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Association of Dietary Copper and Zinc Levels with Hepatic Copper and Zinc Concentration in Labrador Retrievers

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Background: Copper-associated hepatitis is an inherited disease in the Labrador Retriever. Apart from genetic factors, dietary intake of copper and zinc are suspected to play a role in the pathogenesis.

Objectives: To investigate whether dietary copper and zinc levels of commercially available dry diets are associated with hepatic copper and zinc concentrations in Labrador Retrievers.

Animals: Fifty-five Labrador Retrievers that were fed a single brand and type of commercial dry food for at least 1 year. Of these, 44 dogs were family members of Labrador Retrievers with copper-associated hepatitis.

Methods: Liver biopsies, blood samples, and diet samples were obtained. Liver specimens were scored histologically and copper and zinc concentrations were quantified. Dietary concentrations of copper and zinc were measured. The association between dietary intake of copper and zinc and hepatic copper and zinc concentrations was investigated by linear regression analysis.

Results: High dietary copper and low dietary zinc levels were significantly associated with high hepatic copper levels. No association between dietary intake and hepatic zinc was present.

Conclusions and Clinical Relevance: Dietary copper and zinc at current levels in commercially available dry dog food can influence hepatic copper and can be a risk factor for the development of copper-associated hepatitis in Labrador Retrievers with a genetic susceptibility to copper.

Key words: Diet; Dog; Hepatitis; Liver; Wilson's disease.

Introduction

Copper-associated hepatitis in the Labrador Retriever is a complex hereditary disease recently characterized in the Dutch[1] and American Labrador Retriever populations.[2,3] Copper accumulation in the canine liver progresses over several years without apparent clinical signs. Eventually, chronic hepatitis and cirrhosis result in liver failure at middle or old age. When diagnosed in the clinical phase, the disease often has a fatal course within a few months.

The National Research Council (NRC) provides recommendations for pet food nutrient composition with the aim to meet requirements of dogs with minimal risk of deficiency or toxicity.[4] Recommendations are often based on values that are either extrapolated from data in growing puppies or composition of commercial maintenance diets that have not been associated with signs of deficiencies. There is no reference in these guidelines to appropriate dietary intake for animals with metal metabolism abnormalities.

Abbreviations:

aPTT	activated partial thromboplastin time
dwl	dry weight liver
ME	metabolizable energy
NRC	National Research Council
n-s	nonspecific
PTT	prothrombin time

Labrador Retrievers with an increased hepatic copper concentration after D-penicillamine treatment that were fed a diet with a copper content of 1.23 mg/1,000 kcal metabolizable energy (ME) had a significant decrease in their hepatic copper concentration.[5]

Zinc salts are often used in the treatment of Wilson's disease,[6] the best described human form of copper toxicosis. Zinc does not promote cupriuresis, like the copper chelators D-penicillamine or trientene, but creates a negative copper balance by blocking copper uptake in the enterocytes. Therefore, zinc is often used in presymptomatic patients and for maintenance therapy.[6] Zinc induces the endogenous copper chelator metallothionein when it is absorbed into the enterocytes. Here it forms a complex with metallothionein in the cytosol. When copper enters the enterocyte, zinc is displaced by copper from the metallothionein binding site, forming a copper-metallothionein complex, which remains in the enterocyte and does not pass to the portal circulation. The enterocyte including copper will be shed into the feces.[7] The effect of high-dose zinc salts to decrease hepatic copper in dogs has been described previously.[8]

Currently, no data are available on the potential influence of dietary copper and zinc concentrations

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that are regularly present in commercial dry diets on hepatic copper and zinc concentration in Labrador Retrievers.

In this study, we collected pedigree information and analyzed liver biopsy specimens and diet samples from 55 Labrador Retrievers to investigate whether dietary copper and zinc content at levels present in commercially available dry diets were associated with hepatic copper and zinc concentration and could therefore be a potential risk factor for copper-associated hepatitis in Labrador Retrievers with a genetic predisposition for this disease.

Materials and Methods

Animals

Recruitment of Labrador Retrievers was performed at the Department of Clinical Sciences of Companion Animals, Faculty of Veterinary Medicine, Utrecht University, The Netherlands, in 3 different ways between July 2008 and July 2009.

First, an invitation for participation was sent via the Dutch Labrador Retriever breed club to owners of Labrador Retrievers that were first degree relatives (descendents, siblings, or parents) of 11 Labrador Retrievers that were previously diagnosed with copper-associated hepatitis by evaluation of a liver biopsy specimen. Second, an appeal to participate to owners of Labrador Retrievers over 2 years of age was placed in the Dutch Labrador Retriever breed club's magazine. Third, owners of Labrador Retrievers that were referred to our department during the study period were invited to participate with their dog.

Inclusion criteria were that the dog should be fed a single type and brand of commercially available dry diet for a minimum period of 1 year at the time of investigation, that the daily intake of food should consist for at least 90% of this diet, and that the recipe of the diet should not have changed in the year previous to the sample collection for this study.

A medical history was obtained from the owners, and a physical examination was performed on all Labrador Retrievers by 1 examiner (HF). Blood was collected in sodium citrate for analysis of a coagulation profile, including prothrombin time (PTT), activated partial thromboplastin time (aPTT), and fibrinogen concentration. Platelets were counted in EDTA-preserved samples.

Liver Biopsy

When coagulation test results were within normal limits,[a] at least 3 liver biopsies were obtained with a 14G needle using a Tru-cut device under local anesthesia and ultrasound guidance as described previously.[9] Two biopsy specimens were fixed for 3 hours in 4% neutral buffered formalin and then transferred to 70% ethanol. Specimens were embedded in paraffin and 4 μm thick slides were cut and mounted on glass slides. Sections were stained with hematoxylin and eosin, rubeanic acid for copper and according to Gordon and Sweet for reticulin. All samples were evaluated by 1 board certified pathologist (TSGAMvdI) according to the World Small Animal Veterinary Association standards.[10] The pathologist was blinded to the hepatic copper concentrations of the dogs.

One biopsy specimen was collected in a copper free container for quantitative copper determination. It was freeze dried for 24 hours and copper was measured by Instrumental Neutron Activation Analysis.[11] Copper concentrations are reported in mg/kg dry weight liver (dwl).

Diet Examination

The feeding regime of the dogs was investigated by a questionnaire for the owners. Reported information included the period over which the current diet had been fed and the type and amount of other food and supplements or herbals that the dog received during the previous year. Owners were asked to bring a sample (250 g) and the content information from the package of the dog food. Dog food manufacturers were interviewed to establish whether there had been a change in the composition of the dog food brands that were collected in this study.

Food samples were analyzed for moisture, crude protein, crude fat, crude fiber, ash content, copper, and zinc levels in a commercial laboratory (Royal Canin Research Centre, Aimargues, France) according to AFNOR procedures.[12] Samples were sent with a code, so that laboratory technicians were blinded to the brand name of the sample. Samples were analyzed in 3 different batches in July 2008, May 2009, and January 2010. ME density was calculated by modified Atwater factors.[13] The amounts of copper and zinc are reported in mg/1,000 kcal.

Statistical Analysis

Data were analyzed in R version 14.0.[14] Linear regression was used to study the association between hepatic zinc (in all dogs) and hepatic copper (from dogs with increased hepatic copper levels) with inflammatory lesions in the canine liver. Results from dogs with nonspecific (n-s) reactive hepatitis, acute hepatitis, and chronic hepatitis were compared with the results from Labrador Retrievers with normal histology. Hepatic copper was log transformed to ensure normality and constance of variance. A Bonferroni corrected P-value < 0.05 was considered significant.

The association between hepatic copper and zinc concentration as outcome variable and age, sex, and dietary copper and zinc as explaining factors was studied by stepwise backward linear regression. The best fitting model was determined based on Akaike's information criterium. The validity of the final model was checked by studying the residuals on normality and constance of variance. Hepatic copper concentration was log transformed because it did not show a normal distribution. The coefficient of determination was calculated by the following formula: 1−(residual deviance/null deviance) × 100%.

A Wilcoxon rank sum test with continuity correction was used to test the difference between hepatic copper concentrations in dogs fed diet nr. 28, which was selected because it was the most commonly fed diet, and dogs fed any other diet. A Fisher's exact test was used to test the difference in proportion of family members of the affected dogs in the group of dogs that was fed diet nr. 28 versus the group of dogs fed any of the other diets. A Chi-square test was used to test the differences in sex distribution among the 4 groups of histologic diagnosis. A P-value $< .05$ was considered significant.

Normality of the data was ensured by inspecting the histograms. Normally distributed data were reported as mean ± standard deviation and non-normally distributed data were reported as median and range.

Ethics

The study was approved by the Utrecht University Institutional Animal Care and Use Committee. Informed consent was obtained from owners of dogs participating in the study. Owners received the diagnostic results and, where necessary, a treatment plan for their dogs.

Results

Study Population

Fifty-five client-owned Labrador Retrievers participated in the study. Forty-four Labrador Retrievers were first degree relatives to Labrador Retrievers diagnosed with copper-associated hepatitis, including 26 descendents, 16 siblings, and 2 parents. Seven Labrador Retrievers were recruited upon reading the appeal in the Labrador post. In one of these dogs, the owner reported a suspicion of liver disease of a related dog; however, that was not confirmed histopathologically. Four of the Labrador Retrievers were referred to our clinic for clinical signs of liver disease during the study period.

The complete study population consisted of 40 female dogs and 15 male dogs. The mean age of the dogs was 5.7 ± 2.3 years. Of the female dogs, 80% showed a hepatic copper concentration >400 mg/kg dwl, whereas in the males this fraction was 60%. Female Labrador Retrievers had slightly higher hepatic copper concentrations (757 ± 628 mg/kg dwl) than male dogs (642 ± 454 mg/kg dwl). There was no significant difference in the proportion of males and females in the 4 groups of histologic diagnosis.

Clinical Signs and Histopathologic Examination of Liver Biopsy Specimens

The 4 referred dogs showed clinical signs including icterus, polyuria/polydipsia, anorexia, vomiting, and lethargy. Histologic evaluation of liver biopsy specimens of these dogs showed in 2 dogs acute hepatitis and in 2 dogs chronic hepatitis. All other dogs (n = 51) were clinically healthy and had no previous signs of hepatic disease.

In Table 1, the histopathologic diagnosis as well as hepatic copper and zinc concentrations of the 55 Labrador Retrievers are summarized. In 75% of the dogs, hepatic copper concentrations were higher than 400 mg/kg dwl. In dogs with acute or chronic hepatitis, hepatic copper concentration was significantly higher than in dogs with normal histology (Table 1). In these cases, histopathologic evaluation of rubeanic stained slides showed copper loaded hepatocytes and macrophages in the centrolobular areas accompanied by an inflammatory infiltrate of lymphocytes and plasma cells.

The mean hepatic zinc concentration in this study population was 178 ± 32 mg/kg dwl. Two dogs had hepatic zinc concentrations that were slightly below the reference range (120–300 mg/kg dwl)[15] with values of 104 and 118 mg/kg dwl, respectively. All other dogs had hepatic zinc concentrations within the reference range. Hepatic zinc concentrations were not significantly different among different groups of histologic diagnosis (Table 1).

Diet Analysis

Fifty-five Labrador Retrievers met the inclusion criteria to enter the diet survey. One dog had received a glucosamine preparation for her arthrosis in the year previous to the sample collection, no supplements or herbals were reported to be administered to the other dogs.

Four owners participated in the study with 2 dogs being fed the same diet. The mean ME of the diet samples was 3,460 ± 210 Kcal/kg as fed. Mean dietary copper and zinc levels were 4.2 ± 1.4 and 52.4 ± 17.8 mg/1,000 Kcal, respectively. A summary of the analysis of diet samples is provided in Table 2.

From 7 brands of diets, more than 1 sample was analyzed within this study. There were no indications that there was a change in dietary composition in these brands during the study period. We confirmed by telephone interview with the different dog food manufacturers that there had been no change in diet composition in the year preceding the collection of the dog food samples in this study either.

Relation between Diet Composition and Hepatic Copper and Zinc Concentration

In this study group, age and sex did not have a significant influence on hepatic copper concentrations. A significant positive association between dietary copper and hepatic copper and negative association between dietary zinc and hepatic copper were found (Fig 1).

Table 1. Summary of histologic diagnosis and hepatic copper and zinc concentrations in Labrador Retrievers.

Histologic Diagnosis	Copper Status*	Number of Dogs	Hepatic Copper (mg/kg dwl) (mean ± SD)	Hepatic Zinc (mg/kg dwl) (mean ± SD)
Normal	Normal copper	6	240 ± 68	140 ± 25
	High copper	15	724 ± 251	190 ± 26
Reactive hepatitis	Normal copper	2	340 ± 56	156 ± 27
	High copper	12	660 ± 188	187 ± 23
Acute hepatitis	Normal copper	3	307 ± 80	163 ± 15
	High copper	8	1,256 ± 1,122	184 ± 30
Chronic hepatitis	Normal copper	3	340 ± 71	133 ± 19
	High copper	6	1,169 ± 651	192 ± 35

*Data for hepatic copper and zinc concentration in different histologic groups subdivided in a group of dogs with normal copper (<400 mg/kg dwl) and high copper (>400 mg/kg dwl).

Table 2. Results of diet analysis.

Diet	N	ME as Fed (kcal/kg) (mean ± SD)	Cu (mg/1,000 kcal) (mean ± SD)	Zn (mg/1,000 kcal) (mean ± SD)	Zn/Cu Ratio
1	2	3,472 ± 60	4.3 ± 0.4	38.6 ± 3.4	8.9 ± 0.1
2	1	3,212	5.7	56.7	10.0
3	1	3,588	3.0	35.1	11.8
4	1	3,522	2.6	29.5	11.6
5	1	3,180	3.4	37.7	11.1
6	1(2)	3,566	4.7	62.8	13.5
7	4(6)	3,550 ± 83	5.9 ± 0.2	73.0 ± 1.8	12.1 ± 0.7
8	1	3,357	5.5	72.4	13.3
9	1	3,375	5.6	77.3	13.8
10	1	3,375	5.8	77.6	13.4
11	1	3,410	5.6	65.7	11.8
12	1(2)	3,234	4.3	50.7	11.9
13	1	3,137	4.5	41.4	9.3
14	1	3,832	6.0	54.3	9.0
15	1	3,233	3.9	43.3	11.1
16	1	3,501	2.4	38.0	15.6
17	1	3,953	3.0	39.5	13.0
18	1	3,673	3.5	46.6	13.2
19	1	3,600	2.6	33.9	13.0
20	1(2)	3,377	2.9	32.9	11.3
21	1	3,192	5.6	56.1	9.9
22	1	2,812	5.0	51.2	10.3
23	1	3,191	4.3	80.2	18.8
24	3	3,208 ± 41	4.8 ± 0.09	43.9 ± 2.6	9.2 ± 0.7
25	1	3,430	5.8	61.7	10.6
26	3	3,749 ± 82	4.2 ± 0.7	54.2 ± 7.6	12.9 ± 0.4
27	3	3,455 ± 51	4.6 ± 0.3	52.2 ± 0.6	11.4 ± 0.8
28	8	3,459 ± 80	1.9 ± 0.3	62.1 ± 4.8	32.5 ± 7.0
29	2	3,711 ± 48	5.0 ± 0.5	61.6 ± 4.8	12.3 ± 0.4
30	1	378	5.0	61.6	12.3
31	1	367	5.7	66.5	11.6
32	1	345	5.6	71.5	12.8

ME, metabolizable energy as calculated by modified Atwater equation; Cu, copper; Zn, zinc; SD, standard deviation; N, number of samples from specific brand, in brackets: number of dogs fed this diet (in the case of 1 owner with 2 dogs on the same diet, 1 sample of diet was analyzed).

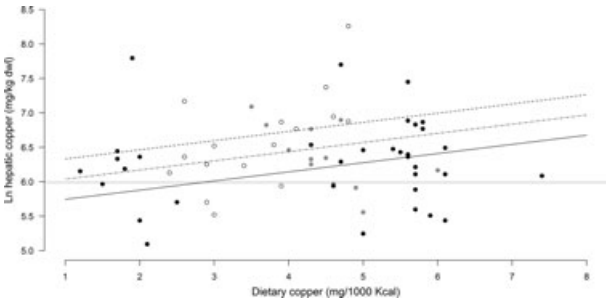


Fig 1. Scatter plot of dietary copper against hepatic copper. Levels of dietary zinc content of the same samples are indicated with open dots (dietary zinc <45 mg/1,000 kcal), gray dots (dietary zinc 45–55 mg/1,000 kcal), and black dots (dietary zinc >55 mg/1,000 kcal). The diagonal lines in the plot indicate the fitted values, with the parameters of the linear model, for the relationship between dietary copper and hepatic copper at different levels of dietary zinc. The dashed line indicates the fitted value for a diet with a zinc level of 30 mg/1,000 kcal, the dot-dash line indicated the fitted value for a diet with a zinc level of 50 mg/1,000 kcal, and the solid lines indicates the fitted values for a diet containing 70 mg/1,000 kcal. The horizontal dotted line depicts the upper limit of normal hepatic copper (natural logarithm of 400 mg/kg dwl).

The estimate for an increase in hepatic copper was 14.2% (95% CI: 0.8–29.3%) with every increase of 1 mg/1,000 kcal in dietary copper, and the estimate for an increase in hepatic copper was 1.5% (95% CI: 0.2–2.8%) for every decrease of 1 mg/1,000 kcal dietary zinc (Fig 1). The coefficient of determination for this model was 11.4%. We noticed that diet nr. 28 was clearly different from the other diets for copper and zinc content and more specifically for the zinc/copper ratio (Table 2). This diet had a mean zinc/copper ratio of 32.5, whereas all other diets in this study had zinc to copper ratio ranging from 8.9 to 18.8. When removing the 8 dogs that were fed this diet from the analysis, the associations were still present and the direction of the effect was the same, but the results did no longer reach significance. The estimate for an increase in hepatic copper when diet nr. 28 samples were removed was 11.2% (95% CI: −19.1–47.4%) with every increase of 1 mg/1,000 kcal in dietary copper, and the estimate for an increase in hepatic copper was 1.3% (95% CI: −0.8–3.4%) for every decrease of 1 mg/1,000 kcal dietary zinc. The coefficient of determination for this model was 3.3%.

Dogs that were fed diet nr. 28 (n = 8) had a significantly lower hepatic copper concentration of 419 ± 174 mg/kg dwl compared with dogs fed any of the other diets (n = 47) with a mean hepatic copper level of 778 ± 613 mg/kg dwl. This difference was significant (P = .02). There was no overrepresentation of family members of affected dogs in the group fed diet nr. 28 compared with the group fed one of the other diets (P = .92).

We did not detect a significant association between hepatic zinc concentration and age, sex, dietary copper concentration, or dietary zinc concentration.

Discussion

The relationship between copper and zinc in commercially available dry diet and hepatic copper concentrations was studied in Labrador Retrievers. Despite the fact that all but 4 dogs in this study population were clinically healthy, 75% had an abnormally high hepatic copper concentration. The majority of dogs in our dataset were first-generation relatives of Labrador Retrievers affected with copper toxicosis and thus might be at risk for inherited copper toxicosis.[1]

A positive association between dietary copper levels and hepatic copper concentration and a negative association between dietary zinc levels and hepatic copper concentration were identified in this study. Dogs that were fed diet nr. 28 had a significantly lower hepatic

copper concentration than dogs fed any of the other diets. This study indicates that the level of copper in pet food, as well as this level relative to zinc content, may be a factor in the accumulation of hepatic copper in Labrador Retrievers with a family history of copper-associated hepatitis.

Hepatic copper concentration in this study was measured in Tru-cut needle biopsy specimens taken with a 14G needle and an automated spring-triggered device. Biopsy specimens collected in this way are less invasive compared with biopsy specimens obtained during laparoscopy or laparotomy because they can be collected under local anesthesia causing little distress to the dog. Previous studies that compared the reliability of hepatic needle biopsies in dogs and cats to wedge biopsy specimens concluded that results from needle biopsies must be interpreted with caution.[16,17] In those studies, 18G needle biopsies were used, that result in a much smaller biopsy specimen volume compared with the 14G biopsy needles that are used in this study. Copper accumulation in primary metabolic copper toxicosis is present in the centrolobular areas of the canine liver, but can extend to portal areas in severe cases. This distribution pattern is consistent throughout the canine liver. For a reliable estimate of copper, both centrolobular and portal areas should be present in a biopsy specimen, and a typical 14G needle biopsy specimen contains on average 15 centrolobular and portal areas. For quantitative copper measurement by instrumental neutron activation analysis we used biopsy specimens of approximately 5 mg dry weight, which meets the standard requirements when performing metal analysis of needle-core biopsy specimens.[18,19] In advanced stages of copper-associated hepatitis, when liver cirrhosis is present, results from hepatic copper determination can become less reliable because there can be a heterogeneous distribution of tissue with fibrotic regions and regenerative nodules. Fibrotic tissue does not contain copper, and regenerative nodules usually contain less copper, depending when the biopsy sample was taken in relation to the formation of the nodule. Indeed, copper accumulation in newly formed hepatocytes takes time. Overall, in cases with chronic copper-associated hepatitis, hepatic copper concentrations are generally lower than in cases of acute copper-associated hepatitis. In this study population, 9 of 55 dogs were diagnosed with chronic hepatitis. Six of these cases had increased hepatic copper concentrations and were independently histologically classified as copper-associated chronic hepatitis by evaluation of H&E and rubeanic acid staining. The rubeanic acid staining scores correlated well with the measured hepatic copper concentrations in these cases. However, in these 6 cases, measurements of hepatic copper concentrations may have been less reliable owing to an increased chance for sampling error.

This study was set up in a cross-sectional way, which may not be the most appropriate study design to investigate a causal relationship between dietary content and hepatic copper concentrations. We optimized the reliability of the data by including only dogs that were fed commercially available dry diet for at least 1 year because hepatic copper concentrations change gradually over time and will take several months to become measurable.

In this set-up, we were able to obtain an unbiased sample of a range of dog foods and to investigate the dietary content of copper and zinc. In dogs, NRC 2006 recommended allowance for copper and zinc are 1.5 and 15 mg/1,000 kcal, respectively. NRC 2006 recommended allowance is the minimum level of an essential nutrient that should be present in commercial dog food to take into account nutrient bioavailability as well as ingredient and individual animal variabilities.[4]

For this study we made the assumption that the dogs were fed according to their caloric needs and did not gain or lose weight in the period of 1 year preceding collection of the liver and dietary sample. To be able to compare the different types of diet in this study, we used the copper and zinc content expressed as mg/1,000 kcal energy density of the diet. Pet food companies may provide data about the copper and zinc content of their diet on the labels. This data are usually expressed as mg/kg diet as fed. The range of copper and zinc content in our dataset was 5–23 mg copper and 104–265 mg zinc/kg diet as fed, respectively. Owners should be aware of the fact that when copper and zinc content is stated on the package label, this is often the amount of copper and zinc that is added to the diet as a premix and does not include the copper or zinc that was already present in the raw foodstuff.

We choose to analyze diet composition instead of relying on the package information as this is often not accurate.[20] The sample size of 250 g that was analyzed may not always be representative for the different production batches of diet. However, when more samples of a diet from different participants in the study were available, there appeared to be little variation (Table 2). This indicates that the conclusions drawn are likely valid.

Here we show that Labrador Retrievers fed a diet with a relatively high level of zinc and low level of copper did have significantly lower hepatic copper concentrations than dogs fed any of the other diets. The results from this study are consistent with results from a previous randomized double-blind placebo-controlled clinical trial that showed a decrease in hepatic copper concentration in dogs fed a low copper diet.[5] Zinc supplementation did not have any additional effect on the reduction in hepatic copper concentrations. In this study, dietary zinc showed a significant negative correlation with copper concentrations in the canine liver. This is in agreement with results from studies in dogs and humans in which zinc supplementation is used in treatment of copper toxicosis.[7,8] However, zinc concentrations used in treatment regimes are far above the supplemented amount present in canine diets. Investigation of the relative influence of dietary copper or zinc concentration or the ratio of their combination on hepatic copper and zinc level requires a prospective clinical trial.

Increased hepatic copper concentrations caused by high copper intake has been described before in several animal species, including dogs[21] and ruminants.[22,23] Also a positive association between dietary copper and hepatic copper concentration in people with or without a genetic predisposition is well documented.[24–28] Overall hepatic copper concentration in this study population was almost twice the upper limit for normal hepatic copper concentration in dogs. This may indicate that dietary copper content as currently present in commercial dry diets is at the high range of what dogs predisposed to hepatic copper accumulation can tolerate to keep their body copper composition within a reference range. The majority of dogs in this study population were family members of Labrador Retrievers with histologically diagnosed copper toxicosis, implying that our results may not be translatable to the general Labrador Retriever or canine population. The effect of dietary copper on hepatic copper concentration may be restricted to dogs with a genetic predisposition.

The dogs fed diet nr. 28 had significantly lower hepatic copper concentrations compared with dogs fed the other diets. Two of these dogs had normal histology, 3 showed nonspecific reactive hepatitis, 1 was a clinical case diagnosed with acute hepatitis, and 2 had subclinical chronic hepatitis. These dogs did not show histologic signs of copper-associated hepatitis. Like any other dog, the Labrador Retrievers can suffer from infectious hepatitis and there may be immune-related factors involved in some forms of hepatitis in the Labrador. This observation indicates that dogs that were fed diet nr. 28 were not in general protected from liver inflammation, but were rather protected from liver inflammation that is caused by high hepatic copper concentrations.

It was striking that despite the fact that 51 of 55 dogs in this population appeared clinically healthy, there was serious copper accumulation, which was associated with hepatitis, in the majority of the dogs. These results may indicate that copper-associated hepatitis is an underestimated health problem in the Labrador Retriever and requires more awareness of owners and veterinarians.

From pedigree analysis it is clear that copper-associated hepatitis in Labrador Retrievers is not a monogenic recessive trait as it is in the Bedlington Terrier.[29] In Labrador Retrievers there is a complex pattern of inheritance, with a female predisposition.[30] We believe that multiple gene variations, each with a small effect determine the phenotype in conjunction with environmental factors, like diet. Currently it is impossible to determine the risk in clinically healthy Labrador Retrievers because there are no genetic markers or biomarkers available. Further prospective evaluation of the role of dietary copper and zinc in the Labrador Retriever population requires elucidation of the underlying genotypic variation so that well-defined study groups with regard to genetic susceptibility can be formed.

Footnote

[a] Reference values used for coagulation tests in our laboratory: PTT (7.2–9.9 second), aPTT (13.2–18.2 second), fibrinogen (1.0–2.7), thrombocyte count (144–603 $\times$ 10^9/L)

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Conflict of Interest Declaration: Dr Biourge is employed by Royal Canin. Royal Canin analyzed the diet samples.

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