated the potential for biofortification of Se content for Saskatchewan-grown lentils as a means of improving human nutrition. The dual objectives of this study were to (1) measure the total Se content of seeds of 19 lentil genotypes grown in 8 key lentil-growing regions in Saskatchewan, Canada, in 2005 and 2006 and (2) identify the chemical forms of Se in extra small red lentil cultivar CDC Robin grown in Saskatoon in 2005.

Table 2. Experimental Design and Sample Protocol

year	2005, 2006
no. of study locations per year	8
study locations (soil zones)	1. Saskatoon (moist dark brown) 2. Kyle (brown) 3. Swift Current (brown) 4. Wilkie (dark brown) 5. Melfort (black) 6. Hodgeville (brown) 7. Rosthern (thin black) 8. Rouleau (moist dark brown)
no. of soil samples per location	4 ($n = 32$)
no. of lentil genotypes per location	19
no. of replications	3 per genotype
no. of lentil seed samples per location (total Se analysis)	114
total no. of lentil samples tested for total Se content	912

EXPERIMENTAL METHODS

Materials. Se standards and chemicals used for digestion and for total Se measurements were purchased from VWR International (Canada) and Sigma-Aldrich Co. (Canada). High-purity chemicals and solvents for HPLC analysis were purchased from Sigma-Aldrich Co. and were used without further purification.

Soil Samples. Locations of the field research sites in Saskatchewan and sample protocol are listed in **Table 2**. These locations cover the major lentil-growing areas in Saskatchewan. Four soil cores were collected at each site from the 0−30 cm soil layer. They were air-dried (≤40 °C), passed through a 2 mm sieve, homogenized into one composite sample, and stored in plastic vials at −20 °C until analysis. The soil samples were collected in October 2005, about 1 month after the lentil plots were harvested.

Approximately 1 g of soil underwent primary organic digestion in 3 mL of HNO_3 (70%) at 90 °C followed by 1 mL of 30% H_2O_2 and further digestion in 3 mL of 70% HNO_3 and 9 mL of 35% HCl at 90 °C over several hours (24 h). The resulting slurry was filtered and made up to 50 mL in deionized water. Measurements of total Se using this modified method were validated using NIST standard reference material 2586 (soils; [Se] = 0.6 ± 0.005 mg kg^{-1}). Soils from the South Saskatchewan River bank ([Se] = 110 mg kg^{-1}), where the Se

hyperaccumulator *Astragalus bisulcatus* grows naturally, were used as a laboratory reference material and measured periodically to ensure consistency in the method. The total Se concentrations of different soils are indicated as the mean of three replicates with standard error.

Lentil Seed Samples. Lentil seeds were obtained from regional variety trials conducted in 2005 and 2006 by the Crop Development Centre (CDC), University of Saskatchewan, Canada. The selected lentil genotypes, market class, and major consuming countries are listed in **Table 1**. For the genotype × environment study, samples of between 10 and 20 g of dry lentil seeds (14% moisture) were collected from each location with three replicates. Each replicated seed sample was prepared by standard HNO_3 H_2O_2 digestion as described previously (*21*). Measurements of total Se concentration using this modified method were validated using NIST standard reference material 1573a (tomato leaves; [Se] = 0.054 ± 0.003 mg kg^{-1}). Total Se was measured by hydride generation flame atomic absorption spectroscopy (HGAAS) on a Varian SpectrAA150 equipped with a hydride generation apparatus (Varian Canada Inc., Mississauga, ON, Canada). Measurements were made on the digested sample solutions outlined above.

Se Speciation. For the Se speciation study, seed samples of 250−500 g were obtained from three replicated plots of the variety CDC Robin (*27*) grown at the Saskatoon location in 2005. The seeds were dehulled in a Satake TM-05 grain-testing mill (Satake Engineering Co. Ltd. Japan) and then carefully separated by hand into seed coat, embryo, and cotyledon fractions. Se species were separated on a BioCAD Sprint perfusion chromatograph fitted with a 100 μL sample loop using an anion-exchange column (Hamilton PRP-X100, Reno, Nevada, NV) and a reverse-phase C18 column (Varian, Lake Forest, CA) using previously developed and reported methods (*28*). Anionic exchange was carried out with 10 mM citric acid, 1 mM potassium hydrogen phthalate (KHP), and 1 μM rubidium nitrate made to pH 4.5 with ammonium hydroxide in water and 2% v/v methanol. Other Se compounds were confirmed using the C18 column with 10 mM triethylamine and 1 mM KHP at pH 9 in water and 2% v/v methanol. Relative concentrations of Se species in natural samples were determined by ICP-MS (Saskatchewan Research Council, Saskatoon, SK, Canada) normalized to rubidium-spiked HPLC solvent.

Lentil samples were ground to a fine powder, and a 250 mg subsample was suspended in 4 mL of Millipore water. Samples were digested by 10 mg of protease XIV (*Streptomyces griseus*) at 38 °C for 90 min, centrifuged, filtered through a 0.5 μm PTFE membrane, and mixed with 3 equiv of HPLC solvent. Standards (SeMet, Se-methylselenocysteine, selenate, selenite) were used after simple dilution to 40 ng Se mL^{-1}. SeCys was prepared from CysSeSeCys by dissolution at pH 11, followed by sodium borohydride reduction. CysSeSeCys was dissolved with 6 M HCl before dilution with solvent.

Statistical Analysis. The experimental design was a randomized complete block design with 3 replicates, at 8 locations for 19 genotypes over 2 years. Subsamples of lentil seeds for the determination of total Se were randomly taken from the entire harvested sample of each of the field plots. Data from both years and 8 locations were combined, and data error variances were tested for homogeneity. Locations, replications, years, and genotypes were considered as random factors. Class variables were year, location, replication, and genotype. Mixed-model analysis of variance was performed using the PROC GLM procedure of SAS version 8.2. Means were separated by Fisher's protected LSD at $P < 0.05$ (*29*). For each location−year data were analyzed separately using the General Linear Model procedure (PROC GLM) of SAS version 8.2 (*29*). Means were separated by Fisher's protected least significant difference (LSD) at $P < 0.05$. The broad sense heritability (H^2) of Se concentration in lentil seeds was calculated from the error mean squares from PROC GLM of SAS version 8.2 (*30*).

RESULTS

Soil Se Concentrations and Conditions. Se availability in soils depends upon soil pH, aeration, organic carbon, and iron levels. In acidic soils, Se is relatively unavailable to plants and occurs mainly as insoluble selenides and elemental forms. This study examined soils from eight different locations in Saskatchewan covering major soil zones where the lentil crop is grown. The total soil Se concentration ranged from 37 to 301 μg of Se kg^{-1}, or equivalent to 0.5−3.8 μmol of Se g^{-1} (**Table 3**). The Saskatoon location showed significantly higher total soil Se concentration than Rouleau or Swift Current. The Wilkie and Melfort locations were the second highest in total soil Se concentration. The most predominant soil texture at these locations was clay loam, and soil pH ranged from 5.9 to 7.9 (**Table 3**). The soil at Rouleau was more alkaline, and at Wilkie it was slightly acidic and poorly aerated.

Table 3. Total Soil Se Concentration, Soil Texture, and pH from Various Locations in Saskatchewan, Canada

location	soil texture	soil pH	total soil Se (μg kg^{-1})	SE[a]
Saskatoon	clay loam	6.3	301	5
Wilkie	clay loam	5.9	262	5
Melfort	clay loam	7.3	213	7
Kyle	clay loam	6.3	75	3
Rosthern	silt loam	6.5	71	5
Hodgeville	clay loam	7.1	70	5
Swift Current	clay loam	6.4	45	5
Rouleau	heavy clay	7.9	37	3

[a] Standard error ($n = 4$).

Table 4. Summary of Combined Analysis of Variance for Total Se Concentration of 19 Lentil Genotypes Grown at Different Locations in Saskatchewan, Canada

source	df	mean square[a]
year	1	1845855*
location	7	19770258*
genotype	18	156536**
replication (year, location)	32	306369*
year × location	7	8191592*
genotype × year	18	101913
genotype × location	126	72551
error	576	32043

[a] Mean square was significantly different at *, $P < 0.05$, and **, $P < 0.1$, respectively.

Total Se Concentration and Se Species (Beneficial Forms) in Lentil Seeds. Combined statistical analysis (mixed model) over the years and locations showed that variation in total Se concentration in lentil seeds was significant ($P < 0.05$ and $P < 0.1$) for years, locations, genotypes, and the interaction between location and year (**Table 4**). As expected with most quantitative traits, the interaction between the genotype and location shows that most of the variation in the total Se concentration in the lentil seeds may have been due to environmental variation such as soil Se content, soil moisture, and other crop management practices. Therefore, data were analyzed and presented in this paper separately for each location−year (**Table 5**). Significant genotypic differences in total Se in lentil seeds were observed at all but two locations: Rosthern and Wilkie in 2005 (**Table 5**). Lentils grown at Saskatoon and Kyle had the greatest mean total Se concentration (643−1884 μg of Se kg^{-1}) compared to those from Swift Current (139−233 μg of Se kg^{-1}), Rosthern (87−305 μg of Se kg^{-1}), and Wilkie (206−392 μg of Se kg^{-1}) (**Table 5**). Furthermore, it was found that the Se concentration of lentil seeds from Wilkie and Melfort was not influenced by soil Se concentration. High levels of seed Se were not observed, despite high soil Se concentration at these locations. The soil moisture conditions, weather patterns, and soil Se available to plants might explain these differences (**Table 3**). Lentils grown at Wilkie may have had lower concentrations of Se due to soil

Table 5. Mean Total Se Concentration of 19 Lentil Genotypes Grown at Different Locations in Saskatchewan, Canada in 2005 and 2006

		total Se concn in lentils (μg kg^{-1})			
year	location	min	max	mean (SE)[a]	genotype effect[b]
2005	Saskatoon	900	2104	1324(7)	*
	Kyle	553	851	643(2)	*
	Hodgeville	232	1403	536(3)	*
	Rosthern	162	560	305(3)	NS
	Melfort	108	619	298(3)	*
	Rouleau	149	403	269(1)	*
	Swift Current	137	327	233(1)	*
	Wilkie	76	505	206(3)	NS
2006	Kyle	1236	2609	1884(5)	*
	Saskatoon	510	1662	885(4)	*
	Rouleau	442	990	633(1)	*
	Melfort	160	507	308(2)	*
	Hodgeville	128	380	220(1)	*
	Wilkie	121	614	392(3)	*
	Swift Current	61	254	139(1)	*
	Rosthern	32	185	87(1)	*

[a] SE, pooled standard error of mean calculated from mean square of ANOVA for each location ($n = 57$). [b] Genotype effect was significantly different at $P < 0.05$. NS, not significant at $P < 0.05$.

acidity, lower soil aeration, and high soil iron concentrations. Comparison of Se concentrations of Saskatoon and Kyle reflected the influence of soil moisture. In 2005, Saskatoon had higher precipitation than in 2006, whereas Kyle experienced greater precipitation in 2006 compared to 2005 (*31*). Between years, seed Se levels at a particular location varied up to 3-fold. The year-to-year variation in Se levels at any specific location can be explained by both soil and weather factors that influence the uptake of Se during grain filling. At each location, the trial fields follow a particular crop rotation and soil properties from field to field can vary substantially in soils derived from glacial till. The weather patterns, particularly temperature and precipitation, are extremely variable in a continental climate and can have a large influence on the availability of soil Se at any time during the growing season.

Se concentration in the lentil seeds varied 4−5-fold across the locations, and on average, seeds of some genotypes had 40−50% more than others (**Table 6**). The extra small red lentil genotype (CDC Robin) and two of the large green lentil genotypes (CDC Sedley and CDC Grandora) had the highest total Se concentrations (612−672 μg of Se kg^{-1}). The small green lentil genotype, Eston, had the lowest (**Table 6**). We calculated that a 35 g serving of CDC Robin lentil (95th percentile of lentil intake per person) grown in Saskatchewan could supply 42% of the current RDA in the United States (55 μg of Se day^{-1}).

Our elemental analysis from seed fractions of CDC Robin lentil from a high Se location (Saskatoon) had mean total Se concentrations as follows: embryo axis, 3600 μg of Se kg^{-1} (45.6 μmol of Se kg^{-1}); cotyledon, 2800 μg of Se kg^{-1} (35.5 μmol of Se kg^{-1}); and seed coat, 2600 μg of Se kg^{-1} (32.9 μmol of Se kg^{-1}). Our previous experiments (*21*) demonstrated that whole seeds of CDC Robin from the Saskatoon location (0.72 μg of Se kg^{-1}; 9.1 nmol of Se kg^{-1}) and those from Swift Current (0.16 μg of Se kg^{-1}; 2 nmol of Se kg^{-1}) showed the greatest range of total Se concentration of the locations tested.

The relative content of Se chemical forms in the lentil seeds was determined by various HPLC-ICP-MS techniques. CDC Robin is a commercially grown cultivar in Saskatchewan because of its early maturity, disease resistance, high yield, and consumer preference. In addition, it was found that lentil seeds from CDC Robin had the highest Se concentration compared to a wide rage of lentil genotypes grown in Saskatchewan. Furthermore, many South Asian consumers (specifically Bangladesh) prefer red cotyledon, extra small seed size (>30 mg) cultivars such as CDC Robin. On the basis of these factors, we chose CDC Robin to study the Se speciation. More than 70% of the Se in the whole lentil sample was present as organic Se with a small fraction (<20%) as inorganic Se (**Table 7**). Small fractions (7%) of SeCys and γ-glutamylselenocysteine were present in the whole lentil seeds, and the concentrations of the other Se species (selenomethionine, dimethylselenoxide, and Se-methylselenocysteine) were not significant. In the embryonic axis, >80% of the Se was present as organic Se with a small fraction (20%) as inorganic Se. SeMet (73%) and selenate (27%) were the major chemical forms of Se present in CDC Robin cotyledon, and inorganic Se (94%) was the major chemical form of Se present in the lentil seed coat (**Table 7**). Our results clearly indicated that the field-grown CDC Robin lentils contained predominately organic Se (80%) as SeMet and SeCys with a minor component of inorganic Se (20%).

Table 6. Comparison of Total Se Concentration in 19 Lentil Genotypes Grown in Saskatchewan, Canada, in 2005 and 2006

	total Se concn (μg kg^{-1})			%RDA[a] (100 g of lentil)	
genotype	Saskatoon (2005)	Kyle (2006)	mean[b] (8 locations, 2 years)	North America (55 μg day^{-1})	Europe (65 μg day^{-1})
CDC Robin	2104 a	2012 bcd	672 a	122	103
CDC Sedley	1446 abcd	2127 abc	612 a	111	94
CDC Grandora	1694 abc	2351 ab	612 a	111	94
Laird	1232 cd	1970 bcde	593 abc	108	91
CDC Greenland	1064 cd	2609 a	544 abcd	99	84
CDC Imperial	1246 cd	1884 bcdef	538 bcde	98	83
CDC Redberry	1947 ab	1583 defg	533 cde	97	82
CDC Sovereign	1503 abcd	2364 ab	533 cde	97	82
CDC Plato	1178 cd	2035 bcd	532 cde	97	82
CDC Meteor	1483 abcd	1470 efg	510 def	93	78
CDC Blaze	1413 bcd	2119 abc	509 def	93	78
CDC Rosetown	1005 d	1942 bcdef	505 def	92	78
CDC Richlea	900 d	2008 bcd	498 defg	91	77
CDC Impact	1136 cd	1844 bcdef	491 defgh	89	76
Red Chief	1429 abcd	1421 fg	472 efgh	86	73
CDC Viceroy	1009 cd	1685 cdefg	471 efgh	86	72
CDC Milestone	1186 cd	1236 g	457 fgh	83	70
CDC Rouleau	1271 cd	1585 defg	431 gh	78	66
Eston	901 d	1555 defg	425 h	77	65
SE[c]	7	5	6		

[a] %RDA was calculated on the basis of the mean total Se concentration across eight locations ($n = 912$) in Saskatchewan. [b] Means within a column followed by different letters are significantly different at $P < 0.05$. [c] SE, pooled standard error of mean calculated from mean square of ANOVA for each location ($n = 57$) and mean of eight locations ($n = 912$).

DISCUSSION

The biological importance of Se and its roles in human health have recently become of great interest in the international community. There is a great necessity for food systems to provide at least 55 μg per day for maximal expression of Se enzymes, and large populations in some parts of the world are Se deficient. Se deficiency compromises the health of developing children and reduces the ability to combat the effects of heavy metals in the human diet (*32*). As a common, universal, and quick-cooking nutritious food source, lentils have the potential to deliver

Table 7. Total Se Concentration and Percentage Composition of Se Species for CDC Robin (Saskatoon, 2005)

			percentage of Se species present in lentil seeds as[a]				
			organic Se			inorganic Se	
seed fraction[b]	contribution to total seed wt (%)	total Se (SE)[c] (μg kg^{-1})	SeMet (±SE)	selenocysteines (±SE)[d]	γ-glutamylselenocysteine (±SE)	selenate (±SE)	selenite (±SE)
CDC Robin whole seed	100	2104	69 ± 2	7 ± 1	2 ± 1	10 ± 1	9 ± 1
CDC Robin embryo	5	3600	19 ± 1	53 ± 2	8 ± 1	3 ± 1	17 ± 1
CDC Robin cotyledon	88	2800	73 ± 2	nd[e]	nd	27 ± 2	nd
CDC Robin seed coat	7	2600	nd	nd	6 ± 1	80 ± 2	14 ± 1

[a] Se speciation as determined using LC and ICP-MS. [b] Lentils were collected from Saskatoon location, 2005 (n = 12). [c] SE, standard error. [d] Selenocysteine and selenocystine. [e] nd, not detected within the limits of quantitation (1.6 parts per trillion in HPLC fraction).

beneficial Se to those who need it. Lentil-growing regions with adequate soil Se play a fundamental role in this mass distribution. We have shown that Saskatchewan-grown lentils contain 425−673 μg of Se kg^{-1} depending upon location, soil characteristics, and growing conditions. This potentially provides 80−120% of the minimum recommended daily Se intake in only 100 g of dry lentils. Our data are derived from small-plot field trials. The Se concentration available in commercial lentil shipments would likely reflect a blended average across many fields in multiple locations.

There is unique potential for Se-rich lentil and other pulse crops to be grown in western Canada without soil supplementation. We conducted a preliminary analysis of the Se content of lentil seeds grown in some other regions of the world (U.S. Pacific Northwest, >50 samples; Australia, >40 samples; Syria, 7 samples; Bangladesh, 12 samples; India, 10 samples; and Nepal, 5 samples). All samples had very low Se concentration, on average, <5% of the Se content of the lowest Se content lentils from Saskatchewan (Swift Current). Many samples from Syria, Bangladesh, India, and Nepal had no detectable Se (<20 ppb) (data not shown).

The chemical species distribution in seeds of Se is important in terms of nutritional benefits. It has long been understood that certain forms of Se are critical to development and self-regulation, whereas others are potential poisons. The biological fate of Se is also determined by the original form and the transformation that occurs during digestion and absorption. The amino acid SeMet is readily incorporated into protein masses, but SeCys, which is found in key regulatory proteins, is tightly controlled and is catabolized into hydrogen selenide. Inorganic forms, such as selenate and selenite, have been studied for their involvement in the treatment of arsenicosis and excretion of mercury (*32*).

The presence of Se in plants grown on soils containing available Se has been reported in many studies. Seleniferous green onion (*Allium cepa* L.) predominantly contained SeMet and small amounts of SeCys (*33*). SeMet and SeCys were the major organic selenides found in sour clover (*Melilotus indica* L.) and alfalfa (*Medicago sativa* L.) grown in seleniferous soils in California (*34*). SeMet is the major organic form of Se found in wheat, common bean, mushroom, and yeast (*35*). Our findings for Se in lentils seed are similar to those reported for seeds of seleniferous wheat (*Triticum aestivum* L.), common bean (*Phaseolus vulgaris* L.), alfalfa, and sour clover, which contain mainly SeMet with a smaller fraction of SeCys (*34, 35*).

HPLC-ICP-MS analysis of the Se species in whole lentils revealed that most of Se was present as SeMet with small amounts of selenate and very small amounts of selenocysteines, selenite, and other selenooligopeptides such as γ-glutamylselenocysteine (gGSeCys) as outlined in **Table 7**. This supports our previous experiments using synchrotron X-ray spectroscopy to identify Se species in lentil seeds and seed tissues (*21*). Synchrotron techniques offer a unique advantage in that samples can be run intact with no pretreatment, but it is difficult to differentiate between chemically similar species such as SeMet and Se-methylselenocysteine (Se-MeSeCys) or to reliably detect smaller components when one type is in great excess. HPLC-ICP-MS methods for Se quantitation can be used to differentiate the forms that X-ray techniques cannot. Conversely, the overlap of other chemical species in the HPLC methods can be differentiated in the related synchrotron experiments. Both methods used in conjunction are sufficient to determine the complete set of Se forms present in seeds.

Our analysis of Se speciation in CDC Robin lentil provided an indication that Se species may vary according to the seed component. The seed coat has a unique Se species profile, largely inorganic. The embryonic axis is enriched for SeCys in comparison to cotyledon tissue. Red lentils are usually decorticated prior to cooking in whole or split form. In terms of Se speciation, split lentils may have lower SeCys content because the embryo fraction is often collected as a byproduct for use in animal feed. In some countries, for example, in Bangladesh, consumers have a distinct preference for decorticated unsplit lentils, which may be beneficial for human nutrition.

Other factors, such as cooking, grinding, and digestion that may affect or transform Se speciation, have been investigated. We found that cooking the lentils in boiling water did not change the total Se content (data not shown). There is a migration of Se from the lentils to the liquid broth, but provided the lentils and broth are consumed as a whole food source, the Se concentration and speciation remain intact. However, we would expect a nearly 50% reduction in Se for lentils that are thermally processed in brine (canned) and consumed after the canning brine is discarded (*36*).

In many parts of the world, lentils with adequate beneficial Se concentration could be considered a natural, whole food source for Se, and a possible solution to Se deficiency-related arsenicosis in Bangladesh and juvenile cardiomyopathy (Keshan disease) in China (*32*). Supplementation of 200 μg per day may help to prevent certain cancers, such as bladder, prostate, liver, colorectal, and lung cancers (*16*). Efforts to optimize the Se in food sources must consider not only the overall concentration but the amounts of the various beneficial forms.

Quantitative traits generally depend on the collective interaction of many genes. The expression of quantitative genes is also influenced by the environment. The phenotypic variance calculation is influenced by the number of years, locations, and replicates used in the experiment, therefore, plant breeders commonly use heritability estimates to distinguish the proportion of total phenotypic variation due to genotype and environmental influences. This estimate is then used to design appropriate genetic improvement strategies In this study, the estimate of

genetic variance was 1135 and that of phenotypic variance was 2877. The broad sense heritability estimate was 0.4, which indicates that Se content in lentil is in the midrange of heritability. An appropriate genetic improvement strategy for increasing Se content lentil would require that environmental influence be kept to a minimum by careful selection of environments with low spatial variability for soil Se content combined with appropriate replication.

Breeding for enhanced Se accumulation and selective speciation may be an effective strategy to help overcome global Se deficiencies. Some studies have suggested the possibility of genetic improvement for Se uptake in *Brassica* vegetables, wheat and soybean (*23*−*25*). By specifically controlling the Se variability in soil Se content, it would be possible to reduce environmental effects as part of our biofortification approach on lentil genetic improvement. It may be possible to screen lentil genotypes for increased Se uptake ability using atomic absorption spectroscopy techniques or possibly using marker-assisted genetic selection. On the basis of our results, we suggest that it may be possible to cost effectively breed lentil cultivars for enhanced Se uptake for specific regions of the world with soils that have lower levels of Se. In regions where Se is highly deficient, it may be necessary to combine this approach with agronomic biofortification using fertilizer with Se additives. This may be particularly important for regions where the rapidly increasing cost of rice may induce further reduction in the land area devoted to pulse production. Further studies are being performed on diverse genotypes, including wild relatives of cultivated lentil, modern commercial cultivars, and genotypes adapted to different geographic locations in Europe, Asia, Africa, and North America.

Se must be available in the soil for uptake and transformation. In general, soil Se is unevenly distributed and varied in availability, ranging from <0.1 to >100 ppm, and most commonly from 1 to 1.5 ppm (*19*). Ultimately, the total Se in the soil depends on the minerals in the rocks from which the soil was derived. Soils of the Northwest, Southeast, and Great Lakes regions of the United States were derived from volcanic deposits and have low soil Se content (<0.05 ppm) (*37*). Soils originating from cretaceous shales, such as those found in South Dakota and Montana, tend to have concentrations upward of 10 ppm (*37*). However, the availability of the Se is greatly dependent on aeration, water availability, pH, and soil texture and composition. In poorly aerated soils, Se is relatively unavailable to plants and occurs mainly as insoluble selenides and elemental forms. Furthermore, wetter soils with alkaline pH have lower Se concentration due to leaching of mobile selenate (*20*).

Our study indicates that uptake of Se in lentil seeds is affected by soil and environmental conditions such as moisture, soil texture, aeration, and soil fertility and irrigation (*7*). The higher Se concentration observed from the Saskatoon area may be due to higher spatial variability in the soil combined with wet weather conditions, which would increase availability of soil Se. Soils at Rouleau had the lowest Se concentration (**Table 3**) and the highest pH. The Rouleau soil was a moist heavy clay with poor aeration, thus reducing the amount of Se that is available to plants. We found that the total soil Se concentration was not the best indicator of plant Se availability, although it is the most commonly used method of reporting Se availability in the literature (*38*). A complete understanding of the biochemistry of Se in soil and lentil plants will require more in-depth studies of plant biochemistry, agronomy, and physiology.

In summary, our present study shows that Saskatchewan soils are naturally rich in Se and that lentils grown in them have great potential as a quick-cooking, Se-rich, natural food product. Significant genotypic differences for Se concentration were observed across the locations. In addition, genotype × environment analysis of the concentration of Se in the lentils indicated that good potential exists for genetic improvement of the concentration of this essential element in lentil. The Se content and the chemical forms of Se within the seed may be altered by conventional plant breeding approaches or by optimizing agricultural production conditions.

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		800.218-sub 1 (b) (6)		
		Case Sample	**Label**	**AAFCO**
		California Naturals Kangaroo & Lentil	**Product Nutrient Analysis (website label)**	**AAFCO-Adult Maint**
(b) (4)	Ca	1.30%	0.83%	0.5 to 2.5%
	Mg	0.13%	0.17%	0.06%
	P	0.74%	0.71%	0.4 to 1.6 %
	Fe	**30 mg/kg**	305 mg/kg	40 mg/kg
	Co	0.12 mg/kg	n/a	25 mg/kg-chicks/rats/sheep max
	Cu	21 mg/kg	13.61 mg/kg	7.3 mg/kg
	Zn	240 mg/kg	193.37 mg/kg	80 mg/kg
	Se	0.7 mg/kg	0.08 mg/kg	0.35 to 2 mg/kg
	Ca:P	1.76:1		1:1 to 2:1
	Cu:Zn	0.09:1		0.09:1-not AAFCO
(b) (4)	Tau	~0.26%		0.1% in Cats
	Cystine	2.32 mg/g = ~0.23%		n/a
	Met	5.78 mg/g = ~0.58%	0.61%	0.33%
	Met-Cys	~0.81%	0.97%	0.65%

		800.218-sub 5		
		Case Sample	**Label**	**AAFCO**
		California Naturals Chicken Meal	**Product Nutrient Analysis (website label)**	**AAFCO-Adult Maint**
(b) (6)	Ca	1.80%	1.98%	0.5 to 2.5%
	Mg	0.14%	0.12%	0.06%
	P	1.30%	1.34%	0.4 to 1.6 %
	Fe	**39 mg/kg**	156 mg/kg	40 mg/kg
	Co	0.14 mg/kg	n/a	25 mg/kg-chicks/rats/sheep max
	Cu	19 mg/kg	18 mg/kg	7.3 mg/kg
	Zn	330 mg/kg	229 mg/kg	80 mg/kg
	Se	0.66 mg/kg	0.78 mg/kg	0.35 to 2 mg/kg
	Ca:P	1.38:1		1:1 to 2:1
	Cu:Zn	0.06:1		0.09:1-not AAFCO
(b) (6)	Tau	1.08 mg/g = ~0.11%		0.1% in Cats
	Cystine	3.2 mg/g = ~0.32%		n/a
	Met	6.2 mg/g = ~0.62%	0.65%	0.33%
	Met-Cys	~0.94%	0.98%	0.65%

		800.218-sub 4 Case Sample Fromm Heartland Gold Grain Free Large Breed Adult	Product Typical Analysis (website label)	AAFCO Growth & Maint
(b) (6)	Ca	1.20%	1.14%	1.2 to 1.8%
	Mg	0.14%	0.17%	0.06%
	P	1%	1.08%	1 to 1.6%
	Fe	**30 mg/kg**	258.26 mg/kg	88 mg/kg
	Co	0.37 mg/kg	n/a	25 mg/kg-chicks/rats/sheep max
	Cu	25 mg/kg	25.83 mg/kg	12.4 mg/kg
	Zn	170 mg/kg	217.37 mg/kg	100 mg/kg
	Se	0.85 mg/kg	n/a	0.35 to 2 mg/kg
	Ca:P	1.2:1		1:1 to 2:1
	Cu:Zn	0.15:1		0.09:1-not AAFCO
(b) (4)	Tau	1.84 mg/g = ~0.18%	n/a	0.1% in Cats
	Cystine	3.15 mg/g = ~0.32%	n/a	n/a
	Met	4.75 mg/g = ~0.48%	n/a	0.35%
	Met-Cys	~0.79%	n/a	0.70%

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Cardiomyopathy and Myocardial Degeneration in Stranded Pygmy (*Kogia breviceps*) and Dwarf (*Kogia sima*) Sperm Whales

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Abstract

Cardiomyopathy (CMP) has been documented as a disease associated with stranded pygmy (*Kogia breviceps*) and dwarf (*Kogia sima*) sperm whales in the United States and Asia. In this study, hearts from 27 pygmy and two dwarf sperm whales stranded in the coastal U.S. Atlantic Ocean and Gulf of Mexico from 1999 to 2006 were analyzed. Gross and microscopic examinations were conducted according to a standardized protocol designed to ensure systematic examination of tissue and data recording. Hearts were weighed and specific measurements made for selected tissues. Fourteen (48.3%) pygmy sperm whales had a microscopic diagnosis of CMP, 12 (41.4%) showed evidence of mild myocardial degeneration (MCD), one (3.4%) had moderate myocarditis and two (6.9%) had no pathological lesions. One dwarf sperm whale had CMP, and the other had mild MCD. The majority of stranded *Kogia* spp. with cardiac lesions came from the southeast Atlantic region (19/27, 70.3%). Cardiomyopathy and MCD lesions were found predominantly among adult whales. An excess of males was found for CMP and MCD (approximately 75% of both groups). The predominant histological lesions found in both disorders were anisokaryosis with karyomegaly and nuclear rowing, followed in frequency by interstitial edema. Cardiac weight, ventricular wall thickness, and valve circumference were compared between pygmy sperm whales with CMP and those with MCD. The largest differences were found for heart weight and intraventricular septum wall thickness, but none of the differences were statistically significant. Further adjustment for sex and body length did not alter the results. In the aggregate, these findings suggest that CMP in *Kogia* spp. is a chronic, progressive condition that represents a continuum from MCD to the more severe forms of the disorder. The etiology of this complex disorder remains unknown.

Key Words: pygmy sperm whale, *Kogia breviceps*, dwarf sperm whale, *Kogia sima*, cardiomyopathy, myocardial degeneration, stranding, U.S. Atlantic Ocean and Gulf of Mexico

Introduction

Cardiomyopathy (CMP) was first described in pygmy (*Kogia breviceps*) and dwarf (*Kogia sima*) sperm whales in 1985 in a study group of 29 beached whales (Bossart et al., 1985). The disease in *Kogia* spp. has been described primarily in whales from the southeastern Atlantic Ocean, but it also occurs in Pacific Ocean whales (Chiu et al., 2003). The etiopathogenesis of the *Kogia* CMP is unknown. Distinct clinical, functional, and pathological patterns of CMP occur in domestic animals and humans, however, and each pattern may be associated with distinct pathogenic mechanisms. While controversies exist with CMP classification schemes, the general clinical, functional, and pathological patterns of CMP are the stress, dilated, hypertrophic, and restrictive forms.

Interest in the etiology and pathogenesis of CMP is ongoing as *Kogia* spp. are the second most common single-stranded cetaceans in the southeastern United States (SEUS) after the bottlenose dolphin (*Tursiops truncatus*). Total annual *Kogia* strandings have ranged from 16 to 69 in the SEUS and from 9 to 40 in Florida (Odell et al., 2004). Annual stranding totals have been highly variable and, at least on the east coast of Florida, may be related to chronic disease and local oceanographic conditions, especially the Gulf Stream (Bossart et al., 1985; Odell et al., 2004). *Kogia* are rarely seen at sea and, despite the relatively high frequency of strandings, very little is known about their biology. In fact, prior to 1966, only one species was recognized (Odell et al., 2004).

The purpose of this study was to further characterize the pathological features of cardiac lesions found in pygmy and dwarf sperm whales using a

newly developed standardized protocol designed to ensure systematic examination of tissue and data recording and to explore potential factors in their etiology.

Materials and Methods

Gross and Microscopic Pathology

The analysis reported here was based on gross and microscopic examination of whole hearts from 27 *K. breviceps* (17 adult males [M], 6 adult females [F], 1 subadult [M], 1 subadult [F], 2 calves [F]) and two *K. sima* (adult [M]) that stranded in the coastal U.S. Atlantic Ocean and Gulf of Mexico between 1999 and 2006 and were submitted to our laboratory for evaluation. A *Kogia* heart dissection manual was developed which describes specific protocols for the collection, fixation, and dissection of heart specimens from *Kogia* spp. (Hensley et al., 2005). Procedures were standardized to ensure systematic gross and microscopic examination of tissue and data recording. *In situ* examination of the heart is detailed in the manual, which also emphasizes the importance of accurately determining the heart weights and specific heart measurements.

Briefly, the formalin-fixed heart was divided into five cross sections of approximately the same width. Cross sections were referred to as Levels 1 through 5, from apex to base, respectively. Heart weights and measurements (right and left ventricular wall thickness at Levels 2 and 4; interventricular septum thickness at Levels 2 and 4; valve circumference [tricuspid, mitral, pulmonary, and aortic]) were determined as described in Hensley et al. (2005). Evaluation included the collection of 12 representative heart sections: septal summit (two blocks), dorsal wall of right ventricle at Level 2, ventral wall of right ventricle at Level 2, dorsal wall of left ventricle at Level 2, ventral wall of left ventricle at Level 2, interventricular septum at Level 2, dorsal wall of right ventricle at Level 4, ventral wall of right ventricle at Level 4, dorsal wall of left ventricle at Level 4, ventral wall of left ventricle at Level 4, and interventricular septum at Level 4. After sectioning, samples were placed into labeled tissue cassettes containing fresh neutral buffered 10% formalin. Tissues were routinely processed, embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin and eosin for examination by light microscopy. Masson trichrome was used as a special stain to demonstrate the presence of collagen.

Myocardial degeneration (MCD) was diagnosed microscopically as (1) mild, multifocal, or diffuse anisokaryosis with karyomegaly, nuclear rowing, and interstitial edema found in all 12 sections described above; and (2) mild, multifocal, or diffuse eosinophilic homogenization of sarcoplasm and vacuolization, myofiber disarray (architectural disorganization), wavy-attenuated myofibers, and/or loss of cross-striations in six or less sections described above. Fibrosis and inflammation were absent.

A diagnosis of CMP consisted of a suite of microscopic lesions, including multifocal or diffuse: (1) moderate to severe anisokaryosis with karyomegaly and nuclear rowing, (2) moderate to severe interstitial edema, (3) mild to severe eosinophilic homogenization of sarcoplasm and vacuolization, (4) mild to severe myofiber disarray (architectural disorganization), (5) mild to severe wavy-attenuated myofibers, (6) mild to severe loss of cross-striations, and (7) mild to severe fibrosis in 11 or more sections as described above.

Statistical Analyses

Statistical analyses were conducted to determine whether heart weights, wall thicknesses, and valve dimensions differed among *K. breviceps* affected with MCD and CMP. Analyses were limited to *K. breviceps* because the number ($n = 2$) of *K. sima* was inadequate. Further, the morphometrics of these two species would be expected to differ, thus they were not combined. In the first phase of the analysis, all *K. breviceps* with CMP were compared to those with MCD using the *t*-test procedure in *SAS*, Version 9.1 (SAS, Cary, NC). A two-way analysis of variance (ANOVA) was then conducted for heart weight and Level 2 intraventricular septum thickness with diagnosis and sex in the model. Finally, PROC GLM in ***SAS*** was used to compare heart weight and Level 2 intraventricular septum thickness adjusting for body length in sperm whales with MCD and CMP. An alpha of $p < 0.05$ was considered statistically significant.

Results

The cardiac lesions found in this sample of 29 stranded *Kogia* spp. are summarized in Table 1 along with the geographic region where they occurred. Fourteen (48.3%) whales had a diagnosis of CMP, 12 (41.4%) showed evidence of mild MCD, one (3.4%) had moderate myocarditis, and two (6.9%) had no pathological lesions. Hearts from two *K. sima* were examined: one had evidence of CMP, the other of MCD. Nineteen of 27 (70.3%) *Kogia* spp. with evidence of cardiac pathology came from the southeast Atlantic region that included the east coasts of Florida, Georgia, South Carolina, and North Carolina.

The age and sex distribution of cardiac lesions is shown in Table 2. Lesions occurred predominantly among adult animals. Twenty-five of 27 (92.6%) affected animals were adults and two

Table 1. Cardiac pathology and geographic distribution in 27 pygmy (*Kogia breviceps*) and 2 dwarf (*Kogia sima*) sperm whales

Region	Cardiomyopathy	Myocardial degeneration	Myocarditis	No lesions
SE Atlantic[1]	9	9	1	2
NE Atlantic[2]	1	--	--	--
Gulf of Mexico[3]	4	3	--	--
Totals	**14**	**12**	**1**	**2**

[1]Florida (east coast), Georgia, South Carolina, North Carolina
[2]Maryland, Delaware, New Jersey, New York, Connecticut, Rhode Island, Massachusetts, New Hampshire, Maine
[3]Florida (west coast), Alabama, Mississippi, Louisiana, Texas

Table 2. Cardiac pathology in 27 pygmy (*Kogia breviceps*) and two dwarf[1] (*Kogia sima*) sperm whales by age group and sex

	Cardiomyopathy		Myocardial degeneration		Myocarditis		No lesions	
Age class	Males	Females	Males	Females	Males	Females	Males	Females
Adults	10	3	8	3	1	--	--	--
Subadults	1	--	--	1	--	--	--	--
Calves	--	--	--	--	--	--	--	2
Totals	**11**	**3**	**8**	**4**	**1**	**0**	**0**	**2**

[1]Includes one *K. sima* male with CMP and one *K. sima* male with MCD

(7.4%) were subadults. No pathological findings were observed in the hearts of the two stranded female calves that were examined. Male whales accounted for the majority of myocardial lesions. Twenty of the 27 (74%) affected whales were male; the excess of males occurred for both CMP (78.6% male) and MCD (66.7% male). Lesions of CMP and MCD were further categorized by severity. Eight of the 14 cases of CMP were categorized as severe, five as moderate, and one as mild. All forms of moderate and severe CMP were observed among adult animals. All cases of MCD were classified as mild.

Among *Kogia* with CMP, anisokaryosis with karyomegaly and nuclear rowing was the most common histological finding (Figures 1, 2 & 3) and was observed in 133 of the 168 (79.2%) tissue blocks examined in the 14 affected whales. In descending frequency, the next most common histological lesions were interstitial edema (61.3%), myofiber disarray (26.2%) (Figure 4), loss of cross-striations (20.2%) (Figure 1), eosinophilic homogenization of sarcoplasm (20.2%), and interstitial edema with fibrosis (17.3%) (Figures 3 & 5). In most cases, the anatomic distribution of each lesion was relatively similar across the 12 sampling sites. There were some exceptions to this observation, however. For example, the frequency of detection of anisokaryosis varied between interventricular septal sites. The distribution of histopathological lesions of CMP in *Kogia* spp. was analyzed according to their anatomical location; the results are shown in Appendix 1.

Anisokaryosis with karyomegaly and nuclear rowing was also the predominant histological lesion in the 12 *Kogia* with MCD. This lesion was observed in 106 of the 144 (73.6%) tissue blocks examined. The frequency of other histological lesions generally followed the pattern observed in whales with CMP, with interstitial edema (37.5%) the next most commonly found lesion. The frequency of myofiber disarray (5.6%), loss of cross-striations (6.9%), eosinophilic homogenization of sarcoplasm (1.4%), and interstitial edema with fibrosis (2.1%) were substantially lower than those observed in cases of CMP. As was found for CMP, the anatomical distribution of each lesion of MCD was relatively similar across the 12 sampling sites. The distribution of MCD lesions by anatomical location is shown in Appendix 2.

A single case of moderate, multifocal chronic myocarditis without concurrent CMP or MCD was also present. The predominant inflammatory cell type was mature lymphocytes. No infectious agents were observed, and the etiology was not determined.

No statistically significant differences were found between pygmy sperm whales with CMP and those with MCD for any of the 11 parameters tested. Differences in heart weight; right and left

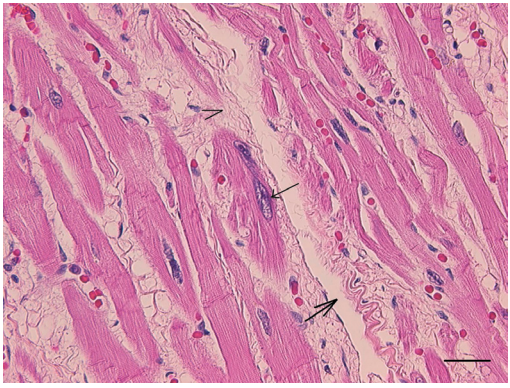

Figure 1. Photomicrograph of heart of a pygmy sperm whale with cardiomyopathy; note the anisokaryosis with karyomegaly (small arrow), loss of cross-striations (arrowhead), and interstitial edema (large arrow). H&E stain; bar = 80 microns.

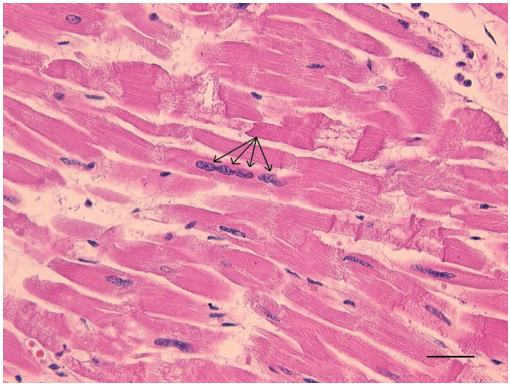

Figure 2. Photomicrograph of heart of a pygmy sperm whale with cardiomyopathy; note the nuclear rowing of cardiomyocytes (arrows). H&E stain; bar = 80 microns.

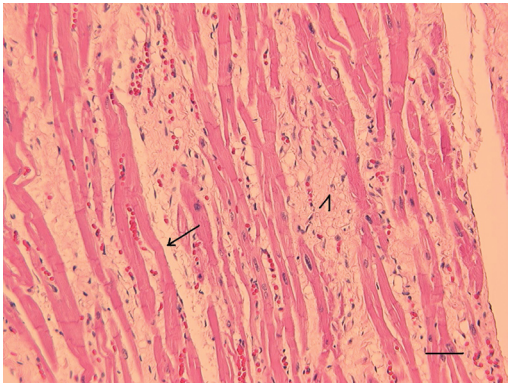

Figure 3. Photomicrograph of heart of a pygmy sperm whale with cardiomyopathy; note the interstitial edema (arrow) and presumptive fibrosis (arrowhead). H&E stain; bar = 150 microns

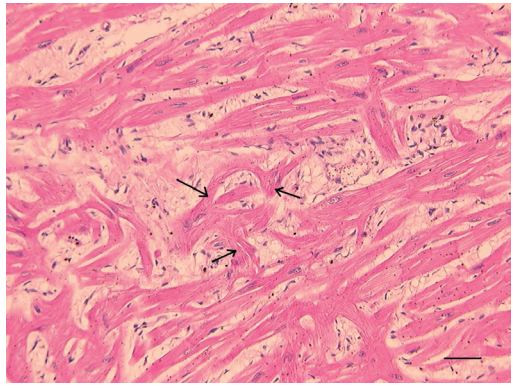

Figure 4. Photomicrograph of heart of a pygmy sperm whale with cardiomyopathy; note the myofiber disarray and architectural disorganization of cardiomyocytes (arrows). H&E stain; bar = 150 microns.

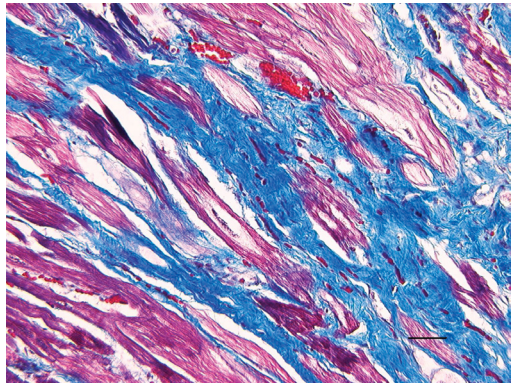

Figure 5. Photomicrograph of heart of a pygmy sperm whale with cardiomyopathy; note the diffuse thickened blue fibrillar deposits of collagen. Masson trichrome stain; bar = 120 microns

ventricular wall thickness at two levels; intraventricular septum thickness at two levels; and circumference of the aortic, pulmonary, mitral, and tricuspid valves were evaluated. The largest differences were found for heart weight and intraventricular septum wall thickness, but none were statistically significant (Table 3). An ANOVA with sex in the model was run to determine whether sex could be acting as a confounder since males would be expected to be larger and were selectively affected. The mean heart weight for *K. breviceps* with CMP was greater than that for MCD for both males and females, but the differences were not statistically significant ($p = 0.14$) (Table 4). Finally, body length was used as a surrogate for body weight to adjust the heart weight data in PROC GLM. The adjusted least square mean heart weight for *K. breviceps* with CMP (1.63 kg)

Table 3. Heart weight, wall thickness, and valve circumference in *Kogia breviceps* with cardiomyopathy and myocardial degeneration

Cardiac parameter	Cardiomyopathy		Myocardial degeneration		P value
	Mean	SD	Mean	SD	
Heart weight (kg)	1.7	0.27	1.4	0.53	0.12
L2 RV wall (cm)	0.5	0.18	0.6	0.25	0.92
L2 LV wall (cm)	1.0	0.47	1.1	0.61	0.54
L2 IV wall (cm)	1.7	0.68	1.3	0.44	0.20
L4 RV wall (cm)	0.7	0.28	0.9	0.60	0.45
L4 LV wall (cm)	1.2	0.58	1.2	0.49	0.86
L4 IV septum (cm)	2.1	0.80	1.8	0.45	0.37
Pulmonary valve (cm)	12.6	1.84	11.8	2.71	0.47
Aortic valve (cm)	11.9	2.34	10.8	2.31	0.38
Tricuspid valve (cm)	19.6	5.24	16.9	2.63	0.26
Mitral valve (cm)	16.3	3.72	14.5	3.28	0.33

Table 4. Heart weight by sex in *Kogia breviceps* with cardiomyopathy and myocardial degeneration

Sex	Cardiomyopathy			Myocardial degeneration		
	Number	Mean weight (kg)	SD	Number	Mean weight (kg)	SD
Males	9	1.7	0.26	8	1.4	0.50
Females	2	1.8	0.42	3	1.4	0.71
Totals	11			11		

*p = 0.14

was greater than that for MCD (1.52 kg), but the difference was not statistically significant (data not shown).

Discussion

The pathological data presented in this report suggest that CMP is a common lesion in *Kogia* spp. that stranded in the coastal waters of the U.S. Atlantic and Gulf of Mexico from 1999 to 2006. These data support and extend a past report of *Kogia* spp. that stranded in the same geographic region from 1980 to 1984 (Bossart et al., 1985). All of the whales in the present study represent single strandings. The consistency, extent, and severity of the pathological lesions suggest that many of these whales were in a state of myocardial decompensation at the time of stranding. Furthermore, this study provides new evidence indicating that *Kogia* CMP is a chronic progressive condition rather that an acute terminal event. Specifically, the similarity in type and distribution of pathological lesions of CMP and MCD implies a continuum of the same process. Thus, MCD may precede and lead to the development of CMP as the whales age. The apparent progressive nature of CMP ultimately would result in a debilitating condition in adults; and the beaching event of the ailing whales would be dependent on the active movement (or lack of purposeful directional movement) and prevailing environmental conditions such as ocean steering currents and weather conditions. The general trend of greater heart weights in *K. breviceps* with CMP compared to those with MCD supports the hypothesis that the disorder is a progressive condition since heart weight may be increased with CMP in other species (see below). It is important to note that none of the heart weight, wall thickness, or valve circumference differences were statistically significant and that the statistical analyses compared whales with CMP to whales with MCD; an unaffected comparison group was not available.

In terrestrial mammals and humans, the general types of CMP include stress, hypertrophic, dilated (congestive), and restrictive forms. The myocardial lesions of the stress, dilated, and hypertrophic CMP types described in other species were observed in the present study.

The stress form represents an acute process mediated by catecholamines, which may lead to sudden death in humans and animals, usually without a history of pre-existing heart disease (Cebilin & Hirsch, 1980; Liu et al., 1982). Elevated endogenous catecholamines produce a distinctive focal hypercontraction and lysis of contractile filaments

in small groups of cardiomyocytes that is termed "contraction band necrosis" (Turnbull & Cowan, 1998). Contraction band necrosis, including loss of cross-striations, interstitial edema, myofiber cytoplasmic hypereosinophilia, and wavy fibers, have been reported previously in the *Kogia* CMP and in other stranded cetaceans (Bossart et al., 1985; Turnbull & Cowan, 1998). Additionally, a more chronic stress hormone component involving cortisol has recently been described in dogs with dilated CMP (Tidholm et al., 2005).

Hypertrophic CMP is well characterized in humans and domestic animals (Liu et al., 1993, 1994; Maron, 1997). The demonstration of specific genetic abnormalities in cardiac energy metabolism or structural and contractile proteins results in approximately half the human cases of hypertrophic CMP (Hughes, 2004). The diagnosis of this form is based on macroscopic enlargement of the heart usually supported by microscopic lesions consisting of myofiber disarray (architectural disorganization). In *Kogia*, heart enlargement is difficult to assess because normal heart weights have not been determined; however, the microscopic lesion of myofiber disarray associated with hypertrophic CMP in other species was seen in 26% of *Kogia* with CMP.

In about half of the human patients with hypertrophic CMP, the disease is familial and is one of the most common causes of a sudden, unexplained death in young male athletes (Schoen, 1999). The preponderance of male whales with myocardial pathology in the current study was a new and interesting finding. In contrast, an earlier study did not find an unusual sex distribution (Bossart et al., 1985). The significance of the excess of CMP among male *Kogia* is unknown but may suggest a sex-linked genetic etiology.

Dilated CMP differs from the hypertrophic form in that the capacity of the ventricle(s) is actually increased, which can impart a "globular" appearance to the heart. In the fresh unfixed heart, the ventricle(s) may feel flabby. Dilated CMP in humans has varied etiologies that may involve complex mechanisms, including postinfectious, autoimmune, and idiopathic factors (Richardson et al., 1996). Dilated CMP also has been associated with L-carnitine and taurine deficiencies in humans, rodents, and domestic animals (Levitan et al., 1987; Keene, 1991; Fascetti et al., 2003; Zaugg et al., 2003). Dilated CMP was recently reported in southern California sea otters (*Enhydra lutris nereis*) and postulated to be associated with domoic acid toxicosis and depletion of myocardial L-carnitine (Kreuder et al., 2005).

The first report of CMP in *Kogia* was a dilated form, which included a grossly dilated flabby right ventricle, generalized myocardial pallor, and chronic passive congestion of the liver (Bossart et al., 1985). The etiology of this case of dilated CMP could not be determined, but nutritional etiologies, including a thiamine deficiency, were postulated. Thiamine deficiency has been reported in captive marine mammals, and myocardial lesions consistent with thiamine deficiency were seen in captive sea lions fed a diet presumably containing high concentrations of thiaminase (Rigdon & Drager, 1955; Worthy, 2001). In this study, it was difficult to assess the occurrence of gross myocardial changes of dilated CMP as all of the examined hearts had already been fixed, thus distorting normal gross morphology. Previously described microscopic heart lesions of the dilated form were found, however, and these consisted of cardiomyocyte degeneration, loss of cross-striations, interstitial edema, and fibrosis (Bossart et al., 1985).

Although each form of CMP is fundamentally different, they are not necessarily mutually exclusive in a given case. Moreover, transitions from one type to another may occur in humans, reflecting chronicity and/or severity of the basic disease process (Hughes & McKenna, 2005). Specifically, hypertrophic CMP may progress to a dilated phase in human patients and resemble dilated CMP (Maron, 2002). Therefore, it appears that the *Kogia* CMP may be best defined as a "mixed form," having microscopic components of all three types. Lesions seen uniformly in all sections included eosinophilic homogenization of sarcoplasm, loss of cross-striations, interstitial edema and fibrosis, anisokaryosis with karyomegaly, myofiber disarray (architectural disorganization), and wavy-attenuated myofibers. Thus, the etiology of CMP in *Kogia* is likely complex and multifactorial. Etiologic components may include metabolic factors, such as excessive repeated sublethal episodes of catecholamine release (repeated acute "stress" reactions) and endogenous glucocorticoid release (chronic "stress" response); nutritional deficiencies; and postinfectious, genetic, and toxic factors (e.g., biotoxins). Further studies may help confirm these hypotheses.

Acknowledgments

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and Megan K. Stolen and Wendy Noke Durden (Hubbs-Sea World Research Institute, Orlando, Florida, USA) for heart acquisition. Special thanks go to Dr. Dan Odell for natural history information and Dr. Ruth Ewing for initial assistance in the heart dissection technique. Additionally, we gratefully acknowledge the volunteer members of the Southeastern Marine Mammal Stranding Network and Harbor Branch marine mammal volunteers for their tireless efforts in advancing the science of marine mammal medicine and pathology.

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Appendix 1. Distribution of histopathological lesions of cardiomyopathy by anatomic site (n = 14)

Histological lesion	SS-1	SS-2	DRV-2	VRV-2	DLV-2	VLV-2	IV-2	DRV-4	VRV-4	DLV-4	VLV-4	IV-4	Total
Anisokaryosis with karyomegaly and nuclear rowing	9 (64%)	9 (64%)	11 (79%)	12 (86%)	12 (86%)	11 (79%)	7 (50%)	13 (93%)	12 (86%)	12 (86%)	11 (79%)	14 (100%)	133 (79.20%)
Interstitial edema	8 (57%)	5 (36%)	9 (64%)	9 (64%)	8 (57%)	9 (64%)	6 (43%)	10 (71%)	9 (64%)	9 (64%)	9 (64%)	12 (86%)	103 (61.30%)
Myofiber disarray (architectural disorganization)	1 (7%)	2 (14%)	4 (29%)	4 (29%)	4 (29%)	5 (36%)	3 (21%)	3 (21%)	3 (21%)	3 (21%)	5 (36%)	7 (50%)	44 (26.20%)
Loss of cross-striations	1 (7%)	2 (14%)	1 (7%)	3 (21%)	5 (36%)	4 (29%)	1 (7%)	2 (14%)	2 (14%)	5 (36%)	4 (29%)	4 (29%)	34 (20.20%)
Myofiber cytoplasmic hypereosinophilia	2 (14%)	2 (14%)	1 (7%)	2 (14%)	2 (14%)	5 (36%)	2 (14%)	3 (21%)	4 (29%)	4 (29%)	4 (29%)	3 (21%)	34 (20.20%)
Interstitial edema and fibrosis	0 (0%)	5 (36%)	2 (14%)	1 (7%)	4 (29%)	3 (21%)	1 (7%)	3 (21%)	2 (14%)	3 (21%)	3 (21%)	2 (14%)	29 (17.30%)
Wavy-attenuated myofibers	0 (0%)	1 (7%)	1 (7%)	2 (14%)	1 (7%)	1 (7%)	2 (14%)	1 (7%)	2 (14%)	2 (14%)	2 (14%)	2 (14%)	17 (10.10%)

SS 1 = Septum summit to the right of the midline
SS-2 = Septum summit to the left of the midline
DRV-2 = Dorsal right ventricle at Level 2
VRV-2 = Ventral right ventricle at Level 2
DLV-2 = Dorsal left ventricle at Level 2
VLV-2 = Ventral left ventricle at Level 2
IV-2 = Interventricular septum at Level 2
DRV-4 = Dorsal right ventricle at Level 4
VRV-4 = Ventral right ventricle at Level 4
DLV-4 = Dorsal left ventricle at Level 4
VLV-4 = Ventral left ventricle at Level 4
IV-4 = Interventricular septum at Level 4

Appendix 2. Distribution of histopathological lesions of myocardial degeneration by anatomical site (n = 12)

Histological lesion	SS-1	SS-2	DRV-2	VRV-2	DLV-2	VLV-2	IV-2	DRV-4	VRV-4	DLV-4	VLV-4	IV-4	Total
Anisokaryosis with karyomegaly and nuclear rowing	9 (75%)	9 (75%)	6 (50%)	7 (58%)	11 (92%)	11 (92%)	10 (83%)	5 (42%)	8 (67%)	9 (75%)	9 (75%)	12 (100%)	106 (73.6%)
Interstitial edema	4 (33%)	3 (25%)	4 (33%)	4 (33%)	5 (42%)	6 (50%)	5 (42%)	2 (17%)	2 (17%)	5 (42%)	6 (50%)	8 (67%)	54 (37.5%)
Myofiber disarray (architectural disorganization)	1 (8%)	0 (0%)	2 (17%)	0 (0%)	1 (8%)	0 (0%)	2 (17%)	0 (0%)	0 (0%)	1 (8%)	1 (8%)	0 (0%)	8 (5.60%)
Loss of cross-striations	1 (8%)	1 (8%)	0 (0%)	0 (0%)	1 (8%)	2 (17%)	2 (17%)	0 (0%)	0 (0%)	1 (8%)	0 (0%)	2 (17%)	10 (6.9%)
Myofiber cytoplasmic hypereosinophilia	1 (8%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (8%)	0 (0%)	0 (0%)	2 (1.40%)
Interstitial edema and fibrosis	1 (8%)	1 (8%)	0 (0%)	0 (0%)	0 (0%)	1 (8%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	3 (2.10%)
Wavy-attenuated myofibers	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (8%)	0 (0%)	1 (0.7%)

SS-1 = Septum summit to the right of the midline
SS-2 = Septum summit to the left of the midline
DRV-2 = Dorsal right ventricle at Level 2
VRV-2 = Ventral right ventricle at Level 2
DLV-2 = Dorsal left ventricle at Level 2
VLV-2 = Ventral left ventricle at Level 2
IV-2 = Interventricular septum at Level 2
DRV-4 = Dorsal right ventricle at Level 4
VRV-4 = Ventral right ventricle at Level 4
DLV-4 = Dorsal left ventricle at Level 4
VLV-4 = Ventral left ventricle at Level 4
IV-4 = Interventricular septum at Level 4

From: Edwards, David
To: Hartogensis, Martine; Murphy, Jeanette
Subject: talk to AFIA Pet Food Conference
Date: Wednesday, January 30, 2019 11:25:00 AM
Attachments: Final Agenda - Pet Food Conference 092718.docx

Hi Martine and Jenny,

I am slated to talk to the AFIA Pet Food Conference on February 12 in Atlanta. They would like an update on DCM and on FSMA inspections. Do you have a recent presentation that I can update to provide the latest? I have 45 minutes total for talking and questions. Greg Aldrich is speaking after me on DCM, and AAFCO is also on the program to provide updates (see attached).

If I need to ping others, please let me know.

Thank you,
Dave

David Edwards, PhD | Director, Division of Animal Feeds
(240)402-6205 | david.edwards@fda.hhs.gov

2019 Pet Food Conference
Tuesday, February 12, 2019
Atlanta, Ga.
7:30 a.m. to 4:00 p.m.

As of 9/27/18

7:30-8:30 a.m.	Breakfast
8:30-8:40 a.m.	Welcome and Overview of the Conference
8:40-9:30 a.m.	Domestic and Global Industry Trends Jared Koerten, Euromonitor International (http://blog.euromonitor.com/)
9:30-10:00 a.m.	Trade Policy and Outlook Gina Tumbarello, American Feed Industry Association
10:00-10:30 a.m.	Break
10:30-11:15 a.m.	Blockchain Management of Pet Food: A Legal Perspective John Dillard, Olsson Frank Weeda Terman Matz PC
11:15-12:00 p.m.	The Healing Power of Man's Best Friend: Opportunities to Save and Enrich Human Lives Robin Ganzert, Ph.D., American Humane
12:00-1:15 p.m.	Networking Lunch (provided)
1:15-2:00 p.m.	FSMA Inspections and Canine Dilated Cardiomyopathy Update David Edwards, Ph.D., FDA Center for Veterinary Medicine
2:00-2:45	Implications of Canine Dilated Cardiomyopathy on Ingredient Categories Greg Aldrich, Ph.D., Kansas State University
2:45-3:15	Break
3:15-4:00	AAFCO Updates on Pet Food Sue Hays, Association of American Feed Control Officials
4:00 p.m.	Wrap up and Adjourn

**** Agenda Subject to Change

(b) (6)

Patient Information

Patient:	(b) (6)	Age: 8	years	Referring Veterinarian:	(b) (6)
Patient Number:	(b) (6)	Weight:(kg)	29.40	Cardiologist:	(b) (6) DVM, DACVIM (Cardiology)
Breed:	Labrador Retriever	Sex:	F	Client Number:	(b) (6)
Exam Date:	(b) (6) 08:22	BSA:	0.96		

History: (b) was presented to the (b) (6) last night for transfer to Cardiology for further evaluation of her heart. She was seen by (b) (6) last week after collapsing last Thursday while playing fetch with her owner. On presentation at (b) (6) she was found to be in atrial fibrillation with evidence of mild heart failure. She was treated with a diltiazem CRI overnight, then oral diltiazem, as well as Lasix, spironolactone, and enalapril. Her heart rhythm converted back to sinus rhythm as of (b) (6) (Friday). She was presented back to (b) on Saturday after collapsing again on Saturday while playing fetch. She was found to still be in a normal heart rhythm and radiographs showed resolution of heart failure at that time. Bloodowrk done at (b) (6) (CBC and chem) was reported as unremarkable.

Physical Examination: Grade 3-4/6 left apical holosystolic murmur. Irregular rhythm consistent with sinus arrhythmia. Clear lungs. Moderate femoral pulses. Normal abdominal palpation. Well hydrated. Normal PLNs. mm pink, CRT normal

Diagnostic Tests: BP: 108mmHg 4cm cuff RR

Echo: see below

Telemetry - (b) (6) heart rhythm was monitored throughout her hospital stay and showed a consistent sinus rhythm/arrhythmia with no significant dysrhythmias.

Renal panel pending

Echocardiographic Report

2D ECHO

LA Systolic Diameter LX	6.3 cm	Aortic Root Diameter	2.3 cm

DOPPLER

AV Peak Velocity	104 cm/s	PV Peak Velocity	72.7 cm/s
AV Peak Gradient	4.3 mmHg	PV Peak Gradient	2.1 mmHg
Mitral E Point Velocity	156 cm/s	TR Peak Velocity	343 cm/s
Mitral E to A Ratio	3	TR Peak Gradient	47.2 mmHg
MR Peak Velocity	521 cm/s		

M-MODE

LV Diastolic Diameter MM	6.8 cm	LVPW Diastolic Thickness MM	0.92 cm
LV Systolic Diameter MM	5.8 cm	LVPW Systolic Thickness MM	1.1 cm
LV Fractional Shortening MM	15.1 %	LVPW Percent Thickening MM	0.16
LV Diastolic Volume Cube	314 cm^3	IVS to PW Ratio MM	1.3
LV Systolic Volume Cube	192 cm^3	LV Mass MM	323 g
LV Ejection Fraction Cube	0.39	LV Mass Normalized MM	336 g/m^2
IVS Diastolic Thickness MM	1.2 cm	LA Systolic Diameter MM	3.8 cm
IVS Systolic Thickness MM	1.4 cm	Aortic Root Diameter MM	2.3 cm
IVS Percent Thickening MM	0.22	MV E Point Septal Separation	1.5 cm

Left Ventricle: Dilated, rounded, and poorly contractile chamber.

Left Atrium: Moderate dilation with marked dilation of right pulmonary vein.

Right Ventricle: Normal.

Right Atrium: Normal.

Mitral Valve: Mildly thickened valve leaflets. 4+ eccentric regurgitation. High inflow velocity with restrictive filling pattern.

Aortic Valve: Normal.

Tricuspid Valve: Thickened valve leaflets with multiple 1+ jets of regurgitation. TR velocity is increased consistent with mild pulmonary hypertension.

Pulmonic Valve: Mild valve thickening. 1+ regurgitation. PI velocity is not suggestive of diastolic pulmonary hypertension.

Aorta: Normal.

Pericardium: Normal.

Diagnosis

Dilated cardiomyopathy - This is a disease characterized by weakening of the heart muscle and dilation of the heart chambers. It is most commonly an inherited disease, but can occur as a consequence of other injuries to the heart. Severe valvular heart disease can sometimes lead to heart muscle failure (cardiomyopathy of overload) and since (b) appears to have severe valve disease as well as heart muscle failure, we cannot be sure whether one led to the other or if there are two completely separate disease processes. As the disease progresses, it can lead to congestive heart failure (fluid in the lungs causing shortness of breath and cough). Abnormal heart rhythms are common and can result in sudden death. Most commonly this is an inherited disease, though it can occur secondary to a deficiency in an amino acid called taurine.

Chronic degenerative valve disease - Degenerative changes in one or more heart valves have caused leaking across these valves. This is the source of the heart murmur. As this disease progresses, the heart enlarges. Eventually this can lead to symptoms of cough and shortness of breath (airway compression and/or congestive heart failure).

Atrial fibrillation on presentation at (b) (6), converted back to sinus rhythm (b) (6) - This is a chaotic and rapid heart rhythm from the upper heart chambers. It most commonly occurs secondary to severe underlying heart diseases, though it can occur in isolation in some giant breed dogs. Our goal medically in treating this arrhythmia is to control the heart rate, but (b) has returned to a normal heart rhythm so no specific medication is indicated for the heart rhythm at this time.

Exertional collapse - I suspect the first episode was likely caused by the new onset of the atrial fibrillation in (b), but the second episode is a little harder to explain. We did not find any evidence while monitoring her in the hospital of other arrhythmia, and she had a normal heart rhythm at the emergency visit after her second collapse as well. It is possible that she collapsed as a result of her severe structural heart disease, though this is a little surprising to see recurrent collapse after starting on medications that had been effective in resolving her heart failure.

Recommendations

Please DISCONTINUE:
Diltiazem - This is a medication to slow the heart rate in atrial fibrillation, but it has not been shown to be effective in decreasing the risk of recurrent atrial fibrillation once an animal converts back to sinus rhythm. Since this is the case with (b) and she is no longer in atrial fibrillation, we do not need to keep her on this medication at this time.

Please CONTINUE:
Furosemide (Lasix) 50mg tablets - Give 1 tablet by mouth once every 12 hours. Furosemide (Lasix, Salix) - This is a diuretic (water pill), that prevents the body from retaining excessive sodium and water. It will cause your pet to drink and urinate more frequently. It is important that fresh water is always available.

Spironolactone (Aldactone) 50mg tablets - Give 1 tablet by mouth once daily. This is a diuretic (water pill) that also blocks a hormone that can injure the heart muscle. It works well in combination with the furosemide and enalapril.

Please INCREASE:
Enalapril (Enacard, Vasotec) 10mg tablets - Give 1 and 1/2 tablets by mouth every 12 hours. This medication is a strong drug that dilates blood vessels, permitting the heart to pump blood more efficiently. It can lower blood pressure (hypotension) and cause changes in kidney function and electrolyte values. If your pet develops weakness or depression, decrease the drug dose by ½ and call. A kidney panel and blood pressure should be reevaluated 7-10 days after beginning this medication.

Please ADD:
Pimobendan (Vetmedin) 5mg tablets - Give 1 and 1/2 tablets by mouth every 12 hours. This is a drug that is approved for the treatment of congestive heart failure secondary to dilated cardiomyopathy or chronic valve disease (endocardiosis). Studies have shown improved quality of life and increase survival time when this drug is added to other standard cardiac medications. In our experience, side effects are uncommon, but it is important that you advise us if you feel your pet is having any potential adverse effects from this medication. The reported potential side effects listed for this medication are increased heart rate, vomiting, diarrhea, inappetance, uneasiness, incoordination, convulsions, increased drinking and increase urinating.
**Please NOTE: When 10mg tablets of Vetmedin become available again, it would be OK to switch to one 10mg tablet in the morning and 1/2 - 10mg tablet at night for the same total daily dose and this would be more cost effective. Currently, however, the 10mg tablets are on backorder and we do not have a confirmed release date.

With advanced heart disease, our biggest dietary concerns are adequate calorie content and low sodium content. We aim for less than 80mg sodium per 100 kilocalories (kcal) in patients that have developed congestive heart failure. We do not advise protein restriction unless there is concurrent kidney disease (i.e. kidney diets are not advised unless there is concurrent kidney disease). Please refer to our diet handouts with a list of currently adequate diets and treats, though this list is not exclusive. If you wish to feed a diet that is not on these lists, you will need to call the manufacturer of the diet to obtain a sodium content.

One thing that can be very helpful for home monitoring is checking sleeping or resting respiratory rates. A recent study showed that even dogs with severe heart disease rarely have resting respiratory rates greater than 30 breaths per minute unless they are starting to decompensate for that disease. Elevated respiratory rates at home may be even more sensitive than chest radiographs at picking up early decompensation. Count your pet's respiratory rate when he/she is at rest or sleeping (not within 20 minutes of being active). If his/her respiratory rate is greater than 30 breaths per minute, recheck again in a couple of hours. If persistently elevated above this level, call.

Exercise is also a concern in advanced heart disease. While cage rest is ideal with active heart failure, some exercise is permissible in asymptomatic disease. However, vigorous or extended exercise should be avoided.

Please call if you have any concerns about (b), if she develops an increase in respiratory rate or effort, has a persistent cough, or has any further collapse episodes. As long as she is doing well, we will plan to recheck her again in another month and will recheck her heart rhythm, chest radiographs, and kidney panel at that time.

(b) (6) (Cardiology)

(Electronically Signed)

Final Date: (b) (6) 16:50

Amended: (b) (6) 17:16

Like us on Facebook!

www.facebook.com/ (b) (6)

Notes to our clients

-Please bring all medications to your pet's scheduled appointments.

-We require a 48 hour notice for all refills. When you call to request a refill, please leave the pharmacy phone number or clearly indicate if you plan on picking up the medication at our facility. PRESCRIPTION REFILLS ARE NOT AVAILABLE AFTER (b) (6) REGULAR BUSINESS HOURS (Evenings, Fridays, holidays and weekends).

-Check out WWW.GOODRX.COM and enter your local zip code to search for the best prices on your medications at your local pharmacies.

-If an emergency arises with your pet, (b) (6) is a 24 hour facility.

Patient Information

Patient:	(b) (6)	Age: 9	years	Referring Veterinarian:	(b) (6)
Patient Number:	(b) (6)	Weight:(kg)	29.30	Cardiologist:	(b) (6) (Cardiology)
Breed:	Labrador Retriever	Sex:	FS	Client Number:	(b) (6)
Exam Date:	05/31/2017 14:13	BSA:	0.96		

History: (b) (6) was presented for reevaluation of dilated cardiomyopathy, chronic degenerative valve disease, historical atrial fibrillation with collapse and historical CHF. (b) (6) continues to do well at home without any episodes of collapse or weakness. (b) (6) has good energy levels, with a normal appetite and eliminations. She is breathing comfortably without an increase in rate or effort and her resting respiratory rates have been averaging 25bpm. Within the last 2-3 days, (b) (6) has been very anxious and not as social due to severe storm anxiety. (b) (6) is also on a daily glucosamine and chondroitin supplement.

Physical Examination: Temp:103.1. Heart Rate:108bpm. RR:120 (panting). Grade 3-4/6 left apical holosystolic murmur. Regular rhythm. Clear lungs. Normal femoral pulses and jugular veins. Normal abdominal palpation. Good hydration. mm pink, normal refill.

Diagnostic Tests: Blood pressure: 90mmHg with 4cm cuff on right forelimb
Thoracic radiographs: Mild progression of cardiac enlargement with no evidence of cardiac decompensation.
Renal panel: SDMA 18, normal BUN and creatinine, mild hypochloremia
Echocardiogram: See below. ECG during echo showed a normal sinus rhythm.

Echocardiographic Report

2D ECHO

LA Systolic Diameter LX	6.1 cm

DOPPLER

AV Peak Velocity	141 cm/s	PV Peak Gradient	3.9 mmHg
AV Peak Gradient	8 mmHg	TR Peak Velocity	261 cm/s
MR Peak Velocity	496 cm/s	TR Peak Gradient	27.2 mmHg
PV Peak Velocity	99.1 cm/s		

M-MODE

LV Diastolic Diameter MM	6.6 cm	LVPW Diastolic Thickness MM	1 cm
LV Systolic Diameter MM	5.4 cm	LVPW Systolic Thickness MM	1.3 cm
LV Fractional Shortening MM	17.7 %	LVPW Percent Thickening MM	0.27
LV Diastolic Volume Cube	285 cm^3	IVS to PW Ratio MM	1.1
LV Systolic Volume Cube	158 cm^3	LV Mass MM	307 g
LV Ejection Fraction Cube	0.44	LV Mass Normalized MM	320 g/m^2
IVS Diastolic Thickness MM	1.1 cm	LA Systolic Diameter MM	4 cm
IVS Systolic Thickness MM	1.5 cm	Aortic Root Diameter MM	2.2 cm
IVS Percent Thickening MM	0.38	MV E Point Septal Separation	1.4 cm

Left Ventricle: Minimal decrease in diastolic dimension with mild decrease in systolic dimension. Persistent moderate decrease in global contractility.

Left Atrium: Moderate dilation, minimal decrease since initial study.

Right Ventricle: Normal.

Right Atrium: Normal.

Mitral Valve: Mildly thickened valve leaflets. 3-4+ regurgitation.

Aortic Valve: Normal.

Tricuspid Valve: 1+ regurgitation. TR velocity consistent with normal pulmonary pressures.

Pulmonic Valve: 1+ regurgitation. Normal PI velocity.

Aorta: Normal.

Pericardium: Normal.

Diagnosis

Dilated Cardiomyopathy
Chronic Degenerative Valve Disease
Historical atrial fibrillation with collaps(b) (6) continues to be in a normal sinus rhythm today
Historical congestive heart failure - no evidence of heart failure today

(b) echo today looks stable to slightly improved from his initial echo in January, though his heart is a little larger today than on the radiographs in February. He is showing no signs of recurrent heart failure and his heart rhythm is still normal. Overall, I am happy with where we are overall.

Recommendations

Please continue to give medications as directed:
Furosemide 40mg tablets- Give 1 tablet by mouth once every 12 hours.
Enalapril 10mg tablets- Give 1 and 1/2 tablets by mouth once every 12 hours.
Spironolactone 50mg tablets- Give 1 tablet by mouth once every 24 hours.
Vetmedin 5mg tablets- Give 1 and 1/2 tablets by mouth once every 12 hours.

ADD:
Trazodone 150mg tablets- Give 1 tablet by mouth up to once every 8 hours as needed for storm anxiety.

As long as(b) (6) continues to do well, we will continue to recheck her every 3-4 months with chest radiographs, renal panel, and blood pressure with periodic echocardiograms. Please call, however, if she develops any new or recurrent clinical symptoms.

(b) (6) (Cardiology)

(Electronically Signed)
Final Date: 31 May 2017 15:11

Like us on Facebook!
www.facebook.com/(b) (6)

Notes to our clients
-Please bring all medications to your pet's scheduled appointments.
-We require a 48 hour notice for all refills. When you call to request a refill, please leave the pharmacy phone number or clearly indicate if you plan on picking up the medication at our facility. PRESCRIPTION REFILLS ARE NOT AVAILABLE AFTER (b) (6) REGULAR BUSINESS HOURS (Evenings, Fridays, holidays and weekends).
-Check out WWW.GOODRX.COM and enter your local zip code to search for the best prices on your medications at your local pharmacies.
-If an emergency arises with your pet, (b) (6) is a 24 hour facility.

(b) (6)

Patient Information

Patient:	(b) (6)	Age: 9	years	Referring Veterinarian:	(b) (6)
Patient Number:	(b) (6)	Weight:(kg)	32.10	Cardiologist:	(b) (6) (Cardiology)
Breed:	Lab	Sex:	F	Client Number:	(b) (6)
Exam Date:	12/11/2017 08:17	BSA:	1.02		

History: Reevaluation of dilated cardiomyopathy with chronic degenerative valve disease, historical atrial fibrillation with collapse, historical congestive heart failure, and urinary incontinence. (b) (6) is doing well at home. Owners do report a new cough with him since his last visit. It is not frequent and is seen at rest and with excitement/activity. She is breathing comfortably. She has a normal appetite and good activity level as well. Owners are transitioning her to a new brand of venison food.

Physical Examination: Temp 102.4. Heart rate 128bpm. RR pant. Grade 3/6 left apical systolic murmur with wide radiation. Regular rhythm. Clear lungs. Fair to moderate femoral pulses. Normal abdominal palpation and PLNs. Well hydrated.

Diagnostic Tests: Chest radiographs: progressive cardiomegaly with VHS 13.5 versus 13 on radiographs in September, normal pulmonary vessels, unchanged lung pattern with no evidence of active heart failure

Renal panel: within normal limits

Echo: see below. ECG during echo showed sinus rhythm.

Echocardiographic Report

2D ECHO

LA Systolic Diameter LX	6.5 cm	Aortic Root Diameter	2 cm

DOPPLER

AV Peak Velocity	161 cm/s	PV Peak Velocity	70.7 cm/s
AV Peak Gradient	10.4 mmHg	PV Peak Gradient	2 mmHg
Mitral E Point Velocity	111 cm/s	TR Peak Velocity	269 cm/s
Mitral E to A Ratio	2	TR Peak Gradient	28.9 mmHg
MR Peak Velocity	487 cm/s		

M-MODE

LV Diastolic Diameter MM	6.6 cm	LVPW Systolic Thickness MM	1.2 cm
LV Systolic Diameter MM	5.9 cm	LVPW Percent Thickening MM	0.049
LV Fractional Shortening MM	11.3 %	IVS to PW Ratio MM	1
LV Diastolic Volume Cube	287 cm^3	LV Mass MM	348 g
LV Systolic Volume Cube	200 cm^3	LV Mass Normalized MM	341 g/m^2
LV Ejection Fraction Cube	0.3	RV Diastolic Diameter MM	0.68 cm
IVS Diastolic Thickness MM	1.2 cm	LA Systolic Diameter MM	3.5 cm
IVS Systolic Thickness MM	1.5 cm	Aortic Root Diameter MM	2.3 cm
IVS Percent Thickening MM	0.25	MV E Point Septal Separation	1.7 cm
LVPW Diastolic Thickness MM	1.1 cm		

Left Ventricle:	Stable diastolic dimension with progressive increase in systolic dimension and decline in myocardial function.
Left Atrium:	Progressive dilation.
Right Ventricle:	Mild dilation.
Right Atrium:	Mild dilation.
Mitral Valve:	Unchanged mild thickening with 3-4+ regurgitation.
Aortic Valve:	Normal. Acceleration slope is decreased.
Tricuspid Valve:	Two jets of 2+ regurgitation. TR velocity consistent with normal pulmonary pressures.
Pulmonic Valve:	Normal. 1+ physiologic regurgitation.
Aorta:	Normal.
Pericardium:	Normal.

Diagnosis

Dilated cardiomyopathy with chronic degenerative valve disease - (b) (6) heart is bigger and does not contract as well as it did at her last two rechecks. However, she is showing no signs of decompensation at this time.
Historical atrial fibrillation with collapse
Historical congestive heart failure
Urinary incontinence

Recommendations

Please give the following medications as directed:
Glucosamine and coat supplement
Incurin - give 2 tablets by mouth once daily in the mornings
Furosemide 40mg tablets - give 1 tablet by mouth every 12 hours
Enalapril 10mg tablets - give 1 and 1/2 tablets by mouth every 12 hours
Spironolactone 50mg tablets - give 1 tablet by mouth once daily in the mornings
Vetmedin 5mg tablets - INCREASE to 1 and 1/2 tablets by mouth EVERY 8 HOURS. (We discussed that we can go to 1/2 of a 10mg tablet + 1/2 of a 5mg tablet to save some in cost for this medication).

Please call if you have any questions or concerns about (b) (6). As long as she continues to do well, we will recheck her again in another 3-4 months. We will do a brief echo and recheck kidney values and blood pressure at that visit +/- chest radiographs (if she is having any respiratory symptoms).

(b) (6), DVM, DACVIM (Cardiology)

(Electronically Signed)
Final Date: 11 December 2017 14:48
Amended: 11 December 2017 14:49

Like us on Facebook!

www.facebook.com/ (b) (6)

Notes to our clients

-Please bring all medications to your pet's scheduled appointments.

-We require a 48 hour notice for all refills. When you call to request a refill, please leave the pharmacy phone number or clearly indicate if you plan on picking up the medication at our facility. PRESCRIPTION REFILLS ARE NOT AVAILABLE AFTER (b) (6) REGULAR BUSINESS HOURS (Evenings, Fridays, holidays and weekends).

-Check out WWW.GOODRX.COM and enter your local zip code to search for the best prices on your medications at your local pharmacies.

-If an emergency arises with your pet, (b) (6) is a 24 hour facility.

From: Palmer, Lee Anne
To: Rotstein, David; Jones, Jennifer L; Carey, Lauren; Queen, Jackie L
Subject: RE: limited ingredient diet with kangaroo as protein source - manufacturer not specified in written history (we have attempted to contact the owner but they do not return phone calls): (b) (6) EON-345965
Date: Thursday, January 25, 2018 1:23:37 PM

Woops, that's 2 from (b) (6) rather than (b) (6) Same household. thx!

From: Rotstein, David
Sent: Thursday, January 25, 2018 1:00 PM
To: Palmer, Lee Anne <LeeAnne.Palmer@fda.hhs gov>; Jones, Jennifer L <Jennifer.Jones@fda.hhs.gov>; Carey, Lauren <Lauren.Carey@fda.hhs gov>; Queen, Jackie L <Jackie.Queen@fda.hhs gov>
Subject: RE: limited ingredient diet with kangaroo as protein source - manufacturer not specified in written history (we have attempted to contact the owner but they do not return phone calls): (b) (6) - EON-345965

Fantastic!

Hope it goes well!

From: Palmer, Lee Anne
Sent: Thursday, January 25, 2018 12:57 PM
To: Rotstein, David <David.Rotstein@fda.hhs.gov>; Jones, Jennifer L <Jennifer.Jones@fda.hhs.gov>; Carey, Lauren <Lauren.Carey@fda.hhs.gov>; Queen, Jackie L <Jackie.Queen@fda.hhs gov>
Subject: RE: limited ingredient diet with kangaroo as protein source - manufacturer not specified in written history (we have attempted to contact the owner but they do not return phone calls): (b) (6) - EON-345965

Hi – trying out the epi summary in EON to give it a dry run (Dave, I've temporarily assigned it to myself). FYI – this makes 6 cases now, 5 from (b) (6) 1 from (b) (6) (involving 2 dogs, 1 death) all eating LID type diet with Kangaroo and Red Lentil, onset dates between 1/20/2017 and 12/30/2017). I'll double check that info as I read through the reports and summarize it in the incident. Just had to try out the new functionality, needed some fun today.

From: Rotstein, David
Sent: Thursday, January 25, 2018 12:27 PM
To: Jones, Jennifer L <Jennifer Jones@fda.hhs gov>; Palmer, Lee Anne <LeeAnne.Palmer@fda.hhs.gov>; Carey, Lauren <Lauren.Carey@fda.hhs gov>; Queen, Jackie L <Jackie Queen@fda hhs gov>
Subject: Fwd: limited ingredient diet with kangaroo as protein source - manufacturer not specified in written history (we have attempted to contact the owner but they do not return phone calls): (b) (6) - EON-345965

Another one from (b) (6)

From: PFR Event <pfreventcreation@fda.hhs.gov>
Date: January 25, 2018 at 12:25:12 PM EST
To: Cleary, Michael * <Michael.Cleary@fda.hhs.gov>, HQ Pet Food Report Notification <HQPetFoodReportNotification@fda.hhs.gov>, (b) (5) (b) (5)
Subject: limited ingredient diet with kangaroo as protein source - manufacturer not specified in written history (we have attempted to contact the owner but they do not return phone calls): (b) (6) - EON-345965

A PFR Report has been received and PFR Event [EON-345965] has been created in the EON System

A "PDF" report by name "2040808-report pdf" is attached to this email notification for your reference Please note that all documents received in the report are compressed into a zip file by name "2040808-attachments zip" and is attached to this email notification

Below is the summary of the report:

EON Key: EON-345965
ICSR #: 2040808
EON Title: PFR Event created for limited ingredient diet with kangaroo as protein source - manufacturer not specified in written history (we have attempted to contact the owner but they do not return phone calls); 2040808

AE Date	04/24/2017	**Number Fed/Exposed**	5
Best By Date		**Number Reacted**	5
Animal Species	Dog	**Outcome to Date**	Unknown
Breed	Shih Tzu		
Age	8 Years		
District Involved	(b) (6)		

Product information
Individual Case Safety Report Number: 2040808
Product Group: Pet Food

Product Name: limited ingredient diet with kangaroo as protein source - manufacturer not specified in written history (we have attempted to contact the owner but they do not return phone calls)
Description: At his scheduled visit to my clinic, thoracic radiographs showed generalized cardiomegaly which had been progressive compared to prior chest radiographs from his regular veterinarian but there was no evidence of cardiogenic edema Echocardiogram was performed which showed dilated cardiomyopathy Fundic exam was abnormal with a suspected partial retinal detachment OS Diet history revealed that (b) (6) was eating a kangaroo based diet At this time the patient was continued on the Cough-tabs, Lasix was discontinued, and Vetmedin (2 5mg a m , 1 25mg p m), enalapril (1 25mg BID), and taurine (500mg BID) were started Taurine was discontinued after a normal taurine level was received Cough persisted despite these changes and a course of doxycycline was prescribed (50mg BID x 10days) The cough improved significantly but did not completely resolve so the doxycycline was continued an additional 14 days The dog has since been lost to follow-up I have attempted to contact the owner and am waiting for a response I did contact the referring veterinarian and to their knowledge the dog is still alive
Submission Type: Initial
Report Type: Adverse Event (a symptom, reaction or disease associated with the product)
Outcome of reaction/event at the time of last observation: Unknown
Number of Animals Treated With Product: 5
Number of Animals Reacted With Product: 5

Product Name	Lot Number or ID	Best By Date
limited ingredient diet with kangaroo as protein source - manufacturer not specified in written history (we have attempted to contact the owner but they do not return phone calls)		

Sender information
(b) (6)

Owner information
(b) (6)

To view this PFR Event, please click the link below:
https://eon fda gov/eon//browse/EON-345965

To view the PFR Event Report, please click the link below:
(b) (6)

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From: Rotstein, David
To: Hartogensis, Martine; DeLancey, Siobhan
Cc: Norris, Anne
Subject: RE: List of firms with city/state?
Date: Friday, June 21, 2019 5:32:39 AM
Attachments: image002.png
image004.jpg
image006.jpg
image008.jpg
image010.jpg
image012.jpg
image013.png
image014.jpg
image015.jpg
image016.jpg
image017.jpg
image018.jpg

I'd consider this to be the final list. We may learn of co-packers, but it won't change the actual firm marketing the product.

Internally for FDA, the Divisions (HAF) is aware of the update and the FDA National Consumer Complaint Coordinator is also aware. Hopefully having all of this internal awareness will help should firms or consumers contact FDA.

D.

David Rotstein, DVM, MPVM, Dipl. ACVP
CVM Vet-LIRN Liaison
CVM OSC/DC/CERRT
7519 Standish Place
(b) (6) **(BB)**

From: Hartogensis, Martine
Sent: Thursday, June 20, 2019 10:06 PM
To: DeLancey, Siobhan <Siobhan.Delancey@fda.hhs.gov>
Cc: Norris, Anne <Anne.Norris@fda.hhs.gov>; Rotstein, David <David.Rotstein@fda.hhs.gov>
Subject: RE: List of firms with city/state?

Hi Siobhan,

Yes, I am happy to be the SME. Sending you the latest list from Dave.

Thanks again!
Martine

From: DeLancey, Siobhan
Sent: Thursday, June 20, 2019 3:18 PM
To: Hartogensis, Martine <Martine.Hartogensis@fda.hhs.gov>
Cc: Norris, Anne <Anne.Norris@fda.hhs.gov>
Subject: List of firms with city/state?

Hi Martine,

[redacted] (b) (5)

Thanks!

Siobhan DeLancey, RVT, MPH
Senior Advisor for Strategic Initiatives
Center for Veterinary Medicine
U.S. Food and Drug Administration
O: 240-402-9973
M: [redacted] (b) (6)
Siobhan.DeLancey@fda.hhs.gov

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ANATOMIC PATHOLOGY LABORATORY
http://www.cvm.ncsu.edu/dphp/labs/histologylab.htm
Room C-310 1060 William Moore Drive
RALEIGH, NC 27607
Phone #: 919-513-6390 Fax #: 919-513-6703

Owner: (b) (6)

Accession Number: (b) (6)
Reference Number:
Case Coordinator: (b) (6)
Received: (b) (6) **Finalized:** 04/18/2017
Sampled:

To: (b) (6)
(b) (6) VETERINARY HEALTH COMPLEX

Phone # (b) (6)

Addended Report

ANATOMIC PATHOLOGY RESULTS

SMALLANIMAL NECROPSY

ANIMAL ID	(b) (6)
REF CASE NO	(b) (6)
SPECIES	Canine
BREED	Schnauzer
SEX	Mc
AGE	3y
SPECIMEN DESC	Body

GROSS

An 8.2 kg, 2.5-year-old, castrated male miniature schnauzer dog is presented for postmortem examination. The animal was euthanized and the body is in fair postmortem condition with a euthanasia?to?necropsy interval of approximately 16 hours. The body appears moderately dehydrated. The hair coat is thick and shiny, and no ectoparasites are seen. Throughout the body, subcutaneous and visceral fat stores are adequate and the body condition score is 4/9. Throughout the dentition, a mild amount of dental calculus is present. Multifocally over the left lateral brachium, left craniolateral tibia, and left temporalis muscle, there are multiple approximately 4-6 cm long linear skin incisions that have been closed with suture. The left antebrachium is shaved and there are a few ecchymotic hemorrhages in this region. The right antebrachium, dorsal metatarsals, and ventral neck are also shaved. All skeletal muscle is grossly unremarkable.

Free within the thoracic cavity are approximately 15 mL of red, watery fluid. The lungs diffusely fail to collapse and contain multiple rib impressions along the pulmonary pleural surface. They are diffusely pink to red. On cut surface, these ooze a moderate to large amount of frothy, white fluid. The heart is subjectively enlarged. The heart measures as follows: right atrium circumference – 6 cm; right ventricular free wall thickness – 0.5 cm; left atrium circumference – 7 cm; left ventricular free wall thickness – 1 cm; interventricular wall thickness – 0.6 cm; pulmonic valve circumference – 4.5 cm; aortic valve circumference – 3 cm; total weight – 99 g (1.2% of the total body weight); right and left atria weight –

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RALEIGH, NC 27607
Phone #: 919-513-6390 Fax #: 919-513-6703

Addended Report **Accession Number:** (b) (6)

ANATOMIC PATHOLOGY RESULTS

15 g; right ventricle weigh 22 g; left ventricle and interventricular septum weight – 62 g. Multifocally expanding the free edge of the leaflets of the mitral valve, there are a few small, less than 2 mm diameter, smooth, shiny, white nodules.

Focally within the peripheral left limb of the pancreas, there is a small, 2 mm diameter, firm, white nodule. Multifocally along the capsular surface of the spleen are a few siderotic plaques.

GROSS DIAGNOSIS
1. Lungs: moderate to marked, diffuse pulmonary edema
2. Heart: mild cardiomegaly with mild mitral valve endocardiosis and mild left ventricular hypertrophy and left atrial dilation
3. Thorax: mild pleural effusion
4. Pancreas: focal nodular hyperplasia

REPORT STATUS PRELIMINARY REPORT-HISTOLOGY PENDING

COMMENTS
Except for gross evidence of moderate to marked pulmonary edema and mild cardiac changes, gross examination is otherwise mostly unremarkable. The heart is mildly enlarged with mild mitral valve endocardiosis and associated mild left atrial dilation and left ventricular hypertrophy. This mild degree of cardiac changes does not fit well with the moderate to severe degree of pulmonary edema present. In addition to the mild structural changes, a functional cardiac abnormality may have been contributing to clinical disease in this patient. Samples of lung and heart were rushed and examined histologically. In addition to the pulmonary edema, there is also histologic evidence of diffuse, acute alveolar injury. These pulmonary changes can explain this patient's signs of respiratory distress. However, the heart is histologically unremarkable and the skeletal muscle was grossly unremarkable; as such, a cause for the markedly elevated CK is not yet identified. Histology of skeletal muscle is pending.

PATH RESIDENT (b) (6) DVM
SENIOR PATH (b) (6) DVM PHD DACVP
DATE (b) (6)

Final Necropsy Report

MICROSCOPIC
Lung (slide 2): Diffusely throughout all sections, there is evidence of alveolar injury characterized by frequent deposition of bright eosinophilic fibrin within alveolar lumina. This fibrin is often forming hyaline membranes lining alveolar septal walls. There is also frequent deposition of fibrin within the alveolar walls and sometimes effacing and replacing the septa. There are also high numbers of scattered foamy, alveolar macrophages. Multifocally scattered throughout multiple sections, there is occasional type II pneumocyte hyperplasia.

Liver (slide 4): There is diffuse mild to moderate, acute congestion of hepatic sinusoids. Low numbers of individually scattered hemosiderin-

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Phone #: 919-513-6390 Fax #: 919-513-6703

Addended Report **Accession Number:** (b) (6)

ANATOMIC PATHOLOGY RESULTS

laden Kupffer cells are present.

Heart (slide 1), Skeletal muscle (slide 3), Kidneys (slide 3), Spleen (slide 3), Stomach (slide 4), Small intestine (slide 4), Colon (slide 4), Pancreas (slide 4), Adrenal gland (slide 4): No remarkable histologic abnormalities are appreciated throughout the tissue sections examined. PAS stains with and without diastase treatment are applied to sections of heart. No evidence of glycogen-storage is appreciated.

FINAL DIAGNOSIS

1. Lungs:
a. Severe, dififuse alveolar injury with marked fibrin deposition (hyaline membranes) and marked alveolar histiocytosis and multifocal type II pneumocyte hyperplasia
b. Moderate to marked, diffuse pulmonary edema
2. Heart: mild cardiomegaly with mild mitral valve endocardiosis and mild left ventricular hypertrophy and left atrial dilation
3. Thorax: mild pleural effusion

COMMENTS

Histology reveals severe, diffuse alveolar injury and alveolar histiocytosis throughout all examined sections of lung. This pattern of injury is often seen in cases of acute respiratory distress syndrome (ARDS). However, ARDS is a clinical diagnosis. The degree of injury present is severe enough to explain this dog's reported clinical disease of respiratory distress. However, an underlying etiology for this alveolar injury is not identified. The heart and skeletal muscle are histologically unremarkable and, as such, a primary noncardiogenic etiology is suspected. However, if there was clinical evidence of cardiac dysfunction then a functional cardiac abnormality in the absence of a correlating structural change cannot be ruled-out based on postmortem examination. Survey sections of other major organs are also histologically unremarkable. Common causes of ARDS in dogs include aspiration of sterile stomach contents, smoke or other toxin inhalation, near drowning, infection and systemic inflammation (sepsis), drug reaction, etc. In this case, there is no overt evidence of sepsis throughout the other tissues examined.

PATH RESIDENT (b) (6) DVM
SENIOR PATH (b) (6) DVM PHD DACVP
FINALIZED DATE (b) (6)

Report Details - EON-323515

ICSR:	2023228	
Type Of Submission:	Initial	
Report Version:	FPSR.FDA.PETF.V.V1	
Type Of Report:	Adverse Event (a symptom, reaction or disease associated with the product)	
Reporting Type:	Voluntary	
Report Submission Date:	2017-07-11 17:06:59 EDT	
Reported Problem:	**Problem Description:**	Please note: Dr. Jennifer Jones was consulted prior to submission of this report. She would like to be involved in the case review 3 week history of cough treated unsuccessfully with doxycycline and prednisone. 3 day history of inappetence and vomiting prior to presentation to NCSU emergency service for dyspnea. Radiographs showed severe pulmonary edema and echocardiogram showed severe Dilated Cardiomyopathy. There was an initial response to diuretic therapy however, he declined and was placed on the ventilator for respiratory support and continued CHF treatment. Attempts to wean off the ventilator were unsuccessful and aquaphoresis was performed. He continued to decline despite aggressive therapy and was euthanized. Infectious disease testing was negative and taurine and carnitine analysis showed adequate levels. Necropsy initially did not reveal a cause for DCM and supported alveolar injury (possibly ventilator related). A re-review of the myocardial histopathology by one of our pathologist showed myofiber vacuoles reminiscent of the changes seen in doxorubicin toxicity. Since the dog had not received doxorubicin, the pathologist recommended culturing the food for Streptomyces peucetius - the bacterium which produces doxorubicin. He also recommended testing for Fusarium spp. a fungus which produces Fumonisin B1, a toxin that produces heart failure in pigs. (b) (6) had been fed California Naturals Adult - both kangaroo with lentils and venison with lentils along with Milo's kitchen treats. We have samples of these foods from 6/17 but not the original bags from when he was presented 2/17. These samples were provided at the time his housemate, (b) (6) (unrelated, older miniature schnauzer) also presented with severe DCM and CHF. I will enter this dog as a separate affected patient. Both dogs had extensive infectious disease testing which was negative and nutritional amino acid deficiencies were ruled out. Because of this, their unrelated lineages (although the same breed, they were from different lines), different ages but similar time of presentation ((b) had clinical signs at the time (b) (6) was treated, but didn't present with CHF for several months), we are considering common environmental factors which could precipitate DCM, including food contamination or toxin exposure.
	Date Problem Started:	(b) (6)
	Concurrent Medical Problem:	No
	Outcome to Date:	Died Euthanized
	Date of Death:	(b) (6)
Product Information:	**Product Name:**	Alternated between: -California Natural Adult Limited Ingredient Grain Free Venison & Green Lentils and Kangaroo & Red Lentils Recipe
	Product Type:	Pet Food
	Lot Number:	
	UPC:	not available
	Package Type:	BAG
	Package Size:	26 Pound
	Purchase Date:	06/01/2017
	Possess Unopened Product:	No
	Possess Opened Product:	Yes
	Storage Conditions:	In a cabinet, in the original bag
	Product Use Information:	**Description:** twice daily feeding The sample we have is from 6/17, however, we do not have food samples from 2/17 when both dogs started with clinical signs.
		Time Interval between Product 2 Years

		Use and Adverse Event:	
		Product Use Stopped After the Onset of the Adverse Event:	No
		Perceived Relatedness to Adverse Event:	Possibly related
		Other Foods or Products Given to the Animal During This Time Period:	Yes
	Manufacturer /Distributor Information:		
	Purchase Location Information:	**Address:**	United States
Animal Information:	**Name:**	(b) (6)	
	Type Of Species:	Dog	
	Type Of Breed:	Schnauzer - Miniature	
	Gender:	Male	
	Reproductive Status:	Neutered	
	Weight:	8.2 Kilogram	
	Age:	2.5 Years	
	Assessment of Prior Health:	Excellent	
	Number of Animals Given the Product:	2	
	Number of Animals Reacted:	2	
	Owner Information:	**Owner Information provided:**	Yes
		Contact:	**Name:** (b) (6)
			Phone: (b) (6)
			Email: (b) (6)
		Address:	(b) (6) United States
	Healthcare Professional Information:	**Practice Name:**	North Carolina State University, College of Veterinary Medicine
		Contact:	**Name:** Darcy Adin
			Phone: (919) 513-6694
			Other Phone: 6145829798
			Email: dbadin@ncsu.edu
		Address:	1060 William Moore Dr Raleigh New York 27607 United States
		Practice Name:	North Carolina State University College of Veterinary Medici
		Contact:	**Name:** (b) (6)
			Phone: 9195136694

			Other Phone:	6145829798
		Practice Name:	North Carolina state University, College of Veterinary Medic	
		Contact:	Name:	(b) (6)
			Phone:	919 513-6694
			Other Phone:	6145829798
Sender Information:	Name:	Darcy Adin		
	Address:	1060 William Moore Dr Raleigh New York 27607 United States		
	Contact:	Phone:	9195136694	
		Other Phone:	6145829798	
		Email:	dbadin@ncsu.edu	
	Permission To Contact Sender:	Yes		
	Preferred Method Of Contact:	Email		
	Reported to Other Parties:	Manufacturer		
Additional Documents:				

Report Details - EON-323519

ICSR:	2023230	
Type Of Submission:	Initial	
Report Version:	FPSR.FDA.PETF.V.V1	
Type Of Report:	Adverse Event (a symptom, reaction or disease associated with the product)	
Reporting Type:	Voluntary	
Report Submission Date:	2017-07-11 17:32:14 EDT	
Reported Problem:	**Problem Description:**	Please note: Dr. Jennifer Jones was consulted prior to submission of this report. She would like to be involved in the case review. (b) (6) housemate, (b) (6) (separate report submitted) was diagnosed with DCM and CHF (b) (6) and was euthanized after aggressive treatment of CHF. At that time (b) had 2 syncopal events closely related to each other. His appetite for dog food declined but he would eat it if tempted with treats mixed in. He was presented 6/22/17 for more syncopal events and was similarly diagnosed with severe DCM and CHF. He was able to be successfully treated however and is clinically doing well on CHF medications as of 7/10/17. A re-review of the myocardial histopathology for (b) (6) housemate (b) (6) was requested at this time because of the unusual diagnosis of DCM in a small breed dog living in the same house as another dog similarly diagnosed a few months ago. This re-review by one of our pathologists showed myofiber vacuoles reminiscent of the changes seen in doxorubicin toxicity. Since the dog had not received doxorubicin, the pathologist recommended culturing the food for Streptomyces peucetius - the bacterium which produces doxorubicin. He also recommended testing for Fusarium spp. a fungus which produces Fumonisin B1, a toxin that produces heart failure in pigs. Like (b) (4) (unrelated, younger miniature schnauzer), (b) (6) had been fed California Naturals Adult - both kangaroo with lentils and venison with lentils along with Milo's kitchen treats. We have samples of these foods from 6/17 but not the original bags from when he was presented 2/17. These samples were provided at the time (b) (6) also presented with severe DCM and CHF. Like (b) (6) had extensive infectious disease testing which was negative and nutritional amino acid deficiencies were ruled out. Because of this, their unrelated lineages (although the same breed, they were from different lines), different ages but similar time of presentation, we are considering common environmental factors which could precipitate DCM, including food contamination or toxin exposure. We have plasma, serum, urine and myocardial tissue samples (latter only for (b) (6)) stored at -80 Celsius in addition to food and treat samples.
	Date Problem Started:	(b) (6)
	Date of Recovery:	07/10/2017
	Concurrent Medical Problem:	No
	Outcome to Date:	Stable
Product Information:	**Product Name:**	Alternated feedings between: California Natural Adult Limited Ingredient Grain Free Venison & Green Lentils Recipe Dog Food and Kangaroo and Lentils
	Product Type:	Pet Food
	Lot Number:	
	Package Type:	BAG
	Package Size:	26 Pound
	Storage Conditions:	In the bag in a cabinet
	Product Use Information:	**Description:** the Venison and Kangaroo types were alternated depending on store availability and were fed twice daily along with Milo's kitchen treats
		Perceived Relatedness to Adverse Event: Possibly related
	Manufacturer /Distributor Information:	
	Purchase Location Information:	
Animal Information:	**Name:**	(b)

Type Of Species:	Dog	
Type Of Breed:	Schnauzer - Miniature	
Gender:	Male	
Reproductive Status:	Neutered	
Weight:	9.6 Kilogram	
Age:	7 Years	
Assessment of Prior Health:	Excellent	
Number of Animals Given the Product:	2	
Number of Animals Reacted:	2	
Owner Information:	**Owner Information provided:**	Yes
	Contact:	**Name:** (b) (6)
		Phone: (b) (6)
		Email: (b) (6)
	Address:	(b) (6) United States
Healthcare Professional Information:	**Practice Name:**	North Carolina State University, College of Veterinary Medicine
	Contact:	**Name:** Darcy Adin
		Phone: (919) 513-6694
		Other Phone: 6145829798
		Email: dbadin@ncsu.edu
	Address:	1060 William Moore Dr Raleigh New York 27607 United States
	Practice Name:	North Carolina State University, College of Veterinary Medic
	Contact:	**Name:** (b) (6)
		Phone: 919-513-6694
		Other Phone: 6145829798
	Practice Name:	North Carolina State University, College of Veterinary Medic
	Contact:	**Name:** (b) (6)
		Phone: 919-513-6694
		Other Phone: 6145829798
Sender Information:	**Name:**	Darcy Adin
	Address:	1060 William Moore Dr Raleigh New York 27607 United States
	Contact:	**Phone:** 9195136694
		Other Phone: 6145829798
		Email: dbadin@ncsu.edu
	Permission To Contact Sender:	Yes

	Preferred Method Of Contact:	Email
	Reported to Other Parties:	
Additional Documents:		

EON #	Last Name	Product	Lot	BB Date	Signalment	Clinical Signs	Open Product?	Closed Product?	MedRec?	Vet-LIRN Open Product Testing	
EON-345822	(b) (6)	California Naturals Kangaroo & Lentil	unknown	unknown	(b) (6)-6 yr FS Lab	cough, dyspnea, collapse, CHF; hx atopy	Yes	No	DCM with left CHF, WB Tau wnl; mild inc ALT/AST/CK	*collection in progress*	
EON-345831	(b) (6)	California Naturals Kangaroo & Lentil; began new Venison Food (Brand?)	unknown	unknown	(b) (6)-8 yr F Lab	collapse, cough, urinary incontinence	No	No	DCM with Chronic Degen Valve Disease, Atrial Fib (resolved), Exertional Collapse; WB Tau: wnl	n/a	
EON-345833	(b) (6)	California Naturals Kangaroo & Lentil; began new Venison Food (Brand?)	unknown	unknown	(b) (6)-5 yr FS Lab **euthanized**	dyspnea, cough-productive, urinary incontinence	No	No	DCM with Endocardiosis, CHF, inc SDMA	n/a	
EON-345835	(b) (6)	Zignature Kangaroo Limited Ingredient	unknown	unknown	(b) (6)-4 yr MC Cocker Spaniel	cough	No	No	DCM, CHF, WB Tau: LOW, low blood Alb with proteinuria	n/a	
EON-345965	(b) (6)	Kangaroo	unknown	unknown	(b) (6)-8 yr MC Shih Tzu	lethargy, cough	unknown	unknown	DCM, WB Tau: wnl	n/a	

Vet-LIRN Case Summary Document

Vet-LIRN Case Number:	800.218
EON/CC #:	Multiple
Owner LAST Name:	(b) (6)
Vet LAST Name:	(b) (7)(A)
Vet-LIRN Initiation Date:	1/23/2018
MedRec: Requested:	already received
MedRec: Received:	
MedRec: Significant finding:	All dogs w/ DCM, 2 Tau wnl, 1 not measured, 1 Tau low
Vet-LIRN Tests (planned):	• MRx Review • Open Product (b) (6) for Metals and Cys/Met • (b) (6) Blood Fe Panel
Vet-LIRN Test Results:	Low product Fe results
Result Interpretation:	Fe Panel: all wnl Iodine: wnl
IF NFA, justification:	

COMPLAINT Narrative: see xls and multiple PFRs

1/23/2018
JJ-MRx summaries.

The listserve BLUF:

- Diet:
  - Kangaroo: low Tau & Met, high Carn
  - Lentil: Low Cys, Met
- In Cats, plasma Taurine (Tau)
  - falsely increases with damaged WBC and Platelets, short half-life
  - Whole blood Taurine a better measurement b/c longer half-life
  - Skeletal muscle even longer half-life and thought to parallel cardiac muscle Tau content
- From one vet dealing with Golden Retrievers eating designer diets (high quality meats) with pea or lentils-most dogs with low or Low Normal Whole Blood Tau → most recovered with Tau supplementation → Hypothesis was low Cys & Met plus Golden Retriever Tau handling may cause the illness

(b) (6)-6 yr FS Lab
Presenting complaint 1/10/2018: cough, dyspnea, multiple episodes of collapse, cardiomegaly, suspected CHF; seen by rDVM 12/30 for cough → by 1/4/2018: collapse episode-circled, fell over, flopped ~1 min, 2nd less severe episode 1/5; → continued shortness of breath, easily tires; heartworm negative (9/9/2017), eats California Naturals Kangaroo and Lentil with veggies and 2 tbsp canned pumpkin; on Apoquel BID and SLIT;

PE 1/10: T 102.7, P 208, R 150, Gr III/VI left apical systolic murmur with gallop, regular tachycardia, quiet heart sounds, localized fine crackles left cranial hilar region with dry cough, poor femoral pulses, mm pale pink; → hospitalized → occ short paroxysms of slow ventricular tachycardia

Labs: 1/5/2018 Chem: mild inc ALT, AST, and CK
T4: low normal
1/10 BP: 152 mmHg
Echo: sinus tachycardia, DCM with left sided CHF
Whole Blood Tau: 292 (200-350), >150 not known risk of Tau Deficiency;
ECG: HR 189 bpm, sinus tachycardia, atrial enlargement, LVE

1/11 T-Rads: mild decrease in severity of cardiomegaly, resolving cardiogenic edema

(b) (6): 8 yr F Lab

Presenting (b) (6): ER for heart evaluation, seen week prior (Thurs) for collapse while playing fetch; at vet-atrial fibrillation with mild heart failure; normal sinus rhythm (nsr) on 1/20 → 1/21 collapse while fetching → nsr with rads showing resolution of HF, CBC/Chem wnl → recheck on 5/31: no episodes of weakness/collapse, last 2-3 anxious and less social b/c storm anxiety; on daily glucosamine/chondroitin → recheck 12/11: new cough, not frequent, at rest and with excitement/activity; owner transitioning to a new venison food, urinary incontinence

PE (b) (6): Gr III to IV/VI left apical holosystolic murmur, sinus arrhythmia, moderate femoral pulses
5/31: T 103.1, RR 120, Gr III-IV/VI left apical holosystolic murmur
12/11: HR 128, pant, Gr III/VI left apical systolic murmur with wide radiation, fair-moderate femoral pulse

Labs: (b) (6)
Whole Blood Taur: 236 (200-350) (>150 = no risk for Tau deficiency)
BP: 108 mmHg
-5/31: 90 mmHg
Echo: DCM, Chronic degenerative valve disease, atrial fibrillation, exertional collapse
-5/31: slight improvement though heart a little larger
-12/11: heart bigger with decreased contractility,
Telemetry: consistent sinus rhythm/arrhythmia
Renal panel: no results listed
-5/31: SDMA 18, normal BUN/Ct, mild low Cl
-12/11: wnl

5/31 T Rads: mild progression of cardiac enlargement
-12/11: progressive cardiomegaly

(b) (6): 5 yr FS Lab → genetic niece of (b) (6)

Presenting complaint (b) (6): new heart murmur, diagnosed with CHF on (b) (6) seen by rDVM for heavy breathing and cough; rads and labs showed enlarged heart, CHF → (b) (6) to ER, increased meds with some improvement → panting and on low sodium Kangaroo and Lentil diet; other dog (b) (6) is (b) (6) aunt, urinary incontinence began prior to CHF treatment/diagnosis; less social and active; on Apoquel → (b) (6) tachypneic, coughed up pink tinged fluid, episode of collapse, dry heaving on way to ER → on Venison and Lentil diet → respiratory rate continue to increase despite Lasix CRI → euthanized

PE (b) (6): HR 150, pant, Gr IV/VI left apical systolic murmur with radiation to right, adequate femoral pulse
(b) (6) HR 180, pant, pale pk, Gr IV/VI murmur, fine crackles right dorsal lung fields

Labs: (b) (6) BP: 110
(b) (6) Renal Panel: mild inc SDMA (19 ug/dL)

(b) (6) Echo: endocardiosis, DCM
(b) (6) ECG: nsr
(b) (6) T Rads: persistent cardiomegaly with mild decreased severity
-9/9-9/10: generally enlarged, unstructured interstitial pulmonary pattern within right middle and cd lobes, mild enlargement cranial lobar pulmonary veins

(b) (6)-4 yr MC Cocker Spaniel
Presenting complaint 9/5/2017: evaluation of cardiomegaly and CHF, diagnosed by rads 8/22; to rDVM 8/22 for week long progressive cough-2x/day with progressive severity; t-rads and labs: CHF; treated with antibiotics and heart meds (concern initially for pneumonia) → improved cough but still occurring; PE 9/5: T 104.4, HR 150, pant, quiet/distant heart sounds with gallop, Gr I/VI systolic murmur on left, fine bilateral crackles, patient shivering so femoral pulse difficult to assess but suspect decreased, palpable jugular pulsation, suspected oral epulis;
-9/19: HR 178-180, T 102.4, quiet heart sounds, gallop, fair femoral pulses, palpable hepatomegaly, epidermal collarettes with exudative crust on ventral abdomen
-11/2: T 103.2, HR 168, RR 110

Labs: 9/5 BP 160 mmHg
-9/19: 152 mmHg
9/5 Chem: Alb 2.5 (inc from 2.1)
-9/19 or 11/2: Renal Panel: BUN 32
9/5 UA: 1.015, pH 8, pro tr
Echo: DCM, CHF
ECG: sinus tachycardia, HR ~200
9/5 Whole Blood Tau: 10 (200-350)

T Rads (9/19 or 11/2?): decreased heart size

PLAN: I Recommend collecting open product from (b) (6) case (only one available) and testing for metals with the other cases. Also based on message board-recommend testing cysteine and methionine from all the DCM cases. If we find something "Low" in the foods for amino acids, we can look at the case from last summer and measure cardiac muscle-Cys, Met, Tau. For (b) (6) case (EON-345822) if dog dies-will ask to save heart tissue for potential testing and histopath. Vet-LIRN can purchase storebought Zignature Essentials for testing based on other results.

DR agrees-If testing confirms an issue with the Amino acids-DAF could have a chat with the firm.
JJ-Contacting the vet to request open product for testing and tissue if dog dies.

1/24/2018
JJ-Vet is collecting the leftover food from (b) (6). The vet mentions additional cases:

> For your information, we have pulled all our cases of dilated cardiomyopathy that we diagnosed in 2017 and identified a fifth animal on kangaroo and lentil diet (out of 23 total) - I will submit an FDA case report for this-, and another one on a lentil-containing Vegan diet. There are some cases in which the diet is not specified that we still need to contact, but at least 11 of the cases are on grain-free diets, including all of the cases in "atypical" breeds. As you may have seen from my listserve query to other cardiologists, not only have

Cardiologists been seeing cases in dogs fed kangaroo and lentil, but there were a number of cases in dogs of "atypical" breeds on lentil-based Vegan diets, and a series of Golden retrievers with taurine deficiency on grain-free diets. Grain-free diets are a fad right now and lots of clients are feeding them, so the incidence of grain-free diets may simply be coincidence. Interestingly however, (b) (6), who I saw recently and re-echoed after several months on another diet with no documented improvement in echo findings is still being fed a grain-free diet. I have asked her owner to switch her diet once again to something not "grain-free" and we will see how things go over the next several months.

Emailed the vet-we're sending a kit to collect the food.

1/30/2018
JJ-Vet submitted an additional PFR but it was unclear which brand kangaroo diet was fed.

LAP did an epi summary: FYI – this makes 6 cases now, 5 from Ohio, 1 from NC (involving 2 dogs, 1 death) all eating LID type diet with Kangaroo and Red Lentil, onset dates between 1/20/2017 and 12/30/2017).

MRx summary:

(b) (6)-8 yr MC Shih Tzu
Presenting complaint 6/19/2017: lethargy for past few weeks and progressive coughing that began 6 months ago; started on cough suppressant and Lasix without improvement; RR/effort normal, no episodes weakness/collapse; on limited ingredient diet with Kangaroo as sole protein source; monthly Sentinel, HW negative February; → recheck 7/6-frequently travelling, dog not liking car rides as much (RR 80's), forceful cough-significant increase; bad night-unable to sleep comfortably; 3 urinary accidents in house since starting new meds
PE 6/19: P 128, Gr III-IV/VI left apical systolic murmur with radiation to the right base, fair femoral pulses, excessive bruising after jugular venipuncture; thin body condition, mm light pink/slightly tacky; fundic-suspect partial retinal detachment on nasal aspect of retina OS w/ hyper-reflectivity around edge;
-7/6: Grade IV/VI murmur, 99.6F, HR 165, RR 56

Labs:
- 6/19 BP: 102 mmHg
  -7/6: 138 mmHg
- 6/19 Rads: generalized cardiomegaly (progressive), mild diffuse bronchointerstitial pattern
  -7/6: unchanged cardiomegaly
- 6/19 CBC: mild non-pre-regenerative anemia, Retic 115 (10-110)
- 6/19 Chem: Na 154 (142-152), K 5.4 (4-5.4), Cl 107 (108-119), Bicarb 30 (13-27), SDMA 14 (0-14), Alb 4.1 (2.7-3.9), AST 83 (16-55), CK 1162 (10-200)
  -7/6 Renal panel: nsf
- 6/19 Whole Blood Taurine: 276 (200-350); >150 no known risk for Tau deficiency
- 6/19 Echo: DCM, mild PV insufficiency, mild tricuspid regurgitation & thickening, mild MV thickening with +3 regurgitation

1/30/18
JG – Shipped food sample collection kit to Vet and informed Vet with tracking information

Note: Vet hospital's zip code was incorrect. updated.

2/2/2018
JJ-Emailed [redacted] requesting testing. Make lab submission forms.

JG – Received treats (kibble) in the provided pink zip bag. Also received original package (empty). Seal the original package with tape.
Log in the treat as 800.218-sub6

2/5/2018
JJ-prepared the sample and packaged box to send to [redacted] (b) (4).

2/22/2018
JJ-We received the results from [redacted] (b) (4). The only significant finding was all products contained inadequate Fe content, below the AAFCO claim. Fe has been associated with idiopathic DCM (Marinescu and McCullough 2011) in people. According to the article, anemia was found in 12 to 55% of patients in heart failure, and the anemia's severity correlated to the degree of heart failure.

Looking at our cases, a CBC was done for 3 dogs according to their discharge summaries. One dog had a non/pre-regenerative anemia, and the other two had no mention of RBC abnormalities. It's possible that chronically feeding a diet containing low Fe could cause a heart issue, but I would also expect to see anemias in these dogs.

Dr. Adin at NCSU emailed additional theories. She continues to see more cases.

- [redacted] (b) (5)
- Just thought I would forward this email chain to you as the growing concern about grain-free diets in the veterinary cardiology community continues to expand. [redacted] (b) (5)
    - [redacted] (b) (5)

- (b) (5) [redacted].

The Vet Cardiology Community is discussing this quite a bit.

2/23/2018
JJ-DR replied- (b) (5) [redacted]

Send feed test results to NCSU and (b) (6). I'll ask Dr. Adin if an iron panel has been done on any of the dogs. If not, we can request a full iron panel for one dog at NCSU and one from (b) (6). Will get estimates for the test from each lab. If the dogs are low Fe, we can test a myocardial sample from the (b) (6) case.

2/27/2018
JJ-The vet will perform a blood iron panel. I received an estimate and submitted a PO. This will be an unfasted blood sample.

Per the vet: "We had not done a CBC as one was completed through the referring vet, prior to her initial referral, and was completely normal dated 1/5/18 through (b) (6)

3/13/2018
JJ-The veterinarian sent the iron panel results. Iron 179 (73-245), TIBC 396 (270-530), Ferritin 443 (89-489). This was an unfasted sample and may be after the dog was switched to a new food??

From the vet: I would love to discuss our findings on dilated cardiomyopathy and dietary relationships in our clinic over the past year. In particular, 75% of our DCM cases in 2017 for which we have adequate dietary histories were on grain free diets. We have started to do a survey of other cardiac patients during the same time period to see if we can get an idea of what percentage of our referral population were feeding these type of diets so we can see if this is truly significant. As we have been looking harder at these cases, we have found some other interesting things. For instance, two patients that were found to be taurine deficient were being fed Zignature diets. One was on the kangaroo variety and the other the pork variety. There continues to be a lot of discussion about diets and dilated cardiomyopathy on our list serve, but I can see it degenerating into I diagnosed DCM and the dog is on this diet so therefore this is a problem, which may or may not be true evidence to support a causative role of the diet. I am not sure what information the FDA would want on all this at this point. We are certainly concerned that there may be a wider concern than simply the kangaroo and lentil diets that we first identified as a potential problem.

3/20/2018
JJ-The invoice is still pending.

4/9/2018
JJ-I'll ask the vet if they received the lab invoice.

4/12/2018
JJ-The vet sent the invoice, and I submitted.
I prepared the food samples and they should ship to MSU for iodine screening.

4/23/2018
JJ-We received the MSU results. All products contained less than 10 ppm. NFA.

From: Forfa, Tracey
To: Hartogensis, Martine
Subject: FW: DCM Plan
Date: Tuesday, June 18, 2019 10:07:15 AM
Attachments: DCM Project Plan.docx

Here you go! The "script" is embedded in the chart. Let me know if you have any questions….and please feel free to tweak it.

From: Forfa, Tracey
Sent: Tuesday, June 4, 2019 12:22 PM
To: Steinberg, Nadine (Nadine.Steinberg@fda.hhs.gov) <Nadine.Steinberg@fda.hhs.gov>; DeLancey, Siobhan (Siobhan.Delancey@fda.hhs.gov) <Siobhan.Delancey@fda.hhs.gov>; Norris, Anne <Anne.Norris@fda.hhs.gov>; Hartogensis, Martine <Martine.Hartogensis@fda.hhs.gov>; Palmer, Lee Anne <LeeAnne.Palmer@fda.hhs.gov>; Carey, Lauren <Lauren.Carey@fda.hhs.gov>
Cc: Dewitt, Susan J (Susan.Dewitt@fda.hhs.gov) <Susan.Dewitt@fda.hhs.gov>; Cepeda, Sandra <Sandra.Cepeda@fda.hhs.gov>; Murphy, Jeanette (Jenny.Murphy@fda.hhs.gov) <Jenny.Murphy@fda.hhs.gov>; Conway, Charlotte <Charlotte.Conway@fda.hhs.gov>
Subject: DCM Plan

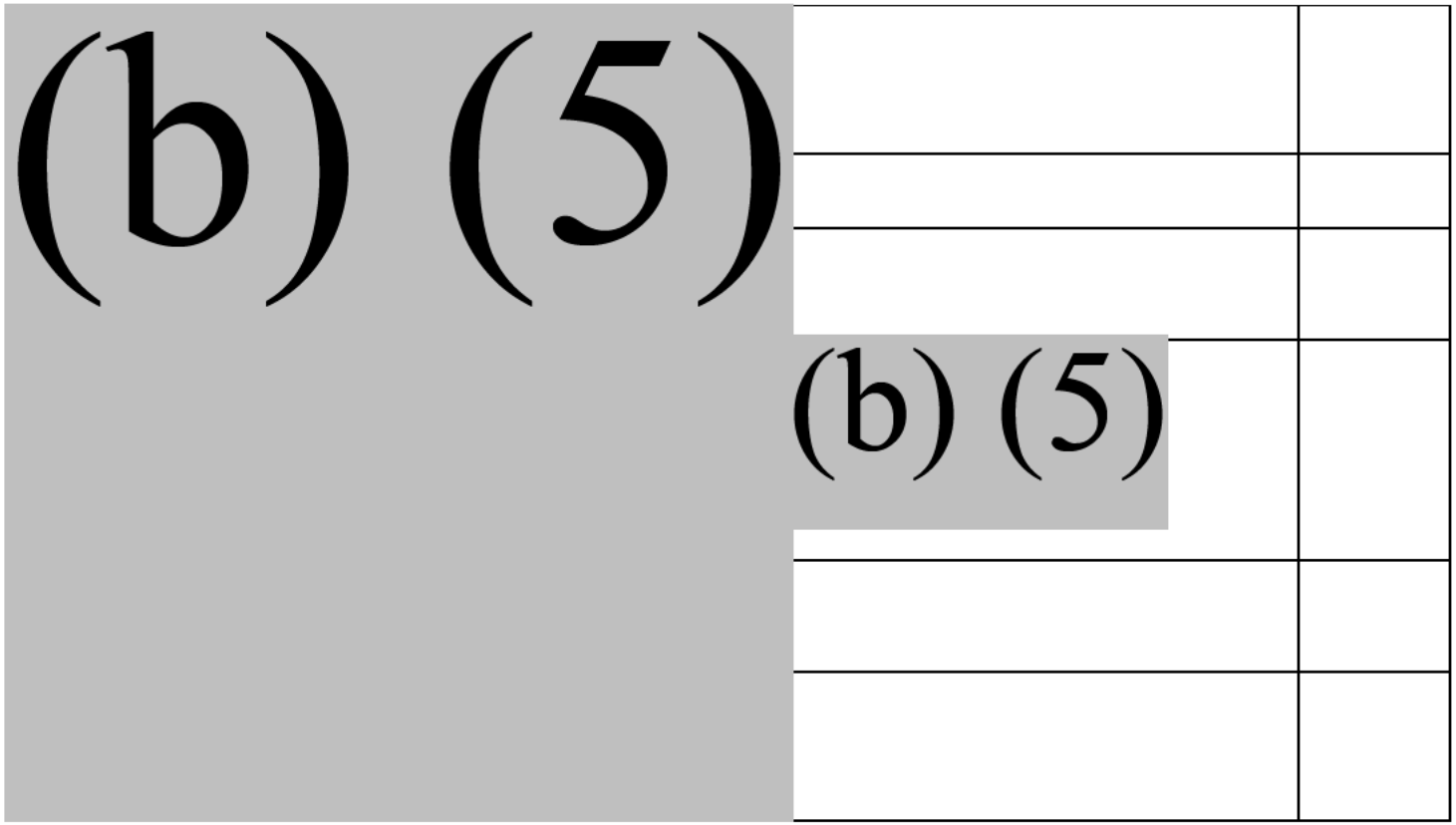
Hi All – As discussed (b) (5)

(b) (5)

Thank you!!!

DCM Plan

Activity	Lead	Notes	Timing
(b) (5)	(b) (5)		
	(b) (5)		
	(b) (5)		


(b) (5)
(b) (5)

From: Rotstein, David
To: Reinschmeed, Kersten; Queen, Jackie; Palmer, Lee Anne; Jones, Jennifer L; Ceric, Olgica; Carey, Lauren
Subject: Fwd: Alternated feedings between:-California Natural Adult Limited Ingredient Grain Free Venison & Green Lentils Recipe Dog Food and Kangaroo and Lentils: Darcy Adin - EON-323519
Date: Tuesday, July 11, 2017 5:45:51 PM
Attachments: 2023230-report.pdf

David Rotstein, DVM, MPVM, Dipl ACVP
CVM Vet-LIRN Liaison
CVM OSC/DC/CERT
7519 Standish Place
(b) (6) (88)

From: PFR Event <pfreventcreation@fda.hhs.gov>
Date: July 11, 2017 at 5:36:16 PM EDT
To: HQPet Food Report Notification <HQPetFoodReportNotification@fda.hhs.gov>, (b) (6) Cleary, Michael * <Michael.Cleary@fda.hhs.gov>
Subject: Alternated feedings between:-California Natural Adult Limited Ingredient Grain Free Venison & Green Lentils Recipe Dog Food and Kangaroo and Lentils: Darcy Adin - EON-323519

A PFR Report has been received and PFR Event [EON-323519] has been created in the EON System

A "PDF" report by name "2023230-report.pdf" is attached to this email notification for your reference

Below is the summary of the report:

EON Key: EON-323519
ICSR #: 2023230
EON Title: PFR Event created for Alternated feedings between: California Natural Adult Limited Ingredient Grain Free Venison & Green Lentils Recipe Dog Food and Kangaroo and Lentils; 2023230

AE Date	(b) (6)	**Number Fed/Exposed**	2
Best By Date		**Number Reacted**	2
Animal Species	Dog	**Outcome to Date**	Stable
Breed	Schnauzer - Miniature		
Age	7 Years		
District Involved	PFR-New York DO		

Product information
Individual Case Safety Report Number: 2023230
Product Group: Pet Food
Product Name: Alternated feedings between: California Natural Adult Limited Ingredient Grain Free Venison & Green Lentils Recipe Dog Food and Kangaroo and Lentils
Description: Please note: Dr Jennifer Jones was consulted prior to submission of this report She would like to be involved in the case review (b) (6) housemate, (b) (6) (separate report submitted) was diagnosed with DCM and CHF (b) (6) and was euthanized after aggressive treatment of CHF At that time (b) (6) had 2 syncopal events closely related to each other His appetite for dog food declined but he would eat it if tempted with treats mixed in He was presented (b) (6) for more syncopal events and was similarly diagnosed with severe DCM and CHF He was able to be successfully treated however and is clinically doing well on CHF medications as of 7/10/17 A re-review of the myocardial histopathology fo (b) (6) housemate (b) (6) was requested at this time because of the unusual diagnosis of DCM in a small breed dog living in the same house as another dog similarly diagnosed a few months ago This re-review by one of our pathologists showed myofiber vacuoles reminiscent of the changes seen in doxorubicin toxicity Since the dog had not received doxorubicin, the pathologist recommended culturing the food for Streptomyces peucetius - the bacter um which produces doxorubicin He also recommended testing for Fusarium spp a fungus which produces Fumonisin B1, a toxin that produces heart failure in pigs Like (b) (6) (unrelated, younger miniature schnauzer), (b) (6) had been fed California Naturals Adult - both kangaroo with lentils and venison with lentils along with Milo's kitchen treats We have samples of these foods from 6/17 but not the original bags from when he was presented (b) (6) These samples were provided at the time (b) (6) also presented with severe DCM and CHF Like (b) (6) had extensive infectious disease testing which was negative and nutritional amino acid deficiencies were ruled out Because of this, their unrelated lineages (although the same breed, they were from different lines), different ages but similar time of presentation, we are considering common environmental factors which could precipitate DCM, including food contamination or toxin exposure We have plasma, serum, urine and myocardial tissue samples (latter only for (b)) stored at -80 Celsius in addition to food and treat samples
Submission Type: Initial
Report Type: Adverse Event (a symptom, reaction or disease associated with the product)
Outcome of reaction/event at the time of last observation: Stable
Number of Animals Treated With Product: 2
Number of Animals Reacted With Product: 2

Product Name	Lot Number or ID	Best By Date
Alternated feedings between: California Natural Adult Limited Ingredient Grain Free Venison & Green Lentils Recipe Dog Food and Kangaroo and Lentils		

Sender information
Darcy Adin
1060 William Moore Dr
Raleigh, NY 27607
USA

Owner information
(b) (6)

To view this PFR Event, please click the link below:
https://eon.fda.gov/eon//browse/EON323519

To view the PFR Event Report, please click the link below:
https://eon.fda.gov/eon (b) (6)

From: Rotstein, David
To: Queen, Jackie L; Jones, Jennifer L; Reimschuessel, Renate; Cerk, Ashley; Palmer, Lee Anne; Carey, Lauren
Subject: Fwd: Alternated between: --California Natural Adult Limited Ingredient Grain Free Venison & Green Lentils and Kangaroo & Red Lentils Recipe: Darcy Adin - EON-323515
Date: Tuesday, July 11, 2017 5:17:06 PM
Attachments: 2023228-report.pdf

David Rotstein, DVM, MPVM, Dipl.ACVP
CVM Vet-LIRN Liaison
CVM OSC/DC/CERT
7519 Standish Place
(b) (6) (Bin)

From: PFR Event <pfreventcreation@fda.hhs.gov>
Date: July 11, 2017 at 5:16:15 PM EDT
To: Cleary, Michael * <Michael.Cleary@fda.hhs.gov>, HQ Pet Food Report Notification <HQPetFoodReportNotification@fda.hhs.gov>, (b) (6)
Subject: Alternated between: --California Natural Adult Limited Ingredient Grain Free Venison & Green Lentils and Kangaroo & Red Lentils Recipe: Darcy Adin - EON-323515

A PFR Report has been received and PFR Event [EON-323515] has been created in the EON System

A "PDF" report by name "2023228report.pdf" is attached to this email notification for your reference

Below is the summary of the report:

EON Key: EON-323515
ICSR #: 2023228
EON Title: PFR Event created for Alternated between: -California Natural Adult Limited Ingredient Grain Free Venison & Green Lentils and Kangaroo & Red Lentils Recipe: 2023228

AE Date	(b) (6)	**Number Fed/Exposed**	2
Best By Date		**Number Reacted**	2
Animal Species	Dog	**Outcome to Date**	Died Euthanized
Breed	Schnauzer - Miniature		
Age	2.5 Years		
District Involved	PFR-New York DO		

Product information
Individual Case Safety Report Number: 2023228
Product Group: Pet Food
Product Name: Alternated between: -California Natural Adult Limited Ingredient Grain Free Venison & Green Lentils and Kangaroo & Red Lentils Recipe
Description: Please note: Dr Jennifer Jones was consulted prior to submission of this report. She would like to be involved in the case review. 3 week history of cough treated unsuccessfully with doxycycline and prednisone. 3 day history of inappetence and vomiting prior to presentation to NCSU emergency service for dyspnea. Radiographs showed severe pulmonary edema and echocardiogram showed severe Dilated Cardiomyopathy. There was an initial response to diuretic therapy however, he declined and was placed on the ventilator for respiratory support and continued CHF treatment. Attempts to wean off the ventilator were unsuccessful and aquaphoresis was performed. He continued to decline despite aggressive therapy and was euthanized. Infectious disease testing was negative and taurine and carnitine analysis showed adequate levels. Necropsy initially did not reveal a cause for DCM and supported alveolar injury (possibly ventilator related). A re-review of the myocardial histopathology by one of our pathologist showed myofiber vacuoles reminiscent of the changes seen in doxorubicin toxicity. Since the dog had not received doxorubicin, the pathologist recommended culturing the food for Streptomyces peucetius - the bacterium which produces doxorubicin. He also recommended testing for Fusarium spp. a fungus which produces Fumonisin B1, a toxin that produces heart failure in pigs. (b) (6) had been fed California Naturals Adult - both kangaroo with lentils and venison with lentils along with Milo's kitchen treats. We have samples of these foods from 6/17 but not the original bags from when he was presented (b) (6). These samples were provided at the time his housemate (b) (6) (unrelated, older miniature schnauzer) also presented with severe DCM and CHF. I will enter this dog as a separate affected patient. Both dogs had extensive infectious disease testing which was negative and nutritional amino acid deficiencies were ruled out. Because of this, their unrelated lineages (although the same breed, they were from different lines), different ages but similar time of presentation (b) had clinical signs at the time (b) was treated, but didn't present with CHF for several months), we are considering common environmental factors which could precipitate DCM, including food contamination or toxin exposure
Submission Type: Initial
Report Type: Adverse Event (a symptom, reaction or disease associated with the product)
Outcome of reaction/event at the time of last observation: Died Euthanized
Number of Animals Treated With Product: 2
Number of Animals Reacted With Product: 2

Product Name	Lot Number or	Best By

	ID	Date
Alternated between: -California Natural Adult Limited Ingredient Grain Free Venison & Green Lentils and Kangaroo & Red Lentils Recipe		

Sender information
Darcy Adin
1060 William Moore Dr
Raleigh, NY 27607
USA

Owner information
(b) (6)

To view this PFR Event, please click the link below:
https://eon fda gov/eon//browse/EON-323515

To view the PFR Event Report, please click the link below:
https://eon fda gov (b) (6)

==========

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From: Jones, Jennifer L
To: Rotstein, David; Palmer, Lee Anne; Carey, Lauren; Queen, Jackie L
Cc: Ceric, Olgica; Reimschuessel, Renate
Subject: Head"s up-potential DCM case-Dr. Adin-NCSU-2 Schnauzers
Date: Tuesday, July 11, 2017 11:38:00 AM
Attachments: image001.png
image002.png

Vet will submit PFR online →
2 dogs-unrelated miniature schnauzers

Dog 1: 2 yr → presented 2/2017 with fulminant CHF → severe DCM on echo, taurine/carnitine normal, infectious disease testing negative, died on the ventilator, necropsy done-myocardial changes were subtle but could be similar to moldy corn toxicity in pigs → plasma, urine, serum, and myocardial tissue available

Dog 2: 7 yr, had a syncopal episode ~2/2017 but presented to vet for progressive frequency of syncopal episodes → 6/2017 for CHF, diagnosed with DCM similar to housemate, nearly same image on Echo, taurine/carnitine normal, infectious disease testing negative, they have changed the diet (Hill's) and dog is responding to treatment; plasma, urine, and serum available

Dogs were eating California Naturals (different bag than from 2/2017) and treats (Milo's Kitchen); Vet has samples of food and treats

Jennifer L. A. Jones, DVM
Veterinary Medical Officer
U.S. Food & Drug Administration
Center for Veterinary Medicine
Office of Research
Veterinary Laboratory Investigation and Response Network (Vet-LIRN)
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new tel: 240-402-5421
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e-mail: *jennifer.jones@fda.hhs.gov*
Web: http://www.fda.gov/AnimalVeterinary/ScienceResearch/ucm247334.htm


Food and Drug Administration Office of Regulatory Affairs

Collection Report

For Sample Number: 9585●1

This is an accurate reproduction of the original electronic record as of ●7/27/2●16

Flag | **Flag Remarks**

Episode Number	Origin	Basis	Sample Type	FIS Smpl Num	Status
	Domestic	Surveillance	Official	1626●362	Completed

FEI	Date Collected	Product Code	Responsible Firm	PAC	Hours
3●●4211953	●6/23/2●16	72AYT●2	Manufacturer	71R8●1	11

Compliance Num	Country of Origin
	United States

Related Smpl Num	Position Class	Sampling District	NDC Number	Permit Number	Storage Rqrmnt.
9585●●	INV	NWJ-DO			Ambient

Dealer is Consumer	Crx/DEA Schedule	Recall Num	Consumer Compl. Num	Brand Name
No			146●48	Merrick

Product Description
See Remarks

Product Label
See continuation.

Reason for Collection
Sample collected per FACTS Assignment ID #11650647 and OP ID # 866●426 referencing Consumer Complaint #146048 reporting the illness of multiple cats from the same household. Sample testing request: Taurine.

MFG Codes
"16●25DL1 3831● 14133"

Expiration Date
●7/26/17

Firm Legal Name	Address	Type of Firm	Firm FEI	FCE
Merrick Pet Care, Inc.	3275 Tierra Blanca Rd Hereford, TX 79●45-7823 US	Manufacturer	3●●4211953	02944
(b) (6)	(b) (6)	Dealer	(b) (6)	

Size of Lot	Est. Value	Rcpt Type	Carrier Name	Date Shipped
One paper bag weighing 5.4kg	$.●●	FDA 484		

Description of Sample
One unopened bag of Merrick Purrfect Bistro Grain Free Real Chicken Recipe weighing 5.4kg

Method of Collection
See continuation.

How Prepared
See continuation.

Collector's Identification on Package and/or Label
9585●1 ●6/23/2●16 EB

Collector's Identification on Seal
9585●1 ●6/23/2●16 Esteban Beltran Investigator

Sample Delivered To
SRL-ACNA

Date Delivered	Orig C/R & Records To
●6/28/2●16	NWJ-DO

Lab w/Split Sample	Lab
●	SRL

Document Number	Document Date	Document Type	Document Remarks
1	06/23/2016	Other	Copy FDA 484, Receipt for Sample, 1 pg.
2	06/23/2016	Other	Copy FDA 484, Receipt for Sample Amend, 1 pg.
3	06/23/2016	Other	Photos of Product labeling, 3 pages

Food and Drug Administration Office of Regulatory Affairs

Collection Report

For Sample Number: 958501

This is an accurate reproduction of the original electronic record as of 07/27/2016

Remarks
See continuation.

Payment Amount	Payment Method	704(d) Sample	702(b) Portion	Collector's Name
$27.99	Cash	No	No	Esteban Beltran

Name of Signer	Date & Time of Signature			Meaning
Esteban Beltran	07/07/2016	08:55 AM	ET	Collector

Food and Drug Administration Office of Regulatory Affairs

Collection Report

For Sample Number: 958501

This is an accurate reproduction of the original electronic record as of 07/27/2016

Continuation:

Product Label
Finished Product. Label on bag read in parts: "***Lot #:16025DL1 38310 14133***Merrick Whole Health Made Right Purrfect Bistro Grain Free *** REAL CHICKEN RECIPE FISH-FREE *** Net Wt 12Lb (5.4kg) *** WHOLE HEALTH MADE RIGHT! *** SQF INSTITUE CERTIFIED *** MERRICK PET CARE, INC. P.O. BOX 9800 AMARILLO, TX 79105 USA WWW.MERRICKCARE.COM *** Best By: July 26, 2017***"

Method of Collection
On 06/23/2016, I collected a sample from the storage area of a retail store. The sample was placed in a clear plastic bag. I officially sealed the clear plastic bag containing the sample with a FDA415a on site. The sample was transported via GOV to the NBRP sample prep room.

How Prepared
On 06/28/16 I placed the sample into a shipping box with bubble wrap. I secured the shipping box for shipment to SRL-ACNA via UPS (Tracking #: 1Z A47 51E 01 9888 4498). I delivered the sample inside to the NBRP reception area for UPS pickup.

Remarks
Product Description: Poultry based dry cat food packed in a dark orange paper bag with brown letters and an image of a cat on the front.

An amendment to the original FDA 484 was done in order to further describe what each sample number consists of and to identify what lot number of the product pertains to the sample number. CSO Gobiga Vanniyasingam assisted during the assignment. Related samples include 958500, 958502, 958503, 958504.

IVYSPRING INTERNATIONAL PUBLISHER

International Journal of Medical Sciences

2012; 9(6):506-512. doi: 10.7150/ijms.4787

Research Paper

Mannose Binding Lectin and Macrophage Migration Inhibitory Factor Gene Polymorphisms in Turkish Children with Cardiomyopathy: No Association with MBL2 Codon 54 A/B Genotype, but an Association between MIF -173 CC Genotype

Nilgun Col-Araz[1], Sibel Oguzkan-Balci[2] ✉, Osman Baspinar[3], Tugce Sever[2], Ayse Balat[4], Sacide Pehlivan[2],

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Received: 2012.06.27; Accepted: 2012.08.19; Published: 2012.08.22

Abstract

Myocardial inflammation is one of the commonest mechanisms in cardiomyopathy (CMP). Mannose binding lectin (MBL) is a key molecule in innate immunity, while macrophage migration inhibitory factor (MIF) is a constitutive element of the host defenses. We investigated the possible association between polymorphisms of MBL2 and MIF genes and CMP in Turkish children. Twenty-children with CMP and 30 healthy controls were analyzed for codon 54 A/B polymorphism in MBL, and -173 G/C polymorphism in MIF genes by using PCR-RFLP methods. No significant difference was found between genotypes and alleles of MBL2 gene codon 54 A/B polymorphism in patients and controls ($p>0.05$). However, serum uric acid levels was found higher in dilated CMP patients with AA genotype. Frequency of MIF -173 CC genotype was significantly higher in patients ($p<0.05$), and sodium levels were higher in patients with MIF -173 CC genotype. This study is the first to investigate the MBL and MIF gene polymorphisms in Turkish children with CMP. We conclude that CC genotype of MIF (-173) polymorphism may be a risk factor for CMP patients. However, further studies with larger samples are needed to address the exact role of this polymorphism in CMP.

Key words: Cardiomyopathy, Children, Macrophage migration inhibitory factor, Mannose binding lectin, polymorphism.

Introduction

Cardiomyopathy (CMP) is defined as "diseases of the myocardium associated with cardiac disfunction" by World Health Organization (WHO), and it is an important cause of chronic congestive cardiac failure in children. The reported incidence for cardiomyopathies is 1,13-1,24 per 100,000 children [1, 2]. Although the pathogenesis of disease is not fully understood, disturbances of the cellular and humoral immune system are frequently observed in CMPs, and myocardial inflammation is one of the commonest mechanisms in cardiomyopathy [3].

Mannose binding lectin (MBL) and Macrophage

migration inhibitory factor (MIF) play substantial roles in the pathogenesis of several inflammatory and autoimmune disorders [4, 5]. Mannose binding lectin is a key molecule in innate immunity with the capacity to bind to microorganisms and kill them by initiating the lectin pathway of complement activation [5]. Furthermore, MBL has a major role in the modulation of inflammation but the mechanisms responsible for MBL interactions with inflammatory pathways is remain unclear [6]. Several studies suggest that, there is a modulatory role of MBL in autoimmune disease such as rheumatoid arthritis and systemic lupus erythematosus [6-8]. Previous studies show that the absence of MBL may affect occurrence of cardiovascular complications and myocardial ischemia/reperfussion injury, and CMP in MBL null animal models (9-11). Mannose Binding Lectin deficiency has been reported by three single nucleotide polymorphisms (SNPs) in codon 52, 54 and 57 of exon 1 in the MBL2 gene [6]. These SNPs are frequently referred to as variants B, C, and D (B, C, and D, denoting the substitution of aspartic acid for glysine codon 54, the substitution of gutamic acid for glycine codon 57, and the substitution of cysteine for arginine codon 52, respectively). Each of these variant alleles affect the stability of the final protein product, resulting in decreased serum levels and a dysfunctional MBL variant with a lower molecular weight than the normal MBL [6, 12].

Macrophage migration inhibitory factor is a constitutive element of the host antimicrobial defences and stress response that promotes proinflammatory function of the innate and acquired immune system. Macrophage migration inhibitory factor plays a regulator role in the immune response system and promotes proinflammatory biological activities. MIF is constitutively expressed in variety types of tissue and cells, including innate immune cells such as monocytes and macrophages [4]. Recently, it has been shown that MIF gene expression is higher in the heart with impaired glucose tolerance with cardiac dysfunction in rats, and elevated levels of MIF were associated with cardiac dysfunction in diabetic patients [13]. Mutations of the human MIF gene would predispose affected hosts to altered to sensibility or severity of inflammatory diseases such as juvenile idiopathic rheumatoid arthritis, and glomerulonephritis [4, 14, 15].

To our knowledge, no studies have investigated the possible roles of MBL2 and MIF gene polymorphisms in children with CMP. The aim of the present study is to investigate any possible association between polymorphisms of MBL2 and MIF genes and CMP in a group of Turkish children, and to investigate the association between the identified genotypes and their clinical features.

Materials and Methods

Patients and controls: Twenty unrelated Turkish children with CMP, followed up in the Paediatric Cardiology Clinic of the Gaziantep University, Medical Faculty, were compared with 30 age-and sex-matched healthy controls. Relatives of CMP patients did not included as healthy controls. The diagnosis of CMP were made by signs and symptoms (irritability, feeding difficulties, weakness, fatigue, dizziness, syncope, tachypnea, tachycardia, hepatomegaly, and evidence of fluid retension), chest X ray (cardiomegaly, pulmonary venous congestion, pulmonary oedema), electrocardiography (hypertrophy of left ventricle with strain, low voltage complexes) and echocardiographic signs. Cardiomyopathies were classified according to their structural and functional abnormalities such as dilated, in the setting of reduced left ventricular systolic function; hypertrophic, in the presence of unexplained septal hypertrophy of the left ventricule; restrictive, when impaired diastolic filling with preserved systolic function and normal ventricular wall thickness [1].The study was approved by the Local Ethics Committee of the Faculty of Medicine, and informed consents were obtained from the parents of children. The medical records of all children with CMP were reviewed for information about age, sex, and to document clinical presentation including symptoms, family history, laboratory and echocardiographic findings.

Genotyping: All patients and controls were analyzed for codon 54 A/B (gly54asp) variation in exon 1 of MBL2 gene and -173 G/C polymorphism in MIF gene. Genomic DNA was extracted from peripheral blood samples using the salting out procedure [16].

Genotyping of MBL2 gene codon 54 A/B: Polymerase Chain reaction (PCR) was performed using a forward (5′-TAGGACAGAGGGCATGCTC-3′) and a reverse (5′-CAGGCAGTTTCCTCTGGAAGG-3′) primers in a 25 µl volume containing 50 ng DNA, 2 mM dNTPs, 2 nmol of each primer, 1.5 mM $MgCl_2$ and 3U Taq polymerase. The product 349 bp was digested with restriction enzyme *Ban*I (Fermentas) identify codon 54 polymorphism, respectively. BanI digestion was performed at 50 ^{o}C for 60 minutes with 5 U enzyme. After enzyme digestion, products were visualized by electrophoresis on 3% agarose gel. The BanI restriction site is present on wild type allele A and absent on variant allele B [17].

Genotyping of MIF gene -173 G/C: PCR was performed using a forward (5′-ACTAAGAAAGACCCGAGGC-3′) and reverse (5′-GGGGCACGTTGGTGTTTAC-3′) primers. For

MIF (-173), a 330 bp fragment was amplified, which was then digested with AluI restriction enzyme (Fermentas), overnight at 37 °C. The products were then separated on 3% agarose gel. The PCR product contains two restriction site for allele C and one of these sites is destroyed when the presence of allele G [18].

Statistical Analysis: All statistical analyses were performed with the Statistical Package for the Social Science for Windows (version 18.0; SPSS Inc, Chicago, IL, U.S.A.).Results are given as mean±SD, while allele frequencies and the distribution of genotype are given as %. Clinical features and MBL/MIF gene polymorphisms were compared using the chi-square and the Fisher's exact tests. Differences between groups were compared by Kruskal-Wallis variant analysis and the Mann-Whitney U-test. Statistical significance was considered at $p<0.05$. Hardy-Weinberg equilibrium (HWE) was calculated using De-finetti program [19]. Differences in allele and genotype distributions were assessed using odds ratios (ORs) and 95% confidence intervals. Sample size was estimated using a power calculation based on other studies [20]. The minimum sample size was determined as 44 person in each group at the 80% power level with an α error of 5%.

Results

Clinical features: The age ranged from 3 months to 13 years (mean**:** 3.47 ± 3.38 years; median: 2.00-IR:3.00) in patients with CMP (n=20, 10 females/10 males). According to the echocardiographic evaluation, 80% (n=16) of the patients had dilated cardiomyopathy, 15% (n=3) hypertrophic cardiomyopathy and 5% (n=1) restrictive cardiomyopathy. All patients had clinical findings of CMP. Echocardiographic findings of CMP patients were shown in Table 1.

Genotype frequencies of MBL2 and MIF genes

The distribution of AA, AB, and BB genotypes for MBL codon 54 were 65 % (13), 25 % (5) and 10 % (2) in CMP compared with 76.7 % (23), 23.3 % (7) and 0 % (0) in the controls. The allele frequency of A/B in MBL was 77.5 % (31), and 22.5 % (9) in CMP compared with 88.3 % (53), and 11.7 % (7) in the controls. No significant difference was found between genotypes and alleles of MBL2 gene in patients and controls ($p>0.05$).

The distribution of GG, GC, and CC genotypes for MIF (-173) were 50 %, 30 %, and 20 % in CMP compared with 56.7 %, 43.3 % and 0 % in the controls (Table 2). CC genotype was significantly higher in patient group ($p=0.0210$, Table 2). The allele frequency of G/C in MIF was 65 %, and 35 % in CMP compared with 78.3 %, and 21.7 % in the controls. The observed genotype counts were not deviated significantly from those expected according to the Hardy-Weinberg Equilibrium for MBL and MIF gene polymorphisms ($p>0.05$).

Genotype frequencies of MBL2 and MIF genes

The distribution of AA, AB, and BB genotypes for MBL codon 54 were 65 % (13), 25 % (5) and 10 % (2) in CMP compared with 76.7 % (23), 23.3 % (7) and 0 % (0) in the controls. The allele frequency of A/B in MBL was 77.5 % (31), and 22.5 % (9) in CMP compared with 88.3 % (53), and 11.7 % (7) in the controls. No significant difference was found between genotypes and alleles of MBL2 gene in patients and controls ($p>0.05$).

The distribution of GG, GC, and CC genotypes for MIF (-173) were 50 %, 30 %, and 20 % in CMP compared with 56.7 %, 43.3 % and 0 % in the controls (Table 2). CC genotype was significantly higher in patient group ($p=0.0210$, Table 2). The allele frequency of G/C in MIF was 65 %, and 35 % in CMP compared with 78.3 %, and 21.7 % in the controls. The observed genotype counts were not deviated significantly from those expected according to the Hardy-Weinberg Equilibrium for MBL and MIF gene polymorphisms ($p>0.05$).

Association between the identified genotypes and patients clinical/laboratory characteristics: We investigated correlations of MBL/MIF genotypes with clinical and laboratory findings of patients such as duration of symptoms, ejection fraction (EF), fractional shortening (FS), left ventricle end diastolic diameter (LVEDD), left ventricle end systolic diameter (LVESD), left ventricle end diastolic volume (LVEDV), and left ventricle end systolic volume (LVESV). No relationship was found between MBL/MIF genotypes and these parameters (data not shown).

In children with dilated CMP (n=16), serum uric acid levels were higher in patients with MBL AA genotype ($p=0.033$, Table 3), while plasma sodium (Na) levels were higher in patients with MIF CC genotype ($p=0.042$, Table 4).

Table 1. Echocardiographic signs of children with cardiomyopathy (CMP).

	Dilated CMP Mean ± SD (min-max) (n=16)	Hypertrophic CMP Mean ± SD (min-max) (n=3)	Restrictive CMP (n=1)
Ejection fraction (EF) (%)	33.75±11.13 (19-58)	77.00±3.60 (74-81)	58.00
Fractional shortening (FS) (%)	15.07±5.89 (8-29)	35.67±6.02 (30-42)	29.00
Left ventricle end diastolic diameter (LVEDD) (cm)	4.75±1.04 (2.40-5.90)	2.83±0.72 (2.00-3.30)	2.40
Left ventricle end systolic diameter (LVESD) (cm)	3.98±1.07 (1.70-5.40)	1.63±0.40 (1.20-2.00)	1.70
Left ventricle end diastolic volume (LVEDV) (ml)	119.94±42.08 (29.60-173.00)	32.600±17.30 (12.70-44.10)	45.00
Left ventricle end systolic volume (LVESV) (ml)	82.15±37.53 (18.10-141.00)	8.15±4.67 (3.36-12.70)	22.00

Table 2. Genotype and allele frequencies of Macrophage Migration Inhibitory Factor (MIF) gene -173 G/C polymorphism in children with cardiomyopathy (CMP).

MIF Genotype	Control n(%)	CMP Patients n(%)	Odds Ratio (95%C.I.)	p
GG	17 (56.7)	10 (50)	0.823 (0.266 - 2.541)	0.4793[a]
GC	13 (43.3)	6 (30)	0.560 (0.169 - 1.858)	0.2578[a]
CC	0 (0)	4 (20)	16.636 (0.842 - 328.60)	0.0210[a]
MIF Allele				
G	47 (78.3)	26 (65)	0.513 (0.210 - 1.256)	0.1072
C	13 (21.7)	14 (35)	1.947 (0.796 - 4.761)	0.1072
HWE (p)	0.129	0.127		

[a]Fisher exact test, HWE: Hardy-Weinberg Equilibrium.

Table 3. Association with serum uric acid levels and Mannose Binding Lectin (MBL2) gene codon 54 A/B polymorphism in children with dilated cardiomyopathy.

MBL genotypes	Serum uric acid level (mg/dL) Mean ± SD (min-max)	95% CI	p
AA	6,139±1,508 (4,5-8,4)	4,979- 7,299	0.033
AB	3,000±0,989 (2,3-3.7)	- 5,894- 11, 894	

Table 4. Association with plasma sodium (Na) levels and Macrophage Migration Inhibitory Factor (MIF) gene -173 G/C polymorphism in children with dilated cardiomyopathy.

MIF genotypes	Plasma Na level (mEq/L) Mean ± SD (min-max)	95% CI	p
CC	137,50 ±2,121 (136-139)	118,44-156,56	0.042
GC	131,00±1,414 (130-132)	118,29- 143,71	

Discussion

Although the pathogenesis of CMP is not fully understood, cellular as well as humoral autoimmun responses are critically associated with the pathogenesis and progression of the disease. Furthermore, disturbances of the cellular and humoral immune system are frequently observed, and myocardial inflammation is one of the commonest mechanisms in cardiomyopathy [3]. Two of the postulated factors are; firstly, myocardial inflammation mediated by the effector cells of the immune system; and secondly locoregional effect of inflammatory mediators, released by the infiltrating lymphocytes, macrophages or endothelial cells [21]. Both MIF and MBL play several roles in innate and adaptive immune responses, and changes in levels of MBL and MIF are implicated as playing causative role in many disease states [4, 6, 22].

Better understanding of the molecular genetics underlying CMP may provide a means of early diagnosis, genotype-based therapy, and even prevention of the disease.

Mannose binding lectin deficiency is associated with susceptibility to infectious and autoimmun diseases and serum MBL levels vary substantially because of the variant alleles in exon 1 of the MBL2 gene, located on chromosome 10 in the humans [22]. In the present study, homozygosity for MBL variant allele was observed only two patients with dilated CMP and the A/A genotype was higher than the variant alleles in CMP patients, but these results were not statistically significant.

Messias-Reason et al, suggested that wild type variants of MBL2 gene was significantly higher in rheumatic hearth diseases [23]. Ramasawmy et al. reported that subjects homozygous for the wild type allele had a higher concentration of MBL than heterozygous subjects and than homozygous for the variant MBL2 alleles [24]. Schafranski et al showed that, genotypes associated with a higher level of MBL seem to represent a risk factor for the evolution of rheumatic carditis, and MBL play a substantial role in the progression of the disease to chronic form [25]. However, some studies indicated that there was either no association of MBL gene polymorphisms and systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA), or even an increased risk for RA has been demonstrated in MBL insufficiency [8, 26]. In the present study, homozygosity for MBL variant allele was observed only two patients with dilated CMP and the A/A genotype was higher than the variant alleles in CMP patients, but these results were not statistically significant. These different results in literature highlight the need for further detailed studies to understand the exact role of MBL2 gene polymorphism in several diseases.

We investigated correlations of MBL genotypes with clinical and laboratory findings of the disease, and found that uric acid levels were higher in patients with MBL AA genotype than the other genotypes. Uric acid is a useful marker for the decompensation trigger in chronic heart failure, and might be related to inflammatory responses [27]. It has been shown that the left ventricular hypertrophy has an important potential to increase uric acid level [28]. Gullu et al. measured uric acid levels in idiopathic dilated cardiomyopthy, and they observed that serum uric acid levels are significantly higher in the lower coronary flow reverse group than in the higher coronary flow reverse group [29]. Interestingly, Garred et al reported that homozygous for wild type alleles in exon 1 of MBL2 gene were more likely to show evidence of persistent inflammation [30]. We suggest that the reason of increased uric acid level in our patients may be both inflammation and chronic heart failure.

Macrophage migration inhibitory factor plays an important role in the control of innate immun responses and promotes proinflammatory biological activities. Four polymorphisms of the human MIF gene (-794, -173, +254, +656) have been reported, and this polymorphisms would predispose affected hosts to altered susceptibility to or severity of inflammatory or infectious disease [4]. Patients with -173 C allele (that is, guanine-to-cytosine transition at position -173) had increased levels of MIF, and increased MIF concentrations had been associated with severe clinical manifestations, high severity scores, and poor outcome of inflammatory disease [4, 31, 32]. In the other studies, no association were found in genotype distributions of MIF -173 G/C polymorphism between ulcerative colitis, juvenile rheumatoid arthritis and healthy controls [14, 33]. However, Donn et al showed that MIF -173 C allele was associated with juvenile idiopathic arthritis [31, 32]. Moreover, MIF-173 C allele had a significantly greater number of joints with active arthritis and was associated with a poor response to glucocorticoids in patients with juvenile idiopathic arthritis [31]. Berdeli et al showed that, the MIF -173 C allele was a poor outcome predictor in JRA [14].

Miller et al. demonstrated that MIF released from ischemic cardiomyocytes stimulates adenosine monophosphate-activated protein kinase (AMPK) activation and promotes glucose uptake, and thereby protects the hearth against ischaemia reperfusion injury [34]. Jian et al found that MIF protein is constitutively expressed by cardiomyocytes in vivo and is increased in the myocardium of infants with cyanotic

cardiac defects in myocardial biopsy materials [35]. Tereshchenko et al did not reveal an association of the myocardial infarction with the MIF-173 C allele polymorphism [36]. In the present study, homozygosity for MIF-173 C allele was observed only four patients with dilated CMP. Recently, it has been shown that presence of -173C allele indicates higher MIF levels [37], and cardiac inflammation (autoimmune, viral or post viral) has an important component in the pathogenesis of dilated CMP [38]. Therefore we suggest that, CC genotype in our patients may be partially responsible from inflammation in dilated CMP, and MIF polymorphism may contribute to MIF release from cardiomyocytes in children with CMP. However, we could not find any relationship between MIF genotypes and cardiac functions. Considering the limited number of our patients, we cannot say that MIF polymorphism does not modulate cardiac functions. Further detailed studies with large patient numbers are needed for this suggestion.

It has been shown that, plasma brain natriuretic peptide (BNP) concentrations were increased in various forms of heart disease with impaired left ventricular systolic function including cardiomyopathy [39]. Natriuretic peptides inhibit the transport of sodium and water in proximal tubules and block reabsorption of sodium [40]. In this study, plasma sodium levels were higher in patients with MIF CC genotype than the other genotypes. However, we could not conclude whether CC genotype of MIF has any effect on BNP with this study. This hypothesis needs further evaluation.

Conclusion

This study is the first to investigate the MBL and MIF gene polymorphisms in Turkish children with CMP. We conclude that CC genotype of MIF (-173) gene may be a risk factor for CMP patients. However, further studies with larger samples are needed to address the exact role of this polymorphism in CMP.

Competing Interests

The authors have declared that no competing interest exists.

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EXPERT REVIEWS

Nutritional and micronutrient determinants of idiopathic dilated cardiomyopathy: diagnostic and therapeutic implications

Expert Rev. Cardiovasc. Ther. 9(9), 1161–1170 (2011)

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Idiopathic dilated cardiomyopathy (IDCM) is the term used to describe a group of myocardial diseases of unknown cause whose common clinical presentation is heart failure. The prevalence of IDCM is estimated to be between 7 and 13% of patients with systolic heart failure. Throughout medical history, several nutrient-deficient states have been identified as the root cause of IDCMs, Keshan's disease being one such example, where selenium deficiency-induced heart failure is now well documented. This raises the question of whether a micro- or macro-nutrient imbalance can provide the milieu for inefficient energy expenditure and cardiac metabolism in the context of IDCMs, either causing or exacerbating the condition. To date, there is insufficient evidence in the literature to support this theory, although numerous studies suggest a link between nutrient deficiencies, inefficient energy expenditure and subsequent heart failure. Given the unique metabolic needs of the failing heart, the role of micronutrient testing and supplementation in IDCMs warrants further well-designed studies.

Keywords: heart failure • idiopathic dilated cardiomyopathy • macrominerals • metabolic cardiology • micronutrients • multinutrient supplementation • vitamins

10.1586/ERC.11.95

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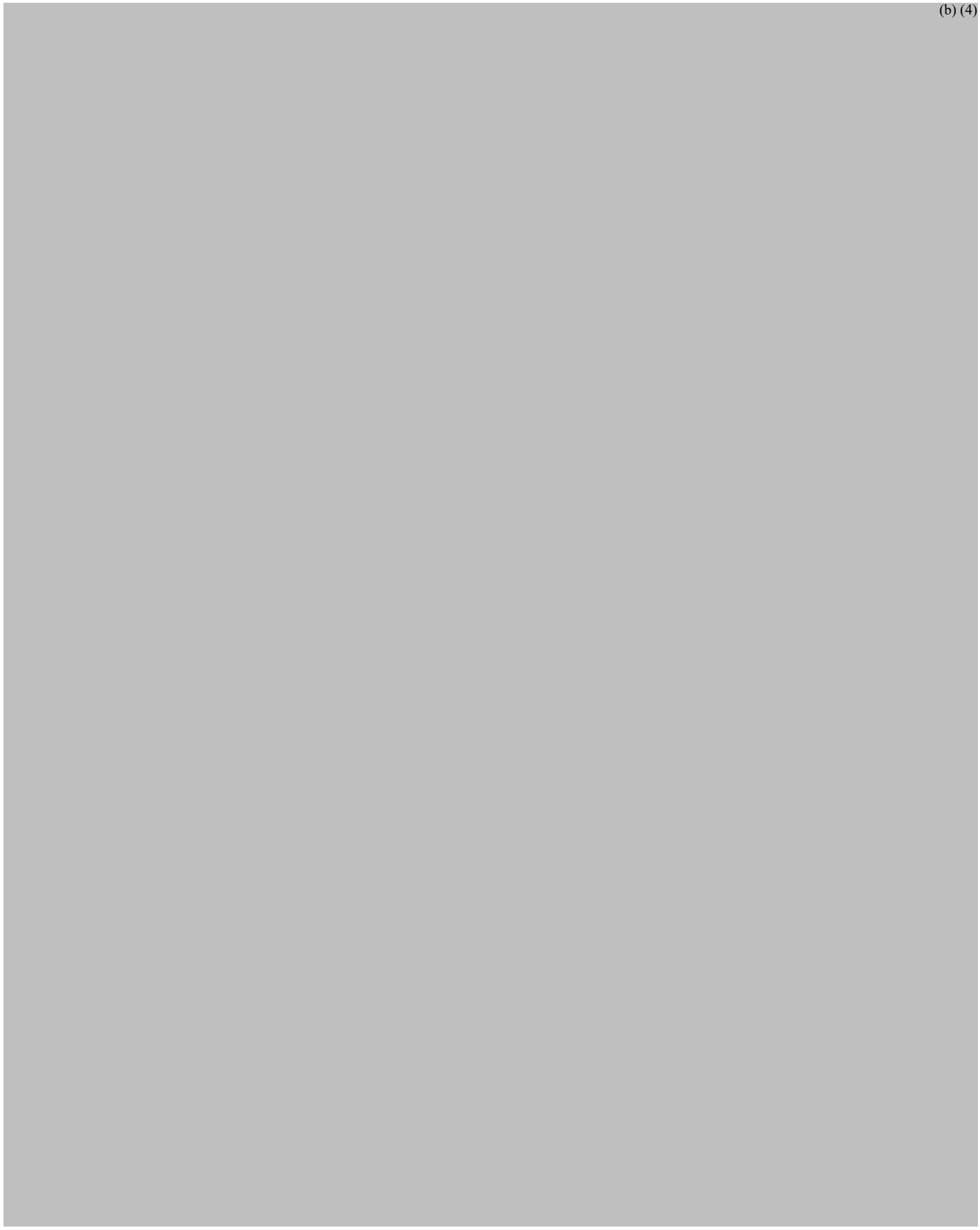

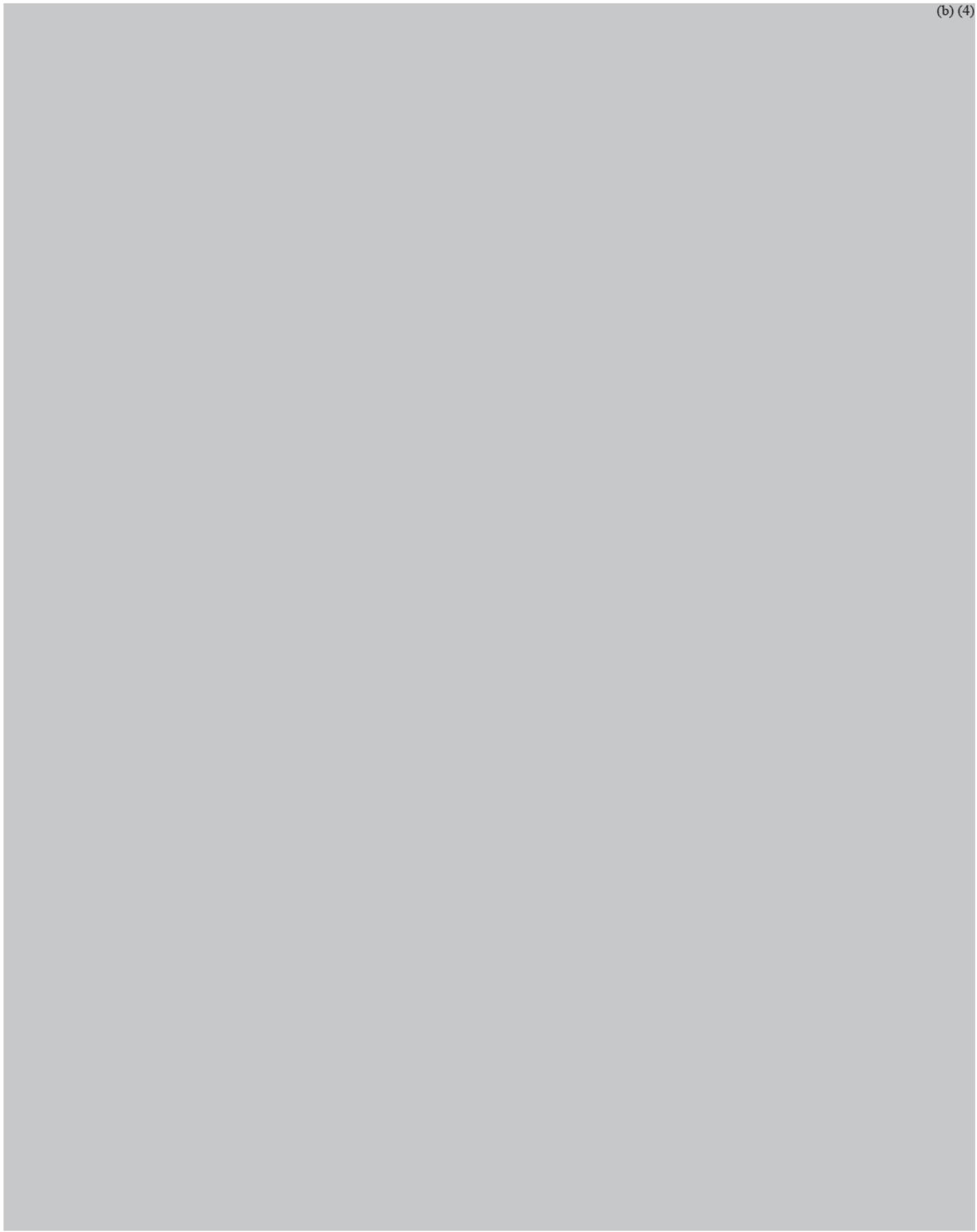

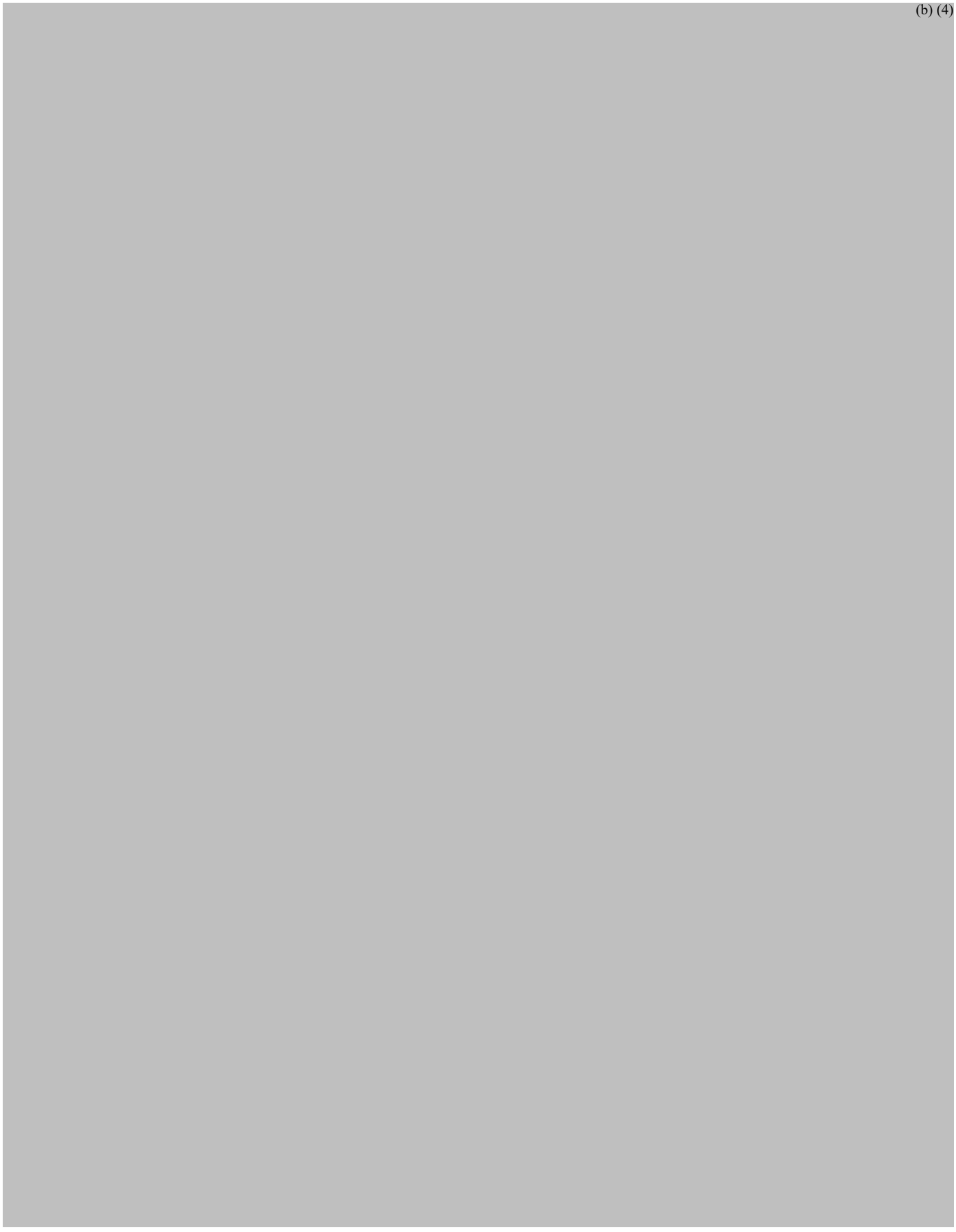

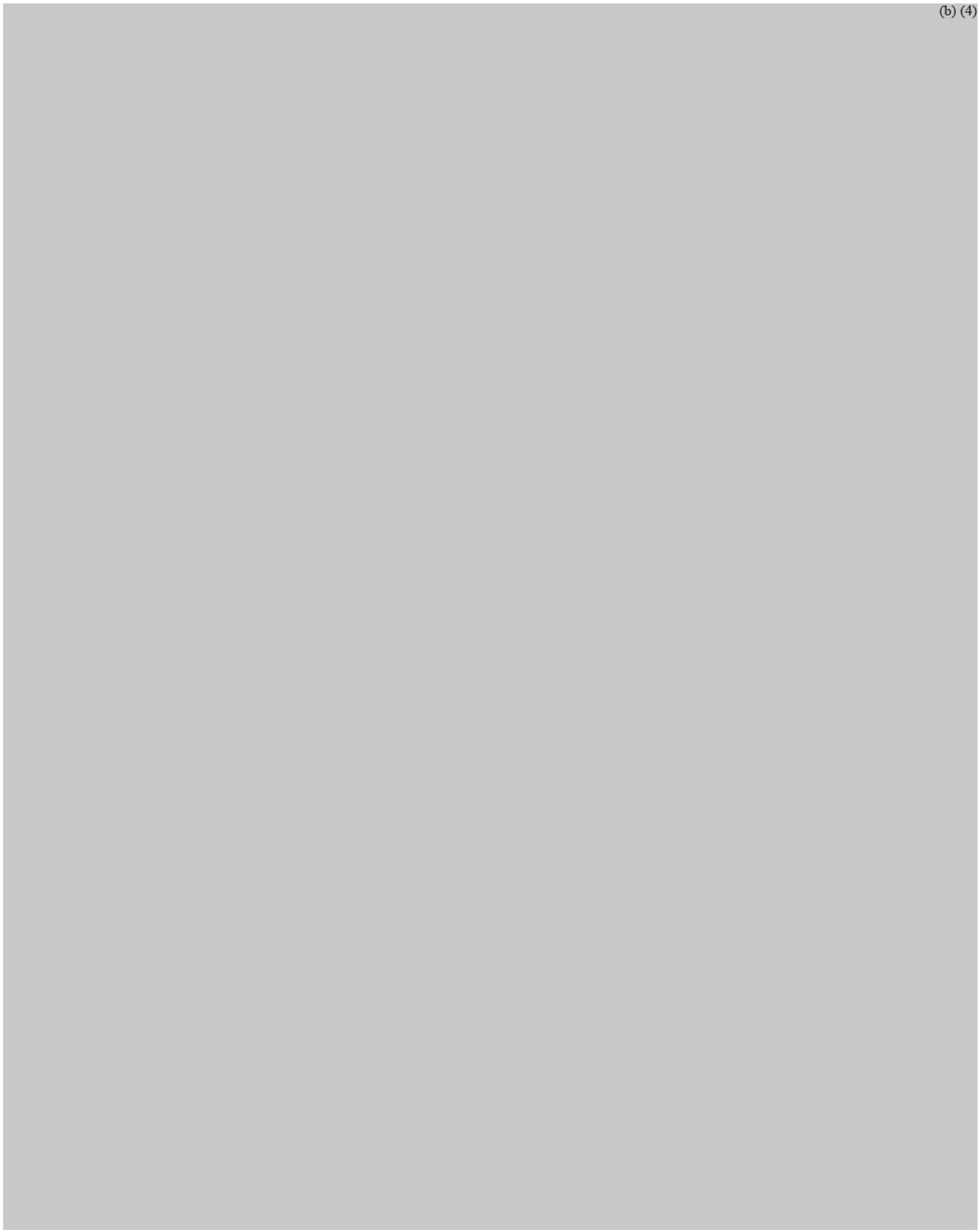

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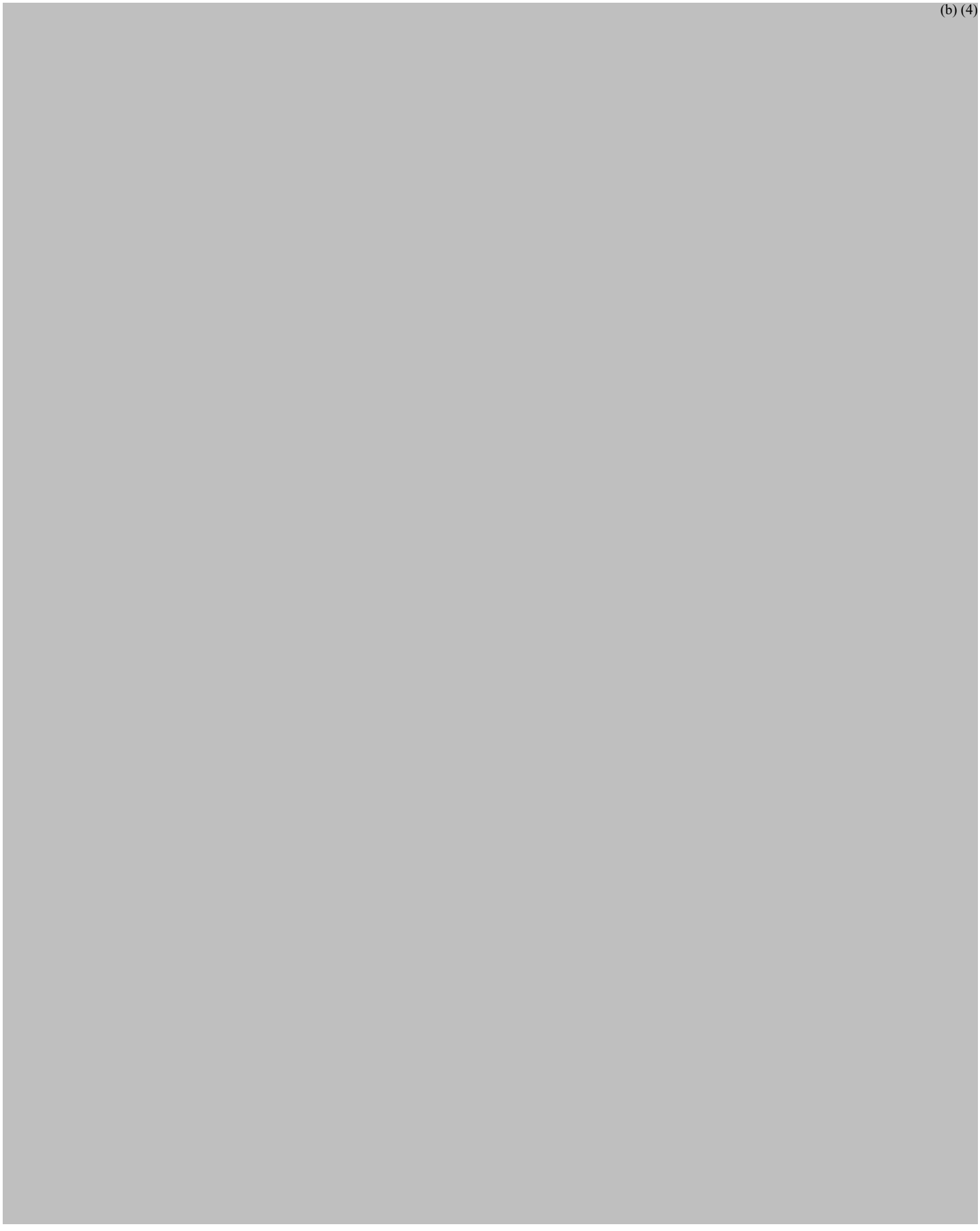

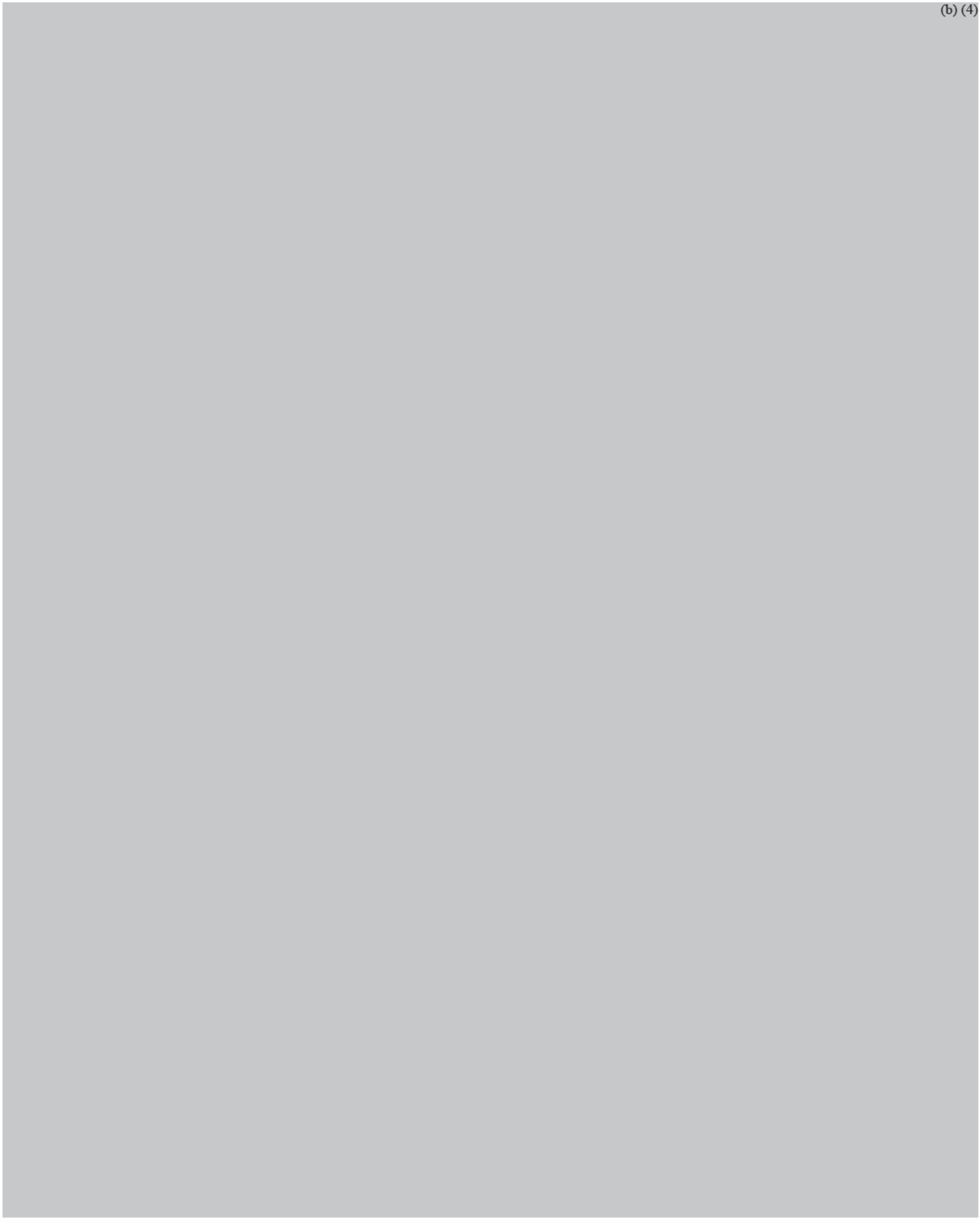

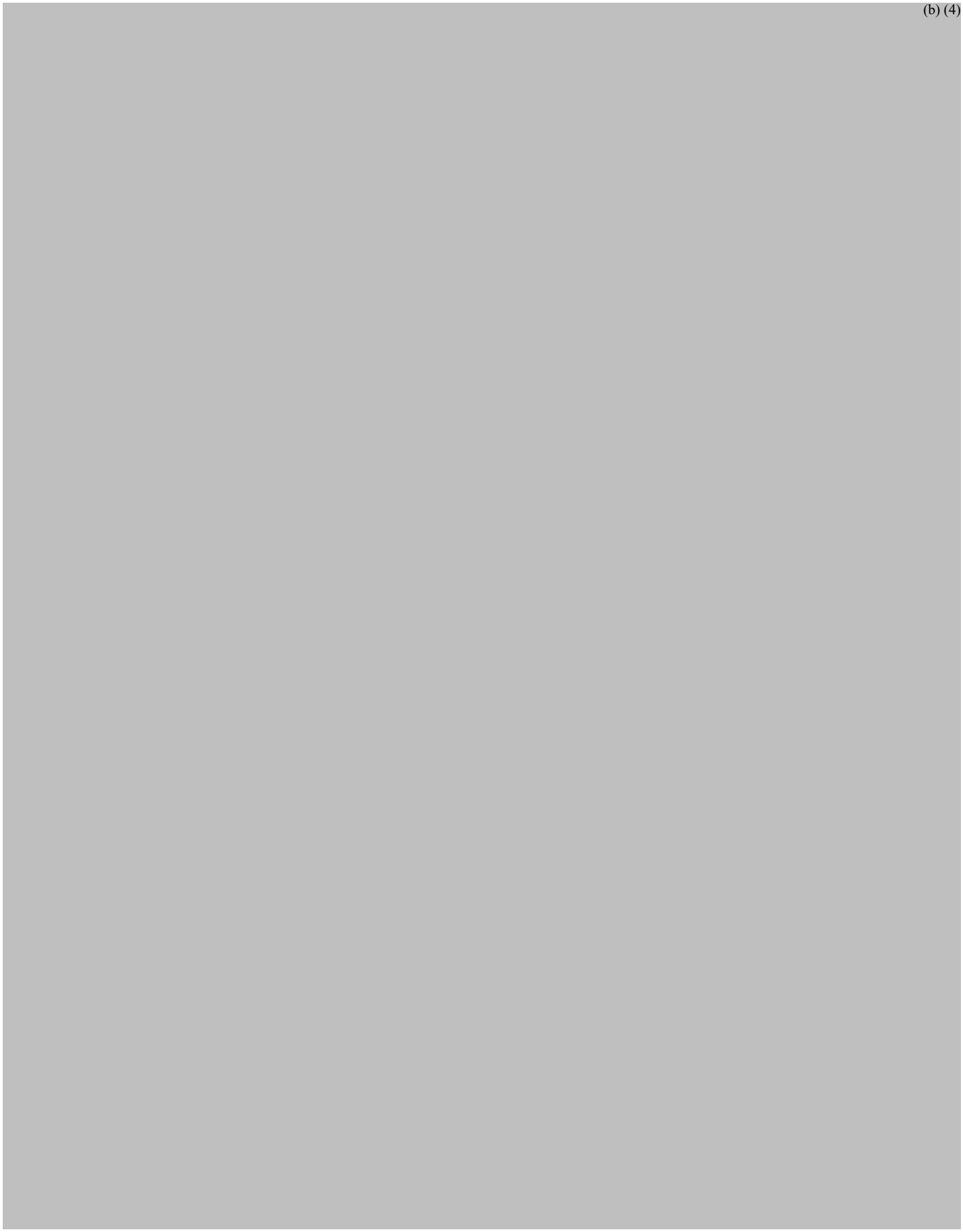

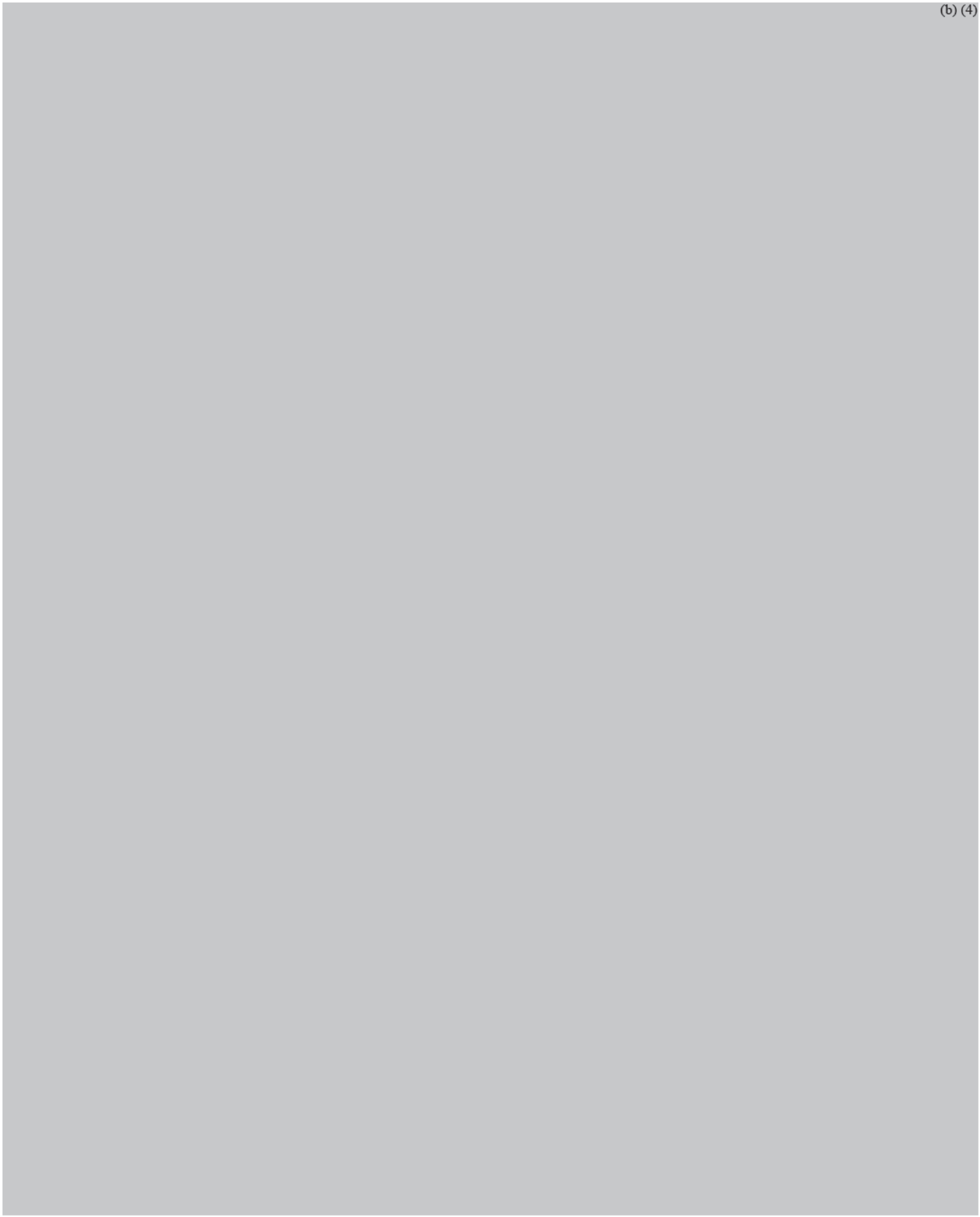

From:	Edwards, David
To:	Burkholder, William
Cc:	Hartogensis, Martine
Subject:	RE: Invitation to participate in panel discussion on DCM investigation at Petfood Forum conference
Date:	Tuesday, February 26, 2019 1:53:50 PM
Attachments:	Mansilla 2019 J Anim Sci.pdf

Also, most of the panel are authors on the attached paper that is to come out in March.
Dave

From: Hartogensis, Martine
Sent: Tuesday, February 26, 2019 1:47 PM
To: Burkholder, William <William.Burkholder@fda.hhs.gov>
Cc: Edwards, David <David.Edwards@fda.hhs.gov>
Subject: FW: Invitation to participate in panel discussion on DCM investigation at Petfood Forum conference

Hi Bill,

[redacted] (b) (5) Jen and Lee Anne can help you with slides.

Does that work for you? Thanks again!

Martine

From: Norris, Anne
Sent: Tuesday, February 26, 2019 1:12 PM
To: Forfa, Tracey <Tracey.Forfa@fda.hhs.gov>; Hartogensis, Martine <Martine.Hartogensis@fda.hhs.gov>; DeLancey, Siobhan <Siobhan.Delancey@fda.hhs.gov>
Subject: RE: Invitation to participate in panel discussion on DCM investigation at Petfood Forum conference

From Petfood Forum web page:

Update on canine DCM investigation: lessons learned, insights for the future

In July 2018, the U.S. Food and Drug Administration (FDA) announced it was investigating cases of canine dilated cardiomyopathy (DCM) in breeds not typically associated with the disease, and that many of the dogs had been fed grain-free pet foods high in pulses, legumes or potatoes. Is there really a link between those ingredients or these pet food formulations and the cases of DCM? This panel will provide the latest updates on the FDA and other investigations, and discuss any lessons learned and insights for the industry going forward.

Greg Aldrich, Ph.D. (moderator), research associate professor at Kansas State University and president of Pet Food Ingredients & Technology
Jennifer Adolphe, R.D., Ph.D., nutrition manager for Petcurean Pet Nutrition
William Burkholder, D.V.M., Ph.D., veterinary medical officer with the Center for Veterinary Medicine, Food and Drug Administration
Chris Marinangeli, Ph.D., director of nutrition, scientific and regulatory affairs for Pulse Canada
Anna Kate Shoveller, Ph.D., assistant professor, Department of Animal Biosciences, at University of Guelph

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From: Forfa, Tracey
Sent: Thursday, February 21, 2019 1:42 PM
To: Hartogensis, Martine <Martine.Hartogensis@fda.hhs.gov>; DeLancey, Siobhan <Siobhan.Delancey@fda.hhs.gov>; Norris, Anne <Anne.Norris@fda.hhs.gov>
Subject: RE: Invitation to participate in panel discussion on DCM investigation at Petfood Forum conference

Thanks! I will set up a time where we can touch base and decide.

From: Hartogensis, Martine
Sent: Thursday, February 21, 2019 1:41 PM
To: DeLancey, Siobhan <Siobhan.Delancey@fda.hhs.gov>; Norris, Anne <Anne.Norris@fda.hhs.gov>
Cc: Forfa, Tracey <Tracey.Forfa@fda.hhs.gov>
Subject: RE: Invitation to participate in panel discussion on DCM investigation at Petfood Forum conference

Ok, thanks!

Martine

From: DeLancey, Siobhan
Sent: Thursday, February 21, 2019 12:19 PM
To: Hartogensis, Martine <Martine.Hartogensis@fda.hhs.gov>; Norris, Anne <Anne.Norris@fda.hhs.gov>
Cc: Forfa, Tracey <Tracey.Forfa@fda.hhs.gov>
Subject: RE: Invitation to participate in panel discussion on DCM investigation at Petfood Forum conference

I'm going to defer to Tracey, but I think it may warrant further discussion. Folding her in here.

From: Hartogensis, Martine
Sent: Thursday, February 21, 2019 12:03 PM
To: DeLancey, Siobhan <Siobhan.Delancey@fda.hhs.gov>; Norris, Anne <Anne.Norris@fda.hhs.gov>

Subject: RE: Invitation to participate in panel discussion on DCM investigation at Petfood Forum conference

Hi Siobhan,

Thank you so much for weighing in. (b) (5)

(b) (5).

Martine

From: DeLancey, Siobhan
Sent: Thursday, February 21, 2019 10:55 AM
To: Hartogensis, Martine <Martine.Hartogensis@fda.hhs.gov>; Norris, Anne <Anne.Norris@fda.hhs.gov>
Subject: RE: Invitation to participate in panel discussion on DCM investigation at Petfood Forum conference

Hmmm. (b) (5)

(b) (5). Also, wondering who the audience would be for this? I assume anyone can attend, whether or not they are members of this organization or PFI? It was a little unfortunate that AFIA got the update overview before our communication went out, and so I think that the other advocates are even more sensitive to the appearance that we are only talking to industry. (b) (5)

From: Hartogensis, Martine
Sent: Thursday, February 21, 2019 9:54 AM
To: Norris, Anne <Anne.Norris@fda.hhs.gov>
Cc: DeLancey, Siobhan <Siobhan.Delancey@fda.hhs.gov>
Subject: RE: Invitation to participate in panel discussion on DCM investigation at Petfood Forum conference

Just circling back on this….Siobhan, any thoughts?

Martine

From: Norris, Anne
Sent: Tuesday, February 19, 2019 10:50 AM
To: Hartogensis, Martine <Martine.Hartogensis@fda.hhs.gov>
Cc: DeLancey, Siobhan <Siobhan.Delancey@fda.hhs.gov>
Subject: RE: Invitation to participate in panel discussion on DCM investigation at Petfood Forum conference

It seems duplicative to do a (b) (5) but defer to Siobhan for her thoughts.

From: Hartogensis, Martine
Sent: Tuesday, February 19, 2019 10:40 AM
To: Norris, Anne <Anne.Norris@fda.hhs.gov>
Cc: DeLancey, Siobhan <Siobhan.Delancey@fda.hhs.gov>
Subject: RE: Invitation to participate in panel discussion on DCM investigation at Petfood Forum conference

Yes, correct! I think this request is for a similar webinar as we did last summer/fall. I believe Bill is still scheduled to go to the Forum.

Let me know your thoughts, of if you would prefer to coordinate.

Thanks again!

Martine

From: Norris, Anne
Sent: Tuesday, February 19, 2019 10:21 AM
To: Hartogensis, Martine <Martine.Hartogensis@fda.hhs.gov>
Cc: DeLancey, Siobhan <Siobhan.Delancey@fda.hhs.gov>
Subject: RE: Invitation to participate in panel discussion on DCM investigation at Petfood Forum conference

This is separate from the request for Bill to present at the Petfood Forum (the same publication's annual conference)? Last I knew, Bill was going to do that because he was doing a labeling workshop at that event as well.

Just making sure I'm not getting my wires crossed!

From: Hartogensis, Martine
Sent: Tuesday, February 19, 2019 9:29 AM
To: Norris, Anne <Anne.Norris@fda.hhs.gov>; Jones, Jennifer L <Jennifer.Jones@fda.hhs.gov>; Palmer, Lee Anne <LeeAnne.Palmer@fda.hhs.gov>; Rotstein, David <David.Rotstein@fda.hhs.gov>; Burkholder, William <William.Burkholder@fda.hhs.gov>
Cc: DeLancey, Siobhan <Siobhan.Delancey@fda.hhs.gov>; Edwards, David <David.Edwards@fda.hhs.gov>; Schell, Timothy <Timothy.Schell@fda.hhs.gov>
Subject: FW: Invitation to participate in panel discussion on DCM investigation at Petfood Forum conference

Hi DCM Team!

See the request below from Watt Global Media. You may recall we participated in a webinar to talk about DCM late last summer (following our update).

My thought was to give our group an opportunity to present updated information (b) (5) (similar to Dave's AFIA slides).

They are looking for a late March/early April timeframe, so please let me know if that would work for you.

Jen and Lee Anne, I would be looking to you for some slides if we need to (b) (5)

Thank you all in advance!

Martine

From: Debbie Phillips <DPhillips@wattglobal.com>
Sent: Monday, February 18, 2019 9:41 AM
To: Hartogensis, Martine <Martine.Hartogensis@fda.hhs.gov>
Subject: FW: Invitation to participate in panel discussion on DCM investigation at Petfood Forum conference

Hello Martine:

I saw your colleague, Dr. Dave Edwards, present last week at the AFIA pet food conference in Atlanta, and it reminded me that I still need to determine if this webinar is a "go." Can you please let me know if you're still available to participate and if you have time during the last week of March or first two weeks of April – which week would be best for you?

As I explained in my email below, we are broadening the topic of the webinar somewhat; it will still include a discussion of the DCM investigation but also cover other, related topics, especially those relevant to novel ingredients and their relative safety in pet foods.

During his presentation, Dr. Edwards gave a brief list of pet food-related issues that CVM is investigating, and I believe your contribution to this webinar could be a brief update on several or all of these:

- *L. monocytogenes, Clostridium, Salmonella* (including recalls in raw pet food)
- DCM
- Elevated thyroid levels in pet foods
- Vitamin D-related recalls
- Pentobarbitol in pet foods

Please let me know if that makes sense to you and your availability. Thank you!

Sincerely,
Debbie

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www.gotomeet.me/DebbiePhillipsDonaldson

From: Debbie Phillips
Sent: Friday, February 1, 2019 9:23 AM
To: 'Hartogensis, Martine' <Martine.Hartogensis@fda.hhs.gov>
Subject: FW: Invitation to participate in panel discussion on DCM investigation at Petfood Forum conference

Hi Martine:

Happy 2019, a month late! I imagine you're very busy considering everything that's been happening (or not happening) in DC recently, but I wanted to follow up on the potential webinar we had corresponded about late last year.

For various reasons, we have decided to broaden the topic of this webinar and cover other aspects of so-called novel pet food ingredients, including benefits, challenges, investigations, etc. That would include the update on the canine DCM investigation and situation –and possibly other situations related to pet food that CVM is involved with now?

Could you please let me know if this is something you could still participate in? Right now we are looking at having the webinar in late March (last week of the month) or first couple of weeks of April. Is there a timeframe that's best for you?

Fyi, I am also following up with Dr. Burkholder about his participating in the DCM panel discussion at our Petfood Forum conference in late April/early May.

Thanks very much,
Debbie

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Check out these upcoming Petfood Forum conferences!
Petfood Forum and Petfood Innovation Workshop 2019: April 29-May 1
Petfood Forum Europe 2019: June 13
Petfood Forum China 2019: August 20
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From: Debbie Phillips
Sent: Friday, November 30, 2018 9:48 AM
To: Hartogensis, Martine <Martine.Hartogensis@fda.hhs.gov>
Subject: RE: Invitation to participate in panel discussion on DCM investigation at Petfood Forum conference

Hi Martine,

Sounds good all around! And yes for the webinar; registration for our webinars is free so you can have as many sign up as you wish.

Thanks again,
Debbie

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From: Hartogensis, Martine <Martine.Hartogensis@fda.hhs.gov>
Sent: Friday, November 30, 2018 8:40 AM
To: Debbie Phillips <DPhillips@wattglobal.com>
Cc: Edwards, David <David.Edwards@fda.hhs.gov>; Murphy, Jeanette <Jenny.Murphy@fda.hhs.gov>; Burkholder, William <William.Burkholder@fda.hhs.gov>; Norris, Anne <Anne.Norris@fda.hhs.gov>
Subject: RE: Invitation to participate in panel discussion on DCM investigation at Petfood Forum conference

Hi Debbie,

Dr. Bill Burkholder is willing to be on the panel in St. Louis.

I am willing to be on the webinar in March as the lead for CVM. We have a wonderful team, so would we be able to include them similar to the last webinar?

Thanks again!

Martine

From: Debbie Phillips <DPhillips@wattglobal.com>
Date: November 30, 2018 at 9:35:42 AM EST
To: Hartogensis, Martine <Martine.Hartogensis@fda.hhs.gov>
Subject: RE: Invitation to participate in panel discussion on DCM investigation at Petfood Forum conference

Hi Martine,

That's great, thank you! And don't worry about the timing; I know it's a very busy time of year.

Not to push my luck, but do you think someone from CVM could also participate in a webinar in March on this? It would be a follow-up/update to September's webinar and seek to inform those in the industry who cannot attend Petfood Forum. We don't know the exact timing in March yet, other than it probably wouldn't be the week of March 18, as there is a major pet show that week.

Thanks again, please let me know if I can answer any questions.

Debbie

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From: Hartogensis, Martine <Martine.Hartogensis@fda.hhs.gov>
Sent: Thursday, November 29, 2018 8:17 PM
To: Debbie Phillips <DPhillips@wattglobal.com>
Cc: Norris, Anne <Anne.Norris@fda.hhs.gov>
Subject: Re: Invitation to participate in panel discussion on DCM investigation at Petfood Forum conference

Hi Debbie!

My sincere apologies for not following up sooner. We did discuss internally and think it would be a wonderful opportunity for CVM.

I am working on finding the right person to represent CVM and will get back to you asap.

Thank you so much for your patience and sending my apologies again!

Martine

From: Debbie Phillips <DPhillips@wattglobal.com>
Date: November 29, 2018 at 4:23:54 PM EST
To: Hartogensis, Martine <Martine.Hartogensis@fda.hhs.gov>
Cc: Norris, Anne <Anne.Norris@fda.hhs.gov>
Subject: FW: Invitation to participate in panel discussion on DCM investigation at Petfood Forum conference

Hi Dr. Hartogensis and Anne:

I hope you both had nice Thanksgiving celebrations last week! And I'm following up on my message below to see if you have had an opportunity to consider this invitation?

Also, please note that we are tentatively planning another webinar about the investigation and situation, probably in March. That would provide a six-month update since the first webinar and also sort of tease the upcoming session at Petfood Forum.

Please let me know if you have any questions. Thank you again for your consideration.

Sincerely,
Debbie

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From: Debbie Phillips
Sent: Friday, November 9, 2018 11:32 AM
To: Martine.Hartogensis@fda.hhs.gov
Cc: Anne.Norris@fda.hhs.gov
Subject: Invitation to participate in panel discussion on DCM investigation at Petfood Forum conference

Hi Dr. Hartogensis:

Thank you again for participating in our webinar in September on the atypical cases of canine DCM and their possible link to certain pet diets. This continues to be a topic of conversation and concern in the industry, as you probably are aware, so we are planning a follow-up/update during our annual conference, Petfood Forum, in late April/early May. Could you or one of your colleagues please consider serving as a panel member for this discussion?

The session is currently scheduled for the afternoon of Wednesday, May 1. I know that seems a long way off, but we prefer to issue invitations in advance, as opposed to the hasty invitation for the webinar!

Other panel members will likely include Dr. Greg Aldrich as moderator (who also participated in the webinar, as you know), plus Dr. Jennifer Adolphe, nutrition manager for Petcurean Pet Food; Dr. Kate Shoveller, assistant professor at the University of Guelph; and Dr. Chris Marinangeli, director or nutrition, scientific and regulatory affairs for Pulse Canada.

In case you are not familiar with Petfood Forum, we just held our 26th edition this past April. It is the only event of its kind for the global pet food industry, drawing more than 3,000 people each year from pet food companies around the world, plus from retailers and related businesses, academia and regulatory organizations, such as AAFCO. In addition to education (concurrent scientific tracks plus panel discussions, general sessions, keynotes and other), it includes a trade show featuring the industry's leading suppliers of ingredients, equipment, packaging materials, testing and other services. This year's show had over 400 booths with more than 250 exhibiting companies.

We offer an honorarium to our speakers and panel members, cover their hotel costs and conference registration and reimburse all other travel expenses.

Please let me know if you have questions about Petfood Forum or this panel discussion. Thank you in advance for considering the request!

Sincerely
Debbie

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The association between pulse ingredients and canine dilated cardiomyopathy: addressing the knowledge gaps before establishing causation

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ABSTRACT

In July 2018, the Food and Drug Administration (FDA) warned about a possible relationship between dilated cardiomyopathy (DCM) in dogs and the consumption of dog food formulated with potatoes and pulse ingredients. This issue may impede utilization of pulse ingredients in dog food or consideration of alternative proteins. Pulse ingredients have been used in the pet food industry for over 2 decades and represent a valuable source of protein to compliment animal-based ingredients. Moreover, individual ingredients used in commercial foods do not represent the final nutrient concentration of the complete diet. Thus, nutritionists formulating dog food must balance complementary ingredients to fulfill the animal's nutrient needs in the final diet. There are multiple factors that should be considered, including differences in nutrient digestibility and overall bioavailability, the fermentability and quantity of fiber, and interactions among food constituents that can increase the risk of DCM development. Taurine is a dispensable amino acid that has been linked to DCM in dogs. As such, adequate supply of taurine and/or precursors for taurine synthesis play an important role in preventing DCM. However, requirements of amino acids in dogs are not well investigated and are presented in total dietary content basis which does not account for bioavailability or digestibility. Similarly, any nutrient (e.g. soluble and fermentable fiber) or physiological condition (e.g. size of the dog, sex, age) that increases the requirement for taurine will also augment the possibility for DCM development. Dog food formulators should have a deep knowledge of processing methodologies and nutrient interactions beyond meeting AAFCO nutrient profiles and should not carelessly follow unsubstantiated market trends. Vegetable ingredients, including pulses, are nutritious and can be used in combination with complementary ingredients to meet the nutritional needs of the dog.

Key words: dilated cardiomyopathy, dogs, feed formulation, grain-free, nutrition, pulse ingredients

INTRODUCTION

In July 2018, the Food and Drug Administration (FDA) issued a statement relating dilated-cardiomyopathy (DCM) in dogs to the consumption of foods that have potatoes and/or pulse ingredients, such as peas and lentils or their co-products, as main ingredients (FDA, 2018). The FDA's statement, as well as media attention, has raised concern in some pet owners, veterinarians, nutritionists, and the pet food manufacturing and retail industry. The underlying cause for concern with pet food and DCM is that there is a link between nutrition that was previously tied to DCM and insufficient circulating taurine (Fascetti et al., 2003; Backus et al., 2006). The result, was an increased need for dietary taurine or its precursor methionine due to higher fermentation of taurine and greater fecal excretion with dietary fermentable fiber (Kim et al., 1996ab). Whether this has any link to dietary pulses or the greater inclusion of pulses in grain-free dog food has yet to be directly demonstrated and mechanistic research is warranted.

Pulses are a subset of legumes, harvested as a dry crop, with low concentrations of lipid. They include peas, lentils, chickpeas, and dry beans (Marinangeli et al. 2017) which have been used as ingredients in dog food for their protein and fiber for more than 2 decades (Butterwick et al., 1994; Rice and Ihle, 1994). As a source of protein, the amino acid (AA) profile in peas, lentils, chickpeas, and beans are generally high in lysine and low in methionine (NRC, 2006) and serve as a complementary protein to both animal and plant-derived ingredients. As an example, soybean meal is derived from defatted soybeans and has an amino acid profile similar to pulses. In a 24-week study that evaluated graded concentrations of soybean meal up to 17 % (as-fed basis) in dog foods, soybean meal inclusion did not affect the nutrient status of dogs as indicated by serum biochemistry analysis (Menniti et al., 2014). However, Yamka et al. (2003) demonstrated that using soybean meal at more than 15 % inclusion on a dry matter basis decreased crude protein digestibility. Based on the authors assessment of current formulas in the market, there is a high likelihood that legume seed use in some foods may be greater than 40 %. This inclusion exceeds concentration of legumes previously investigated in dogs. When used to complement the nutritional profile of other ingredients, pulses can be used as nutrient-rich vehicles to meet the nutritional requirements of dogs and other companion animals. Given that companion animals most often consume static diets for long periods of time, overuse of any ingredient could facilitate higher risk of certain nutrient deficiencies if nutrient balance is not considered in the formulation. Thus, the formulation of static diets that use significant concentrations of a single ingredient, relative to other ingredients in the formulation, requires an in-depth knowledge of nutrient interactions, animal physiology, and effects of processing, beyond that of simply meeting minimum nutrient profiles stipulated in the Official Publication of The Association of American Feed Control Officials (AAFCO, 2018).

The present commentary discusses: 1. The limited data being used to support linkages between DCM and pulse ingredients; 2. The nutritional factors and physiological mechanisms that should

be explored to establish causation between nutritional deficiencies and incidence of DCM; 3. The factors that nutritionists should consider when formulating complete diets destined for long term consumption; and 4. The disadvantages of formulating to protein and minimal AA recommendations rather than to a balanced indispensable AA profile.

The development of canine DCM, historical linkages to taurine deficiency and pulses

Dilated cardiomyopathy is a disease of the myocardium that results in both mechanical dysfunction (enlarged heart cavities and congestion) and/or electrical dysfunction (arrhythmias and sudden death) (Sisson et al., 2000; Maron et al., 2006; Dutton and Alvarez, 2018). Development of DCM is slow and few clinical signs manifest over time. As DCM progresses, signs include lethargy, anorexia, shallow breathing, sudden fainting, and potential death. In some cases, animals may die from irregular heart rhythm without previous signs of the disease. In dogs, DCM can be caused by various factors. Genetic predisposition is thought to play the most important role in the development of DCM in several dog breeds, mostly large and giant breeds. Genetic mutations associated with DCM have been discovered in American lines of Doberman and Boxer dogs (Meurs et al., 2012; Meurs et al., 2013). However, the Doberman variant's association was not upheld in a European population of Dobermans (Owczarek-Lipska et al., 2013). Similarly, a UK population of Boxers did not uphold their published DCM-associated variant (Cattanach et al., 2015). It is becoming increasingly clear that the genetic basis for DCM in dogs is not monogenic, but complex and polygenic. Breeds with the highest prevalence of DCM include Dobermans, Boxers, Great Danes, Newfoundlands, Irish Wolfhounds, English Cocker Spaniels, and Portuguese Water Dogs (Monnet et al., 1995; Borgarelli et al., 2006; Werner et al., 2008; Martin et al., 2009), and the genetic basis of DCM in each of these breeds has been investigated (Dutton and Alvarez, 2018). In addition, Golden Retrievers and American Cocker Spaniels appear to have breed predispositions to taurine deficiency (Kramer et al., 1995; Bélanger et al., 2005). When dogs are not genetically predisposed for developing DCM, diet and physiology are other factors that may be associated with the disease.

The first link between taurine deficiency and DCM was demonstrated in cats in 1987. Cats diagnosed with DCM recovered after taurine supplementation (Pion et al., 1987). Similarly, an inverse association between dietary taurine and the incidence of DCM in a population of foxes was documented by Moise et al. (1991) and established the importance of taurine in the family Canidae. In dogs, DCM diagnoses related to low whole blood taurine concentrations have been reported in Cocker Spaniels, Dalmatians, Boxers, Newfoundlands, Portuguese Water Dogs, English Setters, Alaskan Malamutes, and Scottish Terriers (Freeman et al., 1996; Kittleson et al., 1997; Pion et al., 1998; Alroy et al., 2000; Fascetti et al., 2003; Backus et al., 2006). In all these cases, taurine supplementation improved cardiac function. However, dogs, in contrast to cats, can endogenously synthesize taurine from methionine and cysteine (Figure 1). Therefore, the

abovementioned data does not unequivocally establish taurine intake as the underlying mechanism for the development of DCM in dogs, whether or not they are genetically predisposed. Dietary supply of precursor AAs necessary for taurine synthesis (i.e. methionine and cysteine), metabolic intermediates, and co-factors (such as methyl donors) cannot be ruled out as factors that contribute to the susceptibility of dogs to developing genetic and diet-related DCM. When DCM is diet-related, the formulation and the provision of all nutrients, including indispensable AAs, to facilitate optimum health and wellbeing of dogs should be considered.

Recent reports, including the statement by the FDA (2018), have implicated that lentils, peas and other legumes seeds could be responsible for the development of DCM in dogs not genetically predisposed to this disease. Such statements and associations between pulse ingredients and incidence of DCM are, at the present time, premature. Animals, including dogs, have no minimum or maximum requirements for ingredients. Ingredients serve as the vehicle to providing nutrients to animals. As such, animals have nutrient requirements, not ingredient requirements. In diets that have nutrient deficits, imbalances, or exceed maximums, the final nutrient composition of the diet, not the ingredients, should be critiqued. In addition, animal nutritionists should consider that the nutrient concentration of ingredients can vary, nutrient availability is not 100 %, and diets formulated to marginally meet requirements could actually be deficient. Overall, it is the responsibility of nutritionists to use different ingredients to formulate diets that can be produced and safely meet the nutritional needs of animals.

Taurine deficiency and the development of canine DCM

For dogs, taurine is a dispensable AA synthesized from methionine and cysteine primarily in the liver (Figure 1). Taurine is not incorporated into proteins. Instead, it is used as a mediator for various biological processes and is the most abundant free AA intracellularly (Huxtable, 1992). In the heart, taurine represents ~60% of the total AA free pool (Huxtable, 1992). The high concentration of taurine in cardiac cells may explain the role of a taurine deficiency in the development of DCM. It has been speculated that taurine contributes to the reabsorption of calcium by the sarcoplasmic reticulum and increases the sensitivity of the myofilaments to calcium (Bakker and Berg, 2002). Thus, low dietary taurine intake and/or reduced synthesis of taurine from methionine and cysteine can deplete calcium pools in the cardiac cells and impede proper contraction of the cardiac muscle tissue, resulting in DCM in dogs.

For diagnosing DCM in dogs and cats, among other diagnostic methods including electrocardiograms and echocardiography, it is common to measure taurine concentration in whole blood. Whole blood samples, and not plasma samples, should be used to assess circulating taurine concentrations. In plasma, free taurine concentrations are much lower compared to intracellular taurine. This suggests that the plasma pool is not representative of taurine in other

pools (Schaffer et al., 2010). In platelets, taurine concentration is high and is considered a marker of taurine status. Taurine concentration in platelets is captured when whole blood is analyzed (Huxtable, 1992). However, platelet count can vary depending on the immune status of the animal and whole blood taurine concentration can be affected. In this scenario, whole blood taurine may not represent concentrations of taurine in muscle cells, including cardiac muscle. These additional variables related to the measurement of taurine status may explain why some dogs diagnosed with DCM have normal whole blood taurine concentrations.

As taurine can be synthesized endogenously in dogs, taurine is not considered an indispensable AA for the species Canidae. Thus, there are no recommendations on minimum dietary concentrations of taurine for dogs reported by the National Research Council (NRC, 2006) or AAFCO (2018). The lack of regulation on minimum taurine concentrations in commercial dog foods suggests that endogenous synthesis of taurine can meet the metabolic needs in all dogs and at all life stages. This assumption may not be accurate as studies have determined that synthesis of taurine is related to the size of dog (Ko et al., 2007), and some dietary factors can increase the physiological need for taurine (Story, 1978). Nutritional factors that increase the dietary requirement, reduce the supply, or increase the excretion of taurine in dogs are discussed in subsequent sections of this review and should be considered to avoid taurine deficiency in dogs and the risk of DCM.

Physiological factors can increase taurine utilization in dogs, and endogenous synthesis of taurine could be insufficient for meeting taurine requirements. For example, compared to smaller size dogs, synthesis of taurine in large dog breeds is up to 50% lower per unit of metabolic body weight (Ko et al., 2007). These results demonstrate that larger dogs are at higher risk for insufficient endogenous taurine synthesis, and dietary supplementation or fortification may be required, even when there is no minimum dietary taurine concentration according to current recommendations (AAFCO, 2018). Obesity and diabetes have also been related to lower concentrations of taurine in blood in humans and rats, respectively, (Merheb et al., 2007; Nardelli et al., 2011; Ito et al., 2012) and may increase the requirement for sulfur AAs necessary for endogenous taurine synthesis. This is of importance given that approximately half of dogs in North America are obese (Linder and Mueller, 2014). Data from rats and cats suggests that age and sex could also affect whole body taurine status. Hepatic activity of cysteine sulfonate decarboxylase, the enzyme responsible for taurine synthesis, was shown to be 16× higher in adult male rats versus female rats. In the same study, the activity of cysteine sulfonate decarboxylase was higher in 5-6-week-old kittens compared to 15-month-old cats and in 8-week-old mice compared to 16-week old mice; changes of the enzyme activity in dogs have not been tested (Worden and Stipanuk, 1985). Overall, these studies suggest that, despite some capacity for endogenous synthesis, physiological need of taurine can be heavily dependent on breed, age, sex, and physiological status. These physiological factors could help to predict the risk for developing

DCM when genotypic and environmental factors, such as diet, are simultaneously considered to ensure dogs maintain adequate concentrations of taurine and other sulfur AAs.

Given that there are no recommendations for the minimum concentration of taurine in dog food, the concentration of taurine in dog foods can vary substantially depending on the ingredients used. Taurine is very low in plant-based ingredients (Table 1) but is higher in some algae and fungi species and is ubiquitously found in animal tissues, especially in the heart, brain, and white blood cells (Huxtable, 1992). This is relevant, as many grain-free and/or high legume dog foods attempt to limit the use of animal by-products, which can substantially decrease the levels of dietary taurine. In the context of providing adequate and preventive nutrition, dog foods should include organ meat or animal by-products or be fortified with taurine and/or its precursors (methionine and/or cysteine) to ensure the delivery of sufficient levels of taurine.

Effect of dietary fibre on taurine status and risk of canine DCM

Dietary fiber has been shown to affect the taurine status in dogs. For example, commercial diets formulated with lamb meal and rice bran were shown to cause taurine deficiency in part because of low bioavailable cysteine from lamb meal and possibly more importantly due to the effects of rice bran fiber on gastrointestinal metabolism of taurine (Johnson et al., 1998; Torres et al., 2003). It has been hypothesized that high fiber diets can increase susceptibility to taurine deficiency by 2 mechanisms of action linked to obligatory bile acid conjugation with taurine in dogs (O'Mádille et al., 1965) and reliance on enterohepatic circulation for the reabsorption of bile acids and taurine. First, high fiber diets may increase fecal output and losses of taurine-conjugated bile. This would require higher synthesis rates of bile in the liver, and consequently, higher utilization of taurine (Story, 1978). Second, high consumption of fermentable fibres may increase the abundance of microbial populations that degrade taurine in the intestinal lumen (Kim et al., 1996ab). Either alone or together, increased excretion or degradation of taurine from high fibre diets may decrease enterohepatic circulation and recycling of taurine. Given that taurine is the only AA used for bile acid conjugation in dogs, over time, high fiber diets could increase the risk of taurine insufficiency in dogs and lead to DCM.

This should not be interpreted as dietary fiber being deleterious to the health of dogs. However, there may be a limit to the benefit for soluble fibers. Legume seeds contain an appreciable quantity of oligosaccharides which are known to be fermentable (Tosh and Yada. 2010). Thus, by a similar mechanism as described above, high levels of legume seed oligosaccharides could ostensibly contribute to taurine depletion via excretion in the feces as bile conjugation and degradation by colonic bacteria. In addition to the physiological benefits of high fiber diets in certain dogs, formulators should also be cognizant of possible nutritional risks associated with

high concentrations of fiber in dog foods. Consequently, dog foods with high concentrations of dietary fiber should be accompanied with higher supplies of taurine or sulfur AAs for endogenous taurine synthesis. Overall, the digestibility and bioavailability of taurine in ingredients used and the effect of other nutrients in taurine metabolism should be considered to avoid taurine deficiency and the development of DCM.

Carnitine deficiency and risk of canine DCM

Carnitine is not nutritionally indispensable since it is endogenously produced in the liver and kidneys from lysine and methionine; it can also be attained exogenously from animal-based products. Carnitine is highly abundant in skeletal and cardiac muscles. Together, these represent > 95% of the total carnitine in the body. Carnitine is essential for metabolism of fatty acids used for energy production (Hoppel, 2003). In the heart, where 60% of the energy is derived from fatty acid oxidation, carnitine facilitates the uptake of free fatty acids into the mitochondria to produce ATP (Hoppel, 2003). Plant-based ingredients do not contain carnitine (Table 1). Therefore, in commercial dog foods with reduced inclusion of animal-based ingredients, intakes of carnitine could be decreased if diets are not fortified. Reduced dietary carnitine intake translates into increased reliance on endogenous synthesis to meet physiological requirements.

Given that carnitine is required for sufficient energy production in cardiac muscle, it is not surprising that carnitine deficiency is associated with DCM. In 1991, a family of Boxers diagnosed with DCM were also diagnosed with carnitine deficiency (Keene et al., 1991). In dogs, carnitine deficiency can occur with aberrations of carnitine regulation in disorders such as cardiomyopathy (including DCM), diabetes, sepsis, and malnutrition (Flanagan et al., 2010). However, carnitine deficiency as a causative factor in the development of DCM or a consequence of cardiac malfunction remains as a subject of debate (Freeman and Rush, 2006). Despite the interest in this metabolite, little progress has been made on determining the effect of carnitine supplementation on alleviating risk of DCM. However, both taurine and carnitine are often supplemented in supraphysiological concentrations once DCM is diagnosed. This practice is supported by positive clinical outcomes, albeit without comparison groups (Kittleson et al. 1997; Sanderson et al. 2001). Concentrations of carnitine in the plasma are relatively insensitive to dietary carnitine, and more invasive techniques (biopsies) are required to determine the concentration of carnitine in muscle tissue (Flanagan et al., 2010; Răşanu et al., 2012). The invasive nature of testing for carnitine status is likely the reason why carnitine is rarely explored when investigating possible causes of canine DCM.

Preventing diet-mediated DCM in dogs by providing adequate sulfur AAs and maximizing endogenous taurine synthesis

Although taurine is considered a dispensable AA in dogs, endogenous taurine synthesis requires an adequate supply of bioavailable sulfur AA precursors cysteine or methionine (Figure 1). Thus, providing marginal concentrations of these 2 sulfur AAs, or providing sources with lower bioavailability, could increase the risk of taurine deficiency and facilitate the development of DCM. Contrary to taurine, methionine cannot be synthesized endogenously in dogs (NRC, 2006). Therefore, dogs depend on the provision of dietary methionine to meet daily sulfur AA requirements, which includes production of taurine. From an ingredient perspective, methionine and lysine are usually the first or second limiting AAs in dog diets formulated with soybean meal and rendered meats (NRC, 2006). In addition, methionine is particularly susceptible to damage, and subsequent reduction in bioavailability, secondary to heat processing (Marshall et al. 1982; Hurrell et al. 1983). This suggests that the risk of methionine deficiency is more likely than any other indispensable AA in commercial dog diets. Although the primary role for methionine is protein synthesis, in pigs at least 50% of absorbed methionine acts as a methyl donor and a precursor in the production of cysteine, taurine, sulfate, and pyruvate (Robinson et al., 2016a) (Figure 1). These functions of methionine become more crucial when dietary intake of cysteine, taurine, and/or dietary methyl donors (e.g. folate, betaine, and their precursors) is limited (Robinson et al., 2016b), and they need to be considered when nutritionists set criteria for delivery of sulfur AAs in pet foods.

Methionine and cysteine both contribute to the total sulfur AA requirements for humans and animals. For adult dogs at maintenance, the latest guidelines from the NRC (2006) recommend that adult dog foods contain 0.33% (on dry matter basis) methionine when cysteine is provided in excess, and 0.65% for methionine + cysteine. These NRC (2006) recommendations are not based on dose-response studies, but on a 4-year study where adult dogs were fed low-crude protein diets (Sanderson et al., 2001). In that study, the lowest concentration of methionine in the diet that reported no observable deficiencies was used as the recommended requirement. As companion animals are typically fed a single static diet during adulthood, and for most of their lifespan, it is necessary that AA requirements of dogs should be measured empirically (Baker, 1986). In addition to the lack of empirical data corresponding to the AA requirements of dogs, it is equally important to understand how other dietary (e.g. dietary fiber), environmental, other physiological variables, and breed/genotype may alter AA requirements. The lack of recommendations for taurine in commercial dog food puts a higher stress on accurately meeting requirements for sulfur AAs, not only for protein synthesis, but also for the endogenous synthesis of taurine, for support of optimal methyl status, and for the synthesis of secondary metabolites.

Rethinking indispensable AA targets in commercial dog foods

Currently, the ingredients permitted in pet foods and the corresponding nutrient targets are guided by recommendations made by AAFCO (2018). These recommendations are based on the

peer-reviewed scientific literature and represented in the Nutrient Requirement of Dogs and Cats (NRC, 2006). However, AA recommendations made by AAFCO correspond to total AA content within the formulation and do not consider the true ileal digestibility of ingredients. True ileal digestibility of AAs is more representative of nutrient absorption capacity and bioavailability compared to fecal digestibility or total AA content in the diet (Columbus and de Lange, 2012). To account for the reduced digestibility and bioavailability of protein-bound AAs in food ingredients, AAFCO arbitrarily increases AA recommendations relative to those from the NRC to ensure that an adequate supply of AAs is provided, regardless of the ingredients and effects of processing (Table 2). However, this increment is only applied to lysine, threonine, and tryptophan and not applied to other indispensable AAs, including methionine (AAFCO, 2018). For example, the recommended allowance for lysine reported in NRC (2006) is 0.35% for adult dogs at maintenance, while the minimum content of lysine to meet AAFCO (2018) recommendations is 0.63%. Non-ruminant animals, including dogs, absorb AAs from the duodenum to the terminal ileum (Columbus and de Lange, 2012). Hence, feeding diets with lower ileal digestibility coefficients could decrease actual concentrations of available indispensable AAs, even when meeting AAFCO recommendations. This is of special concern for dietary taurine and other sulfur AAs, considering that there is no regulated minimum threshold for taurine in dog foods and that AAFCO (2018) recommendations for sulfur AAs are not increased compared to NRC (2006) recommendations to account for potential ileal digestibility coefficients. There is a dearth of data in this area to justify empirical adjustments based on different dietary variables. As such, future research should pursue how amino acid requirements change under different dietary variables that can affect small intestinal digestibility and whole body availability.

It is worthwhile to note that minimum dietary nutrient contents for dog foods, as reported in AAFCO (2018), only considers differences between growth/reproduction and adult life stages. This lack of data places the pregnant bitch in the same group as growing animals. Moreover, most studies on nutrient requirements in dogs have been established using Beagles as a proxy for all dogs. Using a single breed creates a homogenous sample and likely does not account for nutritional variability across pure and mixed breeds, or those of different sizes. Unpublished data from Shoveller et al. investigated the minimum methionine (with excess cysteine) requirements of Miniature Dachshunds, Beagles, and Labrador Retrievers as proxies for small, medium, and large dog breeds and found that methionine requirements may differ across breeds or size of dogs and be greater than previously estimated. Thus, given the methods of derivation, single indispensable AA requirements for all dog populations, as presented in AAFCO (2018), may not consider variable AA requirements across dog phenotypes. Moreover, it is widely assumed that endogenous synthesis of dispensable AAs, such as taurine in the dog, is sufficient for meeting metabolic demands. However, recent studies suggest that under some metabolic conditions, dispensable AAs may also be required in diets (Hou et al., 2015). Taurine, as described in this commentary, is a clear example of this paradigm shift. Dietary taurine or the capacity for its

adequate endogenous synthesis, especially in circumstances where excessive losses might occur, should be considered in the final formulation of dog foods to decrease the risk of canine DCM.

Nutritionists and regulatory agencies should be aware that, in the spectrum of nutrient requirements, dog populations with higher AA requirements relative to energy intake and other factors could be at a higher risk for a taurine deficiency. More precise categorization of requirements among different canine populations would help to optimize nutritional adequacy and decrease risk of diseases, such as DCM, that are possibly linked to nutrient deficiencies.

Effect of processing on anti-nutritional factors in plant-based ingredients.

Just as understanding the inherent nutritional characteristics and the interaction between ingredients is important for preventing nutritional imbalances in pet foods, the effects of processing on these factors are equally important. Raw cereals and legumes contain anti-nutritional factors such as trypsin inhibitors, phytates, hematoglutinins, and polyphenols that can decrease protein digestion, nutrient absorption, and/or cause illness. Some of these anti-nutritional factors are thermolabile and, under the right conditions, can be effectively destroyed during the extrusion process improving the overall quality of plant-based ingredients and the final diet (Patterson, et al., 2017). Recent reviews across a variety of legumes and legume-derived ingredients show that the activities of trypsin inhibitor, chymotrypsin inhibitor, and hemagglutinating activity were decreased by up to 95 % across a variety of thermal treatment conditions, including extrusion (Patterson, et al., 2017; Aviles-Gaxiola et al. 2018). Extrusion had modest effects on levels of phytate with reductions ranging from 7 to 26 % and varied by legume and extrusion conditions (Patterson, et al., 2017). **Figure 2** highlights the variability between processing methods and thermic conditions for decreasing anti-nutritional factors. For example, when soybeans were subjected to extrusion at increasing temperatures that ranged from 100 to 150 ^{o}C, trypsin inhibitor levels were incrementally decreased. At 140 ^{o}C, dry extrusion was considerably more effective at decreasing trypsin inhibitors (-91 %) compared to wet extrusion (-44 %). When the dry extrusion temperature was increased to 150 ^{o}C, reductions in trypsin inhibitors were further decreased by 94 % (Zilic et al., 2012). Other thermal treatments, such as micronisation, microwave roasting, and autoclaving also facilitated incremental reductions in trypsin inhibitors with increasing temperatures (Zilic et al., 2012). When formulating foods with higher concentrations of plant-based ingredients, consideration should also be given to the processing methods and the parameters used to effectively optimize the nutritional density and decrease anti-nutritional factors.

It is important to mention that, while temperature and pressure processing can greatly decrease anti-nutritional factors, they can also negatively impact bioavailability of amino acids. The Maillard reaction is a well-known example of heat damaged-protein (Teodorowicz et al., 2017).

In this reaction, lysine interacts with reducing sugars present in the diets forming the Maillard product. The complex formed can be digested and absorbed by the animal but cannot be utilized for metabolic processes (e.g. protein synthesis). Thus, in heat damaged proteins, digestibility of amino acids can greatly overestimate bioavailability (Moehn et al., 2005). Other products of heat damage on proteins include racemization of amino acids (alteration from L to D form) and the formation of cross-linked amino acids. Such components can decrease bioavailability of amino acids and digestibility of proteins, and their effects on protein quality cannot usually be determined using conventional methods of amino acid analysis. Pet foods with higher levels of plant-based ingredients may also require optimization of processing methods to maximize their nutritional density and nutrient bioavailability.

Recommendations for formulating dog food with novel ingredients

Considering the AA profile of dog foods

Feed formulation for agricultural and companion animals should be based on the ideal protein concept (Baker, 1991; Swanson et al., 2013). The ideal protein is defined as that in which all AAs are in perfect balance compared to the animal's AA requirements (mg/g protein). Hence, all indispensable AAs are equally limiting. However, this is impossible to achieve in practical animal feed formulation, and diets should be formulated considering the first limiting indispensable AA. The first limiting indispensable AA refers to the indispensable AA that is present in the lowest proportion compared to the animal's requirement. By meeting the first indispensable limiting AA requirement, requirements for all other indispensable AAs are also inherently satisfied. Moreover, to avoid the formulation of diets with excessive protein concentration or an excess of indispensable AAs relative to the requirements of dogs, animal nutritionists combine multiple ingredients that are complementary in their AA profiles. Commonly, dog foods are formulated with a higher proportion of animal-derived ingredients, and a lower proportion of plant-based ingredients to meet nutrient recommendations. More recently, however, cereal grains have been removed in some diet formulations or the proportion of animal-based ingredients has been reduced. The production of these types of formulations are often driven by consumer perception, rather than scientific evidence. Allowing consumers to direct the ingredient composition of dog foods, or other pet foods, could perpetuate nutrient deficits that affect the health of animals in the long term.

In the formulation of grain-free pet foods, cereal grains are replaced with alternative ingredient(s). Animal-derived ingredients are expensive relative to plant-based ingredients. Thus, pulses, a subset of legumes, are often used as the replacement. In addition to containing substantial fiber, pulses also contain significant concentrations of protein and are used to partly

meet indispensable AA requirements. Of interest, soybean meal and pulses contain 48% and 25% crude protein, respectively, which is substantially greater than the average protein concentration for grains (11%) (Table 1). While the high protein content in soybean meal and pulses is indicative of higher concentration of AAs compared to grains, it does not imply AA balance. Soybean meal and pulses are high in lysine (mg/g protein) but low in sulfur AAs (mg/g protein), while the reverse is true for cereals. Plant-based ingredients tend to have lower ileal digestibility coefficients for protein compared to protein from animal sources (FAO and WHO 1991). Thus, dog foods that contain substantial amounts of pulses, lower proportions of animal-based ingredients, and do not address AA imbalances through the addition of alternate ingredients or fortification, may risk AA deficiencies. To mitigate this risk across the pet food industry and ensure the final pet diets are nutritionally adequate and balanced, it is prudent that the digestibility coefficients of all final pet food products be calculated.

Considering the addition of high fiber ingredients to dog foods

By definition, dietary fiber is carbohydrates that are resistant to digestion by endogenous enzymes in the gastrointestinal tract (NRC, 2006). Typical fibers include arabinoxylan, raffinose, inulin, β-glucan, cellulose, and pectin (NRC, 2006). Common ingredients to increase fiber content in companion animal diets include beet pulp, corn fiber, rice bran, whole grains, and pulse fibers (de Godoy et al., 2013). Achieving an optimal fiber concentration in canine diets has diverse positive physiological effects in the gastrointestinal tract; for example, higher fermentable fiber intake has been shown to slow the transit time of digesta, increasing satiety of the animal (Haber et al., 1977). Moreover, high fiber diets generally have lower energy density making them an important nutritional strategy for controlling body weight (Johnson et al., 2008) and reducing the incidence of diarrhea (Homan et al., 1994). Gut health is also improved with higher consumption of fiber; fermentable fiber can act as a prebiotic and increase the population of health-promoting microbiota including lactobacilli and bifidobacteria (Roberfroid, 2005). Although not required by AAFCO to fulfill the criteria of "complete and balanced", fiber is an important component of the diet, and depending on the type of fiber and the amount consumed, fiber can increase the gut health status. Adding the necessary amount and type of fiber in the diet is crucial for optimal dog nutrition.

Despite the benefits of fiber in the diet, fiber can also affect enterohepatic recycling of taurine (discussed above). In monogastric species, including humans, high dietary fermentable fiber may also decrease digestibility and availability of dietary AAs (Blackburn and Southgate, 1981; Degen et al., 2007) and, in some cases, increase the risk of DCM in dogs fed diets that marginally meet requirements for sulfur AAs. Moreover, higher concentrations of dietary fiber increase the size of the gastrointestinal tract in pigs and poultry (Nyachoti et al., 2000) increasing nutrient utilization in this organ. It has been determined in pigs that on average the gastrointestinal tract catabolizes 30% of dietary indispensable AAs during absorption, and this utilization represents ~50% for sulfur AAs (Stoll et al., 1998; Mansilla et al., 2018), further

reducing precursor availability for taurine synthesis and increasing the risk for taurine deficiency. For some high fiber diets, fortification of specific nutrients, including taurine and other sulfur AAs, might be beneficial to avoid nutrient deficiencies.

Compared to the pet food industry, in other industries where high fiber ingredients (co-products) are routinely used (e.g. swine industry), the effects of fiber on the absorption of nutrients have been given more attention when formulating diets (NRC, 2012). For example, highly fermentable fiber in swine diets increases the threonine requirement to compensate for the increase in mucus (mucin protein) production in the intestinal cell lining (Lien et al., 1997; Mathai et al., 2016). This has underpinned the development of "requirement models" (NRC, 2012) to tailor nutrient requirements for pigs while accounting for the different nutrient interactions. In contrast, in the pet food industry, the only concentrations of nutrients used for comparison are those recommended by AAFCO (2018). Such recommendations are static and may not encompass all the effects of the different nutrient combinations in the final diet. There is a clear need in companion animal nutrition to improve the understanding of the interactions of different ingredients and how these alter nutrient requirements for different breeds, age, and physiological status of dogs.

Other recent publications highlight the need for careful nutrient formulation

Several recent papers, both original research and reviews, likewise highlight the unknowns surrounding grain-free diets (typically legume or pulse-based, but sometimes also with "exotic" ingredients such as kangaroo, bison, or wild boar) and DCM. For example, Adin et al. (2019) examined 48 dogs of many breeds with diagnosed DCM and having a known diet history. Among grain-free diets being consumed in this study, 1 was particularly associated with DCM, possibly underscoring the importance of specific diet formulation. Further, 2 dogs switched from that diet to other grain-free diets showed improvement in their DCM; it is unclear if those dogs were taurine deficient or if they also received taurine and/or carnitine supplementation. This suggests that grain-free composition per se may not be the root cause of DCM. Another recently published case series of 24 Golden Retrievers with DCM and known diet histories were evaluated, and an association between grain-free diets and DCM was suggested (Kaplan et al., 2018). Most dogs (15 out of 24) were fed a single diet which was significantly associated with low blood taurine concentrations, again suggesting that specific diet formulation may play an important role. However, as in the previous study, soluble versus insoluble fiber concentrations were not available for the diets, nor were taurine, methionine, or cysteine concentrations, meaning that the true nutrient profiles of the diets could not be assessed and reinforcing the point that diet formulation for nutrients – not ingredients – is essential. It also suggests that nutrient requirements may vary widely based on breed, diet, and other phenotypic data. Indeed, most of the dogs with DCM in the previously described study were consuming less energy compared to their predicted requirements (Kaplan et al., 2018). It also bears pointing out that the numbers in both studies were very low (representing less than 100 DCM-affected dogs between them),

which surely represents a fraction of the dogs consuming grain-free, pulse-based diets. A recent thoughtful review supports these conclusions by reiterating the crucial need for plant-based diets for dogs to be formulated with sufficient quantities of bioavailable methionine and cysteine to support adequate taurine synthesis (Dodd et al., 2018). This can be achieved with the addition of purified amino acids and other sources that are readily available (Gloaguen et al., 2014). Finally, a recent commentary carefully concludes that a true cause-and-effect relationship between grain-free diets and DCM has not been proven, and other factors may ultimately be more important (Freeman et al., 2018). Taken together, these recent publications may point to faulty nutrient formulation in some, but not all, grain-free diets.

CONCLUSIONS

Recently, it has been suggested that pulse ingredients in commercial dog foods are associated with a limited number of cases of DCM. While pulse ingredients have been implicated for having negative effects on the taurine status in dogs (deficiency of which is a known cause of canine DCM) based on the available evidence, the relationship between pulses and canine DCM remains undefined. However, the FDA statement may harm consideration of protein alternatives, such as pulses, as quality ingredients in pet foods and undermine attempts to diversify ingredients used across the food chain as the global population continues to grow. Ingredients do not represent the nutritional composition of the diet, and therefore, nutrient deficiencies should not be attributed to individual ingredients. The authors of this commentary recognize the important role of endogenous, and perhaps exogenous, taurine in the prevention of DCM in some dogs. The assurance of appropriate concentrations of all indispensable sulfur AAs, including methionine and cysteine, is crucial for ensuring adequate endogenous synthesis of taurine and to meet the metabolic demands of dogs. Additional dietary factors, such as methyl donors required for sulfur AA metabolism, carnitine for energy production in muscle, and dietary fiber, as well as animal factors, such as breed, size, and health status, should also be investigated when nutrient deficiency-related DCM is suspected.

It is the responsibility of animal nutritionists to formulate balanced diets for dogs, and other animals, by looking beyond the goal of meeting AAFCO recommendations or satisfying unsubstantiated market trends. Pulses and other plant-based ingredients can be used to formulate nutritionally adequate dog foods, and final product formulations should be assessed for nutrient balance and bioavailability, especially when using a limited number of ingredients. Although dietary factors are important in the prevention of sulfur AA deficiency and development of DCM, empirical data and mechanistic studies are required to better understand the indispensable AA requirements of dogs and preventing DCM. In diets that contain high concentrations of dietary fiber, compensative inclusion of dietary indispensable sulfur AAs, including exogenous taurine, might be required to offset the possibility of increased fecal excretion or microbial

assimilation of taurine in the large intestine. Processing conditions may also require adjustments to ensure the presence or effects of anti-nutritional factors are minimized and nutrient bioavailability is not compromised. Greater awareness of AA balance is crucial for ensuring that AA requirements are met for dogs consuming static diets.

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Figure 1. *Metabolism of sulfur amino acids. DMG: dimethylglycine, SAH, S-denosylhomocysteine; SAM, S-adenosylmethionine*

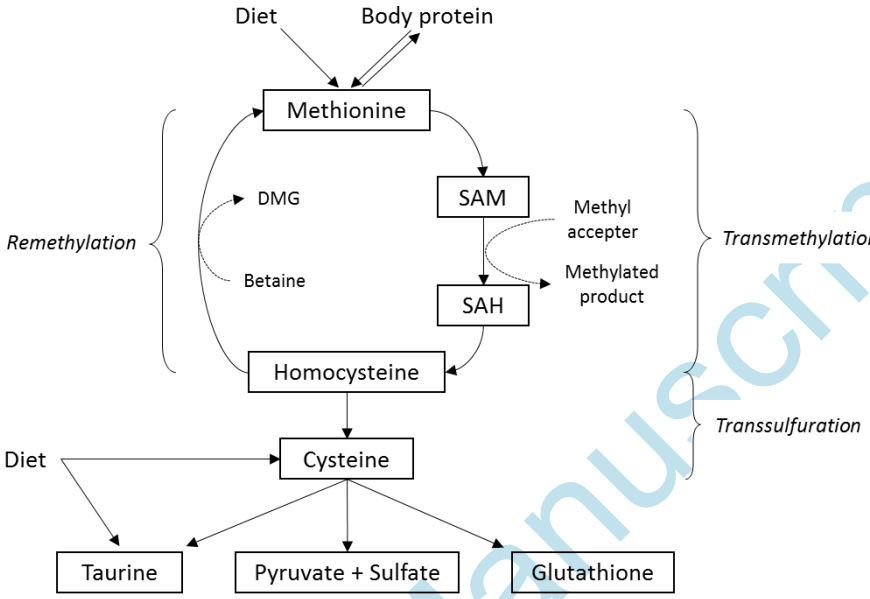

Figure 2. *Effect of thermal processing methods on trypsin inhibitor levels (mg/g) soybean kernel. [1]Treatment conditions: None = no treatment; Dry Extrusion for 25 to 30 sec (1=100 °C; 2=125 °C; 3=140 °C; 4= 150 °C); Wet Extrusion for 25 to 30 sec with 6 to 8 % added moisture (1=100 °C; 2=125 °C; 3=140 °C); Micronisation with near-infrared rays wavelength of 1.8 to 3.4 µm for 90 sec (1=100 °C; 2=125 °C; 3=140 °C; 4= 150 °C); Microwave roasting at 800 W and 2450 MHz (1 = 1 min (kernel temp = 57 °C), 2 = 2 min (kernel temp = 88 °C), 3 = 3 min (kernel temp = 108 °C), 4 =4 min (kernel temp =121 °C), 5 = 5 min (kernel temp = 132 °C)); Autoclaving at 120 °C and 1.2 bars (1 = 10 min, 2 = 20 min, 3 = 30 min). Reprinted with permission from Zilic et al. (2012)*

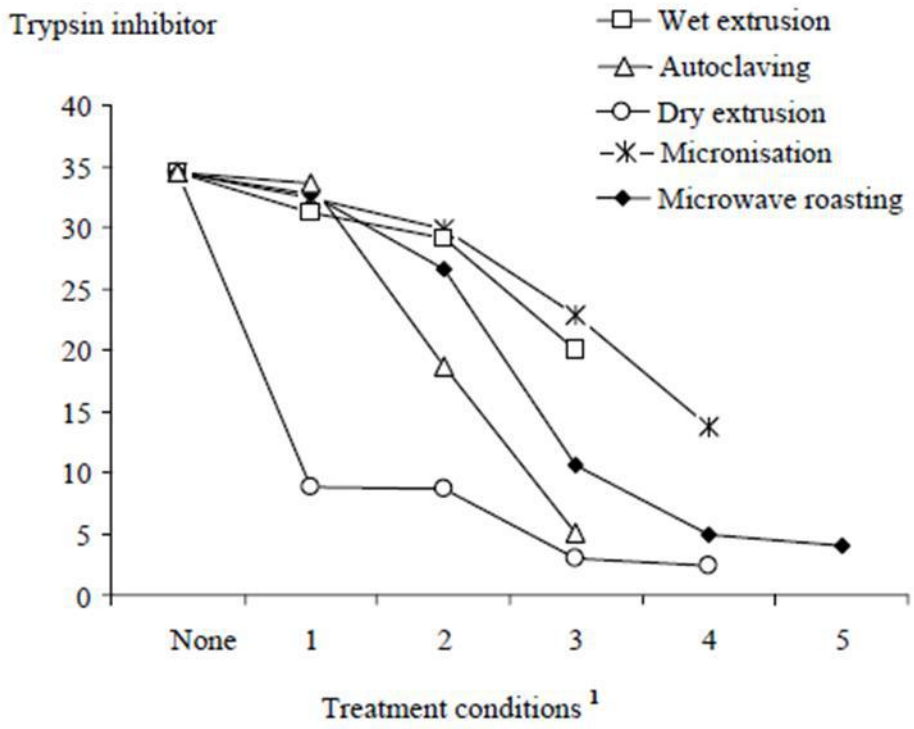

Table 1. *Crude protein (CP), fiber, selected amino acids, and carnitine contents in the principal legumes, cereals, and animal-derived ingredients used in dog food formulation.*[1]

Ingredients		CP, %	Crude fiber,[2] %	α-amino acids, mg/g protein[2]			Tau, mg/kg[3]	Carnitine, mg/kg[4]
				Lys	Met	Cys		
Legumes	Fava Beans	27.2	8.55	23.9	7.0	12.5	--	--
	Phaseolus beans	22.9	NR	72.9	12.7	12.7	--	--
	Kidney beans	20.0	6.40	26.5	14.0	12.0	--	--
	Lentils	26.0	NR	65.8	6.9	10.4	--	--
	Lupins	32.4	14.25	48.7	6.5	14.2	--	--
	Chick peas	20.3	6.16	69.4	14.8	21.6	--	--
	Soybean meal	47.7	3.89	62.0	13.8	14.7	--	--
Grains	Barley	11.3	3.90	35.3	17.7	22.9	--	--
	Corn, yellow dent	8.2	1.98	30.3	21.8	23.1	--	--
	Oats	11.2	2.20	43.9	60.9	32.3	--	--
	Rice	7.9	0.52	44.5	31.8	22.9	--	--
	Rye	11.7	2.71	36.9	13.7	16.3	--	--
	Sorghum	9.4	2.14	21.4	17.1	19.2	--	--
	Wheat hard, red	14.5	2.57	27.0	15.2	22.8	--	--
Animal-derived ingredients	Beef, meat	15.0	--	77.3	28.7	15.3	296	150
	Chicken, meat and skin	17.6	--	81.3	26.7	13.1	159	57
	Chicken, by product	59.0	--	48.1	17.3	16.8	3049	120
	Lamb, ground	16.6	--	88.0	25.9	12.0	473	282.3
	Rendered meat	54.1	2.50	53.8	14.2	11.3	NR	NR

Cys: cysteine, Lys: lysine, Met: methionine, NR: not reported, Tau: taurine.
[1]Values are presented in as-fed basis.
[2] NRC, 2006; NRC, 2012
[3] Spitze et al. 2003
[4] Arslan, 2006

Table 2. *Recommended allowance (RA) and minimum dietary content suggested by AAFCO for crude protein and essential amino acids in dog food, and their physiological roles and potential interactions.*

Nutrient	NRC RA[1], % DM	AAFCO[2], % DM	Important physiological roles and potential interactions
Crude protein	10	18	Necessary for synthesis of non-essential amino acids
Arginine	0.35	--	Competes with lysine absorption, arginine should be increased when high lysine concentrations in the diet
Histidine	0.19	--	
Lysine	0.35	0.63	Highly reactive to reducing sugars during heating (Maillard reaction), reducing bioavailability
Methionine	0.33	0.33	Requirement increases when methyl donors/acceptors and cysteine are reduced in the diet
Methionine + cystine	0.65	0.65	Requirement is increased with low supply of taurine and during immune challenge
Phenylalanine	0.45	0.45	
Phenylalanine + tyrosine	0.74	0.74	
Threonine	0.43	0.48	Abundant in mucosal proteins (mucin), requirement increases when feeding high fermentable fibers
Tryptophan	0.14	0.16	Precursor for serotonin synthesis. Ratio of Trp: LNAA should be considered; lower ratios may deprive appetite
Valine	0.49	0.49	Abnormal Increment of valine, leucine, or isoleucine (BCAA) will cause catabolism of the other BCAA in the muscle
Isoleucine	0.38	--	
Leucine	0.68	0.68	

AAFCO: The Association of American Feed Control Officials, BCAA: branched chain amino acids, DM: dry matter, NRC: National Research Council, RA: recommended allowance, Trp: LNAA: tryptophan to large neutral amino acid ratio.

[1]Recommended Allowance requirements for adult dogs at maintenance, Nutrient Requirements of Dogs and Cats (NRC, 2006).

[2]Miminum dietary content, AAFCO (2018).

From: Norris, Anne
To: Hartogensis, Martine
Cc: DeLancey, Siobhan; Rotstein, David
Subject: RE: DCM Timing
Date: Thursday, June 20, 2019 10:29:48 AM

Just wanted to check – are you planning to (b) (5)

From: Hartogensis, Martine
Sent: Wednesday, June 19, 2019 2:22 PM
To: Norris, Anne <Anne.Norris@fda.hhs.gov>
Cc: DeLancey, Siobhan <Siobhan.Delancey@fda.hhs.gov>; Rotstein, David <David.Rotstein@fda.hhs.gov>
Subject: RE: DCM Timing

Ok, great. We were planning to start (b) (5) .

Let me know if that works for everyone!

Martine

From: Norris, Anne
Sent: Wednesday, June 19, 2019 2:18 PM
To: Hartogensis, Martine <Martine.Hartogensis@fda.hhs.gov>
Cc: DeLancey, Siobhan <Siobhan.Delancey@fda.hhs.gov>; Rotstein, David <David.Rotstein@fda.hhs.gov>
Subject: RE: DCM Timing

Whew, good question! I think we're shooting (b) (5) I don't see it happening sooner than that. Hope that helps!

From: Hartogensis, Martine
Sent: Wednesday, June 19, 2019 2:16 PM
To: Norris, Anne <Anne.Norris@fda.hhs.gov>
Cc: DeLancey, Siobhan <Siobhan.Delancey@fda.hhs.gov>; Rotstein, David <David.Rotstein@fda.hhs.gov>
Subject: DCM Timing

Hi Anne,

Just checking in on your current DCM comms timing..? (b) (5)

Thanks very much in advance!

Martine

From:	Forfa, Tracey
To:	Norris, Anne; Rotstein, David; Jones, Jennifer L; Palmer, Lee Anne; Burkholder, William; Carey, Lauren; Steinberg, Nadine
Cc:	DeLancey, Siobhan; Hartogensis, Martine; Peloquin, Sarah
Subject:	RE: DCM-firm contacts
Date:	Monday, June 03, 2019 3:56:50 PM
Attachments:	image001.png image002.jpg image003.jpg image004.jpg image005.jpg image006.jpg

Hi – That is correct, I have been tasked with (b) (5) Thanks for checking in.

From: Norris, Anne
Sent: Monday, June 3, 2019 3:36 PM
To: Rotstein, David <David.Rotstein@fda.hhs.gov>; Jones, Jennifer L <Jennifer.Jones@fda.hhs.gov>; Palmer, Lee Anne <LeeAnne.Palmer@fda.hhs.gov>; Burkholder, William <William.Burkholder@fda.hhs.gov>; Carey, Lauren <Lauren.Carey@fda.hhs.gov>; Forfa, Tracey <Tracey.Forfa@fda.hhs.gov>; Steinberg, Nadine <Nadine.Steinberg@fda.hhs.gov>
Cc: DeLancey, Siobhan <Siobhan.Delancey@fda.hhs.gov>; Hartogensis, Martine <Martine.Hartogensis@fda.hhs.gov>; Peloquin, Sarah <Sarah.Peloquin@fda.hhs.gov>
Subject: RE: DCM-firm contacts

Adding in Tracey and Nadine because (b) (5)

From: Rotstein, David
Sent: Monday, June 3, 2019 3:27 PM
To: Norris, Anne <Anne.Norris@fda.hhs.gov>; Jones, Jennifer L <Jennifer.Jones@fda.hhs.gov>; Palmer, Lee Anne <LeeAnne.Palmer@fda.hhs.gov>; Burkholder, William <William.Burkholder@fda.hhs.gov>; Carey, Lauren <Lauren.Carey@fda.hhs.gov>
Cc: DeLancey, Siobhan <Siobhan.Delancey@fda.hhs.gov>; Hartogensis, Martine <Martine.Hartogensis@fda.hhs.gov>; Peloquin, Sarah <Sarah.Peloquin@fda.hhs.gov>
Subject: DCM-firm contacts

Everyone,

(b) (5)

(b) (5)

Dave

David Rotstein, DVM, MPVM, Dipl. ACVP
CVM Vet-LIRN Liaison
CVM OSC/DC/CERT
7519 Standish Place
(b) (6) **(BB)**

-----Original Appointment-----
From: Solomon, Steven M
Sent: Wednesday, May 29, 2019 11:40 AM
To: Solomon, Steven M; Flynn, William T; Forfa, Tracey; Norris, Anne; Schell, Timothy; Jones, Jennifer L; Palmer, Lee Anne; Burkholder, William; Carey, Lauren
Cc: DeLancey, Siobhan; Hartogensis, Martine; Murphy, Jeanette; Dewitt, Susan J; Cepeda, Sandra; Steinberg, Nadine; Rotstein, David; Reimschuessel, Renate; Ceric, Olgica; Peloquin, Sarah
Subject: FW: Checkpoint on DCM
When: Thursday, May 30, 2019 12:00 PM-1:00 PM (UTC-05:00) Eastern Time (US & Canada).
Where: CVM 7500 Conf E473 and WebEx

-----Original Appointment-----
From: Solomon, Steven M
Sent: Monday, May 20, 2019 10:36 AM
To: Solomon, Steven M; Flynn, William T; Forfa, Tracey; Norris, Anne; Schell, Timothy; Jones, Jennifer L; Palmer, Lee Anne; Burkholder, William; Carey, Lauren
Cc: DeLancey, Siobhan; Hartogensis, Martine; Murphy, Jeanette; Dewitt, Susan J; Cepeda, Sandra; Steinberg, Nadine

Subject: Checkpoint on DCM
When: Thursday, May 30, 2019 12:00 PM-1:00 PM (UTC-05:00) Eastern Time (US & Canada).
Where: CVM 7500 Conf E473 and WebEx

Purpose: (b) (5)

Meeting materials forthcoming.

Apologies for the lunchtime meeting, but schedules were tight.

Join Webex meeting

Meeting number (access code): (b) (6)
Meeting password: (b) (6)

Join by phone

(b) (6) US Toll
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IMPORTANT NOTICE: Please note that this Webex service allows audio and other information sent during the session to be recorded, which may be discoverable in a legal matter. By joining this session, you automatically consent to such recordings. If you do not consent to being recorded, discuss your concerns with the host or do not join the session.

From: Rotstein, David
To: Jones, Jennifer L
Subject: RE: EON-266814; EON-266821 and EON-266827-Merrick-FW: Moisture Content: RE: Quick Review/Screening: ORA ORS Cat Food Results--Assessment of Taurine Results for Possible Product Action
Date: Wednesday, August 24, 2016 4:31:23 PM
Attachments: 958504-Taurine.pdf
958501-Taurine.pdf
958500-Taurine.pdf

Here you go

David Rotstein, DVM, MPVM, Dipl. ACVP
CVM Vet-LIRN Liaison
CVM OSC/DC/ICERT
7519 Standish Place, RM 120
240-402-5613 (Office) (NEW NUMBER)
(b) (6) (BB)

From: Jones, Jennifer L
Sent: Wednesday, August 24, 2016 7:23 AM
To: Rotstein, David
Subject: RE: EON-266814; EON-266821 and EON-266827-Merrick-FW: Moisture Content: RE: Quick Review/Screening: ORA ORS Cat Food Results--Assessment of Taurine Results for Possible Product Action

Sorry to both you Dave, do you also have the official results report? I just need a copy for the final report, so it's not urgent.

Jennifer Jones, DVM
Veterinary Medical Officer

From: Jones, Jennifer L
Sent: Wednesday, August 24, 2016 7:08 AM
To: Rotstein, David
Subject: RE: EON-266814; EON-266821 and EON-266827-Merrick-FW: Moisture Content: RE: Quick Review/Screening: ORA ORS Cat Food Results--Assessment of Taurine Results for Possible Product Action

Thanks Dave!

Jennifer Jones, DVM
Veterinary Medical Officer

From: Rotstein, David
Sent: Wednesday, August 24, 2016 6:35 AM
To: Jones, Jennifer L
Subject: EON-266814; EON-266821 and EON-266827-Merrick-FW: Moisture Content: RE: Quick

Review/Screening: ORA ORS Cat Food Results--Assessment of Taurine Results for Possible Product Action

Here you go Jen.

David Rotstein, DVM, MPVM, Dipl. ACVP
CVM Vet-LIRN Liaison
CVM OSC/DC/ICERT
7519 Standish Place, RM 120
240-402-5613 (Office) (NEW NUMBER)
(b) (6) (BB)

From: Burkholder, William
Sent: Wednesday, July 27, 2016 4:30 PM
To: Rotstein, David; Benjamin, Linda
Cc: Krieger, Darlene; Queen, Jackie L; Hodges, April; Conway, Charlotte
Subject: RE: Moisture Content: RE: Quick Review/Screening: ORA ORS Cat Food Results--Assessment of Taurine Results for Possible Product Action

OK Everyone. The product appears to be a dry extruded product, for which the AAFCO Cat Food Nutrient Profiles content for taurine is 0.10% on a dry matter basis. Clearly all three samples were analyzed to contain more than that amount of taurine. On a dry matter basis the concentration of taurine in the samples was analyzed to be:

FACTS #	Amount Taurine Found	%Moisture	%Dry Matter	Amount Taurine on a Dry Matter Basis
958500	0.183g/100g ≈ 0.18%	2.20%	100 – 2.20 = 97.80%	0.183/0.9780 = 0.187%
958501	0.153g/100g ≈ 0.15%	1.99%	100 – 1.99 = 98.01%	0.153/0.9801 = 0.156%
958504	0.171g/100g ≈ 0.17%	2.79%	100 – 2.79 = 97.21%	0.171/0.9721 = 0.176%

All of the Dry Matter Taurine percentages are above 0.10%. IF any of the samples were canned cat food, they would not be in compliance with the AAFCO Cat Food Nutrient Profiles for the recommended minimum taurine content and IF the label indicated the product was formulated to meet the AAFCO Cat Food Nutrient Profiles the product would be misbranded.

The answer to the question of consequence/causation of the taurine content in the product from which these three samples originated to the cats in the consumer complaint is that this(ese) lot(s) of product are not indicated to be causative. However, dilated cardiomyopathy from taurine deficiency occurs over a long period of exposure to a deficient diet (months to a year or more), so, if these cats were eating the Merrick Purrfect Bistro Grain Free Real Chicken Recipe feline dry for the 3 years

indicated in the complaint, it is possible that the product was deficient for some long interval of time during that three year period and that a return to "normal" taurine levels in the diet were insufficient to correct the problem in the three cats that developed low blood taurine and the two with dilated cardiomyopathy. Treatment for dilated cardiomyopathy caused by taurine deficiency takes higher daily doses of taurine for several months than normal dietary amounts and is not completely curative.

Recommendations for regulatory steps to consider (b) (5)

Consider recommending the owner have an ophthalmic exam performed on the cat being treated for low blood taurine to see if there are signs of retinal degeneration due to taurine deficiency.

William J. Burkholder, DVM, PhD, DACVN
Leader, Nutrition and Labeling Team I, HFV-228
Division of Animal Feeds
Center for Veterinary Medicine
United States Food and Drug Administration
7519 Standish Place
Rockville, Maryland 20855
Phone: 240-402-5900
Fax: 240-453-6882
E-mail: william.burkholder@fda.hhs.gov

From: Rotstein, David
Sent: Wednesday, July 27, 2016 2:23 PM
To: Benjamin, Linda; Burkholder, William
Cc: Krieger, Darlene; Queen, Jackie L; Hodges, April
Subject: Moisture Content: RE: Quick Review/Screening: ORA ORS Cat Food Results--Assessment of Taurine Results for Possible Product Action

Please see the moisture content below:

The moisture content for the samples are as follows:

FACTS #	Amount Taurine Found	%Moisture
958500	0.183g/100g ≈ 0.18%	2.20%

958501	0.153g/100g ≈ 0.15%	1.99%
958504	0.171g/100g ≈ 0.17%	2.79%

David Rotstein, DVM, MPVM, Dipl. ACVP
CVM Vet-LIRN Liaison
CVM OSC/DC/ICERT
7519 Standish Place, RM 120
240-402-5613 (Office) (NEW NUMBER)
(b) (6) (BB)

From: Benjamin, Linda
Sent: Wednesday, July 27, 2016 7:54 AM
To: Burkholder, William
Cc: Rotstein, David; Krieger, Darlene; Queen, Jackie L; Hodges, April
Subject: FW: Quick Review/Screening: ORA ORS Cat Food Results--Assessment of Taurine Results for Possible Product Action

Hi Bill – Could you please respond to Dave Rotstein.

Dave – As ORS's numbers are very close to the 0.2% guarantee, it might be helpful to know the AV, CV, and/or 95% confidence limit for the analytical method. Additionally, do you know if the numbers below are being reported on a dry matter basis? FYI, the sample description on the collection reports (first 3 attachments) has either "*One unopened bag of Merrick Purrfect Bistro Grain Free Real Chicken Recipe weighing 5.4kg*" or "*Opened bag of Merrick Purrfect Bistro Grain Free Real Chicken Recipe that only had 0.15kg of product. This sample was used by the consumer*" but below [my green highlight] you referenced taurine # for canned products.

Sorry Bill – I just want to make sure you have everything you need.

Thanks for the opportunity to comment,
Linda

From: Rotstein, David
Sent: Wednesday, July 27, 2016 7:22 AM
To: Benjamin, Linda
Cc: Krieger, Darlene; Queen, Jackie L; Hodges, April
Subject: Quick Review/Screening: ORA ORS Cat Food Results--Assessment of Taurine Results for Possible Product Action

Linda,

We received an email from ORS with results for taurine for a cat food. Testing was based on a consumer complaint for 3 cats with cardiac disease and low taurine.

ORS has not finalized the results, but sent on the findings for the DRY cat food and asked whether

CVM considers the results to be low based on the AAFCO requirements for wet cat food.

REQUEST: To answer the following questions:

1) Is the taurine low for a dry cat food based on AAFCO nutrient profiles?
2) If the taurine is low, would it be biologically significant for cats that ate this as their sole/primary diet?

The responses will (b) (5)

REVIEWERS: Bill Burkholder, Krisztina Atkinson, Randall Lovell.

Date Needed: IDEAL—By our Wednesday Pet Food Outbreak Meeting at 11 AM today (7/27). (I will be out on Thursday and Friday and if no one can respond before the meeting today, please include Jackie Queen on the response).

Email from the ORS Lab:

David I hope you can help us this these findings.

We received three consumer complaint Dry Cat Food products for Amino Acid analysis. We assayed the products for the Amino Acid profile and found only Taurine low.

FACTS #	***Amount Taurine Found***
958500	***0.183g/100g ≈ 0.18%***
958501	***0.153g/100g ≈ 0.15%***
958504	***0.171g/100g ≈ 0.17%***

The label for all of the samples are the same and Taurine is declared 0.20% minimum. The AAFCO Nutrient Profile from August 2015 states that the minimum limits for Taurine is 0.20% in canned products. Do you consider these product violated?

Attachments:

Collection Reports
Pet Food Report
Vet-LIRN Summary

Medical records were collected and evaluated by Vet-LIRN. These can be provided by request.

Thank you

David Rotstein, DVM, MPVM, Dipl. ACVP
CVM Vet-LIRN Liaison
CVM OSC/DC/ICERT
7519 Standish Place, RM 120
240-402-5613 (Office) (NEW NUMBER)
(b) (6)(BB)

Food and Drug Administration Office of Regulatory Affairs

Summary Report

For Sample Number: 958500

TD Sample Number: **Import Sample Number**

This is an accurate reproduction of the original electronic record as of 08/24/2016

Sample Class: Normal Everyday Sample
Sample Origin: Domestic
Sample Basis: Surveillance
Sample Flag:
Sample Type: Official
Collecting District: NWJ-DO
Home District:
Orig C/R and Records To: NWJ-DO
Collection PACs: 71R801

Product Name: Poultry Prod Pet Cat Food; Not Elsewhere Classified (NEC); Packaged Food (Not Commercially Sterile)

Product Description: See Remarks Section.

Collection Reason: Sample collected per FACTS Assignment ID #11650647 and OP ID # 8660426 referencing Consumer Complaint #146048 reporting the illness of multiple cats from the same household. Sample testing request: Taurine.

Lab: SRL **Split Num:** 0 **Date Received:** 06/29/2016 **Date Out of Lab:** 08/04/2016

District Conclusion: No Action Indicated (NAI) **District Conclusion Made By:** Tweedley, Karen P **District** 08/12/2016

Disposition Reason: NAI By Examining District **Disposition Authorized By:** Tweedley, Karen P **Disposition Authorized Date:** 08/12/2016

Performing Org	PAC	LID	PAF	Compliance No	Lab Class-Description	Laboratory Status
ACNA-N	71R801		NAR		1 - In Compliance	Completed

Lab Conclusion

Sample Narrative - Method: AccQTag AAA(Waters) Analysis - Taurine
Amt Found - 0.187% (dry matter basis)
Meets AAFCO minimum requirement of 0.10%

Sample Narrative - Method: Instrument Manual (Denver Instrumentst IR60)/ AOAC 930.15 Analysis - Moisture
Amt Found - 2.20%
Amt Declared - 11.00% max

Lab Conclusion Date	Lab Conclusion Made By
08/04/2016	Hawes,Brian M

Food and Drug Administration Office of Regulatory Affairs

Summary Report

For Sample Number: 958501

TD Sample Number: **Import Sample Number**

This is an accurate reproduction of the original electronic record as of 08/24/2016

Sample Class: Normal Everyday Sample
Sample Origin: Domestic
Sample Basis: Surveillance
Sample Flag:
Sample Type: Official
Collecting District: NWJ-DO
Home District:
Orig C/R and Records To: NWJ-DO
Collection PACs: 71R801

Product Name: Poultry Prod Pet Cat Food; Not Elsewhere Classified (NEC); Packaged Food (Not Commercially Sterile)

Product Description: See Remarks

Collection Reason: Sample collected per FACTS Assignment ID #11650647 and OP ID # 8660426 referencing Consumer Complaint #146048 reporting the illness of multiple cats from the same household. Sample testing request: Taurine.

Lab: SRL **Split Num:** 0 **Date Received:** 06/29/2016 **Date Out of Lab:** 08/04/2016

District Conclusion: No Action Indicated (NAI) **District Conclusion Made By:** Tweedley, Karen P **District** 08/12/2016

Disposition Reason: NAI By Examining District **Disposition Authorized By:** Tweedley, Karen P **Disposition Authorized Date:** 08/12/2016

Performing Org	PAC	LID	PAF	Compliance No	Lab Class-Description	Laboratory Status
ACNA-N	71R801		NAR		1 - In Compliance	Completed

Lab Conclusion

Sample Narrative - Method: AccQTag AAA Analysis - Taurine
Amt Found - 0.156% (dry matter basis)
Meets AAFCO minimum requirement of 0.10%

Sample Narrative - Method: Instrument Manual (Denver Instrumentst IR60)/ AOAC 930.15 Analysis - Moisture
Amt Found - 1.99%
Amt Declared - 11.00% max

Lab Conclusion Date	Lab Conclusion Made By
08/04/2016	Hawes,Brian M

Food and Drug Administration Office of Regulatory Affairs

Summary Report

For Sample Number: 958504

TD Sample Number: **Import Sample Number**

This is an accurate reproduction of the original electronic record as of 08/24/2016

Sample Class: Normal Everyday Sample
Sample Origin: Domestic
Sample Basis: Surveillance
Sample Flag:
Sample Type: Official
Collecting District: NWJ-DO
Home District:
Orig C/R and Records To: NWJ-DO
Collection PACs: 71R801

Product Name: Poultry Prod Pet Cat Food; Not Elsewhere Classified (NEC); Packaged Food (Not Commercially Sterile)

Product Description: See Remarks Section.

Collection Reason: Sample collected per FACTS Assignment ID #11650647 and OP ID # 8660426 referencing Consumer Complaint #146048 reporting the illness of multiple cats from the same household. Sample testing request: Taurine.

Lab: SRL **Split Num:** 0 **Date Received:** 06/29/2016 **Date Out of Lab:** 08/04/2016

District Conclusion: No Action Indicated (NAI) **District Conclusion Made By:** Ciaccia, Andrew **District** 08/17/2016

Disposition Reason: NAI By Home District **Disposition Authorized By:** Ciaccia, Andrew **Disposition Authorized Date:** 08/17/2016

Performing Org	PAC	LID	PAF	Compliance No	Lab Class-Description	Laboratory Status
ACNA-N	71R801		NAR		1 - In Compliance	Completed

Lab Conclusion

Sample Narrative - Method: AccQTag AAA Waters Analysis - Taurine
Amt Found - 0.176% (dry matter basis)
Meets AAFCO minimum requirement of 0.10%

Sample Narrative - Method: Instrument Manual (Denver Instrumentst IR60)/AOAC 930.15 Analysis - Moisture
Amt Found - 2.79%
Amt Declared - 11.00% max

Lab Conclusion Date **Lab Conclusion Made By**

08/04/2016 Hawes,Brian M

From: Lambkin, Sonya
To: Hartogensis, Martine; Wittig, Julianna; Conway, Charlotte; McCoig, Amber
Subject: RE: FYSA - Pet Foods Agenda
Date: Thursday, March 14, 2019 10:03:03 AM

Hello, thanks for the call this morning - (b) (5)

[redacted]

Thanks,
Sonya

From: Hartogensis, Martine <Martine.Hartogensis@fda.hhs.gov>
Date: March 14, 2019 at 8:18:23 AM EDT
To: Wittig, Julianna <Julianna.Wittig@fda.hhs.gov>, Palmer, Lee Anne <LeeAnne.Palmer@fda.hhs.gov>, Carey, Lauren <Lauren.Carey@fda.hhs.gov>, Jones, Jennifer L <Jennifer.Jones@fda.hhs.gov>, Peloquin, Sarah <Sarah.Peloquin@fda.hhs.gov>, Lambkin, Sonya <Sonya.Lambkin@fda.hhs.gov>, Conway, Charlotte <Charlotte.Conway@fda.hhs.gov>
Cc: Ceric, Olgica <Olgica.Ceric@fda.hhs.gov>, Reimschuessel, Renate <Renate.Reimschuessel@fda.hhs.gov>, Duggirala, Hesha Jani <Hesha.Duggirala@fda.hhs.gov>, McCoig, Amber <Amber.McCoig@fda.hhs.gov>, Forfa, Tracey <Tracey.Forfa@fda.hhs.gov>
Subject: RE: FYSA - Pet Foods Agenda

Yes, it shouldn't take up too much time.

Martine

From: Wittig, Julianna
Sent: Thursday, March 14, 2019 8:17 AM
To: Hartogensis, Martine <Martine.Hartogensis@fda.hhs.gov>; Palmer, Lee Anne <LeeAnne.Palmer@fda.hhs.gov>; Carey, Lauren <Lauren.Carey@fda.hhs.gov>; Jones, Jennifer L <Jennifer.Jones@fda.hhs.gov>; Peloquin, Sarah <Sarah.Peloquin@fda.hhs.gov>; Lambkin, Sonya <Sonya.Lambkin@fda.hhs.gov>; Conway, Charlotte <Charlotte.Conway@fda.hhs.gov>
Cc: Ceric, Olgica <Olgica.Ceric@fda.hhs.gov>; Reimschuessel, Renate <Renate.Reimschuessel@fda.hhs.gov>; Duggirala, Hesha Jani <Hesha.Duggirala@fda.hhs.gov>; McCoig, Amber <Amber.McCoig@fda.hhs.gov>; Forfa, Tracey <Tracey.Forfa@fda.hhs.gov>
Subject: RE: FYSA - Pet Foods Agenda

Okay. I was thinking that too based on some feed back re purpose of this meeting. Has it changed at all since we last spoke? More like a reminder?

From: Hartogensis, Martine

Sent: Thursday, March 14, 2019 8:16 AM
To: Wittig, Julianna <Julianna.Wittig@fda.hhs.gov>; Palmer, Lee Anne <LeeAnne.Palmer@fda.hhs.gov>; Carey, Lauren <Lauren.Carey@fda.hhs.gov>; Jones, Jennifer L <Jennifer.Jones@fda.hhs.gov>; Peloquin, Sarah <Sarah.Peloquin@fda.hhs.gov>; Lambkin, Sonya <Sonya.Lambkin@fda.hhs.gov>; Conway, Charlotte <Charlotte.Conway@fda.hhs.gov>
Cc: Ceric, Olgica <Olgica.Ceric@fda.hhs.gov>; Reimschuessel, Renate <Renate.Reimschuessel@fda.hhs.gov>; Duggirala, Hesha Jani <Hesha.Duggirala@fda.hhs.gov>; McCoig, Amber <Amber.McCoig@fda.hhs.gov>; Forfa, Tracey <Tracey.Forfa@fda.hhs.gov>
Subject: RE: FYSA - Pet Foods Agenda

Thanks Julianna!

(b) (5)

Thanks in advance!

Martine

From: Wittig, Julianna
Sent: Wednesday, March 13, 2019 5:52 PM
To: Palmer, Lee Anne <LeeAnne.Palmer@fda.hhs.gov>; Carey, Lauren <Lauren.Carey@fda.hhs.gov>; Jones, Jennifer L <Jennifer.Jones@fda.hhs.gov>; Peloquin, Sarah <Sarah.Peloquin@fda.hhs.gov>; Lambkin, Sonya <Sonya.Lambkin@fda.hhs.gov>; Conway, Charlotte <Charlotte.Conway@fda.hhs.gov>
Cc: Ceric, Olgica <Olgica.Ceric@fda.hhs.gov>; Hartogensis, Martine <Martine.Hartogensis@fda.hhs.gov>; Reimschuessel, Renate <Renate.Reimschuessel@fda.hhs.gov>; Duggirala, Hesha Jani <Hesha.Duggirala@fda.hhs.gov>; McCoig, Amber <Amber.McCoig@fda.hhs.gov>
Subject: FYSA - Pet Foods Agenda

Hi all,

So we are all on the same page, team mbrs shared time frames:

(b) (5)

Thanks,
J

From:	Reimschuessel, Renate
To:	Jones, Jennifer L; Rotstein, David; Palmer, Lee Anne; Carey, Lauren; Queen, Jackie L
Cc:	Ceric, Olgica
Subject:	RE: Head"s up-potential DCM case-Dr. Adin-NCSU-2 Schnauzers
Date:	Tuesday, July 11, 2017 11:50:53 AM
Attachments:	image001.png image002.png

(b) (5)

Renate Reimschuessel V.M.D. Ph.D. Vet-LIRN **Phone 1-240-402-5404**
Fax 301-210-4685
http://www.fda.gov/AnimalVeterinary/ScienceResearch/ucm247334.htm

From: Jones, Jennifer L
Sent: Tuesday, July 11, 2017 11:38 AM
To: Rotstein, David; Palmer, Lee Anne; Carey, Lauren; Queen, Jackie L
Cc: Ceric, Olgica; Reimschuessel, Renate
Subject: Head's up-potential DCM case-Dr. Adin-NCSU-2 Schnauzers

Vet will submit PFR online →
2 dogs-unrelated miniature schnauzers

Dog 1: 2 yr → presented 2/2017 with fulminant CHF → severe DCM on echo, taurine/carnitine normal, infectious disease testing negative, died on the ventilator, necropsy done-myocardial changes were subtle but could be similar to moldy corn toxicity in pigs → plasma, urine, serum, and myocardial tissue available

Dog 2: 7 yr, had a syncopal episode ~2/2017 but presented to vet for progressive frequency of syncopal episodes → 6/2017 for CHF, diagnosed with DCM similar to housemate, nearly same image on Echo, taurine/carnitine normal, infectious disease testing negative, they have changed the diet (Hill's) and dog is responding to treatment; plasma, urine, and serum available

Dogs were eating California Naturals (different bag than from 2/2017) and treats (Milo's Kitchen); Vet has samples of food and treats

Jennifer L. A. Jones, DVM
Veterinary Medical Officer
U.S. Food & Drug Administration
Center for Veterinary Medicine
Office of Research
Veterinary Laboratory Investigation and Response Network (Vet-LIRN)
8401 Muirkirk Road, G704
Laurel, Maryland 20708
new tel: 240-402-5421
fax: 301-210-4685
e-mail: *jennifer.jones@fda.hhs.gov*
Web: http://www.fda.gov/AnimalVeterinary/ScienceResearch/ucm247334.htm

From: Jones, Jennifer L
To: "Darcy Adin"
Cc: Ceric, Olgica
Subject: RE: Pet food concern
Date: Wednesday, July 12, 2017 7:02:00 AM
Attachments: image001.png
image003.png
image004.png

Thank you, Darcy.
We received the complaints on our end, and will be in touch about next steps.
Kind regards,
Jennifer

Jennifer Jones, DVM
Veterinary Medical Officer


From: Darcy Adin [mailto:dbadin@ncsu.edu]
Sent: Tuesday, July 11, 2017 5:44 PM
To: Jones, Jennifer L
Subject: Re: Pet food concern

Hi Jennifer,

I've submitted the reports through the portal - one for each dog. The numbers are:
2023230 (I) for (b) (6)
2023228 (I) for

I've also attached the visit summaries for (b) (6) (2) and (b) (5) (1) as well as (b) (6) necropsy report. I have the biological samples stored at -80 and also have food samples.

Thank you so much for your help and I'll look forward to hearing from you or someone on your team!
Take care
Darcy

On Tue, Jul 11, 2017 at 7:33 AM, Jones, Jennifer L <Jennifer.Jones@fda.hhs.gov> wrote:
Hi Darcy,
I can chat today from (b) (5)
Jen

Jennifer Jones, DVM
Veterinary Medical Officer


From: Darcy Adin [mailto:dbadin@ncsu.edu]
Sent: Monday, July 10, 2017 6:47 PM
To: Jones, Jennifer L
Cc: Reimschuessel, Renate; (b) (6)

Subject: Re: Pet food concern

Thank you Dr. Jones! I'm sorry I am just reading email now so I have missed you! I'd love to chat with you about the cases. I am on clinics this week but can try to call you if you have another block of time tomorrow (or later this week). My number is 614-582-9798 or I can be paged from (b) (6).
Thank you!
Darcy

On Jul 10, 2017, at 1:05 PM, Jones, Jennifer L <Jennifer.Jones@fda.hhs.gov> wrote:

> Hello Dr. Adin,
> Please let me know if you'd like to chat about the case this afternoon. I'll be in the office from 1-3pm (tel: 240-402-5421).
> If you suspect an animal's illness may be due to the food, you can submit a report at www.safetyreporting.hhs.gov
> Please mention Vet-LIRN encouraged you to submit a report. Please email me the ICSR number (similar to a confirmation number), so I can find the report on my end. Thank you,
> Jennifer
>
> Jennifer Jones, DVM
> Veterinary Medical Officer
>
> <image001.png> <image004.png>
>
> **From:** Darcy Adin [mailto:dbadin@ncsu.edu]
> **Sent:** Monday, July 10, 2017 11:31 AM
> **To:** (b) (6)
> **Cc:** Jones, Jennifer L; Reimschuessel, Renate
> **Subject:** Re: Pet food concern
>
> Thank you! I will work on this submission later today. I appreciate your help!
>
> On Mon, Jul 10, 2017 at 10:49 AM, (b) (6)@ncsu.edu> wrote:
> Hi Dr. Adin,
>
> As we discussed this morning, (b) (5) is the FDA program. Here is a website that highlights how you can report a complaint.
>
> https://www.fda.gov/AnimalVeterinary/SafetyHealth/ReportaProblem/ucm182403.htm
>
> We work with the FDA Vet-LIRN program on diagnostics from the pet side, but

they agree to include the case in the program and would coordinate with us (or another laboratory). I have copied Dr. Jones and Dr. Reimschuessel here - they can help let us know the process to see if these cases are eligible.

Regards,
(b) (6)

(b) (6)

Darcy B. Adin, DVM, DACVIM (Cardiology)
Clinical Assistant Professor of Cardiology
North Carolina State University
NC State Veterinary Hospital
1060 William Moore Drive
Raleigh, NC 27607
919-513-6032

--
Darcy B. Adin, DVM, DACVIM (Cardiology)
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Taurine and Carnitine in Canine Cardiomyopathy

Article *in* Veterinary Clinics of North America Small Animal Practice December 2006

DOI: 10.1016/j.cvsm.2006.08.010 Source: PubMed

1 author:

Sherry L Sanderson
University of Georgia
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Vet Clin Small Anim 36 (2006) 1325 1343
VETERINARY CLINICS
SMALL ANIMAL PRACTICE

Taurine and Carnitine in Canine Cardiomyopathy

Sherry Lynn Sanderson, DVM, PhD

Department of Physiology and Pharmacology, University of Georgia, College of Veterinary Medicine, 501 DW Brooks Drive, Athens, GA 30602, USA

Dilated cardiomyopathy (DCM) is one of the most common acquired cardiovascular diseases in dogs [1,4]. Although few studies of the prevalence of DCM in the overall population of dogs have been reported, estimates range from 0.5% to 1.1% [5,6]. Only degenerative valvular disease and, in some regions of the world, heartworm infection are more common causes of cardiac morbidity and mortality in dogs. DCM is seen most commonly in large and giant breeds of dogs, although its frequency seems to be increasing in medium sized breeds, such as the English and American cocker spaniels [4,8]. It has been reported rarely in small and miniature breeds of dogs [9].

DCM is particularly challenging to veterinarians because the cause is often unknown and can vary among dog breeds [10]. Because most cases of DCM in dogs are classified as idiopathic, most therapies can be classified as "Band Aid therapies" that palliate the effects of this disease for a short duration but do little to address the primary disease process. Therefore, DCM is almost always a progressive disease, and most dogs will eventually succumb to their disease. Survival times in dogs with DCM are variable and can be influenced by several factors, including breed. However, the prognosis for survival of dogs with DCM remains poor, with reported survival rates of 17.5% at 1 year and 7.5% at 2 years [11,13]. Until recently, reported cases of DCM reversal in dogs were very rare.

With advancements in echocardiology, diagnostic capabilities in canine cardiology have improved dramatically over the past 2 decades. Therapeutic advances have made surprisingly little progress. Symptomatic treatment is the standard care and outcome remains poor.

Recently, more promising therapies for dogs with DCM have resulted from a clearer understanding of the importance of biochemistry and nutrition in managing this disease. Nutrition is now widely accepted as an important adjunct to medical therapy in dogs with DCM.

E-mail address: sanderso@vet.uga.edu

01955616/06/$ – see front matter
doi:10.1016/j.cvsm.2006.08.010

vetsmall.theclinics.com

The importance of nutrition in managing DCM has changed dramatically in the past 10 to 15 years. Historically, dietary sodium restriction was the most common nutritional recommendation for dogs with DCM. The importance of other nutrients in the origin and management of this disease was largely unknown. More recently, widely accepted beliefs about the role nutrient deficiencies could play in DCM have been proven false, further enhancing the ability to direct therapy at an underlying cause rather than just the symptoms.

This article focuses on two nutrients, taurine and carnitine, that play an important role in the cause and treatment of DCM in some dogs. Known risk factors for developing deficiencies of these nutrients are discussed, along with the use of taurine and carnitine for treating DCM in dogs.

TAURINE

What is Taurine?

Taurine is a sulfur containing amino acid. Unlike most other amino acids, taurine is not incorporated into proteins but rather is one of the most abundant free amino acids in the body. Taurine is found in highest tissue concentrations in cardiac muscle, skeletal muscle, the central nervous system, and platelets [14].

Other than conjugation of bile acids and detoxification of xenobiotics through conjugation and excretion in bile, the function of taurine in mammals is not well understood but is highly diverse [14,15]. Since the mid 1970s, taurine has been known to be essential for normal retinal function in cats [16]. In addition, clinical and experimental evidence collected in the late 1980s documented that taurine is essential for normal myocardial function [17,20].

Taurine is involved with numerous metabolic processes, including antioxidation, retinal photoreceptor activity, development of the nervous systems, stabilization of neural membranes, reduction in platelet aggregation, and reproduction [15,16,21,26]. Although the importance of taurine for normal myocardial function is also well recognized, the mechanisms underlying its effect on the heart remain unknown. Much of the available evidence supports the theory that taurine's major effect on cellular function in the heart is modulating tissue calcium concentrations and availability [14,27,28]. In addition, taurine may inactivate free radicals and protect the heart by changing cellular osmolality [29]. Taurine may also have an effect on osmoregulation in the myocardium. Taurine is a small but highly charged osmotically active molecule, and experts have proposed that alterations in cellular osmolality induced by changes in intracellular taurine concentration are a protective mechanism in nervous tissue and myocardium [29]. Other proposed mechanisms specifically related to myocardial function include *N* methylation of cell membrane phospholipids [30], direct effects on contractile proteins [31,32], and interactions with the renin angiotensin aldosterone system [33]. Taurine is a natural antagonist of angiotension II.

Is Taurine an Essential Amino Acid in Dogs?

Taurine is an essential amino acid in cats, and it is well known that taurine de ficiency can cause DCM, retinal degeneration, and reproductive anomalies in this species [18]. However, taurine is not considered an essential amino acid in dogs. One explanation for the differences in taurine requirements between cats and dogs is that the activity of cysteine sulfinic acid decarboxylase (the rate limiting enzyme in the synthesis of taurine from cysteine and methionine) is higher in dogs than cats [34]. However, the difference in activity of this en zyme between dogs and cats does not fully explain the difference in require ments. The activity of this enzyme in humans is even lower than in cats, and taurine is not considered an essential amino acid in healthy adult humans. Therefore, cats and dogs may have additional differences that may explain why taurine is an essential amino acid in cats and not in dogs.

A study in dogs conducted in the 1980s at the University of California at Davis showed that feeding taurine free diets or diets found to be taurine deplet ing in cats [35] did not result in taurine depletion when fed to a group of eight healthy beagles [36]. In addition, results of an early clinical study in dogs, also conducted at this University soon after the relationship between taurine defi ciency and DCM was discovered in cats, were unrewarding. These studies showed that dogs could not become taurine depleted from diet alone, and that taurine did not play a considerable role in the development of DCM in dogs.

Emergence of Taurine Deficiency in Dogs with Dilated Cardiomyopathy

The belief that taurine deficiency could not cause DCM in dogs was challenged in 1989 when taurine deficiency was linked to DCM in foxes [37]. This study reopened taurine's possible role in DCM in dogs, and a collaborative study be tween the University of California at Davis and the Animal Medical Center in New York City was initiated [38]. In this study, plasma taurine levels were eval uated in dogs with DCM and in those with chronic degenerative mitral valve disease. Surprisingly, results of this study showed that plasma taurine concen tration was low in 17% of 75 dogs with DCM, and this deficiency occurred in breeds not commonly afflicted with DCM, such as American cocker spaniels and golden retrievers. However, because the plasma taurine concentration in breeds more commonly affected with DCM were within the reference range, experts concluded that taurine deficiency was unlikely to play an important role in the etiopathogenesis or therapy of DCM in dogs.

Multicenter Spaniel Trial (MUST) Study

Anecdotal reports emerged regarding supplementing American cocker spaniels diagnosed with DCM with taurine; however, initial reports of taurine supple mentation were unrewarding. When Kittelson and colleagues [8] gave taurine and L carnitine supplements to two American cocker spaniels with DCM, both dogs experienced response. These findings initiated the Multicenter Spaniel Trial (MUST) study. In this study, baseline plasma taurine concentrations and echocardiograms were collected in 11 American cocker spaniels diagnosed

with DCM. All dogs were found to have low plasma taurine concentrations at baseline (<50 nmol/mL). After baseline information was collected, dogs were randomly assigned to receive supplementation with both taurine (500 mg by mouth every 8 hours) and *L* carnitine (1000 mg by mouth every 8 hours) or a placebo for 4 months, and echocardiograms were reevaluated after 2 and 4 months of therapy. The group supplemented with both taurine and carnitine showed significant echocardiographic improvement, whereas dogs receiving the placebo did not.

After this initial 4 month period, dogs that had received the placebo initially received supplements of both taurine and carnitine, and subsequently showed echocardiographic improvement after 2 to 4 months of therapy. The magnitude of echocardiographic improvement in the American cocker spaniels was not as dramatic as that seen after taurine supplementation in cats with taurine deficiency DCM. Nonetheless, after 4 months of supplementation, the improvement in myocardial function in each dog was significant enough to allow discontinuation of cardiovascular drug therapy. Improvements were seen in not only cardiovascular function but also survival times. The mean survival time for dogs in this study was 28.3 ± 19.1 months, compared with an average life expectancy for dogs treated with conventional drug therapy of approximately 6 months. Based on results from this study, the current recommendation is to supplement American cocker spaniels diagnosed with DCM with both taurine and carnitine at the doses mentioned earlier.

University of Minnesota Study in Urolith-forming Dogs Diagnosed with Dilated Cardiomyopathy

Around the same time the MUST study was initiated, a separate clinical study was initiated at the University of Minnesota. The population of dogs studied consisted of those with either cystine or urate urolithiasis that developed DCM after long term consumption of a protein restricted diet that was being used to manage their stone disease (Sherry L. Sanderson, DVM, PhD, unpublished data, 1998). Dogs in group 1 underwent only conventional drug therapy for their heart disease, whereas those in group 2 underwent and taurine and/or carnitine supplementation in addition to conventional drug therapy as needed. Dogs in group 1 that were in Modified New York Heart Association (MNYHA) functional class I and II heart failure received enalapril (0.25 mg/kg by mouth every 12 hours) and digoxin (0.01 0.02 mg/kg by mouth divided twice a day), and dogs in MNYHA functional class III and IV received furosemide (dose varied depending on severity of heart disease) in addition to enalapril and digoxin. The population of dogs in group 1 (N 6) consisted of five English bulldogs (four with cystine urolithiasis, one with urate urolithiasis) and one Dalmatian with urate urolithiasis. The population of dogs in Group 2 (N 8) consisted of five English bulldogs (three with cystine urolithiasis, two with urate urolithiasis), two Dalmatians with urate urolithiasis, and one miniature Dachshund with cystine urolithiasis. Because when this study was initiated experts believed that dogs with DCM did not have low plasma taurine

concentrations, none of the dogs in group 1 had these concentrations evaluated at baseline. Plasma taurine concentrations evaluated before supplementation in seven of eight dogs in group 2 ranged from 2 nmol/mL to 45 nmol/mL (mean, 20.9 nmol/mL). These results were below the reference range of 41 nmol/mL to 97 nmol/mL that the investigators established from healthy adult beagles. Echocardiography was performed at baseline and once every 2 months. Details from this study will be published later, but a few interesting and important results were noted:

1. The average life expectancy for dogs in group 1 was 10.5 months, and all dogs were euthanized because of progressive congestive heart failure that became refractory to therapy. The average life expectancy for dogs in group 2 was 47.1 months, and only three of eight dogs were euthanized because of progressive congestive heart failure. In addition, three of five dogs that did not succumb to their heart disease received only taurine and/or carnitine supplementation and no conventional drug therapy for the management of their heart disease.
2. DCM reversed in three of eight dogs in group 2. DCM returned in one dog after the owner discontinued taurine and carnitine supplementation on their own, and in an additional dog when the dose of carnitine was reduced because of diarrhea associated with carnitine supplementation.
3. Dogs consuming a protein-restricted diet long-term could develop taurine deficiency, in contrast to results from previous studies that concluded that a diet could not induce taurine deficiency in dogs. This finding provided an impetus for further examining the effects on plasma and whole blood taurine levels in healthy adult dogs consuming a protein-restricted diet long-term.

Diet-Induced Taurine Deficiency in Healthy Adult Dogs

Previous reports indicated that dogs could not develop diet induced taurine deficiency, even when fed a diet devoid of taurine. However, based on the finding of University of Minnesota study that dogs developed low plasma taurine levels after consuming a protein restricted diet long term, a more controlled study was undertaken to determine the cause of this problem and evaluate the effects of long term taurine deficiency on cardiac function in healthy adult dogs [39].

This study involved 17 healthy adult beagles. Baseline plasma and whole blood taurine levels were evaluated, and echocardiography was performed to assess cardiac function. Once baseline data was collected, dogs were fed one of three protein restricted diets for 48 months. All three diets had similar levels of protein; one diet was also low in fat, a second was high in fat, and a third was high in fat and supplemented with *L* carnitine at 200 mg/kg of diet. All diets contained methionine and cystine concentrations at or above recommended minimum requirements established by the Association of American Feed Control Officials (AAFCO) [40]. After diet assignment, plasma taurine and whole blood taurine concentrations and echocardiography were evaluated every 6 months.

All three dietary treatments caused a significant decrease in whole blood taurine concentration compared with baseline concentrations. Dogs in the high fat

group also experienced a significant decrease in plasma taurine concentration. This study was the first to show that diet could induce taurine deficiency in healthy adult dogs, in contrast to previous studies.

Another important observation was that one dog with taurine deficiency developed DCM, and that taurine supplementation resulted in almost complete reversal of the disease. This study was also the first to clearly document in dogs that taurine deficiency preceded DCM, and that taurine supplementation resulted in substantially improved cardiac function, similar to cats.

Why Did Dogs Develop Taurine Deficiency While Consuming a Protein-Restricted Diet?

The exact mechanism for this problem is unknown. However, this study showed that the AAFCO recommended minimum requirements for amino acids may need to be modified in dogs consuming a protein restricted diet long term. Many therapeutic diets for dogs are now supplemented with taurine.

Additional Examples of Diet-Induced Taurine Deficiency in Dogs

Soybean based diets

Taurine deficiency was identified in two unrelated dogs fed a tofu based diet [41]. Although the diet was low in protein, it met the National Research Council's published requirements for protein and other nutrients in dogs [42]. The authors attributed taurine deficiency to the fact that the primary protein source was soybean curd, which is low in sulfur containing amino acids and devoid of taurine compared with meat proteins [43]. In addition, soybean curd has been shown to accelerate the loss of bile acids in cats [44].

Lamb meal and rice diets

Taurine deficiency was also identified in 12 Newfoundlands consuming two different commercially available lamb meal and rice diets [41]. Echocardiography was performed in six of the dogs, and none were diagnosed with DCM. The taurine deficiency was reversed when the diet was either changed or when the lamb meal and rice diets were supplemented with methionine. This study did not identify the exact mechanism for the development of taurine deficiency in the dogs consuming the lamb meal and rice diets.

In a study by Fascetti and colleagues [45], DCM and taurine deficiency were identified in 12 large and giant breed dogs consuming commercially available diets that contained lamb meal, rice, or both as primary ingredients. All dogs received supplements of with taurine (1000 3000 mg by mouth every 24 hours), and significant echocardiographic improvement occurred in 9 of the 12 dogs that underwent an echocardiogram repeated after taurine supplementation. The authors hypothesized that taurine deficiency caused DCM and was caused by inadequate or unavailable dietary sulfur amino acids, which are essential precursors of taurine synthesis.

In a similar report, five related golden retrievers were diagnosed with taurine deficiency and DCM [46]. Three of five dogs were consuming lamb meal and rice or lamb and rice diets. All showed significant improvement after taurine

supplementation (500 mg by mouth every 12 hours), and all five dogs survived for more than 3 years. The authors attribute the DCM to a suspected autosomal recessive mode of inheritance; however, the potential role diet played in the development of taurine deficiency warrants mentioning.

Potential Causes of Taurine Deficiency in Dogs Consuming Lamb Meal and Rice or Lamb and Rice Diets

Torres and colleagues [47] compared the effects of consuming a lamb meal and rice based diet with effects of consuming a poultry by product based diet in 12 beagles aged 5 to 5.5 months. Although the differences in plasma and whole blood taurine concentrations did not differ among diet groups, dogs consuming the lamb meal and rice based diet excreted less taurine in their urine than dogs consuming the poultry by product based diet. When the lamb meal and rice diet was supplemented with methionine, urinary taurine excretion increased by 54%. Because taurine homeostasis in dogs is achieved primarily through regulating renal taurine excretion, the amount of taurine excreted in urine is a sensitive indicator of the adequacy of either taurine synthesis or absorption of dietary precursor amino acids. The authors concluded that reduced bioavailability of sulfur amino acids in the lamb meal and rice diet is a likely cause of taurine deficiency. This finding is supported by the increase in urine taurine concentrations after supplementation with methionine. Johnson and colleagues [48] showed that ileal digestibility of amino acids in dogs depends on the raw material sources and the temperature used to process feeds and provides a mechanism for these specific dietary effects.

A second potential, although related, cause of taurine deficiency in dogs consuming lamb meal and rice diets was proposed [49,50]. When dietary protein is low in quality, undigested protein reaches the colon, where it serves as a substrate for bacterial growth. Some bacteria produce cholyltaurine hydrolase, an enzyme that causes release of taurine from taurocholic and other bile acids that are normally conserved in the enterohepatic circulation, resulting in increased fecal loss of taurine. Studies in dogs [49] and cats [50] have found that diets containing rice bran and whole rice products provide a source of moderately fermentable fiber and high amounts of fat. These fermentable fibers may increase the number of bacteria in the colon and result in a greater loss of taurine in the feces similar to the mechanism for undigested protein. The fat content of the diet can also affect taurine metabolism through altering intestinal bacteria and subsequent changes in the excretion of bile acids.

How Should Samples be Collected to Evaluate Plasma and Whole Blood Taurine Concentrations?

Fasting versus postprandial blood samples

Although fasting has no effect on plasma taurine concentrations in humans [51], food deprivation causes a small but significant reduction in plasma taurine concentrations in cats [52]. In a study by Torres and colleagues [47], plasma taurine concentrations were significantly reduced in food restricted dogs compared with ad libitum fed dogs. Whole blood taurine concentrations were

also reduced, although the whole blood taurine results were not statistically significant between the two groups. Because of the potential for food intake to affect plasma and whole blood taurine concentrations in dogs, withholding food, but not water, is recommended for 8 hours before sampling.

Anticoagulant used for plasma sample collection

Paired analysis of samples comparing taurine concentrations in plasma collected in lithium heparin with those collected in sodium citrate showed that plasma taurine concentrations are higher when lithium heparin is used as the anticoagulant [38]. Because most studies have used heparinized plasma samples to evaluate plasma taurine levels in dogs, these are recommended rather than sodium citrate plasma samples.

Plasma taurine sample collection

Heparinized, nonhemolyzed blood samples should be obtained and stored on ice until they are processed. After centrifuging, the plasma should be separated immediately from the cellular components, and a small amount of plasma should be left above the buffy coat to prevent contamination of the plasma with cells. Hemolysis and platelet or white blood cell contamination falsely elevates plasma taurine concentrations. Samples should be frozen until analyzed for plasma taurine concentrations.

Whole blood taurine sample collection

Heparinized whole blood should be frozen until samples can be analyzed. Because the red blood cells are lysed before analysis, hemolyzed samples do not adversely affect whole blood taurine analysis.

Plasma and whole blood taurine samples can be sent to the Department of Molecular Biosciences at the School of Veterinary Medicine, University of California, Davis, for analysis.

Which is Better: Plasma Taurine Concentrations or Whole Blood Taurine Concentrations

Earlier studies evaluating the relationship between taurine deficiency and DCM in dogs relied primarily on plasma taurine concentrations to predict tissue taurine concentrations. Studies conducted in dogs by this author showed findings similar to those reported in cats [53]. Relying on plasma taurine concentrations alone does not reliably assess tissue taurine concentrations in dogs. Simultaneously evaluating plasma and whole blood taurine concentrations predicts skeletal and cardiac muscle taurine concentrations better than evaluating either test alone. Therefore, when evaluating taurine status in dogs with DCM, plasma and whole blood taurine concentrations should be assessed simultaneously.

Reference Ranges for Plasma and Whole Blood Taurine Concentrations in Dogs

The reference range used in earlier studies evaluating plasma and whole blood taurine concentrations in dogs was extrapolated from the reference range use in

cats. However, reference ranges for plasma and whole blood taurine concentrations in dogs were published recently (Table 1).

Delaney and colleagues [49] have also suggested that plasma taurine concentrations less than 40 nmol/mL are critically low, as are whole blood taurine concentrations less than 150 nmol/mL. In addition, Sanderson and colleagues [53] found that low plasma taurine concentrations can exist without the presence of DCM.

Therefore, results showed that the onset of clinical signs in dogs, just as in cats, was variable when taurine concentrations declined markedly below the normal range [18].

Which Dogs Diagnosed with Dilated Cardiomyopathy Should Receive Taurine Supplementation?

Evaluation of plasma and whole blood taurine concentrations is recommended for all dogs diagnosed with DCM. An association between taurine deficiency and DCM was found in various breeds of dogs, including American cocker spaniels, Newfoundlands, golden retrievers, Labrador retrievers, Dalmatians, English bulldogs, and Portuguese water dogs. Taurine supplementation is highly recommended in any of these breeds that develop DCM.

Not all dogs with DCM will show dramatic improvement with taurine supplementation. However, even if plasma and whole blood taurine concentrations are within the reference range, giving taurine supplements to dogs diagnosed with DCM may still have some benefits. Because taurine is extremely safe and inexpensive, the risks and costs of supplementation are minimal, even if dogs have normal levels of plasma and whole blood. Proposed mechanisms for the beneficial actions of taurine on the myocardium include modulating tissue calcium concentrations and availability in the heart; inactivating free radicals and protecting the heart through altering cellular osmolality; osmoregulating the myocardium; directly affecting contractile proteins; and serving as a natural antagonist of angiotension II. Dogs with DCM that do not have taurine deficiency may still benefit from some of these proposed mechanisms of action for taurine.

Table 1
Normal concentrations of taurine in dogs

Plasma (nmol/mL)	Whole blood (nmol/mL)
41–97[a]	155–347[a]
72.8–81.2[b]	255.8–276.2[b]

[a]Reference range established from 18 healthy adult beagles consuming a canned commercial maintenance diet. *Data from* 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;62:1616–23.

[b]Reference range established from 131 healthy adult dogs of various breeds consuming a variety of commercial adult maintenance diets. *Data from* Delaney SJ, Kass PH, Rogers QR, et al. Plasma and whole blood taurine in normal dogs of varying size fed commercially prepared food. J Anim Physiol 2003;87:236–44.

Recommended dose for taurine supplementation

This author has successfully used doses of 500 to 1000 mg of taurine administered orally two to three times per day for small dogs (<25 kg), and 1 to 2 g of taurine administered orally two to three times per day for large dogs (25 40 kg). These doses have been shown to normalize plasma and whole blood taurine levels in taurine deficient dogs. Many other doses for taurine are reported in the literature. Whether a smaller or less frequent dose of taurine than what this author recommends can be used successfully remains to be determined. If doses are used that differ from those this author recommends, plasma and whole blood taurine concentrations must be reevaluated after taurine supplementation is initiated to determine if the dose being given is effective and appropriate. Another important point is that echocardiographic improvement in myocardial function is not usually documented before 2 months of supplementation, and often no improvement is documented before 4 months of supplementation. However, the dogs may feel better clinically and be more active before improvement in cardiac function is documented. Owners must not withdraw taurine supplementation prematurely before deciding if their dogs benefit.

Where Can Taurine be Purchased?

Taurine can be purchased through several retail outlets. If taurine is purchased through a health food store, consumers must look for a product that contains a USP certification symbol on the label. This symbol ensures that what is listed on the label is exactly what is found in the product.

LEVOCARNITINE (*L*-CARNITINE)

What is *L*-Carnitine?

L carnitine (β hydroxy γ trimethylaminobutyric acid) is a small water soluble molecule with a molecular weight of 160. In dogs, carnitine is obtained either from dietary protein or endogenous synthesis in the liver using the essential precursor amino acids lysine and methionine. Synthesis also requires iron, vitamin C, and vitamin B_6 as cofactors [54]. Although carnitine is classified as an amino acid derivative, it is not an α amino acid and the amino group is not free. Therefore carnitine is not used for protein synthesis [55].

Carnitine is found in the body either as free carnitine, short chain acyl carnitine, or long chain acylcarnitine. Acylcarnitine is carnitine bound to a fatty acid. Total carnitine is the sum of all the individual carnitine fractions. The free carnitine fraction is normally higher than either the short chain acylcarnitine fraction or the long chain acylcarnitine fraction.

Cardiac and skeletal muscles are significant storage sites, containing 95% to 98% of the carnitine in the body [56], and carnitine is concentrated in these tissues through an active membrane transport mechanism. The heart is unable to synthesize carnitine and depends on transport of carnitine from the circulation into cardiac muscle, which results in up to a 100 gradient between extracellular and intracellular concentrations.

Only the *L* form of carnitine exists naturally in the body. The *D* form competitively inhibits the actions of the *L* form, thereby inhibiting carnitine enzyme systems. In addition, mammals are unable to convert *D* carnitine to *L* carnitine, and therefore this discussion focuses on *L* carnitine.

Why is *L*-Carnitine Important for Normal Myocardial Function?

The normal heart obtains approximately 60% of its total energy production from oxidation of long chain fatty acids [57]. Long chain fatty acids in the cytosol of myocardial cells combine with coenzyme A (CoA) as the first step toward beta oxidation. However, long chain fatty acids must be transported across the inner mitochondrial membrane to generate energy, and the inner mitochondrial membrane is normally impermeable to such bulky polar molecules. Therefore, transport is accomplished through a "carnitine shuttle." In the carnitine shuttle, the activated fatty acid in the cytosol reacts with carnitine to form a more permeable molecule. This reaction occurs on the outer surface of the inner mitochondrial membrane and is catalyzed by the enzyme carnitine acyltransferase I. The newly formed long chain acyl carnitine ester molecule is permeable to the inner mitochondrial membrane and is transported across this membrane, where the enzyme acyltransferase II converts the long chain acyl carnitine back to free carnitine and the long chain fatty acid. Therefore, carnitine functions as a cofactor of several important enzymes necessary for transport of long chain fatty acids from the cytosol into the mitochondrial matrix [58,59]. Once inside the mitochondria, fatty acids undergo beta oxidation to generate energy [60].

Another important function of carnitine is its buffering capacity, which modulates the intramitochondrial acyl CoA:CoA ratio [58]. This process is important because acyl CoA is the activated form of fatty acids used for beta oxidation and lipid synthesis. However, buildup of acyl CoA derivatives in the mitochondria results in decreased free CoA, which inhibits oxidative metabolism. Acyl CoA derivatives also act as detergents at high concentrations. Carnitine also facilitates removal of accumulating short and medium chain organic acids from the mitochondria. Therefore carnitine also has a role in detoxification in the mitochondria.

What Causes *L*-Carnitine Deficiency?

Carnitine deficiency can be a primary or secondary disorder. Primary carnitine deficiencies may arise from genetic defects in synthesis, renal transport, intestinal absorption, transmembrane uptake mechanisms, or excessive degradation of carnitine [61]. In humans, primary carnitine deficiencies have been associated with cardiomyopathies that are usually not present at birth but take 3 to 4 years to develop. *L* carnitine therapy can prevent and reverse cardiac dysfunction in some patients.

Secondary carnitine deficiencies are believed to be much more common in humans and can have many causes [61]. In humans, carnitine deficiency can result from inborn errors of metabolism or develop in patients undergoing long term total parenteral nutrition, vegetarians, and infants fed formulas not

supplemented with carnitine. Carnitine deficiencies are recognized in dogs, but the incidence is not known.

What are the Consequences of *L*-Carnitine Deficiency?

Carnitine deficiency has been shown to cause or be associated with DCM in humans [62,64], hamsters [65,66], and dogs [36,67,69]. More widespread studies have not been undertaken in dogs because carnitine status is difficult to thoroughly assess.

What Types of Carnitine Deficiency Exist in Dogs?

Carnitine deficiency in dogs is classified as either (1) plasma carnitine deficiency, characterized by low concentrations of free plasma carnitine; (2) systemic carnitine deficiency, characterized by low concentrations of free plasma and tissue carnitine; or (3) myopathic carnitine deficiency, characterized by low free myocardial carnitine concentrations in the presence of normal and sometimes elevated plasma carnitine concentrations. Plasma carnitine deficiency alone is not a well documented state and is included to account for the fact that plasma carnitine, but not tissue carnitine sampling, is often pursued in veterinary medicine.

For example, if plasma carnitine concentration is used to assess carnitine status of a dog, it can help diagnose carnitine deficiency when it is low. However, if plasma carnitine concentration is normal, it does not rule out the possibility of the myopathic form of carnitine deficiency, and the myopathic form of carnitine deficiency is estimated to occur in 17% to 60% of dogs with DCM. Evaluating cardiac muscle carnitine concentrations requires a fluoroscopy guided endomyocardial biopsy, which is not practical to perform in most private practice situations and is not without risk. Therefore, diagnosing and determining the incidence of myopathic carnitine deficiency in dogs with cardiac disease remains elusive, but may be an underdiagnosed cause of DCM in dogs.

L-Carnitine Deficiency and Associated Myocardial Disease States in Dogs

Carnitine deficiency was associated with DCM in dogs in a limited number of clinical reports [8,9,68,70] . The first reported case of carnitine deficiency was in a family of boxers [69]. The sire, dam, and two littermates were diagnosed with DCM. One offspring had a low plasma carnitine concentration and low myocardial carnitine concentration at DCM diagnosis. After undergoing treatment with high dose *L* carnitine (220 mg/kg/d orally), this dog's fractional shortening (FS) increased from 18% to 28%. This dog's littermate had low myocardial and normal plasma carnitine concentrations and responded similarly to high dose *L* carnitine supplementation, with its FS increasing from 2% to 24%. The latter dog experienced a decline in myocardial function after *L* carnitine therapy was withdrawn. Both parents of these littermates had normal plasma and low myocardial carnitine concentrations. Unfortunately, both parents died soon after beginning *L* carnitine supplementation.

Costa and Labuc [70] presented another case report of two boxers with DCM. One was treated with 250 mg/kg/d of *L* carnitine orally, and the other was not treated. The myocardial concentration of carnitine was found to be low in the dog that did not receive supplementation and elevated in the dog that did.

Concurrent supplementation with carnitine and taurine has shown benefit in American cocker spaniels with DCM [8]. An unpublished study by this author in 1998 showed beneficial effects from carnitine supplementation in urolith forming dogs diagnosed with DCM while consuming a protein restricted diet (Sherry Lynn Sanderson, DVM, PhD, unpublished material). Both studies showed dramatic improvement in myocardial function and survival times in dogs that received supplementation.

Which Came First: Carnitine Deficiency or Dilated Cardiomyopathy?

A common argument made against the role of carnitine deficiency in dogs di agnosed with DCM is that if carnitine deficiency is diagnosed after the onset of DCM, whether carnitine deficiency caused the DCM or DCM caused the car nitine deficiency is unclear. When myocardial cells are damaged, as may occur with DCM, carnitine can leak out of the cells, resulting in low myocardial car nitine levels. In this situation, the DCM caused the carnitine deficiency. Most published studies linking carnitine deficiency to DCM in dogs have shown this scenario when carnitine deficiency was diagnosed after the onset of DCM.

In an unpublished study conducted at the University of Minnesota, this au thor documented carnitine deficiency before the onset of DCM in three dogs (Sherry Lynn Sanderson, DVM, PhD, unpublished material, 1998). Therefore, the association of carnitine deficiency with DCM at diagnosis may not always imply a cause and effect relationship. However, this study indicates that carni tine deficiency can cause DCM in dogs.

Which Dogs with Dilated Cardiomyopathy Should Receive Carnitine Supplementation?

The importance of carnitine supplementation in the treatment and survival times of some dogs with DCM should not be overlooked. In the first reported study linking carnitine deficiency to DCM in boxers, two of four dogs experi enced good response to carnitine supplementation [69]. Considering the gener ally poor prognosis of this disease in boxers, carnitine supplementation provides owners one additional option for treating this disease, and has made a dramatic difference in the survival times and quality of life of some dogs.

The importance of carnitine supplementation in American cocker spaniels with DCM and urolith forming dogs with DCM should also not be over looked. Although a few anecdotal reports exist in which American cocker span iels with DCM experienced good response to taurine supplementation alone, most cases have shown response to combined supplementation with taurine and carnitine. In the above study by this author, a miniature Dachshund diag nosed with carnitine deficiency before the onset of DCM underwent treatment

only with carnitine supplementation, and its heart disease reversed. Although DCM in many dogs is not associated with carnitine deficiency, carnitine and taurine supplementation offer the most promising hope for improved quality of life and survival times in dogs that experience response.

How is Carnitine Deficiency Diagnosed?

Because performing endomyocardial biopsies is impractical for most clinicians in private practice, most screening for carnitine deficiency relies solely on plasma carnitine levels. The method for plasma carnitine sample collection is almost identical to that used for plasma taurine sample collection. Fasting, heparinized, nonhemolyzed blood samples should be obtained and stored on ice until they are processed. The plasma should be immediately separated from the cellular components ideally in a cold centrifuge, and a small amount of plasma should be left above the buffy coat to prevent contamination of the plasma with cells. Samples should be frozen immediately until analyzed for plasma carnitine concentrations.

What is the Recommended Dose for Carnitine Supplementation in Dogs?

The doses of carnitine being administered may contribute to the lack of favorable results with carnitine supplementation that some investigators observed. The recommended doses for carnitine supplementation in dogs with DCM vary widely in the literature. Although most authors recommend a carnitine dose of 50 to 100 mg/kg orally every 8 hours, the effective dose may depend on the form of carnitine deficiency. In a limited number of cases studied at the University of Minnesota, where pre and post carnitine supplemented plasma and cardiac muscle carnitine levels were obtained, this author's clinical impression was that the effective therapeutic dose in dogs with systemic carnitine deficiency was much lower than the effective dose in dogs with myopathic carnitine deficiency.

Some experts speculate that the myopathic form of carnitine deficiency may be caused by a carnitine transport defect in the heart, and much higher plasma levels of carnitine seem to be needed to overcome this defect and achieve normal concentrations of carnitine in the heart than for the systemic form of carnitine deficiency. Based on this work, the dose of carnitine recommended by this author for systemic carnitine deficiency is 100 mg/kg orally every 8 hours. However, if the myopathic form of carnitine deficiency is present or suspected, the author recommends starting carnitine supplementation at 200 mg/kg orally every 8 hours to maximize the chances that carnitine supplementation will improve myocardial function.

Carnitine is a very safe substance. Diarrhea was the only adverse effect of high doses of carnitine, reported in approximately two thirds of dogs. If diarrhea occurs, the highest dose of carnitine that the dog will tolerate without causing diarrhea should be administered. Therefore, like taurine, *L* carnitine is a safe substance to administer, and, except for the expense, few drawbacks exist to supplementing a dog with DCM with carnitine (carnitine is much more expensive than taurine. Another important point is that the time it takes for

improvement in myocardial function to occur is very similar to that for taurine supplementation. Echocardiographic improvement in myocardial function is not usually documented before 2 months of supplementation with carnitine, and often improvement is not documented for up to 4 months. However, dogs may feel better clinically and be more active before improvement in cardiac function is documented. Owners must not withdraw carnitine supplementation prematurely before determining whether their dogs benefit.

Where Can *L*-Carnitine Be Purchased?

Although *L* carnitine can be purchased from health food stores, this source is extremely expensive. Purity of the sample is also of great importance. Therefore, only products that contain the USP certification seal should be purchased from health food stores.

L Carnitine can also be purchased less expensively in bulk. Bulk carnitine can be purchased from Ajinamotousa, Inc (500 Frank W Burr Boulevard; Park Central West; Teaneck, New Jersey). At last check, the company required a minimum purchase of 10 kg at one time. However, the individual expense can be reduced if several owners split an order. If carnitine is purchased in bulk, owners must measure out the carnitine they are giving to their dogs. One teaspoon of carnitine is equivalent to 2 g of carnitine. Therefore, fractions of a teaspoon can be administered if necessary. Owners must be sure to purchase *L* carnitine, not *D* or the *DL* isomers, because *D* carnitine interferes with *L* carnitine use.

Which Dogs with Dilated Cardiomyopathy Should be Supplemented With Carnitine?

Carnitine supplementation should be recommended for boxers, American cocker spaniels, and dogs with cystine or urate urolithiasis that are diagnosed with DCM. Even if carnitine deficiency did not cause DCM, supplementing dogs with carnitine does not hurt them, and supplementation may be beneficial even if carnitine deficiency is not present. The major drawback to supplementing dogs with carnitine is the expense and occasional gastrointestinal upset.

What are the Reference Ranges for Carnitine Concentrations in Dogs?

The reference ranges for carnitine concentrations in dogs are listed in Table 2 [69].

SUMMARY

Some newer more promising therapies for dogs with DCM do not involve drugs but rather nutritional supplements. Two of the more common nutritional supplements administered to dogs with DCM are taurine and carnitine. Deficiencies of these nutrients have been shown to cause DCM in dogs, and some breeds have been shown to experience dramatic improvement in myocardial function after supplementation with one or both nutrients. Although most dogs diagnosed with DCM do not have a documented taurine or carnitine deficiency, they may still benefit from supplementation. Both nutrients are very

Table 2
Normal concentrations of carnitine in dogs

Carnitine fraction	Plasma carnitine (nmol/mL)	Cardiac muscle carnitine (nmol/mg of NCP)
Free	8–36	4–11
Esterified	0–7	0-4
Total	12–38	5–13

Abbreviation: NCP, noncollagenous protein.

safe to administer to dogs. For some owners, the high cost of carnitine is the only deterrent to giving their dogs supplements of both nutrients.

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Sample Submission Form

Amino Acid Laboratory
University of California, Davis
1020 Vet Med 3B
1089 Veterinary Medicine Drive
Davis, CA 95616
Tel: (530)752-5058, Fax: (530)752-4698

http://www.vetmed.ucdavis.edu/vmb/labs/aal/index.cfm

UC CUSTOMERS ONLY:
Non-federal funds ID/Account Number to bill:_____

Vet/Tech Conta (b) (6)
Company Nam
Address:

Email: (b) (6)
Tel: **Fax:** (b) (6)

Billing Contact: (b) (6) **TAX ID:**
Email: (b) (6) **Tel:** (b) (6)

Patient Name: (b) (6)
Species: Feline
Owner's Name: (b) (6)

Sample Type: ☐ Plasma ☒ Whole Blood ☐ Urine ☐ Food ☐ Other:_____
Test Items: ☐ Taurine ☐ Complete Amino Acid ☐ Other:_____

Taurine Results (nmol/ml)

Plasma:_____ Whole Blood: 368 Urine:_____ Food:_____

Reference Ranges (nmol/ml)

	Plasma		Whole Blood	
	Normal Range	No Known Risk for Taurine Deficiency	Normal Range	No Known Risk for Taurine Deficiency
Cat	80-120	>40	300-600	>200
Dog	60-120	>40	200-350	>150

NC State University
Veterinary Hospital

Fax: Admin
Fax: Referral

1052 William Moore Drive
Raleigh, NC 27607

Small Animal (919) 513-6500
Large Animal (919) 513-6630

Discharge Comments

Client	Patient			
(b) (6)	(b) (6) SCHNAUZER MC BLACK CANINE	Case # (b) (6) 8.2 kg	Attending DVM Student Discharging DVM Referring DVM	(b) (6) (b) (6) (b) (6) (b) (6)

Admission Date/Time (b) (6) 09:55 PM **Discharge Date/Time:** (b) (6) 10:57 AM **Discharge Status:**

**********NOTICE OF EUTHANSIA**********

Case Summary

Diagnosis:

1) Biventricular congestive heart failure (left significantly worse than right)
2) Cardiomyopathy (suspect secondary) vs. myocarditis vs. tachycardia-induced cardiomyopathy vs. other

History:
(b) (6) is a 2 and ½ year old male castrated Miniature Schnauzer who presented the NCSU ER on Thursday, (b) (6) for labored breathing and was subsequently transferred to NCSU Cardiology. (b) (6) initially developed a cough three weeks ago; (b) (6) describes the cough as a wheezing-type cough that occurred more frequently at night. When (b) (6) showed no signs of improvement, (b) (6) presented to his primary veterinarian on Thursday (b) (6). Kennel cough was suspected as the underlying cause of his cough and prednisone and doxycycline were prescribed. On Monday (b) (6) (b) (6) became uninterested in his food and began vomiting. The following day (b) (6) continued vomiting and developed labored breathing and subsequently re-presented to the rDVM for evaluation. Bloodwork and thoracic radiographs were performed. Bloodwork was reportedly unremarkable at this time and there was concern for aspiration pneumonia on his radiographs. Nebulization was performed and subcutaneous fluids, enrofloxacin, unasyn, and gentamicin were administered for treatment. While in-hospital, (b) (6) regurgitated. On Wednesday (b) (6), (b) (6) had improvement in respiratory effort but he still was not eating; subcutaneous fluids, enrofloxacin, unasyn, and gentamicin were performed again on an outpatient basis. The day of presentation (b) (6), (b) (6) syringe fed (b) (6), but as she attempted he immediately regurgitated. (b) (6) developed marked labored breathing following this and was presented to an emergency hospital. Thoracic radiographs were performed (uploaded in eFilm) and revealed cardiomegaly; a diffuse, severe mixed interstitial to alveolar pattern that is most severe caudodorsally; hepatomegaly; and decreased abdominal serosal contrast. (b) (6) was referred to NCSU for further care and ventilation if indicated.

(b) (6) has a history of developing coffee brown urine, sometimes with clumping, after strenuous activity when it is hot outside. The discolored urine typically develops after the activity and lasts 24-36 hours before clearing up. A urinalysis was performed and (b) (6) primary veterinarian detected crystals in the urine. (b) (6) diet was changed to an unknown diet to decrease the amount of crystals prevent stone formation; this diet was ultimately discontinued. Other than this, prior to the coughing that began 3 weeks ago (b) (6) was a normal, healthy dog with no significant medical history. He previously had no respiratory signs or changes in drinking/appetite/urination/defecation. (b) (6) lives with one other dog (not a relative) who is healthy and is currently up to date on his vaccinations (b) (6) is not current on any flea/tick prevention but receives heartworm prevention. (b) (6) is fed California Natural dog food.

Physical Exam Findings (on presentation):
Weight: 8.0 kg BCS: 5/9
T: 100.2 F
P: 160 bpm
R: 64 breaths/min
MMs/CRT: pale pink / <2 sec
Attitude: Alert, responsive
Hydration: Adequate

EENT/Oral: No ocular discharge noted, clear ear canals AU, no crusting/erythema noted; moist/flat nasal planum, no nasal discharge noted
PLN: No peripheral lymphadenopathy noted
CV/R: Grade I-II/VI left apical systolic murmur, femoral pulses hypokinetic but synchronous; jugular venous distention present; normal, albeit tachycardic, rhythm auscultes; dyspneic, inspiratory crackles in all lung fields on bilateral auscultation
GI/GU: Abdomen tense but non-painful on palpation; cranial abdominal organomegaly noted; no obvious fluid wave or masses noted
MSK: Appropriate and symmetrical muscling; ambulatory with no observed lameness; full ortho exam not performed due to patient's status
INTEG: Clean hair coat, no evidence of ectoparasites

NEURO: Mentally appropriate, intact cranial nerve reflexes, limited exam performed due to patient's status

Main Diagnostics (b) (6)
1. Big 4 - Glu: 135, Azo: 15-20, PCV: 40%, TS: 5.0 g/dL
2. Venous blood gas: pH 7.34, PCO2 46, lactate 2.4, HCO3 24.8
3. CBC - WBC 9.4, PCV 45, Seg 7.9, Band 0.18, Plt 157
4. Chemistry - BUN 19, creat 0.4, Phos 6.2, K 4.9, Na 140, TP 4.2
5. Blood Pressure - 90 mmHg systolic via Doppler
6. Urinalysis (post-lasix) - USG 1.011, otherwise unremarkable
7. Cardiac Troponin I - 0.79
8. Bap GM - pending
9. Vector borne panel - pending
10. Taurine levels - pending
11. Carnitine Levels - pending
12. Echocardiogram - Severely dilated and hypocontractile left and right ventricles, severely dilated left and right atria. Changes consistent with DCM (primary vs. secondary) vs. myocarditis vs. pacing-induced vs. other

Main Diagnostics (b) (6)

1. Chemistry - Gluc 225, BUN 29, Creat 0.7, phos 11.7, TP 5.0, ALT 53, CK 709, K 3.3, Cl 95, Na 144
2. Chest radiographs (9:15 AM) - final report pending - Severe generalized cardiomegaly with biventricular heart failure; improved from rDVM radiographs taken prior to presentation
3. Chest radiographs (5:00 PM) - final report pending - Progressive severe diffuse alveolar pattern consistent with worsening cardiogenic pulmonary edema; cannot exclude ventilator - induced lung injury and/or pneumonia

Main Diagnostics (b) (6)

1. CBC - WBC 9.9, Plt (clumping), PCV 44, Seg 8.0, Band 0.7
2. Chem - Gluc 136, BUN 12, Creat 0.7, Phos 4.6, ALT 88, CK 13,621, K 4.3, Na 151, Cl 109, AST 577
3. Coag - PT 9.1, PTT 14, Dimer 189, Fib 539, INR 1.09
4. Chest radiographs (1:30 AM, immediately post-ultrafiltration) - final report pending - markedly improved pulmonary infiltrates consistent with improved cardiogenic edema; residual interstitial to patchy alveolar pattern, decreased caudal cava size consistent with hypovolemia
5. Chest radiographs (11:00 AM) - final report pending - overall improved pulmonary pattern with persistent and in some areas slightly more condensed interstitial to patchy alveolar pattern. Consistent with continued improvement of congestive heart failure with possible concurrent bronchopneumonia; left lateral image shows suspected hiatal hernia

Main Diagnostics (b) (6)
1. CBC - WBC 6.8, PCV 39, Prot 6.9, Seg 4.2, Band 0.54, Toxic Neut Mild, Plt 97
2. Chem - Gluc 165, BUN 37, Creat 0.9, Phos 8.1, ALT 147, AST 1006, CK 35,930, Na 135, K 3.8, Cl 90
3. Urine Creat - 27.9
4. Urine Sodium - pending
5. Chest radiographs (10:00 AM) - final report pending - markedly progressive alveolar pattern consistent with significantly worsened cardiogenic edema; cannot exclude less likely differentials such as ARDS, ALI, hemorrhage, PTE, and pneumonia
6. ECG - suspect atrial tachycardia

Main Therapeutics Throughout Hospitalization (PO medications given through NG tube)

1. Furosemide - 2 boluses given on presentation followed by 1mg/kg/hr CRI with intermittent boluses given as needed
2. Pimobendan - initially 2.5mg PO TID increased to 5mg PO QID throughout hospitalization
3. Dobutamine - titrated between 7.5 mcg/kg/min increasing up to 20mcg/kg/min throughout almost entirety of hospitalziation
4. Maropotant - 1mg/kg IV SID
5. Meropenem - 1.06mg/kg/hr CRI
6. Cisapride - 7.4mg PO TID
7. Pantoporazole - 7.4mg IV BID
8. Nitroglycerin Paste
9. Torsemide - 2.5-5mg PO BID
10. Hydrochlorothiazide - 6.25mg PO BID (started (b) (6))
11. Diltiazem (started (b) (6)) - 0.25mg IV (given slowly over 25 minutes) followed by CRI of 2-5mcg/kg/min (titrated PRN)
12. Triple antibiotic OD
13. NG tube feeding - as recommended per NCSU Nutrition service with supplements of Fish oil, taurine, and carnitine

Brief Daily Summary:

(b) (6) (b) (6) presented late in the evening on (b) (6) to the ER and after a TFAST was performed showing severe cardiomegaly with hypocontractility of the ventricles in addition to reviewing the rDVM radiographs, pimobendan and Lasix were given. An

echocardiogram was then performed (performed sternally cage-side given patient status) and a diagnosis of severe cardiomyopathy (primary vs. secondary DCM vs. myocarditis, vs. pacing-induced cardiomyopathy vs. other) and he was quickly given another dose of Lasix.

(b) (6) After the second bolus of Lasix was given he was immediately placed on a Lasix CRI at 1mg/kg/hr and Dobutamine at 5mcg/kg/min quickly uptitrated to 10mcg/kg/min. After 4-6 hours of clinical worsening and the suspicion for respiratory fatigue, mechanical ventilation was recommended to the owner and pursued. Recheck radiographs (first rads performed at NCSU) after being on ventilation and continued CHF treatment showed continued severe pulmonary infiltrates but significantly improved compared to rDVM rads prior to any intervention. Because of this improvement we continued with aggressive CHF management and mechanical ventilation. Throughout the day he started to show some worsening clinically while on the ventilator and recheck radiographs were performed around 4:30PM. These radiographs showed worsening of the pulmonary infiltrates despite aggressive therapy (dobuatmine, pimobendan, Lasix, etc.). At that time aquapheresis was discussed with the owner and pursued. This was from approximately 7PM-1AM (including setup and moving the patient. This procedure was performed successfully with no significant complications.

(b) (6) Immediately after aquapheresis therapy recheck radiographs were performed (approximately 1:30AM) which showed a marked improvement in terms of pulmonary infiltrates. An interstitial to alveolar pattern persisted, mostly ventrally distributed, but was significantly improved. He was maintained on mechanical ventilation until approximately 5-6:00 AM when he was slowly weaned off ventilation and extubated. He handled this quite well and while sedated as the medications wore off, he clinically was markedly improved from presentation. His congestive heart failure medications were continued at aggressive doses (dobutamine at 15-20mcg/kg/min, Lasix had continued to be at 1mg/kg/hr CRI since presentation, Pimobendan was approximately 0.52mg/kg PO TID etc.). Recheck radiographs were performed around 2:00PM which showed an overall improvement in the pulmonary pattern, although a ventrally distributed alveolar pattern persisted and in some areas were slightly worse. We continued therapy for CHF and the patient was already being covered for pneumonia with meropenum (this antibiotic was chosen based on the patient's recent history of many antibiotics given including doxycycline, enrofloxacin, unasyn, and gentamycin). He continued to do well until acutely worsening was seen during the owner visit approximately 4:00PM (20 minutes into the visit) where his respiratory rate/effort declined. He was given another bolus of Lasix on top the CRI, butorphanol, and an increase in dobutamine. He was able to relax and showed slight improvement, although was unable to breathe as comfortably as prior to the episode. He remained tachycardic throughout the night but otherwise normal.

(b) (6) He remained tachycardic throughout the night at approximately 180bpm and the following morning he showed signs of atrial tachycardia at a rate of 190bpm. He was weaned off his dobutamine and started on diltiazem boluses followed by a CRI which resolved the atrial tach and a sinus tach at 150bpm persisted. He clinically was worse than the day before and repeat chest radiographs were performed that showed severe worsening of his pulmonary infiltrates. After discussion with the owner, we elected to continue even more aggressive diuretic therapy (multiple boluses of Lasix on top of the 1mg/kg/hr CRI he had been on almost since presentation) but by 3:00 PM after only further worsening the decision for euthanasia was made. The owners elected for necropsy with private cremation.

I am sorry for the loss of your patient. Both (b) (6) were absolutely wonderful to work with. If you have any questions at all, please do not hesitate to call us at 919-513-6694.

(b) (6) DVM

NC State University
Veterinary Hospital
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Fax: Referral
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Small Animal (919) 513-6500
Large Animal (919) 513-6630

Discharge Comments

Client	Patient		
(b) (6)	(b) (6) SCHNAUZER MC GRAY&WHITE CANINE	Case # (b) (6) 9.9 kg	Attending DVM (b) (6) Student Discharging DVM (b) (6) Referring DVM (b) (6) (b) (6)

Admission Date/Time: (b) (6) 10:46 AM **Discharge Date/Time:** (b) (6) 06:25 PM **Discharge Status:** UNDETERMINED

CASE SUMMARY:

HISTORY:
(b) (6) a 7 year old male castrated Schnauzer, was presented to NCSU Small Animal Emergency on (b) (6) for episodes of collapse. (b) (6) first collapse episode was in Mid February; he was described to fall over for 6 seconds with no loss of consciousness, and he immediately returned to normal after. (b) (6) had another collapse episode two weeks later, which looked the same and lasted the same amount of time as the first. (b) (6) next episode of collapse was 6/3/17, after a two hour hike, when he collapsed and screamed, which lasted for six seconds, and he was normal after. (b) (6) experienced another collapse a week after, he whined, it lasted six seconds, and was normal after. A week after that, (b) (6) had another collapse episode, where a puppy ran towards him, (b) (6) tried to run with him, stopped, got wobbly, stood still, and was normal after a few seconds, but did not fall to the floor. (b) (6) also reports that he is panting more than usual. He had a good appetite for treats, but seems more reluctant to eat his food since February. He does eventually eat his food if mixed in with some treats. (b) (6) is fed California Natural, Kangaroo or Venison flavor. There has been no change with (b) (6) urination or defecation, and is otherwise healthy. (b) (6) is up to day on flea and tick preventative with a three month Bravecto given on 5/2/17, but is not up to day on Heartworm preventative with Heartgard last given on 4/26/17.

PHYSICAL EXAM FINDINGS:
Weight: 9.9 kgs
T: 100.6 F
P: 130 bpm
R: pant
MMs/CRT: pink/<2 sec
BSC: 5/9
Pain Score: 0/4
Attitude: BAR
Hydration: euhydrated
EENT: Unremarkable cornea/sclera OU, ears free of debris, no nasal discharge, no oral ulcers/lesions/foreign bodies detected
PLN: No lymphadenopathy detected
CV/R: No murmur or arrhythmia auscultated, femoral pulses strong and synchronous, normal bronchovesicular lung sounds noted on bilateral auscultation
GI/GU: Normal on palpation, no fluid wave present, no organomegaly detected
MSK: Ambulatory on all four limbs with no observed lameness.
INTEG: Clean hair coat, no evidence of ectoparasites
NEURO: BAR, normal gait on all four limbs, intact reflexes, cranial nerves intact

DIAGNOSTIC TESTS:
1. Big 4: BG 64 (recheck 79); BUN 15-26; PCV 45; TS 7.2
2. Blood pressure (Doppler): 130 mmHg
3. Echocardiogram: Moderately to severely decreased LV ejection fraction, LV cavity size is severely increased, severely dilated LA, moderate to severe mitral valve regurgitation, anterior and posterior leaflets mild thickened due to endocardiosis, mild tricuspid regurgitation, moderately elevated pulmonary artery systolic pressure. Diagnosis: mitral valve endocardiosis with left atrial enlargement and heart failure, decreased left ventricular systolic function, suspect dilated cardiomyopathy
4. ECG: regular sinus rhythm, complexes suggest LV enlargement, tall R waves
5. Thoracic radiographs: Severe left-sided cardiomegaly with moderate left atrial enlargement, unstructured interstitial pattern, and mild lobar venous distention: most consistent with left-sided congestive heart failure. ***Final report pending***
6. Urinalysis (cytocentesis): USG 1.019, protein negative, quiet sediment

Pending Lab Results:
1. Taurine
2. Carnitine
3. Vector borne panel

4. BAPGM
5. Troponin-I
6. T4
7. Toxoplasma
8. Neospora
9. Chagas
10. Complete amino acids

TREATMENTS:
1. Butorphanol 0.2 mg/kg IV
2. Furosemide 2 mg/kg SQ
3. Pimobendan 2.5 mg PO

ASSESSMENT:
Thank you for bringing (b) (6) in to see us! He is a very cute and sweet boy. (b) (6) presented to us today for evaluation of progressive episodes of collapse. On physical exam today, (b) (6) was stable, but he had mildly increased breath sounds noted in all lung fields. During more excitable moments, (b) (6) showed increased respiratory effort with an abdominal component to his breathing pattern. We consulted with the NCSU Cardiology Service, who performed an ECG and echocardiogram. Unfortunately, (b) (6) echocardiogram revealed evidence of mild mitral valve endocardiosis (i.e. chronic mitral valve disease) and suspected dilated cardiomyopathy. Mitral valve endocardiosis is a chronic, progressive condition in which the valve leaflets become progressively thickened and no longer close appropriately allowing mitral regurgitation. Over time, the left atrium will enlarge and this can lead to congestive heart failure. Typically, in patients with mitral valve disease, the systolic heart function/contractility is maintained until late stages of the disease. Unfortunately, (b) (6) systolic function was significantly decreased, and he showed abnormal dilation of his left atrium and ventricle. These findings were most consistent with a condition called dilated cardiomyopathy (DCM). DCM is a disease of unknown etiology affecting the muscle of the heart and is most commonly seen in large breed dogs (such as Dobermans, Great Danes, and Labrador Retrievers), but there is a small case report of this disease occurring in Standard Schnauzers. Although the exact mechanism of DCM is currently unknown, dietary taurine/carnitine deficiencies, genetics, infectious diseases, and toxins have all been linked to DCM. In order to assess for some of these possible causes, we have submitted testing for multiple nutritional deficiencies and infectious diseases. The NCSU Cardiology Service will call you as these tests become available. DCM leads to poor contractility and low cardiac output, and we suspect that (b) (6) episodes of collapse are most likely due to his low cardiac output during exertion. DCM can also lead to fibrosis and remodeling of the myocardium, which can lead to secondary arrhythmias. Fortunately, we saw no evidence of arrhythmias on (b) (6) ECG today. We performed chest radiographs to evaluate (b) (6) heart and lungs, and (b) (6) had evidence of left-sided congestive heart failure on his radiographs and impending right-sided congestive heart failure on his echocardiogram today. We are starting him on three medications to treat his heart disease and congestive heart failure today, and we may consider adding additional medications and supplements in the future. Please monitor (b) (6) for signs of worsening of his heart failure such as increased exercise intolerance, labored breathing, increased coughing or fainting. Call NCSU Cardiology or your referring veterinarian if any of these signs occur. Also, please start taking respiratory rates when (b) (6) is resting. This number should improve on his medications and remain less than 40 at rest. Please contact us or his primary care veterinarian if his respiratory rate or effort are worsening or you have any other concerns.

INSTRUCTIONS FOR CARE OF YOUR PET:
Medications:
1. Pimobendan 2.5 mg capsules - Please give 1 capsule by mouth every 12 hours as directed. This medication dilates blood vessels (decreasing the workload of the heart) and increases the heart's pumping ability. Possible side effects include vomiting, diarrhea, and arrhythmias. Please begin this medication tonight.

2. Enalapril 5 mg tablets - Please give 1 tablet by mouth every 12 hours as directed. This is an ACE-inhibitor and is used as a cardioprotective medication to decrease remodeling and fibrosis of the heart. This medication will be given lifelong. Possible side effects include decreased blood pressure (hypotension) and decreased blood flow through the kidneys. Blood pressure and kidney bloodwork will need to be periodically monitored while receiving this medication. Please begin this medication tonight.

3. Furosemide 12.5 mg tablets - Please give 1 tablet by mouth every 12 hours as directed. This is a diuretic. Possible side effects include electrolyte abnormalities and decreased blood flow through the kidneys. Kidney bloodwork and electrolytes will need to be periodically monitored while receiving this medication. Please begin this medication tonight.

Activity / Cautions:
Please keep (b) (6) quiet until his recheck exam in several weeks. (b) (6) should be able to regulate his own energy level at home and be taken out for short bathroom breaks.

Diet:
Please continue (b) (6) current diet for the next several weeks. After this, he should be transitioned to a new meat-based commercial diet. We recommend that you consider one of the larger pet food brands (i.e. Royal Canin, Hills, Iams, Purina). Avoid salty treats, such as hot dog or jerky treats.

Monitoring:
1. Please monitor (b) (6) for signs of cardiac decompensation. This would include lethargy, cough, difficulty breathing (increased respiratory rate/effort), worsened abdominal distension, and episodes of collapse or fainting.

2. Please monitor (b) (6) resting respiratory rate. In order to do this, you can count the number of times that (b) (6) breathes in 15 seconds and multiply this number by 4 to get the number of breaths per minute. If you notice that this number is increasing or that it is consistently greater than 35-40 breaths per minute, then you should have (b) (6) evaluated by a veterinarian. A mobile app (Cardalis) is available to record instant rates and trends in respiratory rates.

PLAN FOR RE-EVALUATION OF YOUR PET:
Please call the Cardiology Service tomorrow to schedule an appointment for (b) (6) during the week of July 10th. You can schedule this appointment by calling (b) (6). Please bring a sample of (b) (6) current diet to this appointment for possible testing. He will also likely have a blood pressure, chest radiographs, and a renal panel performed during this appointment to re-evaluate his heart failure.

If you have any questions or concerns before this appointment, please have (b) (6) re-evaluated by your primary care veterinarian or the NCSU Emergency Service. (b) (6) may need recheck bloodwork and chest X-rays prior to this appointment if he is not doing well. He also may need medication adjustments and potentially hospitalization.

In order to help expedite medication refills, please visit us online at www.ncstatevets.org and select Pet Owners, Pharmacy Refills.

(b) (6) (b) (6) (b) (6)

SMALL ANIMAL EMERGENCY SERVICE TEAM:

Faculty:
(b) (6)

Residents/Fellows:
(b) (6)

Interns:
(b) (6)

Supervisor:
(b) (6)

Technicians:
(b) (6)

Client Services:
(b) (6)

Referring Veterinarians - please visit us online at www.ncstatevets.org/veterinarians and fill out our RDVM Feedback Survey!

The following instructions were provided at discharge

CLINICIAN: (b) (6), DVM

Date: (b) (6)

CASE SUMMARY:
Thank you for entrusting us with the care of your companion. The Discharge Summary will be emailed to you and faxed to your RDVM within 24 hours of release/discharge from our facility at (b) (6). If you or your veterinarian do not receive this, please contact the (b) (6) Emergency Service to request a copy. The following will briefly outline the care your companion should receive at home and was explained by our staff at the time of discharge:

DIAGNOSIS (ES):
1. Left-sided congestive heart failure
2. Mitral valve endocardiosis with left atrial enlargement
3. Dilated cardiomyopathy

TREATMENTS:
1. Butorphanol 0.2 mg/kg IV
2. Furosemide 2 mg/kg SQ

3. Pimobendan 2.5 mg PO

INSTRUCTIONS FOR CARE OF YOUR PET:
Medications:
1. Pimobendan 2.5 mg capsules - Please give 1 capsule by mouth every 12 hours as directed. This medication dilates blood vessels (decreasing the workload of the heart) and increases the heart's pumping ability. Possible side effects include vomiting, diarrhea, and arrhythmias. Please begin this medication tonight.

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Activity / Cautions:
Please keep (b) (6) quiet until his recheck exam in several weeks. (b) (6) should be able to regulate his own energy level at home and be taken out for short bathroom breaks.

Diet:
Please continue (b) (6) current diet for the next several weeks. After this, he should be transitioned to a new meat-based commercial diet. We recommend that you consider one of the larger pet food brands (i.e. Royal Canin, Hills, Iams, Purina). Avoid salty treats, such as hot dog or jerky treats.

Monitoring:
1. Please monitor (b) (6) for signs of cardiac decompensation. This would include lethargy, cough, difficulty breathing (increased respiratory rate/effort), worsened abdominal distension, and episodes of collapse or fainting.

2. Please monitor (b) (6) resting respiratory rate. In order to do this, you can count the number of times that (b) (6) breathes in 15 seconds and multiply this number by 4 to get the number of breaths per minute. If you notice that this number is increasing or that it is consistently greater than 35-40 breaths per minute, then you should have (b) (6) evaluated by a veterinarian. A mobile app (Cardalis) is available to record instant rates and trends in respiratory rates.

PLAN FOR RE-EVALUATION OF YOUR PET:
Please call the Cardiology Service tomorrow to schedule an appointment for (b) (6) during the week of July 10th. You can schedule this appointment by calling (b) (6). Please bring a sample of (b) (6) current diet to this appointment for possible testing. He will also likely have a blood pressure, chest radiographs, and a renal panel performed during this appointment to re-evaluate his heart failure.

If you have any questions or concerns before this appointment, please have (b) (6) re-evaluated by your primary care veterinarian or the NCSU Emergency Service. (b) (6) may need recheck bloodwork and chest X-rays prior to this appointment if he is not doing well. He also may need medication adjustments and potentially hospitalization.

IF YOU HAVE ANY QUESTIONS OR PROBLEMS, PLEASE CALL THE SMALL ANIMAL EMERGENCY SERVICE AT (b) (6).

PLEASE CALL TO MAKE YOUR FOLLOW UP APPOINTMENT AS RECOMMENDED

_____ / _____ / _____
Owner/Agent Signature Clinician's Signature Student's Signature

Copy to: Owner / Medical Record / Fax to RDVM

NC State University
Veterinary Hospital
Fax: Admin
Fax: Referral
1052 William Moore Drive
Raleigh, NC 27607
Small Animal (919) 513-6500
Large Animal (919) 513-6630

Discharge Comments

Client	Patient			
(b) (6)	(b) (6)	Case # (b) (6)	Attending DVM	ADIN, DARCY
	SCHNAUZER		Student	(b) (6)
	MC	9.9 kg	Discharging DVM	
	GRAY&WHITE		Referring DVM	(b) (6)
	CANINE			

Admission Date/Time: (b) (6) 10:59 AM **Discharge Date/Time:** (b) (6) 03:16 PM **Discharge Status:**

CASE SUMMARY

DIAGNOSIS:
Dilated Cardiomyopathy (DCM)
Mitral valve endocardiosis and regurgitation

HISTORY: (b) (6) a 7 year old neutered male miniature Schnauzer, was presented to the NCSU cardiology service for re-evaluation of dilated cardiomyopathy. At his prior visit, (b) (6) presented to our Small Animal Emergency on (b) (6) for episodes of collapse. (b) (6) had his first two collapse episodes in mid February; he was described to fall over for ~6 seconds with no loss of consciousness, and he immediately returned to normal after. He later has several more collapsing episodes in June 2017, which prompted his presentation to NCSU SAES. Collapse episodes were preceded by excitement or exertion. He was discharged (b) (6) with Pimobendan 2.5mg PO BID, Enalapril 5mg PO BID and Lasix 12.5mg PO BID. (b) (6) house mate, (b) (6), unfortunately passed away from DCM and CHF in February 2017. Infectious disease testing and amino acid testing did not identify a cause for the DCM. Histopathology was relatively unremarkable, however, findings could have been consistent with a toxic insult.

Since (b) (6) (b) (6) reports (b) (6) is doing much better. He has not had any more collapsing episodes, excepting one moment where he stumbled but did not fall when excited 7/4/17. He is tolerating the medications and eating well. No coughing, sneezing, vomiting or diarrhea. His RR has been very normal since starting medications. His diet has been changed to Science Diet adult small breed from California Naturals.

The tests results from (b) (6) (b) (6) visit were normal, including vector borne testing (IFA, PCR) and taurine and carnitine analysis. A full amino acid profile did not reveal significant abnormaliites, however, consultation with UCD is pending for a full interpretation.

PHYSICAL EXAM FINDINGS:
T- 99.7* P- 136 bpm R- 36 Wt- 9.6kg
MM- pink, slightly tacky but no skin tenting
CRT-<2 seconds
BCS- 6/9
Attitude- BAR
Hydration: equivocal mild dehydration <5%

EENT: Unremarkable cornea/sclera OU, ears free of debris, mild dry crust in both nares, no oral ulcers/lesions/foreign bodies detected
PLN: No lymphadenopathy detected
CV/R: Grade 2/6 left apical systolic murmur, no arrhythmias auscultated, femoral pulses strong and synchronous, normal bronchovesicular lung sounds noted on bilateral auscultation- no crackles or wheezes, eupneic
GI/GU: Soft, non-painful, slightly distended abdomen on palpation, no fluid wave present, no organomegaly detected.
MSK: Ambulatory on all four limbs with no observed lameness.
INTEG: Clean hair coat, no evidence of ectoparasites
NEURO: BAR, normal gait on all four limbs, intact reflexes, cranial nerves intact

RESULTS OF DIAGNOSTIC TESTS:
BLOOD PRESSURE- 110 mmHg systolic
CHEST RADIOGRAPHS- Moderate left sided cardiomegally with no signs of congestive heart failure. Moderate hepamegaly.
RENAL PANEL: all normal (BUN 16, creat 0.6, K 4.0)

ASSESSMENT:
(b) (6) was presented today for a recheck of his recently diagnosed dilated cardiomyopathy (DCM). DCM is a disease of unknown cause affecting the muscle of the heart and is most commonly seen in large breed dogs (such as Dobermans, Great Danes, and Labrador Retrievers). Although the exact mechanism of DCM is currently unknown, dietary taurine/carnitine deficiencies, genetics and toxins have all been linked to DCM. Infectious and nutritional causes of DCM have been ruled out to the best of our ability to test for (b) (6) The overall effect of DCM is a decrease in the contractility (pumping ability) of the heart. Because the heart is unable to pump

with enough vigor to move blood adequately forward into circulation, a volume overload occurs and the heart dilates to accommodate it. As a result, the chambers of the heart become very large, and the walls of the heart become very thin. Ultimately, the heart is unable to accommodate and dilate further; the result is back-up of blood from the heart and into the lungs, known as congestive heart failure. It is important to know that this disease is progressive, and ultimately those patients affected with it will experience congestive heart failure.

Given the unusual timing of (b) (6) both developing dilated cardiomyopathy within the same time frame, their different ages, unrelatedness, same environment and lack of an identifiable cause, we are continuing to hunt for an environmental explanation for (b) (6) DCM. We will keep you up to date as we pursue toxin testing in the food and treats you have brought us. However, negative toxin testing may not completely rule out a toxin since we do not have samples representative of the onset of cardiac signs in both dogs. We have changed (b) (6) food to address this possibility.

We are glad to see that (b) (6) is doing well clinically. His chest xrays showed resolution of congestive heart failure and his renal panel was normal indicating that he is tolerating the medications well. We would like to add spironolactone to his regime because his potassium, while normal, is at the low end of the range, and because this may have some long term benefit through anti-aldosteronism. We have also listed doses for supplements, that while unproven in their benefit, are not harmful and may help his myocardial function. Taurine and carnitine supplementation are unlikely to be helpful since his plasma and whole blood concentrations are normal.

MEDICATIONS:

Plesae continue the following medications:

FUROSEMIDE (12.5 mg tablets): Please give 1 tablet by mouth every 12 hours.

PIMOBENDAN (2.5 mg capsules): Please give 1 capsule by mouth every 12 hours. If (b) (6) continues to have near-collaps episodes, you may increase this to 1 capsule 3 times daily. If the pharmacy gives you 2.5 mg tablets in the future you can also give 1.5 of these tablets twice daily.

ENALAPRIL (5 mg tablets): Please give 1 tablet by mouth every 12 hours.

Please start the following medication:

SPIRONOLACTONE 25 mg tablets: Give 1 tablet by mouth every 24 hours. This is a weak diuretic that can also may have cardioprotective effects. Side effects can include electrolyte abnormalities and decreased blood flow through the kidneys. Kidney bloodwork and electrolytes will need to be periodically monitored while receiving this medication.

(b) (6) taurine and carnitine assessments were normal, making supplementation unlikely to be beneficial. Other supplements with theoretical but unstudied benefits are included below. These are not likely to be harmful and no interactions with his medications are anticipated.
1. D-ribose 5 grams (1 scoop) daily.
2. Coenzyme Q10 (as ubiquinol not ubiquinone) 100 mg capsules: give 1 capsule 2-3 times daily
3. Fish oil 450 mg of EPA and DHA per day. This is an approximate dose and can be rounded up or down depending on the fish oil chosen. Nature Made and Nordic Naturals are high quality brands.

ACTIVITY:
Please avoid strenuous exercise or situations which place undue stress on your pet. In general, pets with congestive heart failure will self-regulate their exercise. Please monitor for any change in exercise capability.

Please continue to monitor (b) (6) respiratory rate and call if this increases. Please also call if he begins coughing or collapse episodes recur.

DIET:
A diet that is moderately restricted in salt is ideal for cardiac patients, as excessive salt load can cause fluid accumulation. A commercial "Senior" diet is formulated with an appropriate amount of salt for your pet. Please also avoid salty treats, such as hot dogs or jerky treats.

NEXT APPOINTMENT:
(b) (6) should have a recheck appointment with NCSU Cardiology in 3-4 months to evaluate chest X-rays, blood pressure, kidney values, echocardiogram, and troponin. If he begins to show signs of heart failure prior to your next recheck, please call NCSU Cardiology so that we can recheck your pet sooner.

CLINICIANS:
(b) (5) Dr. Darcy Adin, (b) (6)

RESIDENTS:
(b) (6)

CLINICAL TECHNICIANS:
(b) (6)

RESEARCH TECHNICIAN:
(b) (6)

CLIENT SERVICES:
(b) (6)

In order to help expedite medication refills, please visit us online at www.ncstatevets.org and select Pet Owners, Pharmacy Refills.

NOTE: If your pet is in need of emergency aid and you are not able to get to the NC State Veterinary Hospital quickly, please seek care at the nearest veterinary emergency facility. Take these discharge instructions and current medications with you so that the treating veterinarian will know as much as possible regarding your pet's medical condition.

Owner - (b) (6) Clinician - Darcy Adin, DVM Student - (b) (6)

Referring Veterinarians - please visit us online at www.ncstatevets.org/veterinarians and fill out our RDVM Feedback Survey!

Follow-up Case Information Uniform Data Entry Form
Vet-LIRN

Date (mm/dd/yy): Jun 9, 2016

EON/CC Number: 266,814

PATIENT INFORMATION

Pet Name: (b) (6)

○ Dog ◉ Cat

Breed: DSH

Age in years (if < 6 months, put 0.5): 12

Gender:

○ M ○ MN ○ F ◉ FS

This form serves as a Uniform Data Entry Form to capture additional case specific information not clear from the Consumer Complaint or Medical Records in a standardized manner. Because each follow-up interview made with owners features questions tailored specifically to the case, each box of information contained in this Uniform Data Entry Form may not be completed.

HISTORY-Additional Comments from Owner

Owner's Description of What Happened: lethargic, difficulty moving, not herself

Any Health Problems Prior to the Event (e.g. allergies, surgeries): (b) (6): none; none in the past; all house cats

Sensitive GI tract (e.g. stomach upset when switching foods, eats a lot of grass) ☐ Yes

Changes to the pet's diet prior to illness ☐ Yes

Date Diet Change:

CLINICAL INFORMATION--Additional Comments from Owner on What Happened

Appetite ☐ Increased ☐ Decreased

Vomiting ☐ Yes

Diarrhea ☐ Yes

Duration of Diarrhea (days):

Blood in Feces ☐ Fresh,Red ☐ Coffee Ground ☐ Black,Tarry

Water Consumption ☐ Increased ☐ Decreased

Urination ☐ Increased ☐ Decreased

Lethargy ☐ Yes

Other:

MEDICATIONS-Taken Prior to the Event and Mentioned by Owner

List medications mentioned by owner (e.g. NSAIDs, steroids, heartworm/flea prevention, antibiotics, etc.)

List probiotics, vitamins, or supplements mentioned by owner:

Continued otherside

Follow-up Case Information Uniform Data Entry Form
Vet-LIRN

EON/CC Number: 266,814

Owner: (b) (6)

Pet's Name: (b) (6)

DIET-Any other foods the owner mentions were given to the animal during this period. (check all that apply)

☒ Commercial Dry — Product Use as Part of Diet: ☐ Primary ☐ Secondary ☐ Occasional

List Product Label Name: Merrick Grain Free Bistro Chicken-started in January of 2014, print out of all pet food purchases thru Jan 2014 --> on the Chicken pretty much the entire time; owner has a listing of all purchases from pet

☐ Commercial Wet-Canned — Product Use as Part of Diet: ☐ Primary ☐ Secondary ☐ Occasional

List Product Label Name: Fancy Feast Wet food given to (b) (6) to stimulate her appetite after her illness onset, but she only licked the gravy. So for ~1 week before the other 4 house cats were tested for taurine, only (b) (6)

☐ Commercial Wet-Pouch — Product Use as Part of Diet: ☐ Primary ☐ Secondary ☐ Occasional

List Product Label Name:

☐ Commercial-Raw — Product Use as Part of Diet: ☐ Primary ☐ Secondary ☐ Occasional

List Product Label Name:

☐ Homemade-Raw — Product Use as Part of Diet: ☐ Primary ☐ Secondary ☐ Occasional

Describe Product Type:

☐ Homemade-Cooked — Product Use as Part of Diet: ☐ Primary ☐ Secondary ☐ Occasional

Describe Product Type:

☐ Table Scraps/Human Food (as an occasional contribution to diet) — Describe Product Type(s): not in past 5 years

☐ Pet Treat Products — Product Use as Part of Diet: ☐ Primary ☐ Secondary ☐ Occasional

☐ Commercial	Product Label Name/Lot:		Date first fed	
	How Product Administered:		Date last fed	
☐ Rawhides or Pig Ears	Product Label Name/Lot:		Date first fed	
	How Product Administered:		Date last fed	
☐ Marrow Bones	Product Label Name/Lot:		Date first fed	
	How Product Administered:		Date last fed	
☐ Chicken Jerky	Product Label Name/Lot:		Date first fed	
	How Product Administered:		Date last fed	
☐ Duck Jerky	Product Label Name/Lot:		Date first fed	
	How Product Administered:		Date last fed	
☐ Sweet Potato Jerky or Treats	Product Label Name/Lot:		Date first fed	
	How Product Administered:		Date last fed	

Continued otherside

Follow-up Case Information Uniform Data Entry Form
Vet-LIRN

EON/CC Number: 266,814

Owner: (b) (6)

Pet's Name: (b) (6)

DIET-*continued*-Any other foods the owner mentions were given to the animal during this period. (check all that apply)

☐ Other Treats

Product Label Name/Lot:

How Product Administered

Date first fed

Date last fed

ENVIRONMENTAL EXPOSURES-Environmental Exposures Mentioned by the Owner Potentially Affecting the Animal's Overall State of Health Prior to the Event . (check all that apply)

☒ Indoor ☐ Outdoor ☐ Indoor & Outdoor ☐ Carrion ☐ Rodents ☐ Grapes or Raisins ☐ Nuts

☐ Plants ☐ Trash ☐ Hunt ☐ Pet Shows ☐ Sporting Events ☐ Pet Recreation Facilities

☐ Livestock ☐ Poultry ☐ Reptiles ☐ Pet Birds ☐ Small Mammals ☐ Untreated Surface Water

☐ Anti-freeze ☐ Mushrooms ☐ Heavy Metals ☐ Ticks ☐ Urban ☐ Suburban ☐ Rural

Comments:

HOUSEHOLD-Signalment of Additional Animals Given the Product mentioned by the owner.

Animal 1 ☐ Reacted

Animal 2 ☐ Reacted

Animal 3 ☐ Reacted

Comments

Submit

Report Details - EON-345831

ICSR:	2040528	
Type Of Submission:	Initial	
Report Version:	FPSR.FDA.PETF.V.V1	
Type Of Report:	Adverse Event (a symptom, reaction or disease associated with the product)	
Reporting Type:	Voluntary	
Report Submission Date:	2018-01-22 18:04:59 EST	
Reported Problem:	**Problem Description:**	One week prior to presentation at a local emergency and specialty clinic, owners noted (b) (6) to be tiring more quickly when playing catch. On the day of her initial presentation to the ER clinic, she had been coughing more than usual and sank to the ground when attempting to chase a ball, but recovered quickly. At the ER clinic she was diagnosed with atrial fibrillation and early congestive heart failure. Treatment was initiated there (b) (6), she was transferred to our clinic on (b) (6) for further evaluation and care. Her arrhythmia converted back to a normal sinus rhythm on (b) (6) prior to transfer and her congestive heart failure resolved with treatment. She had a second collapse episode prior to referral. Echocardiogram showed evidence of dilated cardiomyopathy with concurrent chronic degenerative valve disease.
	Date Problem Started:	(b) (6)
	Concurrent Medical Problem:	Yes
	Pre Existing Conditions:	On supplements - Missing Link and Sea Jerky History of focal facial seizures - owner occasionally uses an herbal supplement for calming when this occurs, unknown
	Outcome to Date:	Better/Improved/Recovering
Product Information:	**Product Name:**	California Natural Grain-Free Kangaroo and Red Lentils Recipe
	Product Type:	Pet Food
	Lot Number:	
	Package Type:	BAG
	Purchase Date:	08/14/2017
	Possess Unopened Product:	No
	Possess Opened Product:	No
	Product Use Information:	**Description:** (b) (6) had eaten this diet for years alongside her housemate who was being fed it for a food allergy.
		Last Exposure Date: 09/01/2017
		Product Use Stopped After the Onset of the Adverse Event: Yes
		Adverse Event Abate After Product Stop: No
		Product Use Started Again: No
		Perceived Relatedness to Adverse Event: Probably related
	Manufacturer /Distributor Information:	
	Purchase Location Information:	
Animal Information:	**Name:**	(b) (6)
	Type Of Species:	Dog

	Type Of Breed:	Retriever - Labrador
	Gender:	Female
	Reproductive Status:	Neutered
	Weight:	32.1 Kilogram
	Age:	8 Years
	Assessment of Prior Health:	Excellent
	Number of Animals Given the Product:	4
	Number of Animals Reacted:	4
	Owner Information:	**Owner Information provided:** Yes
		Contact: **Name:** (b) (6)
		Phone: (b) (6)
		Other Phone: (b) (6)
		Email: (b) (6)
		Address: (b) (6) United States
	Healthcare Professional Information:	**Practice Name:** (b) (6)
		Contact: **Name:** (b) (6)
		Phone: (b) (6)
		Other Phone: (b) (6)
		Email: (b) (6)
		Address: (b) (6) United States
Sender Information:	**Name:**	(b) (6)
	Address:	(b) (6) United States
	Contact:	**Phone:** (b) (6)
		Other Phone: (b) (6)
		Email: (b) (6)
	Permission To Contact Sender:	Yes
	Preferred Method Of Contact:	Phone
	Reported to Other Parties:	Other
Additional Documents:		
	Attachment:	cardio0009.pdf
	Description:	Taurine level
	Type:	Laboratory Report
	Attachment:	cardio0008.pdf

Description:	Echo reports from initial presentation, 5/2017, and 12/2017
Type:	Medical Records

16051

Sample Submission Form

UC CUSTOMERS ONLY:
Non-federal funds ID/Account Number to bill:_____

Amino Acid Laboratory
University of California, Davis
1020 Vet Med 3B
1089 Veterinary Medicine Drive
Davis, CA 95616
Tel: (530)752-5058, Fax: (530)752-4698

http://www.vetmed.ucdavis.edu/vmb/aal/aal.html

Vet/Tech Contact: Account # (b) (6) / Contact: (b) (6) Date: 1-23-17
Company Name: (b) (6)
Address: (b) (6)
Email: (b) (6)
Tel: (b) (6) **Fax:** (b) (6)

Billing Contact: (b) (6) (C) **TAX ID:**
Email: (b) (6) **Tel:** (b) (6)

Patient Name: (b) (6)
Species: hg
Owner's Name: (b) (6)

Sample Type: ☐ Plasma ☑ Whole Blood ☐ Urine ☐ Food ☐ Other:_____
Test Items: ☑ Taurine ☐ Complete Amino Acid ☐ Other:_____

Taurine Results (nmol/ml)

Plasma:_____ Whole Blood: 236 Urine:_____ Food:_____

Reference Ranges (nmol/ml)

	Plasma		Whole Blood	
	Normal Range	No Known Risk for Taurine Deficiency	Normal Range	No Known Risk for Taurine Deficiency
Cat	80-120	>40	300-600	>200
Dog	60-120	>40	200-350	>150

(b) (6)

Patient Information

Patient:	(b) (6)	Age: 5 years		Referring Veterinarian:	(b) (6)
Patient Number:	(b) (6)	Weight:(kg) 25.60		Cardiologist:	(b) (6) (b) (6)
Breed:	Labrador Retriever	Sex: FS		Client Number:	(b) (6)
Exam Date:	(b) (6) 08:19	BSA: 0.88			

History: (b) (6) was presented to (b) today for evaluation of a new heart murmur and evaluation after being diagnosed with congestive heart failure on 8/18/17. (b) (6) was evaluated by her regular vet on 8/18/17 for heavy breathing and coughing. Radiographs and blood work were done at the time and (b) (6) was diagnosed with an enlarged heart and congestive heart failure at that time. (b) (6) was started on Furosemide, Vetmedin and Enalapril by her regular vet. (b) (6) was seen through (b) (6) (b) on (b) (6) for reevaluation of congestive heart failure. The clients report that (b) (6) had improved some but had not improved a lot. Medications were adjusted based on recommendations from (b) (6) on (b) (6) until (b) (6) could get an appointment to be see by (b). The clients report that since the medications were increased (b) (6) has improved, however they do still feel that she is breathing faster than normal at home and she is still panting a lot at home. (b) (6) is still eating very well at home and is currently on a low sodium kangaroo and lentil diet. The clients also report that there other dog, (b), who we also see is (b) (6) aunt (b) (6) mother was a littermate of (b) (b) (6) is also urinary incontinent which started prior to developing congestive heart failure and initiation of treatment. The clients report that (b) (6) was not well controlled on Incurin 1 mg tablets: 1 tablet by mouth once daily so Proin 50 mg tablets: Give 1/2 tablet by mouth every 12 hours was added in. They are unsure if (b) (6) incontinence is now controlled because there other dog has developed incontinence as well. The client feel that (b) (6) is less social and less active at home. (b) (6) is currently receiving furosemide 40 mg tablets: give 1 and 1/2 tablets by mouth every 12 hours, spironolactone 25 mg tablets: give 1 tablet by mouth every 12 hours, enalapril 10 mg tablets: give 1 tablet by mouth every 12 hours, Vetmedin 5 mg tablets: give 1 and 1/2 tablets by mouth every 12 hours, Incurin 1 mg tablets: give 1 tablet by mouth once every 24 hours, Proin 50 mg tablets: give 1/2 tablet by mouth every 12 hours and Apoquel 16 mg tablets: give 3/4 tablet by mouth every 24 hours.

Physical Examination: Temp: 101.5 HR: 150 RR: panting
Grade 4/6 left apical systolic murmur with radiation to the right. Adequate femoral pulses. Regular rhythm. Normal lung sounds. Eupneic. Normal abdominal palpation. PLNs WNL. MM pink/moist. CRT < 2 sec.

Diagnostic Tests: Blood Pressure: 110 mmHg (#4 cuff, left forelimb)
Thoracic Radiographs: Persistent cardiomegaly with mild decrease in severity. No evidence of cardiac decompensation.
Renal Panel: Mildly elevated SDMA (19 ug/dL). Otherwise clinically unremarkable.
Echo: See Below. Sinus rhythm on ECG.

Echocardiographic Report

2D ECHO

LA Systolic Diameter LX	6.3 cm	Aortic Root Diameter	2.1 cm

DOPPLER

AV Peak Velocity	101 cm/s	PV Peak Velocity	76.1 cm/s
AV Peak Gradient	4.1 mmHg	PV Peak Gradient	2.3 mmHg
Mitral E Point Velocity	103 cm/s	TR Peak Velocity	251 cm/s
Mitral E to A Ratio	1.9	TR Peak Gradient	25.3 mmHg
MR Peak Velocity	435 cm/s		

M-MODE

LV Diastolic Diameter MM	7.5 cm	IVS Percent Thickening MM	0.22
LV Systolic Diameter MM	6.6 cm	LVPW Diastolic Thickness MM	0.67 cm
LV Fractional Shortening MM	12.1 %	LVPW Systolic Thickness MM	0.84 cm
LV Diastolic Volume Cube	427 cm^3	LVPW Percent Thickening MM	0.26
LV Systolic Volume Cube	290 cm^3	IVS to PW Ratio MM	1.1
LV Ejection Fraction Cube	0.32	LV Mass MM	238 g
IVS Diastolic Thickness MM	0.74 cm	LV Mass Normalized MM	272 g/m^2
IVS Systolic Thickness MM	0.9 cm	MV E Point Septal Separation	3.4 cm

Left Ventricle: Severe dilation with marked global myocardial dysfunction. Normalized LVIDd 2.9, normalized LVIDs 2.38.

Left Atrium: Severe dilation with septum bowing to the right.

Right Ventricle: Mild to moderate dilation with reduced myocardial function.

Right Atrium: Mild to moderate dilation.

Mitral Valve: Thickened valve leaflets. 3-4+ mitral regurgitation.

Aortic Valve: Mildly thickened valve leaflets. No aortic insufficiency.

Tricuspid Valve: Thickened valve leaflets. Two jets of 2-3+ tricuspid regurgitation. Normal regurgitant velocities.

Pulmonic Valve: Mildly thickened valve leaflets. Mild pulmonic insufficiency.

Aorta: Normal

Pericardium: Normal

Diagnosis

Endocardiosis (chronic degenerative valve disease) - Degenerative changes in one or more heart valves have caused leaking across these valves. This is the source of the heart murmur. As this disease progresses, the heart enlarges. Eventually this can lead to symptoms of cough and shortness of breath (airway compression and/or congestive heart failure). This is usually a slowly progressive disease.

Dilated cardiomyopathy - This is a disease characterized by weakening of the heart muscle and dilation of the heart chambers. As the disease progresses, it can lead to congestive heart failure (fluid in the lungs causing shortness of breath and cough). Abnormal heart rhythms are common and can result in sudden death. Most commonly this is an inherited disease, though it can occur secondary to a deficiency in an amino acid called taurine.

Recommendations

Please continue the following medications as previously directed:

Furosemide 40 mg tablets: Give 1 and 1/2 tablets by mouth every 12 hours.
This is a diuretic (water pill), that prevents the body from retaining excessive sodium and water. It will cause your pet to drink and urinate more frequently. It is important that fresh water is always available.

Enalapril 10 mg tablets: Give 1 tablet by mouth every 12 hours.
This medication is a strong drug that dilates blood vessels, permitting the heart to pump blood more efficiently. It can lower blood pressure (hypotension) and cause changes in kidney function and electrolyte values. If your pet develops weakness or depression, decrease the drug dose by ½ and call. A kidney panel and blood pressure should be reevaluated 7-10 days after beginning this medication.

Vetmedin 5 mg tablets: Give 1 and 1/2 tablets by mouth every 12 hours.
Pimobendan (Vetmedin) in Congestive Heart Failure (CHF) - This is a drug that is approved for the treatment of congestive heart failure secondary to dilated cardiomyopathy or chronic valve disease (endocardiosis). Studies have shown improved quality of life and increase survival time when this drug is added to other standard cardiac medications. In our experience, side effects are uncommon, but it is important that you advise us if you feel your pet is having any potential adverse effects from this medication. The reported potential side effects listed for this medication are increased heart rate, vomiting, diarrhea, inappetence, uneasiness, incoordination, convulsions, increased drinking and increase urinating.

Spironolactone 25 mg tablets: Give 1 tablet by mouth every 12 hours
This is a diuretic (water pill) that also blocks a hormone that can injure the heart muscle. It works well in combination with the furosemide and enalapril.

Proin 50 mg tablets: Give 1/2 tablet by mouth every 12 hours
Apoquel 16 mg tablets: Give 3/4 tablet by mouth once every 24 hours

INCREASE:
Incurin 1 mg tablets: INCREASE to 2 tablets by mouth once every 24 hours.

As we discussed, (b) and (b) (6) unfortunately have very similar structural heart disease. Since they are related, this raises concern for a genetic component. You have expressed that there is no history of heart disease in their lineage. It is possible that the disease has remained silent in other related dogs or is inherited in a way that it is only expressed in certain individuals. The other common denominator that (b) and (b) (6) have is the kangaroo diet. Even though we have not specifically associated this protein source with taurine/carnitine deficiency, it may be warranted to consider a diet with a different protein source since it is a novel protein and both dogs have very similar disease manifestations. Lamb should be avoided as it has been associated with taurine deficiency in dogs.

We did not check (b) (6) blood taurine level today- since (b) was normal it is highly unlikely that (b) (6) will be deficient as they are related and eat the same food.

One thing that can be very helpful for home monitoring is checking sleeping or resting respiratory rates. A recent study showed that even pets with severe heart disease rarely have resting respiratory rates greater than 30 breaths per minute unless they are starting to decompensate for that disease. Elevated respiratory rates at home may be even more sensitive than chest radiographs at picking up early decompensation. Count your pet's respiratory rate when he/she is at rest or sleeping (not within 20 minutes of being active). If his/her respiratory rate is greater than 30 breaths per minute, recheck again in a couple of hours. If persistently elevated above this level, call.

With advanced heart disease, our biggest dietary concerns are adequate calorie content and low sodium content. We aim for less than 80mg sodium per 100 kilocalories (kcal) in patients that have developed congestive heart failure. We do not advise protein restriction unless there is concurrent kidney disease (i.e. kidney diets are not advised unless there is concurrent kidney disease). Please refer to our diet handouts with a list of currently adequate diets and treats, though this list is not exclusive. If you wish to feed a diet that is not on these lists, you will need to call the manufacturer of the diet to obtain a sodium content.

Exercise is also a concern in advanced heart disease. While cage rest is ideal with active heart failure, some exercise is permissible in asymptomatic disease. However, vigorous or extended exercise should be avoided.

***As long as (b) (6) does well at home we would like to re-evaluate her in 4-6 weeks. At this time we will recheck her kidney values/electrolytes and blood pressure as well as repeat chest x-rays.

(b) (6) (Cardiology)

(Electronically Signed)

Final Date:

Like us on Facebook!

www.facebook.com/ (b) (6)

Notes to our clients

-Please bring all medications to your pet's scheduled appointments.

-We require a 48 hour notice for all refills. When you call to request a refill, please leave the pharmacy phone number or clearly indicate if you plan on picking up the medication at our facility. PRESCRIPTION REFILLS ARE NOT AVAILABLE AFTER (b) (6) REGULAR BUSINESS HOURS (Evenings, Fridays, holidays and weekends).

-Check out WWW.GOODRX.COM and enter your local zip code to search for the best prices on your medications at your local pharmacies.

-If an emergency arises with your pet, (b) (6) is a 24 hour facility.

ILR placed in cooler

[redacted]

PATIENT CASE REPORT

Date: [redacted] **Time:** 22:45

Client:	[redacted]	**Patient:**	[redacted]
Address:	[redacted]	**Breed:**	Retriever, Labrador
		Age:	5 Yrs. 2 Mos.

History:
[redacted] returner to [redacted] for increased respiratory rate. The owner reports after the visit yesterday and the lasix bolus, [redacted] did well until evening. Throughout the evening and night her respiratory rate increased to over 40/min. This afternoon she began to cough. The owner reports she coughed up pink tinged fluid. She also had an episode where she was excited and collapsed. She has been taking all of her medications as previously directed. She had her midday dose of Vetmedin and lasix. She is currently on Proin 50 mg 1/2 BID, Apoquel 16 mg 3/4 PO SID PM, Incurin 1 mg 2 PO SID, Vetmedin 5 mg 1 and 1/2 PO TID (for the past 2 days), Enalapril 10 mg 1 PO BID, Furosemide 40 mg 1 and 1/2 PO BID, and Spironalactone 25 mg po BID. She has been dry heaving on the way here this morning. She has a history of allergies and is on a Venison and Lentil diet.

Physical Exam:
Vitals:

	[redacted] 7:05 PM
Vital Sign	211
Weight	27.4 kilograms
Attitude	0 - BAR
Temp	101.4
HR	180
RQ	Panting
Muc Memb	Pale Pink
CRT	<2 sec

Mucous Membranes: Pale pink, moist
Heart and lungs: 4/6 murmur, Fine crackles right dorsal lung fields/no dyspnea, regular rhythm, strong and synchronous femoral pulses
Abdominal Exam: Soft and not painful to palpation,
Musculoskeletal and Integument: Ambulatory with no lameness
Eyes, Ears, Nose, and Throat: No ocular or nasal discharge
Peripheral lymph nodes: No peripheral lymphadenopathy
Urogenital Exam: Unremarkable
Neuro Exam: Intact CN, normal mentation, no ataxia seen
Rectal Examination: Formed stool with no blood
[redacted] [redacted] respiratory rate continued to increase throughout the night despite being on a lasix CRI. Called owner and discussed poor prognosis. Owner elect humane euthanasia. [redacted] also spoke to owner for euthanasia consent per phone consultation. 10 ml fatal plus IV.
Diagnostics:
Radiographs-
The cardiac silhouette is again noted to be generally enlarged. There is an unstructured interstitial pulmonary pattern within the right

middle and right caudal lung lobes. There is mild enlargement of the cranial lobar pulmonary veins. There are no abnormalities of the pleural space.
Conclusion
1. Persistent generalized cardiomegaly with evidence of left-sided congestive heart failure characterized by cardiogenic pulmonary edema and pulmonary venous congestion.
(b) (6), DVM, Diplomate ACVR
The study includes 3 projections of the thorax dated (b) (6). The study is compared with a prior exam from yesterday (b) (6)

The cardiac silhouette is again noted to be generally enlarged. There is a persistent unstructured interstitial pulmonary pattern within the right middle and right caudal lung lobes. This is relatively unchanged since the prior study. There is persistent enlargement of the cranial lobar pulmonary veins. There are no abnormalities of the pleural space.

Conclusion
1. Persistent generalized cardiomegaly with persistent left-sided congestive heart failure characterized by cardiogenic pulmonary edema and pulmonary venous congestion.
(b) (6), DVM, Diplomate ACVR

Diagnosis:
Endocardiosis
Dilated cardiomyopathy

Treatment:
Lasix bolus 80 mg IV
Placed IV catheter
Monitored respiratory rate
Lasix CRI at 0.5 mg/kg/hr throughout the night
Due to poor response to treatment and declining condition owners elected humane euthanasia

Releasing DVM:

_____ (b) (6)
Client Signature Client Name (Print)

(b) (6)

[redacted]

Patient Information

Patient:	(b) (6)	Age: 4 years		Referring Veterinarian:	(b) (6)
Patient Number:	(b) (6)	Weight:(kg) 14.80		Cardiologist:	(b) (6) DVM, DACVIM (Cardiology)
Breed:	Cocker Spaniel	Sex:		Client Number:	(b) (6)
Exam Date:	09/05/2017 08:21	BSA: 0.61			

History: (b) (6) was presented to (b) (6) for evaluation of an enlarged heart and congestive heart failure diagnosed on radiographs on 8/22/17. (b) has no history of a heart murmur. (b) was taken to his regualr vet on 8/22/17 for evaluation of a week long progressive cough. The clients report that (b) was coughing about two times per day and the cough became more severe over the course of the week. (b)'s RDVM ran blood work and took thoracic radiographs at that time. The clients report that initially his RDVM was concerned with pneumonia and prescribed antibiotics, but once the radiographs were reviewed heart failure was diagnosed and furosemide and enalapril were recommended. The clients report that they had not started the antibiotics prior to starting the Lasix and Enalapril. The cough went away after starting the furosemide and enalapril, bu (b) (6) regular vet wanted (b) on the antibiotics as well. The cough is greatly improved, however (b) does still cough every now and then. (b) is currently receiving furosemide 12.5 mgs once daily, enalapril 5 mgs in the morning and 2.5 mgs in the evenings, amoxicillin 500mgs twice daily and doxycycline 100 mgs twice daily.

Physical Examination: Temp: 104.4 HR: 150 RR: panting. Quiet/distant heart sounds with gallop, no audible murmur on left, grade 1/6 systolic murmur left. Regular rhythm. Fine crackles bilaterally. Normal abdominal palpation. Femoral pulses difficult to assess due to shivering, suspect decreased. Palpable jugular pulsation. Good hydration, normal refill, pink mm. Suspect epulis on gingiva associated with left upper canine. Fundic exam WNL.

Diagnostic Tests: Blood Pressure: 160 mmHg with a 4 cm cuff on the left forelimb

Profile: albumin 2.5 (increased from 2.1), otherwise unremarkable

U/A: USG 1.015, pH 8.0, trace protein

Echo: See Below. ECG during echo showed a sinus tachycardia, heart rate averaging about 200bpm.

Echocardiographic Report

2D ECHO

LA Systolic Diameter LX	4.3 cm	Aortic Root Diameter	1.5 cm

DOPPLER

AV Peak Velocity	101 cm/s	PV Peak Velocity	64.6 cm/s
AV Peak Gradient	4.1 mmHg	PV Peak Gradient	1.7 mmHg
Mitral E Point Velocity	166 cm/s	TR Peak Velocity	337 cm/s
MR Peak Velocity	467 cm/s	TR Peak Gradient	45.4 mmHg

M-MODE

LV Diastolic Diameter MM	4.7 cm	LVPW Diastolic Thickness MM	0.71 cm
LV Systolic Diameter MM	4.2 cm	LVPW Systolic Thickness MM	0.82 cm
LV Fractional Shortening MM	11.4 %	LVPW Percent Thickening MM	0.15
LV Diastolic Volume Cube	104 cm^3	IVS to PW Ratio MM	0.99
LV Systolic Volume Cube	72.3 cm^3	LV Mass MM	105 g
LV Ejection Fraction Cube	0.3	LV Mass Normalized MM	173 g/m^2
IVS Diastolic Thickness MM	0.71 cm	LA Systolic Diameter MM	3.1 cm
IVS Systolic Thickness MM	0.71 cm	Aortic Root Diameter MM	1.5 cm
IVS Percent Thickening MM	0.011	MV E Point Septal Separation	1.8 cm

Left Ventricle: Moderate dilation with increased sphericity and severe global decrease in contractility.

Left Atrium: Moderate dilation.

Right Ventricle: Mild dilation with decreased contractility.

Right Atrium: Mild dilation with decreased contractility.

Mitral Valve: 3+ central regurgitation, fused inflow.

Aortic Valve: Normal.

Tricuspid Valve: Multiple 2-3+ jets of regurgitation. TR velocity is increased consistent with moderate pulmonary hypertension.

Pulmonic Valve: Normal.

Aorta: Normal.

Pericardium: Normal. No free fluid in the abdomen, distended hepatic vessels.

Diagnosis

Dilated cardiomyopathy - This is a disease characterized by weakening of the heart muscle and dilation of the heart chambers. As the disease progresses, it can lead to congestive heart failure (fluid in the lungs causing shortness of breath and cough). Abnormal heart rhythms are common and can result in sudden death. Most commonly this is an inherited disease, though it can occur secondary to a deficiency in an amino acid called taurine.

Congestive heart failure - We did not repeat radiographs today, but based on the finding of crackles on physical exam, I suspect that there is still some mild fluid in (b) (6) lungs today.

Mild decrease in blood albumin (protein) - This value is increased from the initial bloodwork, though still just mildly low today. We will keep an eye on this, and if it is persistent or progressive we can evaluate further. It is possible that this could be due to heart failure if there had previously been free fluid in (b) (6) abdomen as well as in his lungs.

Recommendations

Please INCREASE:
Furosemide (Lasix, Salix) 12.5mg tablets - INCREASE to 1 tablet by mouth every 12 hours. This is a diuretic (water pill), that prevents the body from retaining excessive sodium and water. It will cause your pet to drink and urinate more frequently. It is important that fresh water is always available.

Enalapril (Enacard, Vasotec) 5mg tablets - INCREASE to 1 and 1/2 tablets by mouth every 12 hours. This medication is a strong drug that dilates blood vessels, permitting the heart to pump blood more efficiently. It can lower blood pressure (hypotension) and cause changes in kidney function and electrolyte values. If your pet develops weakness or depression, decrease the drug dose by ½ and call. A kidney panel and blood pressure should be reevaluated 7-10 days after beginning this medication.

Please ADD:
Spironolactone (Aldactone) 25mg tablets - Give one tablet by mouth once daily. This is a diuretic (water pill) that also blocks a hormone that can injure the heart muscle. It works well in combination with the furosemide and enalapril.

Pimobendan (Vetmedin) 5mg tablets - Give one tablet by mouth in the morning and 1/2 tablet by mouth in the evening. This is a drug that is approved for the treatment of congestive heart failure secondary to dilated cardiomyopathy or chronic valve disease (endocardiosis). Studies have shown improved quality of life and increase survival time when this drug is added to other standard cardiac medications. In our experience, side effects are uncommon, but it is important that you advise us if you feel your pet is having any potential adverse effects from this medication. The reported potential side effects listed for this medication are increased heart rate, vomiting, diarrhea, inappetance, uneasiness, incoordination, convulsions, increased drinking and increase urinating.

While we are waiting for taurine results, consider supplementing:
Taurine 500mg tablets - Give one tablet by mouth every 12 hours.
L-carnitine 1g - Give 1g by mouth every 8 hours.

One thing that can be very helpful for home monitoring is checking sleeping or resting respiratory rates. A recent study showed that even pets with severe heart disease rarely have resting respiratory rates greater than 30 breaths per minute unless they are starting to decompensate for that disease. Elevated respiratory rates at home may be even more sensitive than chest radiographs at picking up early decompensation. Count your pet's respiratory rate when he/she is at rest or sleeping (not within 20 minutes of being active). If his/her respiratory rate is greater than 30 breaths per minute, recheck again in a couple of hours. If persistently elevated above this level, call.

I also recommend considering a new diet with a different protein source. While I do not know of any documented amino acid deficiencies associated with a kangaroo diet, I also have two littermates that I diagnosed with severe dilated cardiomyopathy that were both fed a kangaroo diet for a long time. In that case, an inherited form of disease is possible, and in (b) , either an inherited or taurine-associated form of disease is possible, but the connection does bother me. With advanced heart disease, our biggest dietary concerns are adequate calorie content and low sodium content. We aim for less than 80mg sodium per 100 kilocalories (kcal) in patients that have developed congestive heart failure. We do not advise protein restriction unless there is concurrent kidney disease (i.e. kidney diets are not advised unless there is concurrent kidney disease). Please refer to our diet handouts with a list of currently adequate diets and treats, though this list is not exclusive. If you wish to feed a diet that is not on these lists, you will need to call the manufacturer of the diet to obtain a sodium content.

Exercise is also a concern in advanced heart disease. While cage rest is ideal with active heart failure, some exercise is permissible in asymptomatic disease. However, vigorous or extended exercise should be avoided.

I would like to recheck (b) again in another 7-10 days for chest radiographs, kidney panel, and bloodwork on the new medications. Please call if you have any questions or concerns in the meantime. We will call when we receive taurine level results (this can take a couple of weeks sometimes).

(b) (6) (Cardiology)

(Electronically Signed)
Final Date: 05 September 2017 12:17
Amended: 05 September 2017 12:28

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www.facebook.com/ (b) (6)

Notes to our clients

-Please bring all medications to your pet's scheduled appointments.

-We require a 48 hour notice for all refills. When you call to request a refill, please leave the pharmacy phone number or clearly indicate if you plan on picking up the medication at our facility. PRESCRIPTION REFILLS ARE NOT AVAILABLE AFTER (b) (6) REGULAR BUSINESS HOURS (Evenings, Fridays, holidays and weekends).

-Check out WWW.GOODRX.COM and enter your local zip code to search for the best prices on your medications at your local pharmacies.

-If an emergency arises with your pet, (b) (6) is a 24 hour facility.

(b) (6)

(b) (6)

Client ID:	(b) (6)	Patient ID:	(b) (6)
Client Name:	(b) (6)	Name:	(b) (6)
Spouse/Other:	(b) (6)	Breed:	Spaniel, Cocker
Address:	(b) (6)	Sex:	Neutered Male
		Color:	Black/ White/ Brown
Telephone:	(b) (6)	Age:	4 Yrs. 3 Mos.
		DOB:	(b) (6)

Referring Veterinarian:	(b) (6)
Practice:	(b) (6)
Phone:	(b) (6)
FAX:	

Cardiology Reevaluation

Reevaluation of:
Congestive heart failure, left sided, Dilated cardiomyopathy, Hypoalbuminemia.

(b) (6) continues to do well at home, without any weakness or collapse. The owners report that (b) (6) has great energy levels and loves to play. The owners have reported resting respiratory rates at 14-16 bpm, without any coughing. (b) (6) has a normal appetite with normal eliminations, though did vomit clear liquid once 1.5 weeks ago. The owner reports that the mass or (b) (6) gums does not seem to affect his chewing anymore.

Physical Exam:

	9/19/2017 3:06 PM	9/19/2017 3:12 PM	11/2/2017 1:03 PM
Vital Sign	CAR	038	399
Weight	15.5 kilograms	15.5 kilograms	16.5 kilograms
Attitude	0 - BAR		0 - BAR
Temp	102.4	102.4	103.2
HR	178	180	168
RR			110
RQ	Panting	Panting	Panting
Muc			Pink
Memb			
CRT	<2 sec		<2 sec
BP	152 #4/LF	152 4/LF	

Quiet heart sounds. Gallop present. Fair femoral pulses. Regular rhythm. Normal lung sounds. Normal jugular veins. Palpable hepatomegaly. Epidermal collarettes with exudative crusting on ventral abdomen. PLNs WNL. Unchanged appearance to growth on gingiva. MM pink/moist. CRT < 2 sec.

Diagnostics:
Thoracic radiographs: Decrease in heart size as compared to previous films. No evidence of cardiac decompensation.
Renal panel: BUN 32 mg/dL, otherwise unremarkable.
Taurine level: pending, with call with results

Diagnosis:
Congestive heart failure, left sided
Dilated cardiomyopathy
Hypoalbuminemia

Superficial dermatitis

Recommendations:
Please give the following medications as directed:

ITEM DESCRIPTION	DIRECTIONS
Vetmedin 5 mg tab	Give 1 tablet by mouth in the mornings and 1/2 tablet by mouth in the evenings.
L-Carnitine 500mg tablets	Give 1 tablet by mouth every 12 hours.
Taurine 500mg tablets	Give 1 tablet by mouth every 8 hours.
Spironolactone 25mg tablets	Give 1 tablet by mouth once every 24 hours.
Enalapril 5mg tablets	Give 1 and 1/2 tablets by mouth every 12 hours.
Furosemide 12.5mg tablet	Give 1 tablet by mouth every 12 hours.

ADD:
Simplicef 100mg tablets - Give 1 tablet by mouth once every 24 hours for 10 days.
(b) (6) has some lesions on his abdomen that are characteristic of a superficial skin infection. Simplicef is a good antibiotic for uncomplicated skin infections. (b) (6) should be re-evaluated by your primary veterinarian or a veterinary dermatologist if he does not improve.

We will call you with (b) (6) bloodwork results when they are available.

Please continue to monitor (b) (6) for cough, lethargy and/or changes in respiratory rate/effort.

*** As long as (b) (6) continues to do well at home we would like to re-evaluate him in 3-4 months. At this time we will recheck his kidney values/electrolytes, repeat chest x-rays and repeat an echocardiogram.

Like us on Facebook!!
www.facebook.com/(b) (6)

Notes to our clients
-Please bring all medications to your pet's scheduled appointments.
-We require a 48 hour notice for all refills. When you call to request a refill, please leave the pharmacy phone number or clearly indicate if you plan on picking up the medication at our facility. PRESCRIPTION REFILLS OUTSIDE OF (b) (6) (b) (6) REGULAR BUSINESS HOURS (Evenings, Fridays, holidays, and weekends) MAY BE ASSOCIATED WITH AN AFTER HOURS FILLING FEE.
-Check out **www.goodrx.com** and enter your local zip code to search for the best prices on your medications at your local pharmacies.
-If an emergency arises with your pet, (b) (6) is only a phone call away. (b) (6) is a 24 hour facility and the emergency veterinarians can always reach the cardiologist on-call.
-Please schedule your recommended recheck as soon as possible. Our schedule tends to book up quite quickly and we want to make sure that we see your pet in a timely manner.

Report Details - EON-345835

ICSR:	2040532	
Type Of Submission:	Initial	
Report Version:	FPSR.FDA.PETF.V.V1	
Type Of Report:	Adverse Event (a symptom, reaction or disease associated with the product)	
Reporting Type:	Voluntary	
Report Submission Date:	2018-01-22 18:42:17 EST	
Reported Problem:	**Problem Description:**	(b) had been presented to his regular veterinarian for a week long history of cough on August 22, 2017. Radiographs were taken and (b) was initially placed on antibiotics for presumed pneumonia (amoxicillin and doxycycline). Radiographs were reviewed by a radiologist who revised the diagnosis to congestive heart failure and furosemide and enalapril were added. I evaluated (b) on September 5, 2017 and diagnosed him with dilated cardiomyopathy with congestive heart failure.
	Date Problem Started:	08/15/2017
	Concurrent Medical Problem:	No
	Outcome to Date:	Better/Improved/Recovering
Product Information:	**Product Name:**	Zignature Kangaroo Limited Ingredient Formula Dry Dog Food
	Product Type:	Pet Food
	Lot Number:	
	Package Type:	BAG
	Possess Unopened Product:	No
	Possess Opened Product:	No
	Product Use Information:	**Description:** (b) had been fed this diet since he was one year of age because it was recommended by Pet People. He was also given grain free treats, carrots and apples.
		Time Interval between Product Use and Adverse Event: 3 Years
		Product Use Stopped After the Onset of the Adverse Event: Yes
		Product Use Started Again: No
		Perceived Relatedness to Adverse Event: Probably related
	Manufacturer /Distributor Information:	
	Purchase Location Information:	
Animal Information:	**Name:**	(b) (6)
	Type Of Species:	Dog
	Type Of Breed:	Spaniel - Cocker American
	Gender:	Male
	Reproductive Status:	Neutered
	Weight:	15.5 Kilogram
	Age:	4 Years
	Assessment of Prior Health:	Excellent

	Number of Animals Given the Product:	4	
	Number of Animals Reacted:	4	
	Owner Information:	Owner Information provided:	Yes
		Contact:	Name: (b) (6)
			Phone: (b) (6)
			Other Phone: (b) (6)
			Email: (b) (6)
		Address:	(b) (6) United States
	Healthcare Professional Information:	Practice Name:	(b) (6)
		Contact:	Name: (b) (6)
			Phone: (b) (6)
			Other Phone: (b) (6)
			Email: (b) (6)
		Address:	(b) (6) United States
Sender Information:	Name:	(b) (6)	
	Address:	(b) (6) United States	
	Contact:	Phone:	(b) (6)
		Other Phone:	(b) (6)
		Email:	(b) (6)
	Permission To Contact Sender:	Yes	
	Preferred Method Of Contact:	Phone	
	Reported to Other Parties:	Other	
Additional Documents:			
	Attachment:	cardio0011.pdf	
	Description:	Initial visit with echo report and most recent recheck medical record	
	Type:	Medical Records	
	Attachment:	cardio0012.pdf	
	Description:	Taurine level	
	Type:	Laboratory Report	

FDA-CVM-FOIA-2019-1704-000487

Report Details - EON-345965

ICSR:	2040808	
Type Of Submission:	Initial	
Report Version:	FPSR.FDA.PETF.V.V1	
Type Of Report:	Adverse Event (a symptom, reaction or disease associated with the product)	
Reporting Type:	Voluntary	
Report Submission Date:	2018-01-25 12:18:44 EST	
Reported Problem:	**Problem Description:**	At his scheduled visit to my clinic, thoracic radiographs showed generalized cardiomegaly which had been progressive compared to prior chest radiographs from his regular veterinarian but there was no evidence of cardiogenic edema. Echocardiogram was performed which showed dilated cardiomyopathy. Fundic exam was abnormal with a suspected partial retinal detachment OS. Diet history revealed that (b) was eating a kangaroo based diet. At this time the patient was continued on the Cough-tabs, Lasix was discontinued, and Vetmedin (2.5mg a.m., 1.25mg p.m.), enalapril (1.25mg BID), and taurine (500mg BID) were started. Taurine was discontinued after a normal taurine level was received. Cough persisted despite these changes and a course of doxycycline was prescribed (50mg BID x 10days). The cough improved significantly but did not completely resolve so the doxycycline was continued an additional 14 days. The dog has since been lost to follow-up. I have attempted to contact the owner and am waiting for a response. I did contact the referring veterinarian and to their knowledge the dog is still alive.
	Date Problem Started:	04/24/2017
	Concurrent Medical Problem:	Yes
	Pre Existing Conditions:	(b) was presented to me for evaluation of lethargy and progressive cough of 6 months duration. He had been treated with a cough suppressant (Cough-tabs 1/2 tab PO BID) and furosemide (5mg once daily) prior to presentation with no response.
	Outcome to Date:	Unknown
Product Information:	**Product Name:**	limited ingredient diet with kangaroo as protein source - manufacturer not specified in written history (we have attempted to contact the owner but they do not return phone calls)
	Product Type:	Pet Food
	Lot Number:	
	Possess Unopened Product:	Unknown
	Possess Opened Product:	Unknown
	Storage Conditions:	Unknown
	Product Use Information:	**Description:** History in medical record describes diet but does not indicate duration of administration. **Product Use Stopped After the Onset of the Adverse Event:** No **Perceived Relatedness to Adverse Event:** Probably related
	Manufacturer /Distributor Information:	
	Purchase Location Information:	
Animal Information:	**Name:**	(b)
	Type Of Species:	Dog
	Type Of Breed:	Shih Tzu
	Gender:	Male

	Reproductive Status:	Neutered
	Weight:	6.08 Kilogram
	Age:	8 Years
	Assessment of Prior Health:	Good
	Number of Animals Given the Product:	5
	Number of Animals Reacted:	5
	Owner Information:	**Owner Information provided:** Yes
		Contact: **Name:** (b) (6)
		Phone: (b) (6)
		Other Phone: (b) (6)
		Email: (b) (6)
		Address: (b) (6) United States
	Healthcare Professional Information:	**Practice Name:** (b) (6)
		Contact: **Name:** (b) (6)
		Phone: (b) (6)
		Other Phone: (b) (6)
		Email: (b) (6)
		Address: (b) (6) United States
Sender Information:	**Name:**	(b) (6)
	Address:	(b) (6) United States
	Contact:	**Phone:** (b) (6)
		Other Phone: (b) (6)
		Email: (b) (6)
	Permission To Contact Sender:	Yes
	Preferred Method Of Contact:	Email
	Reported to Other Parties:	Other
Additional Documents:	**Attachment:**	cardio0030.pdf
	Description:	Labwork including CBC, profile, taurine level and radiology report
	Type:	Laboratory Report
	Attachment:	cardio0029.pdf
	Description:	Medical records from initial and follow-up visit
	Type:	Medical Records

FDA-CVM-FOIA-2019-1704-000490

Report Details - EON-350158

ICSR:	2044632
Type Of Submission:	Initial
Report Version:	FPSR.FDA.PETF.V.V1
Type Of Report:	Adverse Event (a symptom, reaction or disease associated with the product)
Reporting Type:	Voluntary
Report Submission Date:	2018-03-27 15:12:36 EDT

Reported Problem:

Problem Description:	At the time of diagnosis (10/31/17), Lucy was a 13 year old female spayed Labrador retriever who had been maintained on a Zignature Kangaroo formula. She presented with a history of a progressive cough which, prior to presentation, became productive and she coughed up a small volume of pink foam (possible pulmonary edema). On examination she had a 2/6 left apical systolic heart murmur and on echo diagnosed with advanced dilated cardiomyopathy with severe left ventricular dilation, moderate to severe left ventricular systolic dysfunction, and moderate to severe left atrial dilation. Thoracic radiographs were suspicious for early congestive heart failure. A whole blood taurine level was submitted and was low at 168. She was treatment with furosemide, benazepril, pimobendan, spironolactone, taurine and l-carnitine and her diet was changed to Royal Canin Early Cardiac. At her recheck in 2/26/18, (b)(6) heart had improved significantly with now mild dilated cardiomyopathy with normalized left atrial dimensions, mild left ventricular dilation and low normal left ventricular systolic function. The furosemide was able to be discontinued at this time.
Date Problem Started:	10/31/2017
Concurrent Medical Problem:	No
Outcome to Date:	Better/Improved/Recovering

Product Information:

Product Name:	Zignature Kangaroo Formula
Product Type:	Pet Food
Lot Number:	
Package Type:	BAG
Possess Unopened Product:	Unknown
Possess Opened Product:	Unknown

Product Use Information:

Product Use Stopped After the Onset of the Adverse Event:	Yes
Adverse Event Abate After Product Stop:	Yes
Product Use Started Again:	No
Perceived Relatedness to Adverse Event:	Probably related
Other Foods or Products Given to the Animal During This Time Period:	Unknown

Manufacturer /Distributor Information:

Name:	Pets Global - Zignature
Type(s):	Manufacturer
Address:	28334 Industry Dr Valencia California 91355 United States

		Contact:	Phone:	(661) 309-1235
			Web Address:	www.zignature.com
		Possess One or More Labels from This Product:	Yes	
	Purchase Location Information:			
Animal Information:	Name:	(b)		
	Type Of Species:	Dog		
	Type Of Breed:	Retriever - Labrador		
	Gender:	Female		
	Reproductive Status:	Neutered		
	Weight:	33.18 Kilogram		
	Age:	13 Years		
	Assessment of Prior Health:	Good		
	Number of Animals Given the Product:	1		
	Number of Animals Reacted:	1		
	Owner Information:	Owner Information provided:	Yes	
		Contact:	Name:	(b) (6)
			Phone:	(b) (6)
			Other Phone:	(b) (6)
			Email:	(b) (6)
		Address:	(b) (6) United States	
	Healthcare Professional Information:	Practice Name:	(b) (6)	
		Contact:	Name:	(b) (6)
			Phone:	(b) (6)
			Email:	(b) (6) @cvcavets.com
		Address:	(b) (6) United States	
		Practice Name:	(b) (6)	
		Contact:	Name:	(b) (6)
			Phone:	(b) (6)
			Email:	(b) (6) @cvcavets.com
		Address:	(b) (6) United States	
		Type of Veterinarian:	Referred veterinarian	
		Permission to Release Records	Yes	

		to FDA:
Sender Information:	**Name:**	(b) (6)
	Address:	(b) (6) United States
	Contact:	**Phone:** (b) (6) **Email:** (b) (6) @cvcavets.com
	Permission To Contact Sender:	Yes
	Preferred Method Of Contact:	Email
	Reported to Other Parties:	Other
Additional Documents:		
	Attachment:	(b) (6) Echo Report 2017-10-31.pdf
	Description:	Echocardiogram 10-31-2017
	Type:	Echocardiogram
	Attachment:	(b) (6) Echo Report 2018-02-26.pdf
	Description:	Echocardiogram 2-26-2018
	Type:	Echocardiogram
	Attachment:	(b) (6) Taurine Level 2017-11-03.pdf
	Description:	BW Taurine Level 11-3-2017
	Type:	Laboratory Report

Selenium bioavailability: current knowledge and future research requirements[1 5]

Susan J Fairweather-Tait, Rachel Collings, and Rachel Hurst

ABSTRACT

Information on selenium bioavailability is required to derive dietary recommendations and to evaluate and improve the quality of food products. The need for robust data is particularly important in light of recent suggestions of potential health benefits associated with dif ferent intakes of selenium. The issue is not straightforward, however, because of large variations in the selenium content of foods (deter mined by a combination of geologic/environmental factors and selenium supplementation of fertilizers and animal feedstuffs) and the chemical forms of the element, which are absorbed and metab olized differently. Although most dietary selenium is absorbed effi ciently, the retention of organic forms is higher than that of inorganic forms. There are also complications in the assessment and quantifi cation of selenium species within foodstuffs. Often, extraction is only partial, and the process can alter the form or forms present in the food. Efforts to improve, standardize, and make more widely available techniques for species quantification are required. Similarly, reliable and sensitive functional biomarkers of selenium status are required, together with improvements in current biomarker methods. This re quirement is particularly important for the assessment of bioavail ability, because some functional biomarkers respond differently to the various selenium species. The effect of genotype adds a potential further dimension to the process of deriving bioavailability estimates and underlines the need for further research to facilitate the process of deriving dietary recommendations in the future. *Am J Clin Nutr* 2010;91(suppl):1484S 91S.

INTRODUCTION

To derive selenium requirements and establish dietary rec ommendations for optimal health, estimates of selenium bio availability are needed. A literature review on the bioavailability of selenium from foods was published in 2006 (1), and it highlights the dependence of bioavailability on food sources associated with different forms of selenium and emphasizes the importance of the assessment of bioavailability with the use of functional assays. Data on chemical speciation and metabolic transformations (in conjunction with information on the relation between selenium intake and status and health outcomes) are required to assess selenium bioavailability and the longer term health consequences that result from different intakes.

DIETARY REQUIREMENTS

The 1991 UK Dietary Reference Values (2) used data from older literature and estimated that between 55% and 65% of dietary selenium is absorbed. The 1993 Population Reference Intakes published by the European Scientific Committee for Food (3) concluded that for selenium "all usual dietary forms are absorbed quite efficiently." The 2000 report of the US Food and Nutrition Board (4) suggested that most dietary selenium is highly bioavailable: >90% of selenomethionine is absorbed; selenocysteine appears to be absorbed very well; ≈100% of selenate is absorbed, but a significant fraction is lost in the urine; and >50% of selenite is absorbed (depending on luminal in teractions) and is better retained than selenate. There is clearly a need to review dietary recommendations in light of more re cent data, in particular, information on dietary forms of selenium and the relationships between intake and health outcomes.

SELENIUM SPECIATION

A recent review (5) provides information on the forms of selenium in food and associated health effects; technical approaches used for speciation have also been reviewed recently (6, 7). The analysis of forms of selenium in food is a challenging task; there are currently no methods that can reliably extract 100% of the selenium from foods without potentially affecting the species, and the techniques are established in only a few laboratories worldwide. Therefore, care has to be taken to extract as much selenium as possible while still retaining the form that is present in the food as consumed; conditions that are devised to maximize the extraction of selenium from a food matrix may cause changes in chemical form. Ideally, the measurements should be made in food that has gone through processing (eg, cooking) followed by simulated gastrointestinal digestion, be

[1] From the School of Medicine, Health Policy and Practice, University of East Anglia, Norwich, United Kingdom.

[2] Presented at the workshop "Micronutrient Bioavailability: Priorities and Challenges for Setting Dietary Reference Values," held in Barcelona, Spain, 11 12 June 2009.

[3] This article does not necessarily reflect the views of the Commission of the European Communities and in no way anticipates future policy in this area.

[4] Supported by the Commission of the European Communities, specific RTD Programme "Quality of Life and Management of Living Resources," within the 6th Framework Programme (contract no. FP6 036196 2 EURRECA: EURopean micronutrient RECommendations Aligned).

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cause this is the form present in the lumen of the gut that is of interest. Although it has not been possible to produce comprehensive data that describe forms of selenium in food, there are limited data on the percentage distribution of different species (expressed as percentage of extractible or total selenium); examples are given in **Table 1**.

The selenium content and species of both plant and animal foodstuffs depend on environmental conditions, in particular, the quantity and species of selenium to which the animal/plant is exposed (6, 24). Selenomethionine is predominant in cereals, and selenium concentrations vary from 0.01 to 0.55 μg/g fresh weight (5), whereas in other plant foods the content is generally lower, with the exception of Brazil nuts and vegetables, which are selenium-accumulating plants, namely those in the allium and brassica families. The selenium content of Brazil nuts varies depending on soil content and other environmental factors, and nuts from trees in the central part of Brazil contain $\leq$10 times more selenium than those from West Brazil (6). The reason for the high content of selenium in Brazil nuts is that the proteins are high in sulfur-containing amino acids, and selenomethionine can nonspecifically replace methionine. The major species in non–selenium-accumulating plant foods are selenate and selenomethionine, plus smaller amounts of selenocysteine. In contrast, the predominant form of selenium in selenium-accumulating plants is γ-glutamyl methylselenocysteine (13, 14). There are limited data on the forms of selenium in animal foodstuffs, but it appears that the major forms are selenomethionine and selenocysteine, which are incorporated nonspecifically into muscle protein (19). In addition, selenate and selenite have been detected in fish (18, 20) and there appear to be large differences between fish species in relation to selenoproteins (25). In foods of animal origin, supplementation with organic compared with inorganic selenium results in meat of higher selenium concentration. For example, when a comparison is made between the effect of selenium yeast and sodium selenite supplements, skeletal muscle from lambs contained 0.12 and 0.08 μg selenium/g fresh weight, respectively (26), and beef contained 0.41 and 0.30 mg/kg dry weight, respectively (27).

ABSORPTION, RETENTION, AND METABOLISM

Data on selenium metabolism from different foods and selenium supplements indicate differences in the absorption and use of selenium between inorganic and organic forms in humans (28, 29) and rats (30). The absorptive pathways have not yet been fully characterized, but selenium as selenate or selenite appears to be very well absorbed but less well retained in the body than organic forms of selenium, such as selenomethionine and selenocysteine (31–33). The proposed metabolic pathways for different forms of selenium are shown in **Figure 1** (5). Most forms of selenium are efficiently absorbed, but subsequent metabolism depends on the form in which they are present in plasma. Selenomethionine, selenocysteine, selenate, and selenite enter the selenide pool and from here the selenium is either used for selenoprotein synthesis or excreted in the urine as a selenosugar. Selenomethionine can, however, also be incorporated directly (and nonspecifically) into proteins through the replacement of methionine. A separate pathway is followed by the organic compound, γ-glutamyl methylselenocysteine, found in brassica and allium vegetables, whereby it is first converted to Se-methylselenocysteine and then transformed by β-lyase into methylselenol, which is primarily excreted in breath and urine but may also enter the selenide pool.

Several approaches have been used to measure the bioavailability of selenium in various foods, as summarized in **Table 2**. These include the measurement of changes in plasma selenium concentration, measurement of glutathione peroxidase (GPx) enzyme activity, and absorption/retention studies. For the last, intrinsic techniques with the use of stable isotopes of selenium have been developed to label the endogenous forms of selenium in foods (40). In general, selenium is absorbed efficiently, but it is not possible to assign specific figures for retention and use (bioavailability) to individual forms of selenium because of the complexity of many foods (Table 1). However, a study by Bügel et al (39), on the assumption that selenomethionine is the major form in meat, showed that most of the selenium was absorbed and just over half retained in the body (ie, not excreted in the urine). Selenium in Brazil nuts appeared to be better used than selenomethionine, in terms of the response of plasma selenium concentration and red blood cell GPx activities: the plasma selenium increase was similar despite the fact that the daily intake from Brazil nuts was half that from selenomethionine (35). Changes in selenium status that reflect changes in intake occur over a period of several weeks or months, although the feeding trial of Hawkes et al (38) showed a significant difference between a beef, rice, and powdered milk diet with low selenium content and one with high selenium content after only 14 d. In a study by Kirby et al (11), the plasma selenium response in a feeding trial appeared to be related to the form of selenium in wheat flour biscuits: intake of selenomethionine in biofortified wheat-biscuits resulted in a greater increase in plasma selenium after 6 mo than the oxidized selenomethionine (selenomethionine selenoxide) in fortified biscuits (Table 2).

FUNCTIONAL MEASURES

There are 25 known selenoprotein genes in humans (41, 42), which encode selenoproteins with a variety of functions, as summarized in **Table 3**. Several of the selenoproteins, which include selenoproteins P and W and the GPx 1, 3, and 4, have been used widely as biomarkers of selenium status. Functional biomarkers are only useful if they can be measured in readily accessible tissues, such as blood. At present, the most promising biomarker appears to be selenoprotein P, which appears to reach a plateau after 2–4 wk of supplementation (88, 89) and is well correlated with plasma selenium across a wide range of selenium status (90), up to a plasma selenium concentration of $\approx$125 ng/mL (33). Selenoprotein P typically accounts for approximately half of the selenium in plasma (46). It is generally more sensitive than other selenoproteins, such as GPx, in both deficiency (90) and after supplementation (89–91), and, in addition, the response of selenoprotein P to different forms of selenium appears to be similar (92).

Biomarkers of selenium status have recently been the subject of a systematic review (93), in which the response of each biomarker to either depletion or supplementation (only studies that intervened with selenomethionine or selenium-enriched yeast were included) was assessed and evaluated for different population groups. However, for most biomarkers there was

TABLE 1
Examples of forms of selenium (percentage of total or extractable selenium) in foods

Food (reference)	Typical selenium content[1]	Forms of selenium
	μg/g fresh weight	
Selenium-enriched yeast (5, 8)	1200–2200	60–84% Selenomethionine, usual percentage in high-quality commercial preparation of selenium-enriched yeast but values for selenomethionine content can vary: 23–83% Selenomethionine 3–21% Selenocysteine 1–20% γ-Glutamyl-Se-methylselenocysteine 4% Selenate 13–51% Other forms
Brazil nuts (*Bertholletia excelsa*) (9)	2 54 (0 85–6 86)	≈25% Selenomethionine
Wheat (8, 10)	0 1–30 0 08–44	12–19% Selenate/ite 56–83% Selenomethionine 4–12% Selenocysteine 1–4% Se-methylselenocysteine ≈55% Selenomethionine
Wheat (biofortified) (11)	8 3	76–85% Selenomethionine
Wheat-flour (biofortified) biscuits (11)	4 4	76–85% Selenomethionine
Wheat flour (unfortified) soaked in aqueous solution of selenomethionine and baked into biscuits (11)	8 5	55% Selenomethionine selenoxide 5% Selenomethionine
Broccoli (selenium enriched) (12)	62 3[2]	45% Se-methylselenocysteine 20% Selenate 20% Selenate 12% Selenomethionine
Onions (*Allium cepa*) (13)	<0 5	100% Selenate (extractable selenium)
Onions (selenium enriched) (13)	140	63% γ-Glutamyl-Se-methylselenocysteine 10% Selenate 5% Selenomethionine
Garlic (*Allium sativum*) (13)	<0 5	53% Selenomethionine 31% γ-Glutamyl-Se-methylselenocysteine 12% Se-methylselenocysteine 4% Selenate
Garlic (selenium enriched) (14)	296	73% γ-Glutamyl-Se-methylselenocysteine (total eluted selenium) 13% Selenomethionine 4% γ-Glutamyl-selenomethionine 3% Se-methylselenocysteine 2% Selenate
Lentils (*Lens culinaris* L) (15)	0 24–0 36	90% Organic selenium 10% Selenate
Carrots (16)	<0 05	Undetectable
Carrots (selenium enriched) (16)	0 4–2 2	Selenium-enriched with the use of selenate (% extractable): ≈54% Selenomethionine 32% Selenate ≈14% γ-Glutamyl-selenomethionine Selenium-enriched with the use of selenite: ≈71% Selenomethionine 17% Selenite ≈12% γ-Glutamyl-selenomethionine
Potatoes (17)	0 12	50% Selenomethionine (extractable) 50% Selenate (extractable)
Shellfish (18)	0 36–1 33	7 6–44 8% Selenate
Cod (19, 20)	1 5	70% Selenomethionine 12% Selenite
Tuna (canned in water) (21)	5 6	29% Selenomethionine (extractable)
Shark (21)	2 0	56% Selenomethionine (extractable)
Swordfish (22)	Not quantified	Selenomethionine, selenenyl sulfide, selenite
Chicken (23)	0 5	56–66% Selenomethionine (extractable) 20–31% Selenocysteine (extractable)
Lamb (23)	0 4	56–60% Selenomethionine (extractable) 50% Selenocysteine (extractable)

[1] Values are means and/or ranges
[2] μg/g dry weight

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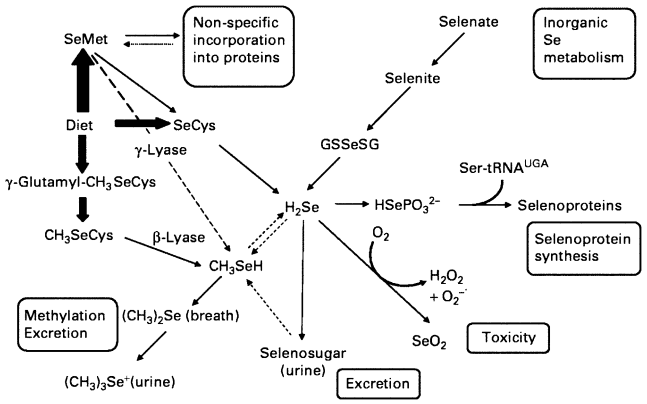

FIGURE 1. Metabolic pathway of dietary selenium in humans. Se, selenium; SeMet, selenomethionine; SeCys, selenocysteine; GSSeSG, selenodiglutathione; γ glutamyl CH_3SeCys, γ glutamyl Se methylselenocysteine; H_2Se, hydrogen selenide; $HSePO_3^{2-}$, selenophosphate; CH_3SeCys, Se methylselenocysteine; CH_3SeH, methylselenol; $(CH_3)_2Se$, dimethyl selenide; SeO_2, selenium dioxide; $(CH_3)_3Se^+$, trimethyl selenonium ion. Reproduced with permission from reference 5.

a paucity of data for meaningful subgroup or dose response analysis. In the included studies plasma selenium was the most commonly measured biomarker, and it responded positively to intervention, as did whole blood and erythrocyte selenium, plasma selenoprotein P, and platelet, plasma, erythrocyte and whole blood GPx activity, albeit with significant heterogeneity in each case. The review concluded that further large scale in terventions are required to assess the usefulness of selenium responsive biomarkers, and these could conceivably include aspects of speciation. Plasma selenium concentration reflects dietary exposure to most forms of selenium, but in the absence of a well described homeostatic regulation there is no absolute plateau, although the concentration will reach a steady state at any constant level of intake after ≈10 12 wk (33, 91, 92, 94 97). In addition to dose, the plasma response to dietary selenium is species dependent, so consumption of 2 different forms may result in different plasma selenium concentrations (33, 92, 95, 96, 98, 99).

EFFECT OF GENOTYPE

The response by individuals to 6 wk of selenium supple mentation with 100 μg sodium selenite/d has been shown to be influenced by genetic polymorphisms in the selenoprotein P gene (*SEPP*) (100) and *GPX4* gene (101). Biomarkers that are commonly used to assess selenium bioavailability (plasma se lenium, selenoprotein P, and GPx3) were associated with 2 common single nucleotide polymorphisms in *SEPP* in both baseline and postsupplementation samples (100). The *GPX4* polymorphism was shown to influence lymphocyte GPx4 con centration and other selenoproteins in vivo (101). A single nu cleotide polymorphism in GPx1 (Pro198Leu) was associated with selenium deficiency and impaired GPx1 activity (102) and also may be associated with a different response of GPx1 ac tivity to selenium (103). This observation raises the issue of whether common polymorphisms in selenoprotein genes, such as *SEPP, GPX1, GPX4*, and selenoprotein S (*SELS*) (92), will

TABLE 2
Bioavailability of selenium from various foods[1]

Food (reference)	Technique used	Results
Selenium (Se) yeast, 300 μg/d for 10 wk, then single dose of ^{77}Se yeast (34)	Absorption from stable isotopically labeled material (327 μg selenium)	89%
	Retention (absorption minus urinary excretion)	74%
Brazil nuts, 53 μg/d for 3 mo (35)	Plasma selenium increase	64.2%
	Plasma GPx increase	8.2%
	Whole blood GPx increase	13.2%
Selenomethionine, 100 μg/d for 3 mo (35)	Plasma selenium increase	61%
	Plasma GPx increase	3.4%
	Whole blood GPx increase	5.3%
Biofortified wheat flour biscuits, mean intake 172 μg/d for 6 mo (11)	Plasma selenium increase after 6 mo feeding trial	72 μg/L increase
Fortified wheat flour biscuits, mean intake 208 μg/d for 6 mo (11)	Plasma selenium increase after 6 mo feeding trial	16 μg/L increase
Basal diet, 52 μg selenium + cow milk, 15 μg selenium (36)	Fractional absorption in ileostomists	65.5% 73.3%
Shrimp, 88 μg/d for 6 wk (37)	Plasma selenium increase	6.3 μg/L increase
	Apparent absorption	83%
Beef, rice, and powdered milk , 14 μg/d (low) compared with 297 μg /d (high) for 14 d (38)	Plasma selenium change	−40 μg/L (low); 97 μg/L (high)
	Muscle selenium	−0.37 μg/g protein (low); 0.57 μg/g protein (high)
	Platelet GPx	−120 nkat/g protein (low); 100 nkat/g protein (high)
	Red blood cell selenium	−42 μg/L (low); 106 μg/L (high)
	Red blood cell GPx	−15 nkat/g protein (low); 13 nkat/g protein (high)
Pork, 106 μg/d for 3 wk; 7 d metabolic balance in final week (39)	Apparent absorption	94%
	Retention	58%

[1] GPx, glutathione peroxidase; nkat, nanokatal.

TABLE 3
Summary of human selenoproteins and their functions[1]

Group/name (reference)	Abbreviation(s)	Location	Main functions
Glutathione peroxidases			
Glutathione peroxidase-1 (43)	GPx1, cGPx, GSH-Px	Widely expressed throughout the body, intracellular enzyme	Cystolic enzyme, antioxidant activity
Glutathione peroxidase-2 (44)	GPx2,GI-GPx	Mainly in gastrointestinal tissue, also in liver	Protection of GI tract from oxidative damage
Glutathione peroxidase-3 (45)	GPx3, eGPx	Plasma [accounts for 10–30% selenium in plasma (46)] and extracellular fluid, expressed in liver, kidney, heart, lung, thyroid, GI tract, and breast (47)	Plasma antioxidant, can decrease lipid hydroperoxides (48)
Glutathione peroxidase-4	GPx4, PHGPx	Widely expressed, high expression in the testes (49, 50); cytosolic and membrane-bound forms (51, 52)	Antioxidant activity, protects membranes from peroxidative degradation (51); can decrease phospholipid, cholesterol and cholesterol ester hydroperoxides to less toxic derivatives (52); protection against oxidatively damaged DNA (53); regulation of 15-lipoxygenase pathway (54) and 5-lipoxygenase (55); important for male fertility and sperm maturation/function/motility (56–59)
Glutathione peroxidase-6 (41)	GPx6	Embryo and olfactory epithelium	Unknown
Thioredoxin reductases			
Thioredoxin reductase-1 (60, 61)	TrxR-1, TR1, Txnrd1	Intracellular (cytosolic/nuclear), widely distributed	Regulation of intracellular redox state, cell signaling; decreases thioredoxin
Thioredoxin reductase-2 (60, 61)	TrxR-2, TR2	Mitochondrial, widely distributed	Regulation of intracellular redox state; decreases thioredoxin
Thioredoxin reductase-3 (60, 61)	TrxR-3, TR3	Testis-specific	Regulation of intracellular redox state
Iodothyronine deiodinases			
Iodothyronine 5′ deiodinase-1, type 1	DIO-1, DI1, 5′IDI	Kidney, liver, thyroid, and brown adipose tissue (62–64)	Thyroid hormone metabolism, converts inactive thyroxine to active 3,3′-5′triiodothyronine; activation of thyroid hormones (65)
Iodothyronine 5′ deiodinase-2, type 2 (63, 66, 67)	DIO-2, DI2	Thyroid, CNS, pituitary, brown adipose tissue, skeletal muscle	Activation of thyroid hormones
Iodothyronine 5 deiodinase-3, type 3 (68)	DIO-3, DI3	Placenta, CNS, fetus	Inactivation of thyroid hormones
Selenoproteins and other			
Selenoprotein-P (69, 70)	SelP, Sepp1	Plasma [accounts for 30–50% of selenium in plasma (46, 71)] and also ubiquitously expressed in most tissues; high expression in brain, liver, and testes	Selenium homeostasis (72) and transport of selenium to tissues; antioxidant activity and decrease of lipid hydroperoxides (73)
Selenoprotein-W (74, 75)	SelW	Most tissues, abundant in brain, colon, heart, skeletal muscle, and prostate	Involved in skeletal and cardiac muscle metabolism/function, antioxidant function
Selenoprotein-N	SelN	Most tissues, ubiquitous expression, transmembrane glycoprotein associated with endoplasmic reticulum (76, 77)	Unknown, may be important in muscle and development (76)
Selenoprotein-S	SelS	Membrane protein, located in the endoplasmic reticulum, widely expressed	Inflammatory response, regulation of inflammatory cytokines (interleukin 1β and 6 and tumor necrosis factor alpha) (78), removal of misfolded proteins from the endoplasmic reticulum (79)
Selenoprotein-K (80)	SelK	Membrane protein, localized to endoplasmic reticulum	Possible antioxidant activity
Selenoprotein-R (81)	SelR / MsrB1	Cytosol and nucleus; widely expressed	Antioxidant, protein repair and methionine metabolism (82)
Selenoprotein-H (83)	SelH	Widely expressed in tissues, localized to the nucleus	DNA binding protein, regulation of glutathione synthesis genes, and phase II detoxification
Selenoprotein-I (41)	SelI	Unknown	Unknown

(Continued)

TABLE 3 (*Continued*)

Group/name (reference)	Abbreviation(s)	Location	Main functions
Selenoprotein-M (84)	SelM	Localized in the endoplasmic reticulum	Protein folding in the endoplasmic reticulum, antioxidant activity
Selenoprotein-O (41)	SelO	Unknown	Unknown
Selenoprotein-T (41)	SelT	Unknown	Unknown
Selenoprotein-V (41)	SelV	Testes	Unknown
15 kD selenoprotein (85)	Sel15	Localized in the endoplasmic reticulum	Thioredoxin-like, role in unfolded protein response (86)
Selenophosphate synthetase-2	SPS-2	Unknown	Selenoprotein biosynthesis (87)

[1] The list of selenoproteins that contain selenocysteine was generated from information on the selenoprotein database SelenoDB (42). Glutathione peroxidases 5, 7, and 8 (GPx5, GPx7, GPx8); selenoproteins R2, R3, and W2 (SelR2, SelR3, SelW2); selenium-binding protein 2 (SBP2); selenophosphate synthetase1 (SPS1); and eukaryotic elongation factor (eEFSec) are not listed in the table of selenoproteins because they are homologs that contain cysteine or other amino acids that do not contain selenocysteine (SelenoDB). CNS, central nervous system; GI, gastrointestinal.

have a significant effect on the metabolism of dietary selenium and will generate different figures for bioavailability. However, it is most likely that the effect of genotype on the biomarkers used to predict bioavailability is subtle and only becomes relevant when longer-term health outcomes are considered (104–106).

RESEARCH REQUIREMENTS

The bioavailability of different selenium species requires further investigation with the use of stable isotope labels, and the mechanism of absorption of the different forms of selenium needs to be elucidated. Further data on selenium species in food are required, but with the well-known extraction constraints it will not be possible to generate comprehensive information for all foods; therefore, dietary intervention studies may be required to study foods that make a major contribution to selenium intake. The native forms of selenium need to be labeled intrinsically with stable isotopes of selenium to measure uptake and retention of food selenium in acute studies, and longer-term studies need to be undertaken to measure changes in functional biomarkers; the most promising at present is selenoprotein P, but other novel biomarkers should be sought. Interactions between selenium and other micronutrients, such as vitamin E, should be taken into consideration [possibly with the use of a network biology approach (107)], particularly in relation to health outcomes that are associated with antioxidant nutrients, such as inflammation. Finally, the effect of common selenoprotein gene polymorphisms on metabolism (and hence requirements) remains to be clarified.

The authors' responsibilities were as follows—SJF-T: first draft of the manuscript; and RC and RH: contribution of sections to the manuscript draft. All authors approved the final manuscript. The authors had no personal or financial conflicts of interest.

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Erratum

Fairweather Tait SJ, Collings R, Hurst R. Selenium bioavailability: current knowledge and future research. Am J Clin Nutr 2010;91(suppl):1484S 91S.

In the print version of this article, the term ''selenium methylselenocysteine'' appears in error. The correct term, as provided by the authors, is ''Se methylselenocysteine.'' This error occurs in Table 1 on page 1486S, in the legend to Figure 1 on page 1487S, and in text on page 1485S. The correct term appears in the online version.

doi: 10.3945/ajcn.2010.30169.

Erratum

Morris MS, Jacques PF, Rosenberg IH, Selhub J. Circulating unmetabolized folic acid and 5 methyltetrahydrofolate in relation to anemia, macrocytosis, and cognitive test performance in American seniors. Am J Clin Nutr 2010;91:1733 44.

On page 1735, the last sentence of the first paragraph of the section entitled ''Classification of subjects according to vitamin B 12 status and folate fractions'' contains a detection limit. The published detection limit for the assay is 0.18 nmol/L, not 0.027 nmol/L. The incorrect limit was also given in the footnotes to Table 1 and Table 4. There were 7 eligible subjects with measured serum folic acid concentrations between 0 and 0.18 nmol/L who were included in the group with detectable unmetabolized folic acid. In addition, in Table 4, the multivariate model was not specified for the row labeled ''Mean cell volume.'' The results in that row were controlled for sex, age, race ethnicity, current smoking, current alcohol intake, BMI, self reported cancer history, and serum concentrations of ferritin, cystatin C, and C reactive protein.

doi: 10.3945/ajcn.2010.30170.

Erratum

Yatabe MS, Yatabe J, Yoneda M, et al. Salt sensitivity is associated with insulin resistance, sympathetic overactivity, and decreased suppression of circulating renin activity in lean patients with essential hypertension. Am J Clin Nutr 2010;92:77 82.

In the print version of this article, 2 lines appear for each group represented in Figure 7; however, only one line should appear for each group. The correct version of Figure 7 appears below. The online version of this figure is correct.

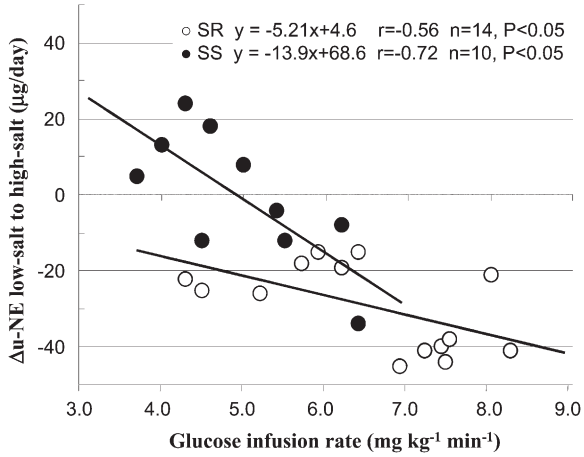

FIGURE 7. Relation between glucose infusion rate and change in urinary norepinephrine (*Δ*u NE) from the low to high salt diet in salt resistant (SR) subjects (*n* 14, open circles) and salt sensitive (SS) subjects (*n* 10, filled circles) with essential hypertension. Statistical significance was estimated with Pearson's correlation coefficient.

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Taurine deficiency in dogs with dilated cardiomyopathy: 12 cases (1997–2001)

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Objective—To determine signalment, history, clinical signs, blood and plasma taurine concentrations, electrocardiographic and echocardiographic findings, treatment, and outcome of dogs with low blood or plasma taurine concentrations and dilated cardiomyopathy (DCM).

Design—Retrospective study.

Animals—12 client-owned dogs with low blood or plasma taurine concentrations and DCM.

Procedure—Medical records were reviewed, and clinical data were obtained.

Results—All 12 dogs were being fed a commercial dry diet containing lamb meal, rice, or both as primary ingredients. Cardiac function and plasma taurine concentration improved with treatment and taurine supplementation. Seven of the 12 dogs that were still alive at the time of the study were receiving no cardiac medications except taurine.

Conclusions and Clinical Relevance—Results suggest that consumption of certain commercial diets may be associated with low blood or plasma taurine concentrations and DCM in dogs. Taurine supplementation may result in prolonged survival times in these dogs, which is not typical for dogs with DCM. Samples should be submitted for measurement of blood and plasma taurine concentrations in dogs with DCM, and taurine supplementation is recommended while results of these analyses are pending. (*J Am Vet Med Assoc* 2003;223:1137–1141)

Large-breed dogs, especially males, are predisposed to developing dilated cardiomyopathy (DCM).[1] Because the long-term prognosis for dogs with this disease is poor, methods for preventing the disease would be beneficial. However, in most affected dogs, the underlying cause is unknown.

In 1987, Pion et al[2] reported an association between low plasma taurine concentrations and DCM in cats. Oral supplementation of affected cats with taurine significantly improved clinical signs, restored myocardial function, and improved survival times.[3] Since then, the addition of taurine to commercial diets for cats has resulted in a marked decrease in the number of cats developing this disease.

Traditionally, dogs have not been recognized as having a dietary need for taurine, because they are able to synthesize taurine from the dietary sulfur amino acids methionine and cysteine.[4] Recently, however, a cardiologist in private practice (JRR) brought to the attention of the authors 4 unrelated, large-breed dogs with DCM. At the time of initial examination, all 4 dogs were found to have low blood taurine concentrations. One common factor among the dogs was consumption of the same lamb meal and rice commercial dry diet. Later, a Border Collie with DCM and low blood taurine concentrations was brought to our attention by a second local cardiologist in private practice. This dog was also consuming a lamb meal and rice diet, but one produced by another manufacturer. The common diet history for these 5 dogs suggested that diet may have had a role in the development of low blood taurine concentrations and DCM in these dogs. The purpose of the study reported here was to determine the signalment, history (including diet history), clinical signs, blood and plasma taurine concentrations, electrocardiographic and echocardiographic findings, treatment, and outcome of dogs with low blood or plasma taurine concentrations and DCM. In addition, we wanted to determine whether diet may have had any role in the development of DCM.

Criteria for Selection of Cases

The cardiology database at the Veterinary Medical Teaching Hospital of the University of California, Davis, was searched for dogs examined between October 1997 and August 2001 in which a diagnosis of DCM had been made. Dogs were included in the study only if DCM had been diagnosed by a veterinary cardiologist; the diagnosis had been confirmed by means of echocardiography; samples had been submitted to the Amino Acid Laboratory at the University of California, Davis, and blood or plasma taurine concentration had been found to be low; and a complete diet history was available. In addition, the 5 dogs brought to the authors' attention by local cardiologists were included in the study.

Procedures

Information collected for all dogs included signalment, history, diet history, initial clinical signs, electrocardiographic and echocardiographic findings, blood and

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plasma taurine concentrations, and treatment. Follow-up information was obtained by reviewing medical records or through telephone conversations with the attending cardiologist or primary care veterinarian.

Measurement of blood and plasma taurine concentrations—In all dogs, blood and plasma taurine concentrations were determined by the Amino Acid Laboratory at the University of California, Davis. At least 1 mL of heparinized blood, plasma, or both was submitted for analysis. Blood and plasma taurine concentrations were determined as described[5] with an automated amino acid analyzer.[a] Plasma taurine concentrations < 40 nmol/mL and blood taurine concentrations < 150 nmol/mL were considered indicative of a taurine deficiency.

Statistical analyses—Descriptive statistics (mean, SD, median, and range) were calculated for taurine concentrations and echocardiographic findings. Paired *t* tests were used to compare taurine concentrations and echocardiographic findings obtained at the time of diagnosis of DCM with values obtained after treatment. All analyses were performed with commercial software[b]; values of $P \leq 0.05$ were considered significant.

Results

From October 1997 through August 2001, 64 dogs with DCM were evaluated at the University of California veterinary teaching hospital. Blood samples from 24 of these dogs were submitted for analysis of taurine concentration, and 14 of the dogs were classified as having a taurine deficiency on the basis of a low blood or plasma taurine concentration. Six of the 14 dogs were excluded from the study, because they were American Cocker Spaniels, a breed well documented to have taurine- and carnitine-responsive DCM.[6] A seventh dog was excluded because of an inadequate diet history. Thus, 12 dogs were included in the study, including the 5 dogs brought to the authors' attention by local cardiologists and the 7 dogs in which DCM was diagnosed at the veterinary teaching hospital during the study period.

The 12 dogs included in the study consisted of 8 males (4 sexually intact) and 4 females (2 sexually intact). There were 3 English Setters, an Alaskan Malamute, a Border Collie, a German Shepherd Dog, a Golden Retriever, a Gordon Setter, a Great Pyrenees, a Labrador Retriever, a Newfoundland, and a Scottish Terrier. Dogs ranged from 4.5 to 11 years old at the time DCM was diagnosed (mean and median, 8.3 years).

Lethargy and anorexia were the 2 most common clinical abnormalities (7 dogs each). Other clinical signs included cough (n = 5), dyspnea (5), weight loss (3), trembling (1), and collapse (1). Three of the dogs were clinically normal at the time of initial examination. In 2 of these dogs, an arrhythmia was detected during a routine examination. The third dog was evaluated at the request of the owner, because 2 of her other dogs had recently been found to have low taurine concentrations and DCM.

Ten of the 12 dogs had ECG abnormalities. Left ventricular enlargement (LVE) was the most common abnormality (n = 7), followed by left atrial enlargement (6), atrial fibrillation (3), left bundle branch block (2), and ventricular premature contractions (1). All of the dogs underwent echocardiography. Mean ± SD E-point to septal separation at the time of initial examination was 14.5 ± 5.6 mm (n = 10; median, 14.5 mm; range, 7.5 to 26 mm). Mean fractional shortening at the time of initial examination was 15.7 ± 7.6% (n = 12; median, 15.9%; range, 7.0 to 28.9%). Mean plasma taurine concentration at the time DCM was diagnosed was 16 ± 20 nmol/mL (n = 12; median, 7 nmol/mL; range, 2 to 64 nmol/mL). Blood taurine concentrations had also been measured in 8 dogs. Mean blood taurine concentration at the time DCM was diagnosed was 121 ± 76 nmol/mL (median, 135 nmol/mL; range, 8 to 229 nmol/mL).

All 12 dogs were consuming a commercial dry diet with lamb meal, rice, or both as primary ingredients. Eight of the 12 dogs were consuming the same commercial lamb meal and rice dry diet[c] (diet A). The remaining 4 dogs were each consuming 1 of 4 other commercial diets[d-g] (diets B through E). Nutrient composition of the 5 diets was determined from the manufacturers' reported data (Table 1).

All 12 dogs were treated with taurine (1,000 to 3,000 mg, PO, q 24 h) beginning at the time DCM was diagnosed or when the low blood or plasma taurine concentration was discovered. In addition, 8 dogs were treated with inotropic agents, 7 were treated with diuretics, 7 were treated with angiotensin-converting enzyme inhibitors, and 1 was treated with a calcium channel blocker to manage cardiac abnormalities. Eleven of the dogs were changed to a different commercial dry diet at the time DCM was diagnosed or when the low blood or plasma taurine concentration was discovered.

Dogs were reevaluated between 1 and 12 months after the time of initial diagnosis (mean ± SD, 4.3 ± 3.5 months; median, 3 months). Electrocardiography was repeated on 4 dogs. Results were normal for 1 of the 4 dogs; abnormalities identified in the other 3 included left ventricular enlargement (n = 1), atrial fibrillation (2), and ventricular premature contractions (1). Echocardiography was repeated on 9 dogs. Mean E-point to septal separation was 8.9 ± 4.1 mm (n = 8; median, 8.3 mm; range, 2.9 to 17 mm). This was significantly ($P = 0.002$) lower than the mean value at the time of initial examination of these 8 dogs. Mean fractional shortening was 22.5 ± 6.3% (n = 9; median, 20%;

Table 1—Proximate dry matter composition of diets fed to dogs with dilated cardiomyopathy and low blood or plasma taurine concentrations

	Diet				
Variable	A	B	C	D	E
Crude protein (%)	24	23	17	22	28
Crude fat (%)	14	13	7	11	13
NFE* (%)	48	57	63	62	46
Crude fiber (%)	2	1	7	5	4
Ash (%)	10	6	6	NR	9
ME (kcal/g)	3.8	3.9	3.4	3.5	3.8

All values were obtained from manufacturers' reported data.
*Calculated or reported nitrogen-free extract.
NR = Not reported. ME = Metabolizable energy.

range, 16.6 to 36%). This was significantly (P = 0.002) greater than the mean value at the time of initial examination of these 9 dogs. Blood and plasma taurine concentrations increased in the 3 dogs in which analyses were repeated. Mean plasma taurine concentration was 226 ± 54 nmol/mL (n = 3; median, 208 nmol/mL; range, 183 to 286 nmol/mL), and mean blood taurine concentration was 455 ± 88 nmol/mL (n = 3; median, 443 nmol/mL; range, 373 to 548 nmol/mL). Plasma taurine concentrations were significantly (P = 0.02) increased in these 3 dogs, compared with concentrations prior to institution of taurine supplementation. Blood taurine concentrations were not significantly increased, but the P value was close to the cutoff for significance (P = 0.06).

Seven of the 12 dogs were alive at the time of the study. Mean ± SD survival time for all 12 dogs was 585 ± 499 days (median, 456 days; range, 1 to 1,460 days). One dog died within 1 day, 3 dogs died within 180 days, and 1 dog died within 365 days of initial examination. Survival times for the 7 dogs still alive at the time of the study ranged from 365 to 1,460 days (913 ± 380 days; median, 1,095 days). None of the 7 surviving dogs were receiving any cardiac medications at the time of the study other than taurine.

Discussion

With the exception of the single Newfoundland, none of the dogs in this study were of breeds predisposed to developing DCM. Breeds recognized to have a high prevalence of DCM include the Scottish Deerhound, Doberman Pinscher, Irish Wolfhound, Great Dane, Boxer, Saint Bernard, Afghan Hound, Newfoundland, and Old English Sheepdog.[7] The dogs in the present study also generally had longer survival times than are typically reported in the literature for dogs with DCM. For instance, 2 recent multibreed retrospective studies[8,9] reported overall probabilities of survival 1 year after diagnosis of DCM of 17.5 and 37.5% and median survival times of 27 and 65 days. In contrast, the median survival time for the 12 dogs in the present study was 456 days, and many of these dogs were still alive at the time of the study. Finally, several of the dogs in the present study regained substantial cardiac function and were weaned off all medications except taurine. This is unusual for most dogs with DCM, in which the disease is typically progressive and fatal. Information on follow-up cardiac evaluations and measurements of taurine concentrations was not available for all dogs in the present study. However, in those dogs in which this information was available, cardiac function improved and taurine concentrations increased to concentrations greater than those considered evidence of a deficiency. Furthermore, in all of the dogs that lived > 1 year, all cardiac medications other than taurine were discontinued. These characteristics suggest that the dogs in the present study did not have the typical form of DCM.

Consumption of commercial dry diets containing lamb meal or rice as a primary ingredient was a common finding among dogs in the present study. Three of the 5 diets that these dogs consumed contained both lamb meal and rice (diets A, C, and E); 1 contained chicken meal and rice (diet B); and 1 contained ground whole wheat, lamb meal, and rice (diet D). Most of the dogs were changed to another diet at the time DCM was diagnosed. However, the owner of the Border Collie decided to keep the dog on the same commercial lamb meal and rice diet (diet D) but supplemented the dog with taurine. Increases in taurine concentration and cardiac function in this dog indicated a response to taurine supplementation and supported the hypothesis that diet played a contributing role in the development of DCM by causing taurine depletion.

Taurine is the most abundant of the free amino acids in tissues. High concentrations of taurine are found in animal tissues, especially muscle, viscera, and brain. In cats, 3 manifestations of taurine deficiency have been identified: central retinal degeneration, reproductive failure and impaired fetal development, and DCM.[10] Taurine deficiency can also cause hearing loss, platelet hyperaggregation, and impaired immune function, although specific clinical disorders have not been recognized.[10] The mechanism of heart failure in taurine-deficient cats and dogs is poorly understood, but in the myocardium, taurine appears to participate in many functions, including cellular osmoregulation, free-radical scavenging, and modulation of contraction strength through regulation of calcium concentration.[3]

In cats, inadequate provision of dietary taurine results in DCM.[3] Cats have a limited ability to synthesize taurine because of low concentrations of the enzymes cysteine sulfinic acid decarboxylase and cysteine dioxygenase.[3] In contrast, dogs have not been generally recognized to have a need for dietary taurine, because they have the metabolic capacity to synthesize taurine from cysteine and methionine.[4] The concentration of taurine necessary to prevent clinical signs of a taurine deficiency in cats varies with diet composition and processing, but ranges from 400 mg of taurine/kg of diet to > 2,000 mg of taurine/kg of diet.[11,12]

Possible causes of the taurine deficiency in the dogs in the present report include insufficient synthesis of taurine, extraordinary loss of taurine or its precursors in urine, extraordinary gastrointestinal tract loss of taurine in bile acid conjugates as found in cats, and a reduction in protein digestibility.[13] On the basis of our clinical findings, in conjunction with the blood and plasma taurine concentrations and common diet histories in these dogs, we hypothesize that the consumption of diets with inadequate or unavailable sulfur amino acid precursors of taurine resulted in taurine deficiency and low blood taurine concentrations that, in turn, led to the development of abnormal cardiac function and DCM.

Recent experimental and clinical observations in dogs are supportive of the possibility that insufficient synthesis of taurine from sulfur amino acid precursors results in the development of DCM. Sanderson et al[14] found low plasma taurine concentrations in Beagles fed an energy-dense, protein-restricted (10% protein on a dry-matter basis) diet for 48 months. One dog developed DCM, and taurine supplementation (500 mg, PO, q 12 h) reversed the cardiac changes in this dog. Prolonged feeding of a commercial prescription diet with a protein-to-calorie ratio similar to one used by

Sanderson et al[14] may have induced development of DCM in Dalmatians,[15] but whether taurine deficiency caused DCM in these dogs is unclear. Blood and plasma taurine concentrations in the Dalmatians were within reference limits at the time of clinical evaluation. However, taurine concentrations in blood and tissues at the time DCM developed were not known. It is possible that a period of taurine deficiency could produce myocardial damage in dogs that cannot be reversed. Following a change in diet, blood taurine concentrations may indicate normal body taurine stores and not reveal that a period of deficiency had occurred.

Alternatively, the rice bran and whole rice products in the commercial diets consumed by most of the dogs in the present study may have been a factor in the development of low blood and plasma taurine concentrations. Rice bran and whole-rice products are sources of moderately soluble fiber and contain relatively high amounts of fat. The fiber, fat, or protein content of the rice bran may accelerate excretion of bile acids, predisposing dogs to taurine deficiency. Stratton-Phelps et al[16] recently demonstrated that cats fed a purified diet with 26% (dry-matter basis) full-fat rice bran had critically low plasma and blood taurine concentrations after 6 and 12 weeks, respectively. Extraordinary intestinal loss of taurine secondary to increases in bacterial populations appears to be contributing to taurine deficiency in these cats.[h] Preliminary results from a dose-response study conducted by the authors of the present study indicate that cats can develop critically low blood taurine concentrations when consuming full-fat rice bran at concentrations as low as 4% (dry-matter basis).

Dogs in the present study were all fed a commercial dry diet containing high quantities of lamb meal or rice products, but why this should be associated with taurine deficiency was not readily apparent, as the diets appeared sufficient in protein and sulfur amino acid contents and had passed testing in accordance with the Association of American Feed Control Officials' feeding trials for all life stages. However, because lamb meals may be of low quality, limited bioavailability of sulfur amino acids required for taurine synthesis in the diet was suspected. Relative to other meat meal sources, lamb meal has been shown to have poor ileal nitrogen and cystine digestibility in dogs.[17] Results of a study involving feeding a lamb meal and rice diet to young Beagles for 8 months indicate that plasma taurine concentrations decreased substantially during the first month after dogs were switched to the diet but did not change thereafter.[18] Thus, the lamb meal and rice diet appeared to have an effect on taurine status, but not to the point of a depletion sufficient to cause DCM.

Recently, low blood taurine concentrations have been identified in Newfoundlands.[19] Reduced reproductive performance, small litter sizes, poor litter growth, and small puppies were reported, and similar findings have been reported for cats with taurine deficiency.[20] Diet appeared to be the cause of the taurine deficiency in these dogs, in that 7 of the 12 dogs with plasma taurine concentrations < 40 nmol/mL were consuming diets containing lamb meal and rice. Methionine supplementation in dogs consuming lamb meal and rice diets resulted in substantial improvement in taurine concentrations, and plasma taurine concentrations increased when a dietary change was instituted but were unchanged when the diet was not changed.

The authors have also examined 2 dogs with taurine deficiency that were being fed a home-prepared, low-protein, tofu-based diet that meet the National Research Council's requirements for adult maintenance.[21] Taurine deficiency in these dogs was attributed to inadequate synthesis, and it was assumed that the low concentrations of protein in general and of sulfur amino acids in particular provided an inadequate supply of precursors for taurine synthesis. An additional contributing factor may have been an increase in taurine loss, as soybean protein augments bile acid loss through microbial degradation and accelerates cholecystokinin-mediated bile acid turnover.[5,22] Similarly, we are aware of 3 Golden Retrievers with taurine deficiency that lived in the same household and were consuming a vegetarian diet formulated by the owner.

Taken together, these findings suggest that taurine deficiency may result in DCM in dogs other than American Cocker Spaniels fed a diet that contains insufficient amino acid precursors for adequate taurine synthesis or that accelerates taurine loss. We recommend that a complete diet history be obtained for every dog each time it is examined by a veterinarian, including the name and amount of food fed, the name and amount of any snacks and treats, a description of the manner in which the dog is fed, whether the dog has access to other food sources, and whether any dietary supplements are given. We also recommend that all home-cooked diets be evaluated by a veterinary nutritionist.

Blood and plasma taurine concentrations should be measured in all dogs with DCM, just as measurement of blood and plasma taurine concentrations is recommended for all cats with DCM.[23] Although blood taurine concentration is only a fraction of the concentration in the tissues, blood and plasma taurine concentrations do change in proportion with tissue concentrations.[4,24] Blood taurine concentrations may be used to substantiate a diagnosis of taurine deficiency when plasma concentrations are equivocal. In addition, blood taurine concentrations are only slightly altered after eating, whereas plasma taurine concentration may change substantially in taurine-depleted animals.[23] A substantial increase in plasma or serum taurine concentration can occur secondary to taurine leakage from granulocytes and platelets, as occurs with clotting or hemolysis, but analysis of blood taurine concentration is not confounded by these effects. Serum taurine concentrations are of questionable clinical value because of the variations in time of clotting and the method of serum separation, and in our experience, the variability in serum taurine concentrations is greater than the variability in plasma taurine concentrations.

Finally, we recommend that taurine be administered to all dogs with DCM, pending results of taurine

analysis. Taurine is inexpensive and readily available and has no reported adverse effects when administered orally. Follow-up measurement of blood and plasma taurine concentrations should be performed after 1 to 2 months of taurine supplementation to confirm that taurine status has improved and verify owner compliance with regard to administration.

One manufacturer produced 3 of the diets (diets A, B, and C) associated with taurine deficiency in the present study. Since identification of these dogs, the manufacturer has added taurine to 1 of the 3 diets (diet A). Diets D and E, each produced by other manufacturers at the time the dogs consuming them developed low taurine concentrations and DCM, have recently been acquired by other companies. The formulation of diet E has been changed, but neither diet D nor diet E includes additional taurine. Nevertheless, we suggest that veterinarians not focus on particular diets but on the issue of taurine deficiency as a whole. Further research is needed to identify dietary factors inducing taurine deficiency and determine the mechanisms of their effects.

[a]System 7300 and Model 121-M automated amino acid analyzers, Beckman Instruments Inc, Palo Alto, Calif.
[b]SYSTAT, version 9.0, SPSS Inc, Chicago, Ill.
[c]Nutro Natural Choice lamb meal and rice formula dog food, Nutro Products Inc, City of Industry, Calif.
[d]Nutro Natural Choice senior dog food, Nutro Products Inc, City of Industry, Calif.
[e]Nutro Natural Choice lite rice and lamb meal formula for overweight dogs, Nutro Products Inc, City of Industry, Calif.
[f]Nature's Recipe adult maintenance lamb meal and rice, Nature's Recipe Pet Foods, Heinz Pet Products, Pittsburgh, Pa.
[g]Sensible Choice adult lamb meal and rice formula, Pet Products Plus Inc, St Charles, Mo.
[h]Stratton-Phelps M, Rogers QR, Backus RC, et al. Oral antibiotics do not replete taurine in cats consuming 26% dietary rice bran (abstr), in *Proceedings*. Fed Am Soc Exp Biol 2002;5055.

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Iron Laboratory Studies in Pediatric Patients With Heart Failure from Dilated Cardiomyopathy

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Iron deficiency (FeD), with or without anemia, in adults with heart failure (HF) is associated with poor outcomes, which can be improved with replacement therapy. A similar therapeutic opportunity may exist for children; however, iron laboratory measurements and FeD have not been described in pediatric patients with HF. A single-center, retrospective study was conducted on 28 patients <21 years old with a diagnosis of dilated cardiomyopathy and HF who had iron laboratories (serum iron, iron saturation, and ferritin) performed. The mean (standard deviation) age at time of laboratory collection was 10.3 (5.5) years. Twenty-seven patients (96.4%) met the criteria for FeD. Serum iron and iron saturation were significantly associated with inpatient hospitalization, being on inotropic medications, or having stage D HF. Low-serum iron was associated with a higher left ventricular end-diastolic dimension and left ventricular end-systolic dimension *z*-score by echocardiography ((β −2.58, 95% confidence interval [CI] −4.76, −0.40, p = 0.02) and (β −2.43, 95% CI −4.70, −0.17, p = 0.04)), respectively. Low ferritin was associated with higher mortality (relative risk 0.29, 95% CI 0.12, 0.70, p = 0.006). In conclusion, FeD was common in this pediatric cohort with more advanced HF. Iron profile abnormalities were associated with worse HF severity and outcomes including mortality. **(Am J Cardiol 2017;120:2049–2055)**

Iron deficiency (FeD) is the most common nutrient deficiency worldwide, and is particularly prevalent in children and females of childbearing age.[1,2] Iron is an essential nutrient and a cofactor that is crucial for multiple cellular processes and is likely even more important in metabolically active cells such as cardiac myocytes.[3] FeD in adult patients with heart failure (HF) has been studied extensively.[4–9] Several studies have estimated the prevalence of FeD in adult patients with HF to be as high as 73%.[10–13] Recent trials of iron supplementation in adult HF patients demonstrated significant improvements in New York Heart Association functional class, quality of life scores, exercise capacity, anemia, and a reduction in the risk of hospitalization.[4–9,14] No studies have investigated the coexistence of FeD with HF in pediatric patients where growth may place a further demand on proper iron metabolism. Therefore, children with HF may be at an especially high risk for FeD and the associated comorbidities. Timely identification of FeD may also allow early intervention. In this study we evaluated the iron profiles in a cohort of children with HF from dilated cardiomyopathy to examine the prevalence and outcomes of FeD in this disease population.

Methods

This was a single-center, retrospective observational study. The study was approved by the local Institutional Review Board. Inclusion criteria were age of <21 years old, the diagnosis of dilated cardiomyopathy, and at least one set of iron laboratories that included ferritin, serum iron, and iron saturation [Tsat] plus a same-day complete blood count. Patients were identified through a systematic search of the institution's electronic medical record for cardiomyopathy or HF based on the International Classification of Diseases-Ninth Revision-Clinical Modification (ICD-9-CM) codes 425.1, 425.11, 425.18, 425.4, 425.8, 425.9, 428.0, 428.1, 428.20, 428.21, 428.22, 428.23, 428.32, 428.41, 428.42, 428.43, 428.9, 428.0, 429.3, 429.89, and 429.9 for the period from 2005 to 2014. This list of identified patients was adjudicated by the investigators to ensure inclusion criteria were met, including the presence of a complete set of iron laboratories and complete blood count. Patients were excluded if they were already receiving iron supplementation, post-cardiac transplantation, receiving chemotherapy or erythropoietin, received a transfusion within 2 weeks before obtaining iron laboratories, exposed to cardiopulmonary bypass 2 weeks before iron laboratories, or have renal dysfunction on renal replacement therapy as these conditions are likely to affect the iron profile. Patients with congenital heart defects leading to HF were excluded as this group is likely to have a significantly different etiology to their HF.

A comprehensive chart review was performed on the 28 patients included in the study to collect their demographics including age, gender, race/ethnicity, location at time of the iron laboratories (outpatient, inpatient, or cardiac intensive

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See page 2054 for disclosure information.

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care unit [ICU]), echocardiogram, presence of anemia and microcytic anemia (based on hemoglobin or MCV less than the 5th percentile for age),[15] and the use of HF medications including beta blockers, angiotensin-converting enzyme inhibitors/angiotensin receptor blockers, diuretics, anticoagulants, or inotropes within 48 hours of laboratory collection. Outcome measures included mortality and use of mechanical circulatory support (MCS) following collection of iron profile laboratories. Patients were followed up until the end of 2014 with a mean (standard deviation [SD]) time from laboratory collection to end of follow-up of 2.05 (2.11) years. FeD was assessed by examining serum iron level (mcg/dL), total iron binding capacity (mcg/dL), Tsat, and ferritin (ng/mL). Because the definition of FeD in healthy adults and children is nearly identical, we used the definition of FeD in adults with HF which has been used consistently in major studies and trials: ferritin <100 ng/mL alone or a ferritin 100 to 300 ng/ml and Tsat <20%.[14]

Echocardiography data to assess the severity of dilated cardiomyopathy were abstracted from the echocardiogram reports at the time of iron laboratories, including the left ventricular shortening fraction (%), left ventricular end-diastolic dimension z-score, and left ventricular end-systolic dimension z-score. The reviewed echocardiograms were performed on average within 14 days of iron laboratory collection. To grade the severity of HF at the time of iron laboratory collection, HF stage,[16,17] plasma B-type natriuretic peptide (BNP), location at time of laboratory collection (outpatient vs inpatient), and use of an inotrope were collected.

Descriptive data were presented as counts with percentages or mean values and SDs where appropriate. Multivariable linear regression models were used to examine the relation between iron laboratory levels with BNP and echocardiographic parameters. Multivariable relative risk regression was used to assess the relation between iron laboratory levels with location of laboratories (inpatient/outpatient), inotrope use, and HF stage D. Binary outcomes for mortality, need for MCS, and a "composite" outcome defined as either mortality or need for MCS were analyzed using univariate relative risk regression as the number of events was not adequate for a multivariable analysis. All linear and relative risk regression models used robust standard errors. Natural log transformations were used for BNP, serum iron, Tsat, and ferritin in the linear regression models. For the relative risk regression models, iron values and BNP were transformed by taking the base-2 logarithm. Multivariable general additive models adjusted for age and race were used to illustrate the relation of left ventricular end-systolic dimension (LVESD) and left ventricular end-diastolic dimension (LVEDD) (z-scores) with iron laboratory values on the nontransformed scale using 3 degrees of freedom. Data analysis was performed using Stata 14.0 (StataCorp LP, College Station, Texas).

Results

A total of 28 patients were included in this study. The baseline characteristics of the study population are listed in Table 1. A majority of patients were hospitalized when laboratories were obtained with 53.6% of patients in the cardiac ICU, 21.4% in the acute care ward (non-ICU), and 25.0% in outpatient clinics. A majority of patients had HF stage D (71.4%), and the mean (SD) plasma BNP for the entire cohort was 745 (912) pg/mL.

The cohort's iron profile is listed in Table 2. Based on the previously published definition of FeD in adult HF patients, 27 patients (96.4%) met the criteria for FeD. Twenty-six patients (92.9%) met criteria for FeD based on ferritin <100 ng/mL alone. Of the 26 patients with low ferritin, 21 (80.8%) also had a Tsat <20%. Anemia for age was found in 11 (39.3%) patients and only 2 of those patients had a microcytic anemia.

Table 1
Summary of patient characteristics

Demographics	Value, % or mean (SD) (n = 28)
Age (years)	10.3 (5.5)
Male	13 (46%)
Race/ethnicity	
White	11 (39%)
Black	4 (14%)
Asian	1 (4%)
Other or "Did not indicate"	12 (43%)
Weight (kg)	41.0 (27)
Weight z-score	–0.4 (2.0)
Height (cm)	134 (36)
Height z-score	–0.7 (1)
BMI	20 (7)
BMI z-score	0.01 (1)
Location at Lab	
Outpatient	7 (25%)
Inpatient (non-ICU)	6 (21%)
CICU	15 (54%)
Heart failure stage D*	20 (71%)
Anemia (Hgb)	11 (39%)
Microcytic anemia	2 (7%)
Heart Failure Medications	
Beta-Blocker	9 (32%)
ACE-I/ARB	25 (89%)
Diuretic	22 (79%)
Anticoagulation	15 (54%)
Inotrope	18 (64%)
BNP (pg/mL)	745 (912)
Clinical Outcomes	
Mortality	4 (14%)
Listed for transplant	12 (43%)
Mechanical Circulatory Support	4 (14%)

* Based on Heart Failure Staging for Infants and Children modifications made to the ACC/AHA staging guidelines.[14,15]

Table 2
Summary of iron profile variables

Iron Status	
Deficiency*	27 (96%)
Ferritin <100 (ng/mL)	26 (93%)
Tsat <20%	22 (79%)
Iron Lab Profile	**mean (SD)**
Serum Iron (g/dL)	60 (35)
Tsat %	17 (10)
Ferritin (ng/mL)	45 (30)

* Iron deficiency defined as ferritin < 100 ng/mL or ferritin = 100–300 ng/mL and iron saturation (Tsat) < 20%.

Table 3
(A) Multivariable relative risk model for clinical heart failure severity with iron profile variables. (B) Multivariable linear regression modeling B-type natriuretic peptide (BNP) and echocardiographic measures of dilated cardiomyopathy with iron profile variables

(A)

Variable†	Inpatient (n = 21/28)		Inotrope (n = 18/28)		Heart failure stage D* (n = 20/28)	
	RR (95% CI)	p	RR (95% CI)	p	RR (95% CI)	p
Log2(Serum Iron)	0.71 [0.56,0.90]	**0.006**	0.67 [0 51,0.87]	**0.003**	0.82 [0.66,1.03]	0.095
Log2 (Tsat)	0.69 [0.53,0.92]	**0.011**	0.63 [0.48,0.81]	**0.001**	0.80 [0.61,1.04]	0.092
Log2 (Ferritin)	1.00 [0.82,1.22]	0.982	0.94 [0.73,1.20]	0.607	0.99 [0.80,1.21]	0.887

(B)

Variable†	Ln(BNP) (n = 28)		LVEDD (z-score) (n = 28)		LVESD (z-score) (n = 28)	
	β (95% CI)	p	β (95% CI)	p	β (95% CI)	p
Ln(Serum Iron)	–1.16 [–2.02,–0.29]	**0.011**	–2.58 [–4.76,–0.40]	**0.023**	–2.43 [–4.70,–0.17]	**0.036**
Ln(Tsat)	–1.04 [–1.99,–0.09]	**0.033**	–2.50 [–4.96,–0.05]	**0.046**	–2.50 [–4.91,–0.09]	**0.043**
Ln(Ferritin)	–0.02 [–0.54,0.50]	0.928	–0.93 [–2.38,0.53]	0.201	–1.33 [–2.95,0.28]	0.101

* Based on Heart Failure Staging for Infants and Children modifications made to the ACC/AHA staging guidelines.[14,15]
† Models adjusted for Age and Race with each Iron Profile variable added one at a time.

Additionally, there was no association between patient weight or body mass index (BMI) z-scores and iron laboratory values.

We compared the degree of iron profile abnormalities with the severity of the cardiomyopathy and HF. Patients were grouped into HF severity based on location (outpatient vs inpatient), the use of inotropes, and HF stage at the time of laboratory collection. Table 3A lists the multivariable analyses between these markers of HF severity and iron laboratory values. While ferritin did not show an independent association with the severity of HF using these measures, both serum iron and Tsat were associated with being inpatient and being on inotropic medications (Table 3).

Echocardiography parameters relevant to dilated cardiomyopathy were also compared with the iron profile. Table 3B lists the independent relation between iron laboratory values and these echocardiographic parameters. Lower levels of serum iron and Tsat were significantly associated with a higher LVEDD z-score and a higher LVESD z-score, but ferritin was not associated with remodeling on echocardiography. Figure 1 provides an illustration of the relation between LVEDD, LVESD, and iron levels. BNP was also examined in relation to iron values. Lower serum iron and Tsat were significantly associated with higher BNP levels, a marker of increased wall stress.

We compared the degree of iron profile abnormalities with clinical outcome measures including mortality and MCS. A total of 8 patients had a poor clinical outcome (death or need for MCS). Table 4 lists the associations between iron laboratory values and these clinical outcome measures. Higher serum iron trended toward not requiring MCS. High ferritin level was significantly associated with lower mortality and lower composite outcome. It is important to note that among the other clinical and laboratory indices including age, weight, BMI, patient location, HF stage, presence of anemia, echocardiographic indices of remodeling, and inotrope use, none were independently associated with clinical outcomes.

Discussion

In this study, we observed a high prevalence of FeD in a pediatric cohort with HF. We also examined individual components of the conventional iron laboratory profile and their relation to left ventricular remodeling by echocardiography, severity of HF, and HF related cardiovascular outcomes. Lower levels of iron storage and availability were in general associated with higher severity of remodeling, HF, and worst clinical outcomes, whereas BNP and clinical or echocardiographic features were not.

In our cohort, the frequency of FeD, 91%, is quite alarming. Although not reported before in the pediatric literature, the prevalence in our study is higher than reports from adult HF studies, and this is likely due to several different factors. First, the cohort studied has higher severity of illness as demonstrated by the proportion admitted to the hospital and on inotropes as well as by the overall mortality and need for MCS, whereas the adult studies focused on ambulatory patients with HF. Additionally, defining FeD in HF patients is challenging, and this difficulty emanates from the fact that FeD can be understood as total body depletion or a functional metabolic deficiency.[18] Because patients with HF are susceptible to both types of deficiency, diagnosing FeD in HF patients is complex.[19] In other medical conditions, the diagnosis of FeD is a ferritin <30 g/L and a Tsat <16%. However, these definitions have serious limitations as well because in long-term illnesses and inflammatory states, ferritin can be increased independently of iron status.[18] For example, similar to the accepted adult definition for iron deficiency, in pediatric chronic kidney disease, a ferritin <100 g/L is also the accepted cutoff for iron deficiency.[20] Furthermore, a study in adult patients with HF demonstrated that 73% had confirmed FeD on bone

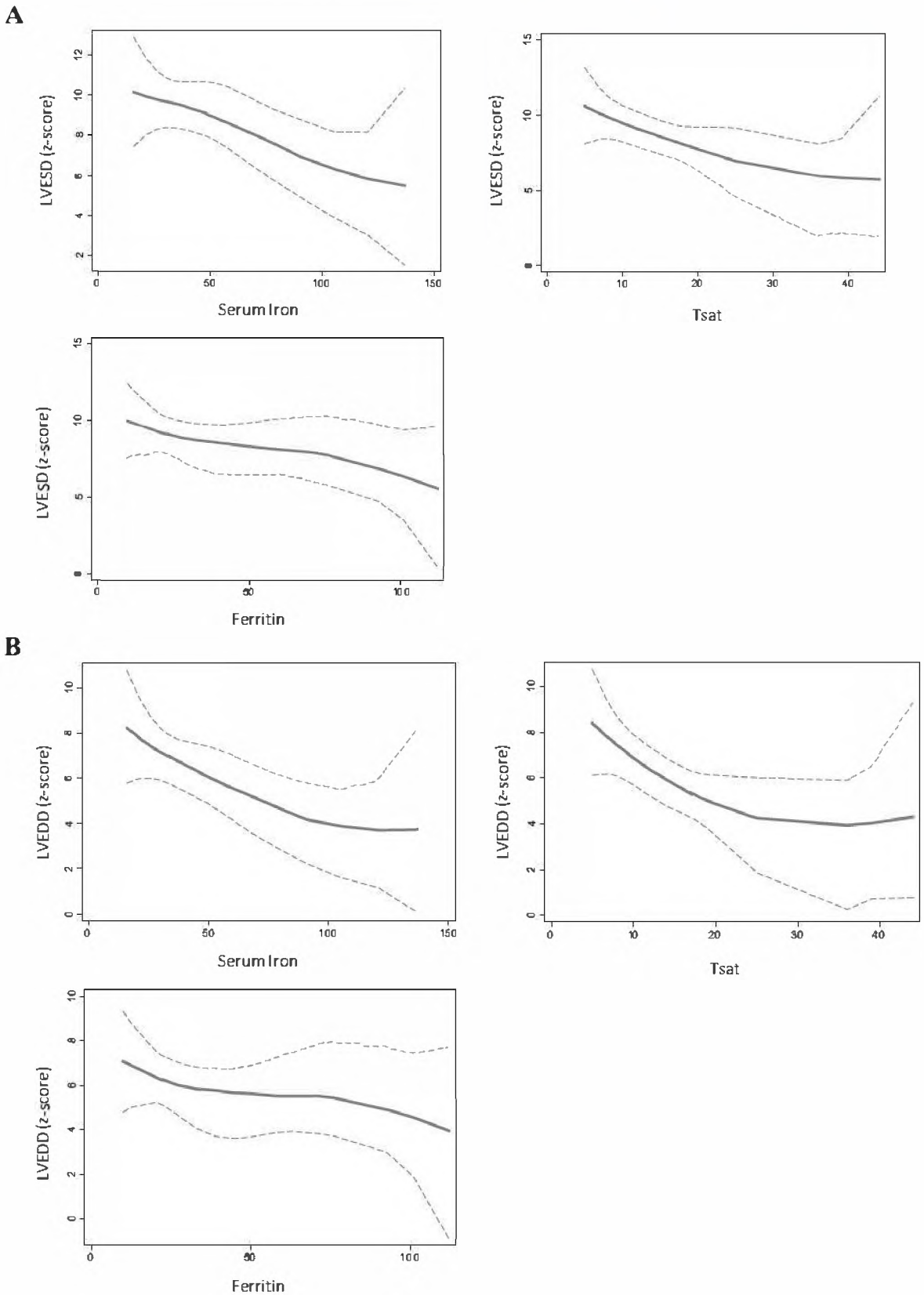

Figure 1. Relation between *(A)* LVESD and *(B)* LVEDD (z-scores) and iron profile values. LVEDD = left ventricular end-diastolic dimension; LVESD = left ventricular end-systolic dimension.

Table 4
Univariate relative risk regression modeling clinical outcomes

Variable	Mortality (n = 4/28)		MCS (n = 4/28)		Composite* (n = 8/28)	
	RR (95% CI)	p	RR (95% CI)	p	RR (95% CI)	p
Age (years)	1.11 [0 95,1.30]	0.197	1.00 [0.86,1.17]	0.954	1.05 [0.95,1.16]	0.357
Male	1.15 [0.18,7.32]	0.879	0.38 [0.04,3.39]	0.390	0.69 [0.20,2.41]	0 563
White	3.00 [0.34,26.48]	0.323	0.33 [0.04,2.94]	0.323	1.00 [0.30,3.30]	1.000
Weight (kg)	1.01 [0 99,1.04]	0.287	1.01 [0.98,1.03]	0.633	1.01 [0.99,1.02]	0.249
Weight z-score	1.09 [0.66,1.78]	0.742	0.97 [0.48,1.95]	0.934	1.03 [0.68,1.56]	0.880
Height (cm)	1.02 [0 99,1.04]	0.1900	1.01 [0.97,1.05]	0.687	1.01 [0.99,1.03]	0.291
Height z-score	1.05 [0.69,1.61]	.809	1.41 [0.46,4.33]	0.549	1.20 [0.73,1.96]	0.471
BMI	1.03 [0 92,1.14]	0.634	1.01 [0.91,1.11]	0.915	1.01 [0.95,1.08]	0.649
BMI z-score	1.01 [0.30,3.43]	0.985	0.93 [0.34,2.52]	0.881	0.96 [0.40,2.31]	0 922
Location at Lab		0.231		—		1.000
Outpatient	ref				ref	
Inpatient	0.33 [0.06,2.01]		All MCS		1.00 [0.25,3.96]	
Heart failure stage D†	0.40 [0.07,2.45]	0.322	All MCS	—	1.20 [0.30,4.86]	0.798
Anemia (Hgb)	1.55 [0.25,9.74]	0.643	0.52 [0.06,4.52]	0.549	0.93 [0.27,3.19]	0 905
Beta-Blocker	6.33 [0.73,54.84]	0.094	No MCS	—	1.27 [0.38,4.26]	0.703
Diuretic	0.82 [0.10,6.77]	0.852	All MCS	—	1.91 [0.28,13.09]	0 510
Anticoagulation	All Mortality	—	All MCS	—	All Composite	—
Inotrope	0.56 [0.09,3.48]	0.530	All MCS	—	1.67 [0.40,6.94]	0.483
Cardiac Function						
Log2(BNP)	0.86 [0.72,1.04]	0.122	1.71 [0.97,3.02]	0.064	1.09 [0.84,1.41]	0 507
LVEDD (z-score)	1.01 [0.85,1.20]	0.939	1.51 [0.57,4.00]	0.403	1.27 [0.84,1.93]	0.256
LVESD (z-score)	0.99 [0.83,1.17]	0.906	1.35 [0.92,1.99]	0.130	1.15 [0.94,1.40]	0.164
Log2(Serum Iron)	1.66 [0.82,3.35]	0.161	0.19 [0.03,1.07]	0.060	0.71 [0.34,1.45]	0.340
Log2(TSAT)	1.33 [0.67,2.65]	0.414	0.19 [0.03,1.31]	0.091	0.53 [0.20,1.38]	0.193
Log2(Ferritin)	0.29 [0.12,0.70]	**0.006**	0.74 [0.48,1.15]	0.177	0.53 [0.35,0.81]	**0.003**

* Composite of mortality or need for MCS.
† Based on Heart Failure Staging for Infants and Children modifications made to the ACC/AHA staging guidelines.[16,17]

marrow aspiration despite a high ferritin.[21] Therefore, traditional definitions in the general population may not be applicable to patients with HF, particularly those who are more advanced, have active symptoms, or are in a decompensated state. For this reason and the fact that most of our cohort has higher severity of HF, we also examined individual iron profile components as continuous variables.

Multiple studies in the adult HF literature have described the definition of FeD in HF as a serum ferritin level <100 g/L (a measure of total body FeD), or serum ferritin level between 100 and 300 g/L combined with Tsat <20% (a measure of functional FeD).[22,23] This definition of FeD based on therapeutic responses has since been included in guidelines on the treatment of adults with HF.[24] Although there is no validated definition of FeD in children with HF, the observed association of lower available iron stores with higher severity of HF suggests that FeD should be further examined, including its repletion as a therapeutic target in HF in children as few exist in children with HF.

Iron has merit as a therapeutic target because it is essential for many cellular functions. For example, the importance of iron homeostasis goes beyond the commonly acknowledged effect on erythropoiesis. In fact, a majority of patients with FeD in this study did not have concurrent anemia, which is a similar observation in adult studies.[18] Iron is a critical part of cellular processes such as oxygen transport, oxygen storage, mitochondrial respiration, and cellular immunity.[3] Hence, it is theoretically plausible that FeD can lead to disease progression and poor outcomes as suggested by the limited preliminary outcome analysis in this study. Even without a well-elucidated mechanism to explain these associations, multiple adult studies have demonstrated FeD as an independent predictor of all-cause mortality,[10,13,25] whereas parenteral iron replacement improved markers of cardiac remodeling and HF.[5,26,27] It is worth noting that weight and BMI were not associated with the iron profile, suggesting FeD may not simply emanate from poor intake in which case parenteral replacement therapy is required.

In our study, there were differences between the various components of the traditional iron laboratory profile that warrant discussion. First, lower serum iron and Tsat were associated with higher BNP and a higher severity of remodeling, whereas the level of ferritin was not. However, ferritin was associated with mortality and composite outcome. These observed differences between markers of iron availability (serum iron and Tsat) and iron storage (ferritin) may be due, in part, to the independent elevation of ferritin with inflammation, which may be heightened and more prevalent during acute decompensated HF.[21,28] However, it is also possible that iron availability is more important than depleted total body stores in acute HF where the cardiomyocyte iron requirements may theoretically increase. Conversely, ferritin as a storage marker may be more related to the duration and hence underlying reserve and reversibility of the diseased heart, which may contribute more to the association with major adverse outcomes other than admission for acute decompensated HF. Future large

studies encompassing a broader group of patients at all stages of HF will be important to determine the significance of these findings.

There are several limitations to this study. In addition to the potential selection bias of chronically ill and hospitalized patients enrolled in the study, the sample size is small. Therefore, prevalence is not likely to be accurate from this study. Pediatric HF encompasses heterogeneity of diagnoses. By keeping the cohort to dilated cardiomyopathy and excluding patients whose iron profile could be affected by transfusions and other disease states that can affect iron metabolism, the sample size was further diminished. We graded severity of HF by how patients were treated instead of symptoms because it is difficult to assign a symptom-based functional class to children, especially in a retrospective study. This is a limitation in the sense that it would be more difficult to compare our findings with the existing literature on FeD in adults with HF, as severity in adults is symptom-based and graded by a well-established and validated functional class system. Because of the potential patient selection bias, and because a numerical cutoff does not adequately define "deficiency" in disease states, we purposely examined the association of iron laboratory values as a continuous variable with severity of HF and cardiomyopathy to demonstrate the presence of robust relations in children.

Despite recognition of the importance of FeD in adults with HF, this study describes FeD as a new, potentially serious comorbidity in children. FeD appears to be common, particularly in sicker, more advanced HF patients. Iron profiling may also serve as biomarkers for clinical outcome. The observations in this study should help guide future studies investigating the effects of iron metabolism on pediatric HF outcomes and whether FeD can be a therapeutic target for HF therapy in this orphan disease population.

Disclosures

The authors have no conflicts of interest to disclose.

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Chronic oral exposure to the aldehyde pollutant acrolein induces dilated cardiomyopathy

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Ismahil MA, Hamid T, Haberzettl P, Gu Y, Chandrasekar B, Srivastava S, Bhatnagar A, Prabhu SD. Chronic oral exposure to the aldehyde pollutant acrolein induces dilated cardiomyopathy. *Am J Physiol Heart Circ Physiol* 301: H2050–H2060, 2011. First published September 9, 2011; doi:10.1152/ajpheart.00120.2011.—Environmental triggers of dilated cardiomyopathy are poorly understood. Acute exposure to acrolein, a ubiquitous aldehyde pollutant, impairs cardiac function and cardioprotective responses in mice. Here, we tested the hypothesis that chronic oral exposure to acrolein induces inflammation and cardiomyopathy. C57BL/6 mice were gavage-fed acrolein (1 mg/kg) or water (vehicle) daily for 48 days. The dose was chosen based on estimates of human daily unsaturated aldehyde consumption. Compared with vehicle-fed mice, acrolein-fed mice exhibited significant ($P < 0.05$) left ventricular (LV) dilatation (LV end-diastolic volume 36 ± 8 vs. 17 ± 5 μl), contractile dysfunction (dP/dt_{max} 4,697 ± 1,498 vs. 7,016 ± 1,757 mmHg/s), and impaired relaxation (tau 15.4 ± 4.3 vs. 10.4 ± 2.2 ms). Histological and biochemical evaluation revealed myocardial oxidative stress (membrane-localized protein-4-hydroxy-*trans*-2-nonenal adducts) and nitrative stress (increased protein-nitrotyrosine) and varying degrees of plasma and myocardial protein-acrolein adduct formation indicative of physical translocation of ingested acrolein to the heart. Acrolein also induced myocyte hypertrophy (~2.2-fold increased myocyte area, $P < 0.05$), increased apoptosis (~7.5-fold), and disrupted endothelial nitric oxide synthase in the heart. DNA binding studies, immunohistochemistry, and PCR revealed significant ($P < 0.05$) activation of nuclear factor-κB in acrolein-exposed hearts, along with upregulated gene expression of proinflammatory cytokines tumor necrosis factor-α and interleukin-1β. Long-term oral exposure to acrolein, at an amount within the range of human unsaturated aldehyde intake, induces a phenotype of dilated cardiomyopathy in the mouse. Human exposure to acrolein may have analogous effects and raise consideration of an environmental, aldehyde-mediated basis for heart failure.

acrolein; oxidative stress; cardiomyopathy; environmental pollution

IDIOPATHIC DILATED CARDIOMYOPATHY (DCM) is the underlying diagnosis in approximately one-third of cases of heart failure (HF) (15). While often attributed to remote infectious, metabolic, or toxic injury to the heart, in most circumstances the etiological factors responsible for DCM are difficult to identify. Epidemiological studies have established that pollution exposure is associated with increased mortality from several cardiovascular diseases, including HF (3, 5, 28). The biological mechanisms proposed to explain these adverse effects have included pollutant-induced alterations in autonomic tone, the elaboration of proinflammatory and prooxidant mediators, and the physical translocation of soluble constituents of pollutants into the circulation that have direct effects on the heart and vasculature. Theoretically, all of these broad mechanisms can unfavorably impact pathogenetic alterations and/or modifiers of DCM and HF (16). Nonetheless, little is known about the potential environmental triggers of DCM and the specific effects induced by individual constituents of the pollutant mix.

Aldehydes are ubiquitous pollutants in air and water generated by burning fossil fuels (10). They are also readily found in food and are natural products of lipid peroxidation and glucose oxidation (10). More than 300 different aldehydes have been identified in various foods, and at least 36 are present in water, often at levels exceeding maximal recommended concentrations (2, 10). Unsaturated aldehydes are highly reactive; form adducts with cell thiols and amine groups in sugars, phospholipids, proteins, and DNA bases (9, 25); and provoke oxidative stress and proinflammatory responses in tissue (30, 38). Nonetheless, the in vivo cardiovascular effects of exposure to aldehyde pollutants are not well defined.

Because toxicological profiles of environmental aldehyde mixtures are difficult to determine, we have previously focused on the cardiac effects of acrolein, a prototypical reactive α,β-unsaturated aldehyde classified by the Environmental Protection Agency (EPA) as a high-priority air and water toxic (7). These studies demonstrated that acute exposure to acrolein at concentrations documented in human disease, or doses approximating human oral total aldehyde intake, impaired cardiac function and intrinsic cardioprotective responses in mice (19, 42). However, the cardiac effects of long-term acrolein exposure, an issue with greater implications for public health, remain unknown. Notably, the abundance of acrolein and other aldehydes derived endogenously from lipid peroxidation (and their protein-aldehyde adducts) are known to be elevated in the failing heart (14, 33, 40, 41). In the current study, we evaluated whether long-term oral exposure to acrolein would engender inflammation, oxidant stress, and cardiomyopathy.

METHODS

Eight-week-old male C57BL/6 mice weighing ~20 g were used. All animal studies were performed in compliance with the National Institutes of Health (NIH) *Guide for the Care and Use of Laboratory Animals* [Department of Health and Human Services Publication No. (NIH) 85-23, revised 1996] and were approved by the University of Louisville Institutional Animal Care and Use Committee.

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Acrolein dosage and administration. Acrolein was prepared daily from the acid hydrolysis of diethyl acetal acrolein as previously described (19, 42) and used within 4 h. In our previous study, we estimated the maximal human daily unsaturated aldehyde consumption to be 5 mg·kg^{-1}·day^{-1} and maximal acrolein exposure to be 0.1–0.2 mg·kg^{-1}·day^{-1} (42). Based on these estimates, and with the intent of using acrolein as a representative unsaturated aldehyde, we tested the chronic effects of 1 mg·kg^{-1}·day^{-1} acrolein, representing a 5- to 10-fold greater dose than the expected human acrolein intake but only 20% of the expected overall unsaturated aldehyde intake. Animals were gavage-fed acrolein (in 200 μl water, n 15) or the same volume of water (vehicle, n 18) daily for 48 days.

Echocardiography. M-mode, two-dimensional, and Doppler echocardiography in mice were performed under tribromoethanol sedation (0.25 mg/g ip) using a Philips Sonos 5500 machine and 15-MHz linear array transducer as previously described (14, 41). The two echocardiographers performing the study were blinded as to the assigned experimental group of each mouse. Measured parameters included end-diastolic (ED) and end-systolic (ES) diameter (D), end-diastolic anterior and posterior wall thickness (AWT and PWT, respectively), and the ejection time (ET) and heart rate as determined from the aortic Doppler trace. Left ventricular (LV) systolic function was indexed by the fractional shortening [FS (EDD − ESD)/EDD] and the mean velocity of circumferential fiber shortening (V_{cf} FS/ET) (34, 35). LV hypertrophy and/or wall thinning was assessed by the relative wall thickness [RWT (AWT PWT)/LVEDD]. Echocardiographic imaging was performed at baseline and after 48 days of acrolein feeding.

LV pressure-volume studies. Closed-chest LV pressure-volume (P-V) studies were performed in adult C57/BL6 mice (n 8/group) anesthetized with 80 μg/g ip pentobarbital and mechanically ventilated (155–160 breaths/min, tidal volume 15 μl/g) as previously described (19). Body temperature was maintained at 37°C using a heating pad and lamps. A Millar 1.4-Fr conductance catheter (SPR-839) was inserted in the LV via the carotid artery, and pressure and conductance signals were visualized on-line using the ARIA-1 system (Millar). A small (<1-cm) abdominal incision was made to gain access to the subdiaphragmatic inferior vena cava (IVC). After hemodynamic stabilization for 15 min, recordings of pressure and conductance were performed under steady-state conditions and during transient mechanical IVC occlusion [to vary load and allow determination of the end-systolic pressure-volume relation (ESPVR)]. Intravenous hypertonic saline (0.5–1 μl/g) was then given to determine parallel conductance, and LV volume (μl) was derived from the parallel conductance and ex vivo cuvette calibration with heparinized, warm blood. LV systolic function was indexed by dP/dt_{max}, stroke work (area bounded by the P-V loop), maximal power (peak value of the product of LV pressure and flow), and end-systolic elastance (E_{es}, the slope of the ESPVR) (19, 41). LV diastolic function was assessed by the LVEDP, dP/dt_{min}, and tau, the time constant of LV relaxation (ms) (19, 33, 41).

Immunohistological studies. Formalin-fixed, paraffin-embedded short-axis LV sections (5 μm) were deparaffinized and rehydrated for histological and immunohistochemical staining using standard techniques as previously described (14, 34, 41). Hematoxylin and eosin-stained sections were used to evaluate cardiomyocyte cross-sectional area. In separate studies, immunostaining was performed for the activated p65 subunit of nuclear factor (NF)-κB using anti-p65 antibody (Chemicon) as described previously (31). Nuclear staining intensity was quantified with a MetaMorph 4.5 imaging system and software (Universal Imaging). Digital images were acquired from six fields at standard intervals in each of five short-axis sections from each group. The threshold for p65 staining was predetermined and held constant for all sections analyzed.

Immunohistochemical staining for protein-nitrotyrosine was performed to index peroxynitrite generation in the heart. Deparaffinized and rehydrated tissue sections were incubated for 20 min with 10 mmol/l citric acid (pH 6.0) and then treated with enzymatic antigen retrieval to recover antigenicity. Nonspecific binding was blocked with 5% normal goat serum and 0.05% saponin (Sigma) in PBS (pH 7.4) for 30 min, followed by incubation with monoclonal anti-nitrotyrosine antibody (1:200; Santa Cruz Biotechnology) in PBS with 1% BSA and 0.05% saponin for 1 h at 37°C. Tissue sections were then incubated for 30 min at room temperature with Alexa fluor-555 anti-mouse IgG (1:500) secondary antibody (Invitrogen), which labeled nitrotyrosinated protein residues red, and counterstained with 4′,6-diamidino-2-phenylindole (DAPI) (Invitrogen), which labels nuclei blue. Images were made with a 40 objective lens at 12 different locations in each tissue section. Mean fluorescence intensity was evaluated using MetaMorph software in 12 images/heart. Sections treated with peroxynitrite (1 mmol/l) were used as positive controls.

Western blotting. Total protein extraction, SDS-PAGE Western blotting, and immunodetection using electrochemiluminescence protocols (Amersham Biosciences) were performed as previously described (19). IgG-purified polyclonal 1:2,000 anti-KLH-acrolein primary antibody and horseradish peroxidase-linked secondary antibody were used to evaluate protein-acrolein adducts (19). Protein adducts with 4-hydroxy-*trans*-2-nonenal (HNE) in the membrane fraction (isolated using differential centrifugation) were probed using both dot blots and Western blotting. Polyclonal anti-KLH-HNE primary antibody was used as previously described (34). For dot blots, protein (1.0 μg) was loaded in the wells of a Bio-Dot apparatus (Bio-Rad) and microfiltered through nitrocellulose membranes under vacuum. Primary antibodies for the detection of endothelial nitric oxide (NO) synthase (eNOS), phospho-eNOS-Ser^{1177}, inhibitor of κBα (IκBα), and α-tubulin were obtained from Santa Cruz Biotechnology.

For immunoblot analysis of the monomeric and dimeric forms of eNOS, equal amounts of total protein lysates were subjected to low-temperature SDS-PAGE (LT-PAGE) (43). Briefly, the gel running buffer, 6% SDS-containing polyacrylamide gels, and the gel assembly were equilibrated to 4°C before running the samples. The samples were mixed with SDS containing gel-loading buffer and were not heated. The temperature of the gels was maintained below 10°C during electrophoresis by immersing the gel tanks in ice. Following LT-PAGE, the gels were transferred, and the blots were probed with anti-eNOS antibody and the corresponding secondary antibody. The intensity of the immunoreactive bands was quantified by ImageQuant TL software.

Electrophoretic mobility shift assay. NF-κB DNA binding activity was quantified by electrophoretic mobility shift assay (EMSA). Nuclear protein extraction from frozen myocardium, the EMSA protocol, autoradiography, and densitometry were all performed as previously described (14). ^{32}P-labeled consensus double-stranded oligonucleotides (sense, 5′-AGTTGAGGGGACTTTCCCAGGC-3′) containing the NF-κB binding site were used as probes. Specificity of NF-κB DNA binding activity was confirmed in competition studies using cold consensus or mutant oligonucleotides.

Real-time PCR and mRNA quantitation. Total RNA isolation from LV tissue, cDNA synthesis, and quantitative real-time PCR were performed as previously described (14). mRNA transcripts for atrial natriuretic factor (ANF), tumor necrosis factor-α (TNF-α), and interleukin (IL)-1β were determined and normalized to glyceraldehyde-3-phosphate dehydrogenase expression using primer pairs previously described (14).

Apoptosis quantitation. Myocardial apoptosis was assessed by using the DeadEnd Fluorometric terminal deoxytransferase-mediated dUTP nick-end labeling (TUNEL) assay kit from Promega, which catalytically incorporates fluorescein-12-dUTP at the 3′-ends of fragmented DNA in apoptotic cells using recombinant terminal deoxynucleotidyl transferase (rTdT). Deparaffinized and rehydrated tissue sections were treated with Proteinase K (20 μg/ml) for 15 min at 37°C and then fixed with 4% methanol-free formaldehyde solution in PBS.

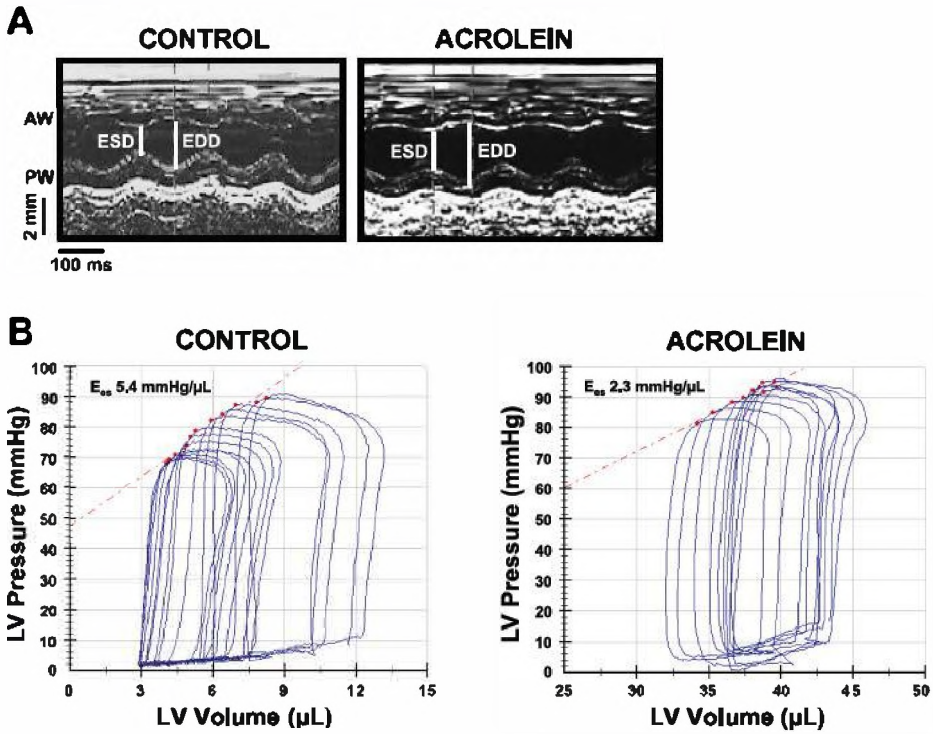

Fig. 1. Chronic acrolein exposure depresses left ventricular (LV) function. *A*: M-mode echocardiograms from two mice, one acrolein-fed and the other vehicle-fed (control). AW and PW, anterior and posterior wall, respectively; ESD and EDD, end-systolic and end-diastolic diameter, respectively. *B*: LV pressure-volume loops and the corresponding end-systolic pressure-volume relations in representative control and acrolein-fed mice. E_{es}, end-systolic elastance.

All subsequent steps were performed following the manufacturer's instructions. All sections were counterstained with DAPI to label nuclei. Cardiomyocytes were identified by staining with anti-troponin I antibody (Santa Cruz Biotechnology) followed by Alexa Fluor 555-conjugated secondary antibody (Invitrogen). TUNEL-positive nuclei (cyan staining) were visualized directly by confocal microscopy (Zeiss LSM510) with nuclear staining confirmed by *z*-axis sections. Images were taken with a ×63 objective lens at six different locations in each tissue section, and nine sections per heart were evaluated to determine the overall apoptotic rate (total 54 fields/heart). DNase (10 U/ml)-treated sections were used as positive controls. Sections without rTdT treatment were considered as negative controls.

Statistical analysis. Continuous variables are presented as means ± SD. Two-group comparisons were performed using an unpaired *t*-test. A P value <0.05 was considered significant.

RESULTS

Chronic acrolein consumption induces LV remodeling and dysfunction. Mice gavage-fed acrolein at 1 mg·kg^{-1}·day^{-1} for 48 days displayed no overt abnormalities or distress and no significant mortality. Body weight was similar between vehicle-fed and acrolein-fed animals after 48 days (control 26.1 ± 2.3 g; acrolein 26.0 ± 2.0 g). Baseline echocardiographic variables (before the start of feeding) were similar between the two groups. M-mode echocardiographic images obtained after the 48-day feeding period are shown in Fig. 1*A*. The acrolein-exposed mouse exhibited increased LV size and decreased FS compared with control. Group echocardiographic data (Table 1) indicate that acrolein exposure induced LV dilatation (increased EDD and ESD), LV systolic dysfunction (reduced FS and V_{cf}), and wall thinning (decreased RWT) consistent with a phenotype of DCM. While these changes were not severe (generally between ~8 and 20% change), they were highly consistent and statistically significant. To evaluate LV function more precisely, P-V analysis was performed. Figure 1*B* shows representative P-V loops from control and acrolein-exposed mice during IVC occlusion, together with the corresponding ESPVRs. Consistent with the echocardiographic results, acrolein exposure induced LV dilatation with increased end-diastolic volume and end-systolic volume and depressed LV systolic function as indicated by the smaller E_{es}. Group data (Table 2) demonstrated consistent LV enlargement and more profound reductions in systolic function with diminished dP/dt_{max}, maximal power, E_{es}, and stroke work. Also evident was impairment of LV relaxation with decreased dP/dt_{min} and increased tau. Hence, chronic acrolein exposure induced pathological remodeling and LV dysfunction.

Chronic acrolein exposure generates myocardial oxidative stress and protein-acrolein adducts. α,β-Unsaturated aldehydes are products of lipid-peroxidation and as such are sensitive markers of oxidative stress (8, 37, 40). Moreover, reac-

Table 1. *Echocardiography in control and acrolein-exposed mice*

	Control (n = 16)	Acrolein (n = 14)	P Value
HR, beats/min	469 ± 60	481 ± 62	0.585
LVEDD, mm	3.7 ± 0.1	4.0 ± 0.2*	<0.001
LVESD, mm	2.1 ± 0.2	2.5 ± 0.2*	<0.001
FS,%	43 ± 4	36 ± 3*	<0.001
ET, ms	51 ± 4	52 ± 7	0.771
V_{cf}, circ/s	8.5 ± 1.1	7.1 ± 1.0*	0.0022
AWT, mm	0.78 ± 0.04	0.74 ± 0.06	0.060
PWT, mm	0.79 ± 0.03	0.76 ± 0.03*	0.0089
RWT	0.42 ± 0.02	0.38 ± 0.02*	<0.001

Values are means ± SD; n, no. of mice. HR, heart rate; LV, left ventricular; EDD, end-diastolic diameter; ESD, end-systolic diameter; FS, fractional shortening; ET, ejection time; V_{cf}, velocity of circumferential fiber shortening; AWT and PWT, anterior and posterior wall thickness at end-diastole, respectively; RWT, relative wall thickness. *Statistical significance.

Table 2. *Pressure-volume parameters in control and acrolein-exposed mice*

	Control (n 8)	Acrolein (n 8)	P Value
HR, beats/min	501 ± 63	451 ± 45	0.073
LVEDV, μl	17 ± 5	36 ± 8*	<0.001
LVESV, μl	8 ± 2	29 ± 7*	<0.001
LVPSP, mmHg	94 ± 9	80 ± 14*	0.028
LVEDP, mmHg	7 ± 3	11 ± 6	0.134
SW, mmHg·μl	601 ± 214	378 ± 153*	0.025
dP/dt_{max}, mmHg/s	7,016 ± 1,757	4,697 ± 1,498*	0.010
Maximal power, mW	3.45 ± 1.61	1.98 ± 0.81*	0.029
E_{es}, mmHg/μl	4.93 ± 1.16	3.35 ± 0.98*	0.049
dP/dt_{min}, mmHg/s	8,002 ± 1,995	5,291 ± 1,957*	0.013
Tau, ms	10.4 ± 2.2	15.4 ± 4.3*	0.0094

Values are means ± SD; n, no. of mice. EDV, end-diastolic volume; ESV, end-systolic volume; PSP, peak systolic pressure; EDP, end-diastolic pressure; SW, stroke work; dP/dt_{max} and dP/dt_{min}, maximal and minimal rate of change in LV pressure, respectively; E_{es}, end-systolic elastance; tau, time constant of LV relaxation. *Statistical significance.

tive aldehydes can induce cellular toxicity by adducting with cysteine, histidine, and lysine residues on proteins (9, 37). To index oxidative stress in the hearts of control and acrolein-exposed mice, we measured protein-HNE adducts. The abundance of protein-HNE adducts in total heart homogenates did not change in acrolein-exposed mice (data not shown). However, examination of the membrane fraction of the cardiac homogenates revealed robust augmentation of protein-HNE adducts as assessed by dot blot and Western blotting (Fig. 2*A*), indicating membrane-localized oxidative stress. We next determined whether acrolein-exposed mice exhibited greater formation of acrolein-protein adducts in serum and heart tissue. Hearts harvested from mice chronically fed acrolein did not exhibit appreciable increases in the abundance of protein-acrolein adducts over control (data not shown). Because these results were not striking, we further examined the abundance of plasma and myocardial acrolein adducts 1 and 24 h after a single oral dose. Plasma protein-acrolein adducts (~150 kDa) increased markedly at both time points with the highest levels seen at 1 h (Fig. 2*B*), suggesting that ingested acrolein reaches the blood. Myocardial protein-acrolein adducts, involving proteins of varying molecular weight, were more modestly increased at 1 h but returned to baseline by 24 h (Fig. 2*C*), approximating the adduct levels observed in the hearts from chronically fed mice. These results suggest that, following oral exposure, sufficient acrolein translocates via the circulation to the heart to modify proteins. However, these adducts accumulate transiently and are then metabolically removed or degraded. Presumably, adduct formation is less pronounced after chronic exposure because of the metabolic disposition of extant tissue adducts.

Chronic acrolein exposure disrupts myocardial eNOS function and induces nitrative stress. We next examined whether acrolein disrupts eNOS function and promotes nitrative stress in the heart. As shown in Fig. 3*A*, a single oral dose of acrolein (5 mg/kg) profoundly suppressed eNOS phosphorylation at Ser^{1177}, an indicator of eNOS activation (4), without affecting overall eNOS abundance in the heart. In contrast, chronic exposure to acrolein significantly diminished eNOS dimers and increased relative levels of eNOS monomers (Fig. 3*B*), suggestive of eNOS uncoupling (36). Uncoupling of eNOS would be expected to promote the generation of reactive oxygen species (ROS) and peroxynitrite (36, 39). Indeed, hearts from mice chronically fed acrolein exhibited significantly greater staining for protein nitrotyrosine, an index of peroxynitrite generation (Fig. 3*C*). These results indicate that chronic acrolein exposure disrupted and uncoupled eNOS and induced nitrative stress in the heart.

Chronic acrolein exposure induces myocyte hypertrophy and apoptosis. As shown in Fig. 4*A*, histological evaluation of acrolein-exposed hearts revealed myocyte hypertrophy, with a twofold increase in myocyte cross-sectional area compared with control hearts. There was no substantial difference in interstitial fibrosis (data not shown). Gene expression of the hypertrophic marker ANF was similarly augmented over twofold in acrolein-exposed hearts compared with control (Fig. 4*B*). Despite these observations, gravimetric analysis of the LV and whole heart did not reveal differences in LV or whole heart weight (normalized to body wt) between the groups. This suggested that the increase in myocyte size was offset by myocyte loss. Indeed, as shown in Fig. 5, we observed a greater frequency of TUNEL-positive nuclei in the hearts of acrolein-exposed mice; these were primarily in cardiomyocytes. Quantitation of the apoptotic rate revealed a more than sixfold increase in TUNEL-positive nuclei compared with control. Hence, chronic oral acrolein exposure induced prohypertrophic and proapoptotic effects in the heart.

Chronic acrolein exposure promotes myocardial inflammation. Reactive aldehydes are known to promote inflammation (30, 38), which is a hallmark of chronic HF (14, 22). NF-κB is a central transcriptional regulator of proinflammatory mediators such as TNF-α and IL-1β. To evaluate NF-κB activation, we performed EMSA using pooled cardiac tissue from animals with either acute (24 h after single dose of 1 mg/kg) or chronic oral acrolein exposure, along with appropriate controls. As seen in Fig. 6*A*, heart tissue from chronically exposed (but not acutely exposed) mice demonstrated robust activation of NF-κB. Figure 6*B* depicts activated NF-κB p65 subunit immunostaining and quantitation of nuclear immunoreactivity from control and acrolein-exposed hearts. Consistent with the DNA binding studies, the hearts from acrolein-exposed mice exhibited a robust (~5-fold) increase in the nuclear localization of p65. Additionally, protein levels of IκBα (which binds cytoplasmic NF-κB thereby preventing its nuclear translocation) were decreased in hearts from acrolein-exposed mice (Fig. 6*C*). Moreover, in parallel with NF-κB activation, hearts from acrolein-exposed mice also exhibited significant (~2-fold) upregulation of TNF-α and IL-1β mRNA expression compared with controls (Fig. 6*D*), which is indicative of sustained inflammation.

DISCUSSION

In this study, we demonstrate for the first time that oral exposure to acrolein, a prototypical α,β-unsaturated aldehyde pollutant, at concentrations within the estimated range of human total unsaturated aldehyde exposure, induces a phenotype of DCM in the mouse. Specifically, 48 days of acrolein exposure induced: *1*) LV dilatation, wall thinning, impairment of LV relaxation, and depressed contractility; *2*) chronic membrane-localized oxidative stress associated with varying degrees of systemic and myocardial protein-acrolein adduct for-

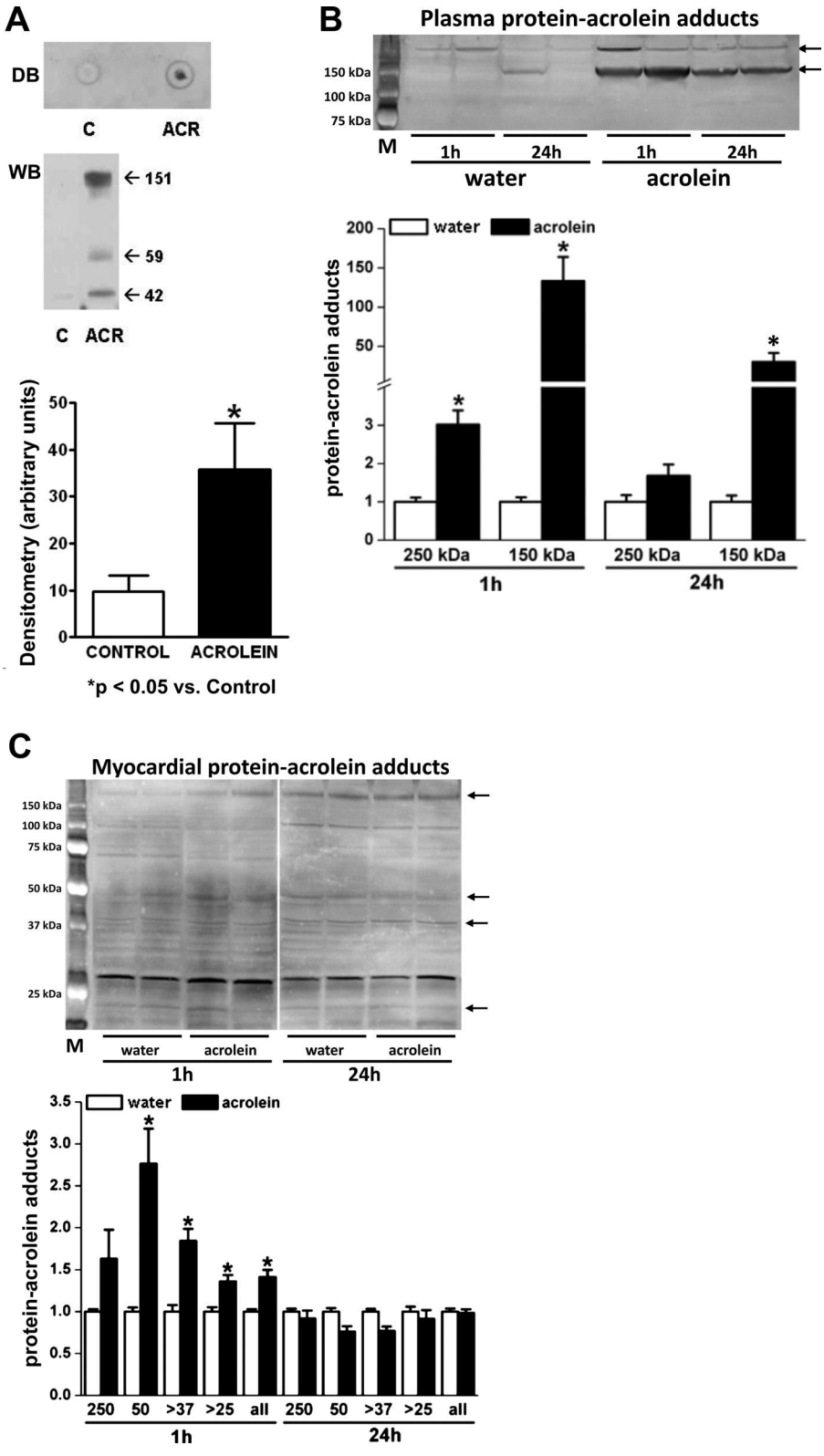

Fig. 2. Chronic acrolein exposure induces cardiac oxidative stress and protein modification. *A*: representative dot blot (DB) and Western blot (WB) performed on the membrane fractions of cardiac homogenates derived from acrolein (ACR)-fed and vehicle-fed [control (C)] mice and corresponding WB densitometry. *B* and C: WB and densitometry for protein-acrolein adducts in plasma (*B*) and myocardium (*C*) from mice fed a single dose of acrolein (1 mg/kg) or water 1 and 24 h after exposure. Augmented protein bands at different molecular weights are indicated by the arrows. M, molecular weight markers. *$P < 0.05$ vs. control; n 4 mice/group.

mation; *3*) diminished levels and uncoupling of eNOS with associated myocardial nitrative stress; *4*) myocyte hypertrophy and apoptosis without fibrosis; and *5*) myocardial inflammation with activation of NF-κB and upregulation of TNF-α and IL-1β. The features of oxidant stress, hypertrophy, apoptosis, and inflammation are pathological hallmarks of the failing heart. Taken together, the results suggest that analogous environmental exposure to acrolein in humans can contribute to the development of DCM and/or exacerbate pathological remodeling in humans with preexisting disease. Our results further

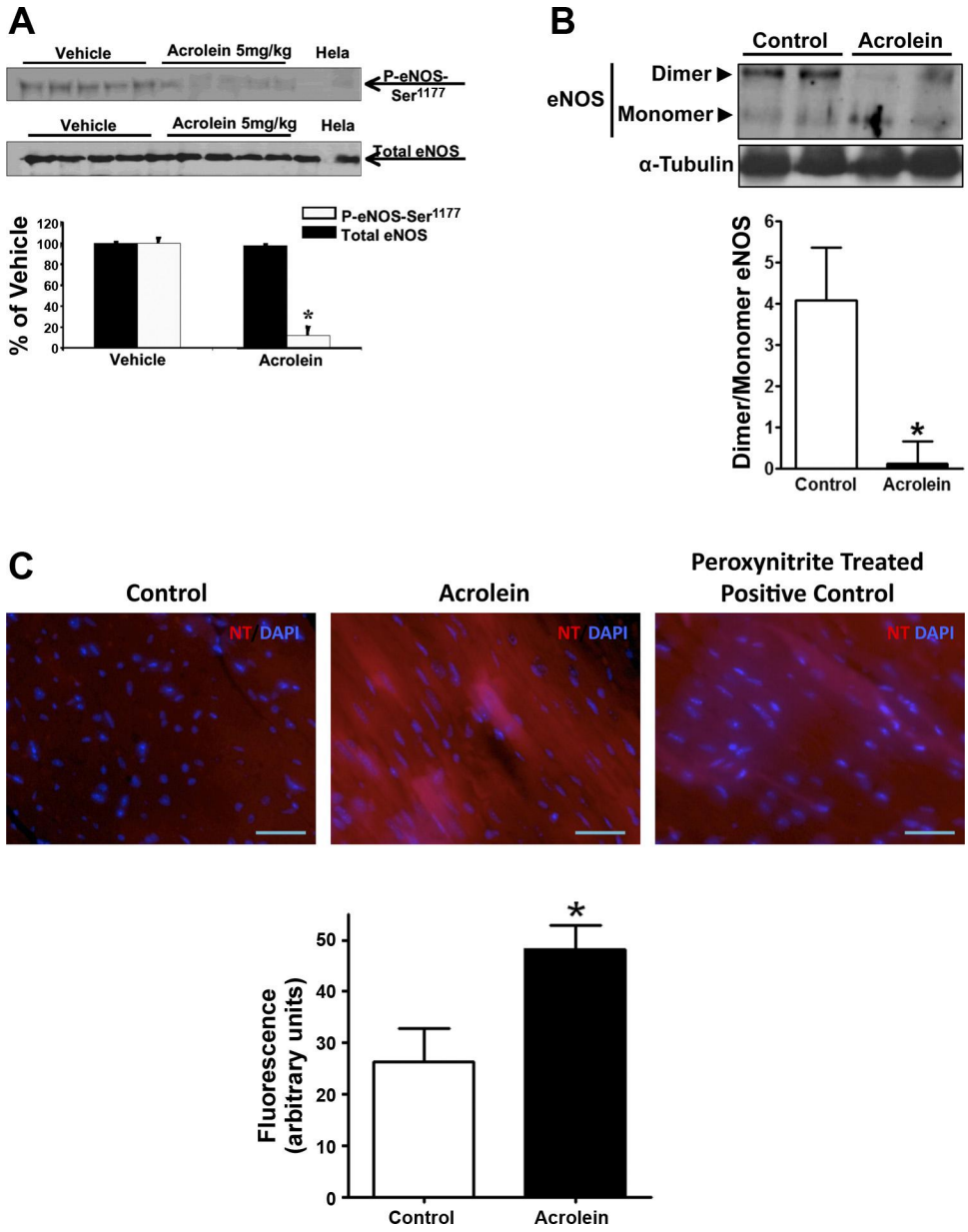

Fig. 3. Acrolein increases myocardial nitrative stress. *A*: WB and quantitation for phospho (P)-endothelial nitric oxide synthase (eNOS)-Ser^{1177} and total eNOS performed on total cardiac homogenates from mice 24 h after a single oral dose of acrolein (5 mg/kg) or vehicle (n 5/group). Hela, Hela cell lysate. *B*: WB and densitometry for eNOS dimer and monomer performed on cardiac homogenates derived from mice chronically fed acrolein or water for 48 days (n 4–5/group). *C*: immunofluorescent stains for protein-nitrotyrosine (NT, red) with 4′,6-diamidino-2-phenylindole (DAPI) costain for nuclei (blue) in hearts harvested from acrolein-fed and control-fed mice as in *B*, along with fluorescence quantitation (control, n 3; acrolein, n 5). Peroxynitrite-treated sections were used as a positive control. *$P <$ 0.05 vs. control.

suggest the possibility that acrolein (and potentially other unsaturated aldehydes) can serve as a dietary xenobiotic mediator and/or modulator of cardiomyopathy.

Epidemiological data indicate that pollution exposure increases cardiovascular morbidity and mortality (3, 5, 28), with the most robust associations related to ischemic heart disease, dysrhythmias, HF, and cardiac arrest (28). A recent study of elderly survivors of acute myocardial infarction revealed that air pollution exposure increased both the risk of mortality and the risk for new-onset HF within four to five years (44). Because the development of new-onset HF following infarction is related to the progression of underlying LV remodeling over time (16), this suggests that exposure to one or a variety of constituent pollutants can exacerbate underlying structural remodeling. One proposed mechanism of pollution-related cardiovascular risk is the physical translocation of soluble pollutant constituents into the heart and vasculature via the circulation (5). However, little is known about the specific pathophysiological responses to individual constituents of source mixtures of environmental pollutants.

Acrolein is a ubiquitous aldehyde pollutant of considerable importance to public health (7). High levels of acrolein have been detected in several foods (ranging from 10 to 600 μg/kg), cigarette smoke (10–140 μg/cigarette), water samples, heated oils, automobile exhaust, coal, and industrial waste (10, 11, 42). Volatile aldehydes such as acrolein are important constituents of the vapor phase of urban air pollution and diesel exhaust and are considered hazardous air pollutants by the EPA (7, 29). Given the large number of environmental sources of acrolein and its potential for long-term toxicity, we sought to determine the effects of chronic acrolein exposure on the heart. In this study, we chose to examine the effects of ingested (as opposed to inhaled) acrolein because, in humans, even in smokers, the highest level of acrolein exposure is through food

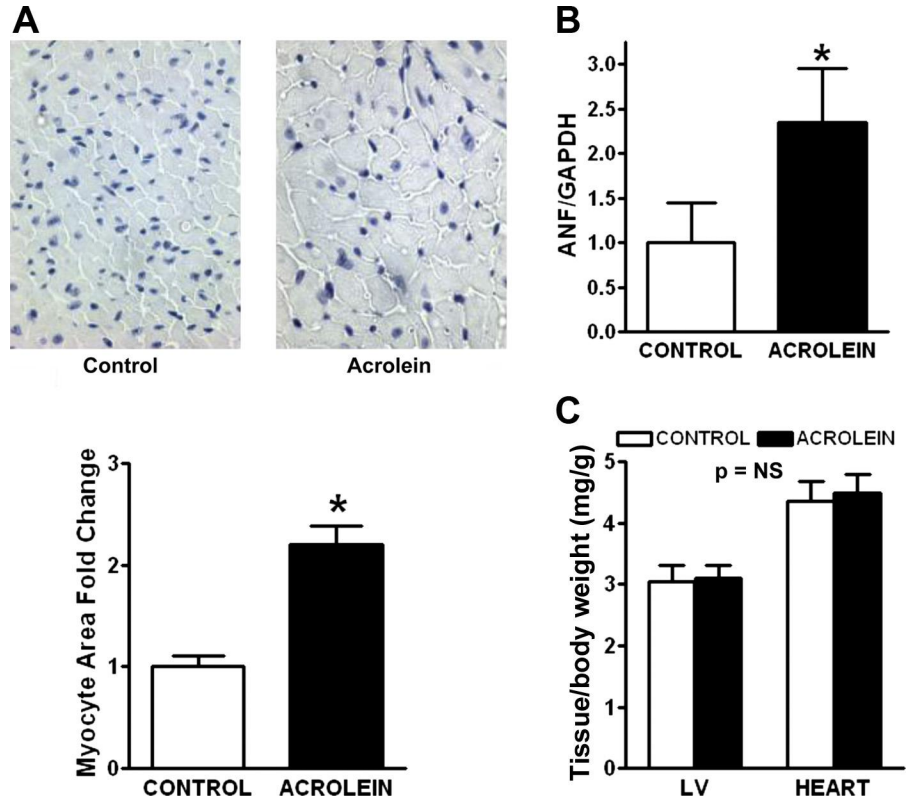

Fig. 4. Chronic acrolein exposure induces myocyte hypertrophy. *A*: representative histomicrographs of heart tissue from control and acrolein-fed mice demonstrating myocytes in cross section and corresponding quantitation of myocyte cross-sectional area. Also shown is the expression of the atrial natriuretic factor (ANF) gene in the heart by quantitative real-time PCR (*B*) and tissue gravimetric data (*C*) from the same experimental groups. GAPDH, glyceraldehyde-3-phosphate dehydrogenase; NS, not significant. *$P < 0.05$ vs. control.

substances (42). Nevertheless, our findings that acrolein translocates to plasma and heart tissue following exposure (evidenced by the formation of adducts) and induces chronic changes in cardiac gene expression suggest the possibility that analogous exposure to acrolein in ambient air may, via physical transport in blood, produce similar responses. This is consistent with the high cardiovascular toxicity associated with the aldehyde-containing components of air pollution, diesel exhaust, and cigarette smoke (3, 18).

We have previously estimated the maximal human acrolein exposure from food and water to be 0.1 $mg \cdot kg^{-1} \cdot day^{-1}$ (with an additional 0.1 $mg \cdot kg^{-1} \cdot day^{-1}$ from cigarette smoking) and the maximal human unsaturated aldehyde consumption to be 5 $mg \cdot kg^{-1} \cdot day^{-1}$ (42). In the current study, we evaluated the chronic effects of 1 $mg \cdot kg^{-1} \cdot day^{-1}$ acrolein, a dose fivefold lower than in our acute studies (42), representing a level 5- to 10-fold greater than maximal human acrolein consumption but only ~20% of total estimated unsaturated aldehyde intake. We chose this intermediate dose given that the sensitivity to acrolein varies among experimental animals; compared with rabbits (LD_{50} 7 mg/kg), mice are relatively less sensitive (LD_{50} 40 mg/kg acrolein) (10). Human sensitivity to acrolein, however, has not been assessed. Whether different acrolein dosing regimens (e.g., lower but more frequent doses) would influence the results differently should be explored in future investigations.

Our results establish that environmental exposure to acrolein, via the oral route, induces a state of inflammation and oxidant stress in the heart, along with LV systolic dysfunction, myocyte hypertrophy, and apoptosis, all consistent with xenobiotic-mediated DCM. These effects are consistent with the known prooxidant and proinflammatory effects of α,β-unsaturated aldehydes, which have been shown to activate inflammatory genes and signaling (including NF-κB) (27, 30, 38) and promote monocyte adhesion to endothelial cells (13). Similarly, in our study, acrolein-exposed hearts exhibited NF-κB activation, proinflammatory cytokine (TNF-α, IL-1β) gene expression, and oxidative and nitrative stress. Furthermore, in our prior study (19), we have shown that oxidative stress is required for acrolein-induced contractile dysfunction, since such effects were prevented by the antioxidant *N*-acetylcysteine. These findings are of significance, since chronic inflammation and oxidant stress are hallmarks of HF and considered to be important mediators of pathological LV remodeling (12, 16, 22). Plasma TNF-α is an independent predictor of patient mortality in HF (6), and, in experimental models, TNF-α induces many aspects of HF, including contractile depression, hypertrophy, apoptosis, matrix metalloproteinase activation, and oxidative stress (14, 22). Similarly, systemic oxidant stress in human HF correlates with the degree of ventricular dysfunction (21). Signaling related to ROS has been strongly implicated in the induction of pathological cardiac hypertrophy, and ROS can also mediate apoptosis, alter calcium channels and calcium flux, and reduce myofilament calcium sensitivity (12, 20, 32). Moreover, in vivo treatment with ROS scavengers improves pathological LV remodeling (17).

The stimulus for inflammatory cytokines and oxidative stress in HF is generally thought to reflect a response to injury, hemodynamic abnormalities, neurohormonal activation, and alterations in tissue perfusion. Our data suggest that environmental triggers may also contribute to this process and thereby exacerbate the course and progression of HF, and that those with preexisting LV dysfunction may be especially sensitive to environmental acrolein exposure. Interestingly, epidemiologi-