Copper-Associated Liver Diseases

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KEYWORDS

• Wilson's disease • Metabolic disease • Centro-lobular copper
• Heritability • Diet

Copper (Cu) is an essential trace element, belonging to the first transition series of elements. Other members of this series include zinc, manganese, cobalt, iron, and chromium. The atomic weight of naturally occurring copper is 63.546.

The liver is essential for copper metabolism because it is the principal recipient of absorbed copper, has the highest stored copper content, delivers copper in protein-bound form to other tissues, and is the principal organ of excessive copper elimination by biliary excretion.[1,2]

Copper transport between organelles and across membranes is much the same for animals, bacteria, fungi, and plants because of the highly conserved cellular copper transport elements (**Fig. 1**).[2]

Trace elements, in general, function as cofactors for antioxidant enzymes. Copper is a transition metal able to cycle between two redox states: oxidized Cu^{2+} (cupric ion, stable) and reduced Cu^{+}(cuprous ion, unstable). Copper can therefore function as an electron acceptor/donor for different enzymes.[3] It plays a role as a cofactor in hydrolytic, electron transfer and oxygen-utilization enzymes in the generation of cellular energy (cytochrome-c-oxidase), detoxification of oxygen-derived radicals (superoxide dismutase), iron metabolism (ceruloplasmin), blood coagulation, neuropeptide modification (dopamine-B-hydroxylase), melanin synthesis (tyrosinase), and connective tissue cross-linking (lysyl-oxidase).[1,4–10]

Free copper ions are able to catalyze the formation of hydroxyl radicals via the *Haber-Weiss reaction*:

$$O_2^{\cdot -} + Cu^{2+} \rightarrow O_2 + Cu^{+} \quad (1)$$

$$Cu^{+} + H_2O_2 \rightarrow Cu^{2+} + OH^{-} + OH^{\cdot} \quad (2)$$

$$O_2^{\cdot -} + H_2O_2 \rightarrow O_2 + OH^{-} + \mathbf{OH^{\cdot}}$$

The final outcome of this reaction is the toxic hydroxyl radical ($OH^{\cdot}$). This radical can directly damage lipids, proteins, and nucleic acids. Oxidative damage can induce

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Vet Clin Small Anim 39 (2009) 489–511
doi:10.1016/j.cvsm.2009.02.001 **vetsmall.theclinics.com**
0195-5616/09/$ – see front matter

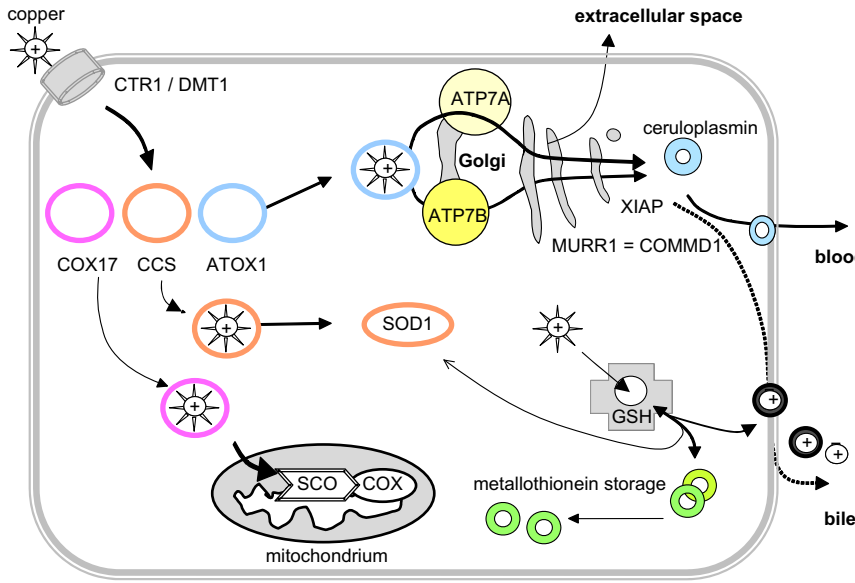

Fig. 1. Copper trafficking within the cell. Several intracellular pathways are involved in normal hepatic copper metabolism. Because of a high potential for oxidative damage, no free copper is present within the cell. Excessive copper is excreted into bile after interaction with COMMD1. CTR1, copper transporter 1; COX17, CCS, ATOX1, SCO, target-specific copper transporters; ATP7A, Menkes disease protein; ATP7B, Wilson's disease protein; SOD1, superoxide-dismutase 1; COX, cytochrome c oxidase; MURR1 = COMMD1, copper metabolism murr1 domain-containing protein 1, associated with copper toxicosis in Bedlington terriers; DMT1, divalent metal transporter 1; XIAP, X-linked inhibitor of apoptosis; GSH, glutathion. (*Data from* Refs.[14,16,22,40,45,73–99]).

inflammation, which ultimately can lead to liver damage. Oxidative stress affects transcription factors, resulting in deregulated gene expressions. In addition, oxidative stress is a major inducer of cytokine production in macrophages and other cells, of which profibrotic cytokines favor the production of collagen.[3,5,11,12]

Normal liver copper concentrations in dogs are higher than in people, mice, and rats.

The daily food intake of copper is about 14 to 15 mg/kg dry weight food in dogs, but considerable variation can be found between brands. Copper is present in vegetables, fruits, grains, nuts, meat, seafood, and drinking water, but to obtain copper concentrations in the above range, copper is commonly added to commercial dog food. Forty percent to 60% of ingested copper is absorbed across the apical membrane of the mucosa of the upper small intestine. The remaining copper leaves the body unabsorbed in feces.[11]

Two proteins are thought to be responsible for the absorption of dietary copper: the divalent metal transporter 1 (DMT1) and the copper transporter 1 (Ctr1). DMT1 transports copper (Cu^{2+}) directly from copper in the diet. Ctr1 is a transporter of Cu^{+}, which is reduced by endogenous plasma membrane reductases and dietary components such as ascorbate.[3] In the bloodstream, copper is bound to albumin (not specific binding), ceruloplasmin or transcuprein (specific binding). Within 2 to 6 hours of absorption, copper from blood enters the liver and the kidneys. In the liver, copper is immediately bound by intracellular chaperones, which are target-specific

transporter proteins. These chaperones deliver copper to specific intracellular target molecules. In a second step, after 4 hours or more, copper is exported from the liver cell by the copper-transporting ATPase, ATP7A, re-enters the blood stream, and is delivered to other organs.[1,3,13–16]

COPPER STORAGE DISORDERS IN HUMANS

Wilson's disease (Online Mendelian Inheritance in Man [OMIM] 277,900) and Menkes disease (OMIM 309,400) are autosomal recessive inherited copper storage disorders. Wilson's disease is the most completely characterized disorder of copper toxicity in humans. Patients with this disorder accumulate copper in various tissues, particularly the liver and brain and, in small amounts, in the cornea and kidney. Reduction or absence of ATP7B-gene expression in these patients reduces the rate of incorporation of copper into ceruloplasmin, and reduces biliary excretion of copper. Progressive hepatic copper accumulation, liver cirrhosis, and basal ganglia degeneration ensue. Ocular accumulation of copper leads to a typical circumferential corneal pigmentation, known as Kayser-Fleisher rings. In the blood, ceruloplasmin concentrations are reduced and nonceruloplasmin-copper is greatly increased.

Other disorders of copper metabolism in humans include Indian childhood cirrhosis and non-Indian childhood cirrhosis (Endemic Tyrolean infantile cirrhosis [OMIM 215,600] and idiopathic copper toxicosis). These disorders of copper toxicity resemble Wilson's disease phenotypically. However, their genetic background is still unsolved, although a complex etiology is suggested, with influencing factors from the environment, such as high copper intake.[1,2,6,9]

Furthermore, copper is involved in a number of diseases without known impact on the pathogenesis, including Parkinson's disease, Alzheimer's disease, and Prion diseases.[17–21]

COPPER STORAGE DISORDERS IN MICE, RAT, AND SHEEP

The toxic milk mouse and the Long-Evans Cinnamon rat (LEC-rat) were the first animal models used to study Wilson's disease with both models having many features in common with their human counterpart. In these animals, mutations in the ATP7B gene lead to copper accumulation in the liver and progressive inflammation and cirrhosis.[12,15]

North Ronaldsay sheep, with an unknown abnormality of copper metabolism, develop liver cirrhosis comparable to idiopathic copper toxicosis in people owing to copper-induced increased lysosomal activity and hepatic stellate cell activation.[22]

COPPER-ASSOCIATED CHRONIC HEPATITIS

Hepatic copper accumulation can result from increased uptake of copper, primary defects in hepatic copper metabolism, or from altered biliary excretion of copper. Toxicity of copper is dependent upon the molecular association and subcellular localization of molecules as well as their total concentration in tissue. In inherited copper storage disorders, copper accumulation is always localized centrolobularly. This is the case in Bedlington terrier copper toxicosis, Wilson's disease in humans, and liver disease in LEC-rats. In contrast to primary copper storage disorders, secondary copper loading of liver cells during cholestasis or cholatestasis, copper is mainly restricted to the periportal parenchyma.[16,23]

Copper-Associated Chronic Hepatitis in Dogs

In the Bedlington terrier, inherited copper toxicosis is a well-described disease. In this breed a deletion of exon 2 in the COMMD1 gene (previously called MURR1) causes accumulation of copper in hepatocytes, resulting in chronic hepatitis.[24–26] Moreover, hepatic copper storage and associated hepatitis are breed associated in the West Highland white terrier, Skye terrier, Doberman pincher, Dalmatian, and Labrador retriever.[12,27–32]

The average canine liver copper concentration is 200 to 400 ppm (ppm = μg/g = mg/kg) per dry weight (dw) of liver tissue.[28–31,33,34] Hepatic copper concentrations in affected dogs of breeds with primary copper storage disease vary between individual animals and between breeds from 600 to above 2200 ppm (**Table 1**).

CLINICAL SIGNS AND LABORATORY RESULTS IN DOGS WITH COPPER-ASSOCIATED CHRONIC HEPATITIS

Dogs with hepatic copper accumulation can appear normal over years before developing clinical signs late in disease, although copper may begin to accumulate by 5 to 6 months of age. One investigator followed dogs with the COMMD1 deletion from birth to 3 years of age, and found excessive copper accumulated in the liver by 1 year of age, although histologic evidence of hepatitis did not occur before affected dogs were 2 years old (R. Favier, 2005, personal communication). Therefore, dogs with inherited copper storage disorders appear to be subject to a prolonged period of several years between severe accumulation of copper and development of histologic signs of inflammation, as well as between the consolidation of histologic signs of inflammation and recognition of clinical signs of disease.

With the exception of hemolysis from copper release into blood, which is only described for Bedlington terriers, symptoms of the disease are all nonspecific, resulting from liver dysfunction. The clinical signs may start with a mild decrease in activity or appetite. In most cases, owners will recognize these intermittent signs only with retrospect. Over weeks to months, dogs may vacillate between periods of decreased activity and periods of normal behavior. After months to years, symptoms become more prominent, and may include salivation with intermittent vomiting and nausea. Polyuria and polydipsia, icterus, diarrhea, and ascites may develop in advanced disease (**Box 1**).

Table 1
Normal range of liver copper concentrations in dogs

Range, ppm dw	Reference Range	Dogs	Breed	Method	Reference
120–304	<400	6	Labrador retriever	NAA	28
100–700	197 ± 113	13	Doberman pinschers	NAA	30
91–358	206 ± 56	22	Bedlington terriers	SP	31
94–270	190 ± 56	15	mixed breed dogs	SP	31
60–270	155 ± 66	13	mixed breed dogs	SP	30
38–650	156 ± 119	37	5 mixed breed dogs + 32 pure breed dogs	SP	34

Abbreviations: NAA, neutron activation analysis; ppm, parts per million (ppm equals μg/g, as well as mg/kg); SP, spectroscopy.

Box 1
Clinical signs of copper-associated chronic hepatitis in dogs

Exercise intolerance
Depression
Anorexia
Vomiting
Weight loss
Polyuria/Polydipsia
Icterus
Diarrhea
Ascites
Salivation

Nonspecific clinical signs of copper-associated chronic hepatitis.

Findings on routine serum biochemical analyses include a greater relative increase in ALT (alanine aminotransferase) activity than ALP (alkaline phosphatase), suggesting primary hepatocellular liver disease.

DIAGNOSIS

Histopathologic evaluation of liver tissue is currently the only means of diagnosis of copper-associated hepatitis. Two or more liver biopsies, taken with a large-core needle (14 gauge), are a required minimum to evaluate liver tissue and determine copper toxicosis quantitatively or semi-quantitatively. Liver biopsy samples containing more than 6 to 8 portal triads are considered adequate for histologic diagnosis of human liver disease.[35] From reports comparing different biopsy techniques in dogs, relatively large-sized biopsies of the liver are required for accurate diagnosis (14 gauge, 1.8-mm diameter, 1-cm length).[35–39] To avoid puncture of adjacent organs, such as the gallbladder, stomach or intestine, the patient should be fasted for 12 hours before the procedure. In people with liver disease, significant hemorrhage after biopsy occurs in approximately 0.2% of patients.[16,35]

The typical magnitude and localization of copper within zone 3 within the liver lobule (centrolobular) are characteristics of primary copper storage disease.[28,40,41] Copper accumulates in hepatocytes, and results in hepatocellular inflammation with copper-laden macrophages and chronic hepatitis. The chronic hepatitis is characterized by hepatocellular apoptosis, necrosis, regeneration, and fibrosis, as well as an inflammatory infiltrate, which can be mononuclear or mixed. Fibrosis is part of the histopathologic definition of chronic hepatitis but may appear delayed in the disease process. Cirrhosis results as the end stage of the disease.[42]

COPPER ASSESSMENT

Copper concentrations in liver tissue can be measured quantitatively by irradiation of small biopsies and measurement of the induced Cu radioactivity in small pieces of liver (2 mg of tissue), or by spectrophotometric methods on fresh frozen liver (1 to 2 g of tissue needed). For the latter method, formalin-fixed tissue can be submitted, but measurement of copper concentrations in wet weight liver tissue is not recommended,

especially in marginally elevated copper concentrations, because the reference ranges for copper are established on dry tissue basis. Alternatively, histochemical stains, such as rubeanic acid and rhodanine, are recommended to evaluate liver tissue semiquantitatively for copper. These stains consistently detect copper in liver biopsy specimens when amounts exceed the normal limit of 400 μg/g dw. It has been suggested that rhodanine demonstrates the protein to which copper binds rather than the copper itself.[43]

A histochemical grading system for evaluation of liver tissue stained with rhodanine for semiquantitative evaluation of hepatic copper concentrations in Bedlington terriers was developed by Johnson and colleagues.[44] The same grading system was applied for assessment of semiquantitative copper scores in rubeanic acid (dithio-oxamide)–stained liver tissue of Bedlington terriers, Doberman pinchers, and Labrador retrievers.[28,44–46] In a grading scale of 0 to 5, with 0 having no copper, scores above 2 are considered abnormal in both staining methods (**Fig. 2**).

Further staining methods, which have been applied for detection of copper include Timm's silver stain, cresyl-violet, dithizone, and orcein for copper-associated

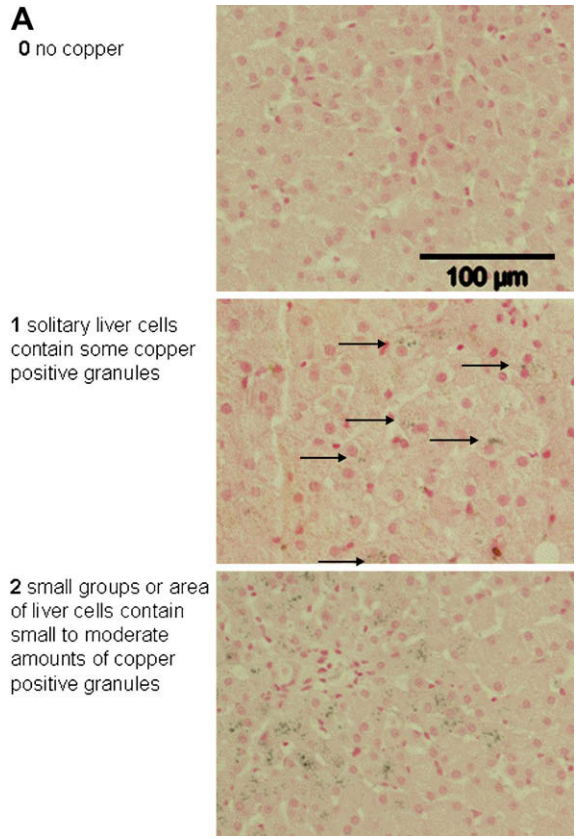

Fig. 2. (*A, B*) A histochemical grading system for evaluation of canine liver tissue stained with rhodanine or rubeanic acid. Copper scores above 2 are considered abnormal. Histology slides of 3-μm thickness of liver tissue from dogs stained with rubeanic acid for copper are shown as example. (*Courtesy of* T.S.G.A.M van den Ingh, TCCI Consultancy BV, Utrecht, The Netherlands.)

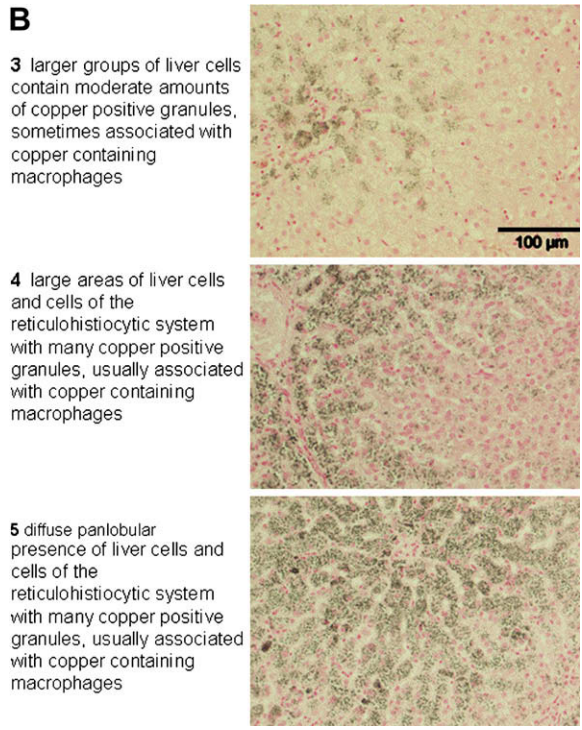

Fig. 2. (*continued*)

protein.[47] These staining methods have not been established for detection of copper in pets, and no grading system is available for veterinary use (**Table 2**).

COPPER ACCUMULATION SECONDARY TO CHOLESTASIS IN DOGS

Copper may accumulate in the liver secondary to cholestatic liver diseases. Because of defective copper excretion in the bile, cholestatic liver diseases often result in copper accumulation in the periportal areas. The accumulation occurs in hepatocytes. The magnitude of copper accumulation from cholestasis is not as high as that found in dogs with inherited copper storage disorders. In a review of 17 liver biopsies from breeds not identified to be affected by inherited copper-associated liver disease, the mean copper concentration was 984 µg/g dry weight liver.[34] Another study revealed that 3+ or higher histochemical detection of copper in the central area of the liver lobule indicates a primary copper storage disease.[42,45] In their study, Spee and colleagues[42] were able to find distinction criteria to determine whether copper accumulation is primary or secondary to hepatitis by comparison of liver biopsies from Bedlington terriers with copper toxicosis with those harvested from non–copper-associated breeds diagnosed with severe chronic hepatitis, and dogs with chronic extrahepatic cholestasis. Copper metabolism was analyzed using histochemical staining and quantitative reverse transcriptase polymerase chain reaction (RT-PCR) by comparison of the gene expressions of *ATOX1*, *COX17*, *ATP7A*, *ATP7B*, *CP*, *MT1A*, *COMMD1*, and *XIAP*. Oxidative stress was measured by determining GSH/GSSG ratios and gene-expression (*SOD1*, *CAT*, *GSHS*, *GPX1*, *CCS*, *p27KIP*, *Bcl-2*).

Table 2
Staining methods for copper in liver tissue

Staining Method	Grading System for Veterinary Use	Copper Color
Rhodanine	Yes	Red to red-yellow
Rubeanic acid (dithiooxamide)	Yes	Deep blue to black
Timms silver stain	No	Black
Orcein	No	Black

BEDLINGTON TERRIER

In 1975, hepatic copper toxicity was first described in Bedlington terriers.[48] It was subsequently shown that affected Bedlington terriers have an inherited autosomal recessive defect of the *MURR1* gene, which was renamed to *COMMD1* (copper metabolism murr1 domain–containing protein 1). The extent of hepatic damage tends to parallel the increasing hepatic copper concentrations, which occur from decreased copper excretion into bile in *COMMD1*-deficient liver cells. The accumulated copper in liver tissue is seen as dense granules in lysosomes and occurs mainly in the centrolobular region of the liver. The histologic changes extend from focal necrosis to chronic hepatitis, which may ultimately lead to cirrhosis. In some cases, acute hepatic necrosis, copper-associated hemolytic anemia, and acute liver failure may occur. Female and male dogs are equally affected.

Copper toxicosis in Bedlington terriers (**Fig. 3**) can clinically be divided into three stages (**Table 3**). In the first stage, hepatic copper concentrations increase from 400 to 1500 ppm dw. Copper accumulation initially occurs in zone 3 of liver lobule (centrolobular hepatocytes). This stage remains clinically silent. A liver biopsy will reveal increased concentrations of copper but the histologic structure of the liver appears normal.

In the second stage, copper concentrations increase further into a range of 1500 to 2000 ppm dw. Histologically, copper accumulation is also found in zones 2 and 1 (midzonal and periportal hepatocytes). A liver biopsy will reveal inflammation with centrilobular mixed cell foci, containing necrotic hepatocytes, lymphocytes, plasma cells, neutrophils, and copper-laden macrophages. In the most advanced stage, dogs become clinically ill. Copper concentrations may exceed 2000 ppm dw and histology reveals hepatitis and cirrhosis. Cholestasis and bile duct proliferation occur along with fibrosis probably because of compression exerted on bile ducts in a distorted fibrotic liver and/or a cytokine-induced proliferation of bile ducts.[31,48–58]

Homozygous affected dogs have the highest copper concentrations. Heterozygous carrier dogs generally have an increase in copper concentrations until the age of 6 to 9 months before concentrations fall back to within the normal range.

The disease can be diagnosed by copper measurement in liver biopsies, as well as with genetic testing. Estimates of the incidence of copper toxicosis in Bedlington terriers varied from 34% to 66% between countries before genetic testing became available. Genetic assays investigate the presence of a particular microsatellite marker, which is in linkage disequilibrium with the COMMD1 mutation, or they detect the deletion of exon 2 of *COMMD1* directly.

DOBERMAN PINSCHER

Copper-associated hepatitis in Dobermans almost exclusively affects female dogs. In young dogs (1 to 3 years), increased serum ALT, centrolobular copper accumulation,

Fig. 3. Bedlington terrier with copper toxicosis. (*Courtesy of* Jan Rothuizen, DVM, PhD, Utrecht, The Netherlands.)

and subclinical hepatitis occur. Clinical evidence of liver disease usually begins around 4 to 7 years of age with chronic hepatitis and cirrhosis. Copper appears to be associated with the disease, because recent studies suggest that copper is often increased before the development of clinical hepatitis. Furthermore, copper excretion studies reveal decreased biliary Cu excretion in affected Doberman pinschers. Moreover, copper chelator (penicillamine) therapy in subclinical dogs normalized copper concentrations with improvement in the grade of histologic damage.[59]

Table 3
Stages of copper toxicosis in Bedlington terriers

Stage	Clinics	Copper	Liver Histology
1	No clinical signs	Copper in zone 3 (centrolobular) from 400–1500 ppm	Normal liver structure
2	No clinical signs	Copper in all zones 1500–2000 ppm	Inflammation
3	Clinical illness	Copper in all zones >2000 ppm dw	Inflammation + cirrhosis

DALMATIAN

In a retrospective study of 10 Dalmatians with copper-associated chronic hepatitis, two of the dogs were related and all presented for gastrointestinal clinical signs.[32] Males were equally affected as females and all dogs had elevated liver enzymes and necro-inflammatory liver changes, as well as centrolobular copper accumulation. In five dogs, hepatic copper concentrations exceeded 2000 μg/d dw liver, with several dogs having copper levels as high as those observed in Bedlington terriers.[32]

WEST HIGHLAND WHITE TERRIER

Affected dogs of this breed were 3 to 7 years of age. Some dogs had elevated hepatic copper concentrations (centrolobular) but no evidence of liver disease, which led to the suspicion that copper was a cause of subsequent chronic hepatitis and cirrhosis. Copper accumulation does not appear to increase with age in the West Highland white terrier, and there is no gender predilection.[34,60] Biliary excretion studies revealed a decreased excretion of radioactive copper in affected dogs.[61]

SKYE TERRIERS

Cholestasis was the suspected etiology of copper-associated chronic hepatitis and cirrhosis in Skye terriers. The 10 described dogs were 1 to 10 years old. Female and male dogs were equally affected, and presented with intermittent signs of anorexia, vomiting, and ascites. At a terminal stage of the disease, the animals developed jaundice and died.[27]

LABRADOR RETRIEVER

Chronic hepatitis is reported to be common in this breed and copper accumulation is associated with about 75%, but not all cases of chronic hepatitis. Females are more commonly affected, and generally are presented at around 7 years of age (range 2 to 10 years). Clinical signs are nonspecific and include anorexia, vomiting, and weight loss. Hepatic copper concentrations generally range from 650 to 3000 μg/g dw (histologically above 2+ with rubeanic acid staining). The histologic localization of copper in the centrolobular region of the liver lobule is an indicator for primary copper accumulation.[23,28,62]

OTHER BREEDS

Publications of other breeds with liver disease (**Table 4**) associated with copper accumulation include reports of an Anatolian shepherd dog, 6 German shepherd dogs, 11 Keeshonden, and a Boxer.

THERAPY

Diet

The goal of medical therapy is to reduce the absorption of copper and to enhance its excretion. Therefore, diets heavily supplemented with copper and copper-containing vitamin/mineral supplements should be avoided. Foods containing large amounts of copper, such as eggs, liver, shellfish, organ meats, beans/legumes, mushrooms, chocolate, nuts, and cereals should be excluded from the diet.

We have investigated the effects of a low-copper diet and zinc gluconate on hepatic copper accumulation in 21 client-owned Labradors that were related to former dogs affected with copper associated chronic hepatitis and that had been diagnosed

with elevated hepatic copper concentrations. We found that feeding of low copper diets to Labradors is effective in reducing hepatic copper concentrations. Hepatic copper concentrations were assessed before and following an average of 8 months and 16 months of treatment. During this time, all dogs were fed exclusively on a low copper diet (hepatic, Royal Canin). In addition, the dogs were assigned to one of two groups in a randomized double-blind manner to receive a supplement of zinc gluconate or a placebo. Hepatic copper concentrations decreased significantly in both groups at control examinations.

Chelation

Chelating agents are commonly used to enhance urinary copper excretion. Chelators compete with binding sites for metals and produce a water-soluble complex with copper, which is then excreted into urine or bile. The standard chelating agent for the treatment of copper storage disorders in people and dogs is penicillamine. Another accepted treatment in people is the use of zinc for induction of intestinal metallothionein for chelation of copper and prevention of intestinal uptake of the metal.[55,63–66]

PENICILLAMINE

Recommended dosage: *10 to 15 mg/kg twice a day orally*

Penicillamine can chelate copper and other metals. The drug leads to mobilization of copper from tissues and promotes copper excretion in urine. Penicillamine also may increase the synthesis of metallothionein, and has anti-inflammatory, immunosuppressive, and antifibrotic effects.[59,67–72] Lifelong therapy might be required. The drug is effective for the treatment of chronic hepatitis owing to copper accumulation. Adverse effects occur in about 20% of dogs as inappetence, vomiting, and diarrhea. These adverse effects can generally be adverted by mixing the drug with food, and dividing the daily dosage into frequent applications. Side effects reported in people include vitamin-B deficiency from increased urinary loss of pyridoxine, fever, cutaneous eruptions, lupuslike symptoms, lymphadenopathy, cytopenias, and proteinuria. Penicillamine is potentially teratogenic and its use during pregnancy is not recommended. Pet owners should be informed about the potential risks of handling the drug for pregnant women.

Clinical improvement from penicillamine treatment might take weeks to months, and large interindividual variations are observed with respect to the effectiveness of the drug in people, as well as in dogs. Follow-up liver biopsies are generally required to determine if a patient will need long-term therapy. One author described an average detoxification rate of around 900 ppm copper decrease per year during penicillamine treatment in Bedlington terriers.[55,66]

Penicillamine was effective for treatment of Doberman pinschers with copper-associated subclinical hepatitis.[59] We have tested copper chelation therapy with penicillamine (10 to 15 mg/kg twice daily orally for 3 to 6 months) in Labrador retrievers in a randomized, double blind, placebo-controlled study and found the drug to be effective for the treatment of hepatic copper accumulation in this breed.

ZINC

Recommended dosage: *200 mg of elemental zinc daily per dog (in divided doses) or 7.5 mg elemental zinc/kg twice a day orally*.

Oral zinc is given to reduce copper absorption from the diet. Zinc induces the production of metallothionein in intestinal mucosal cells. Metallothionein is

Table 4
Literature review of copper-associated hepatitis in different dog breeds

Breed	No. Dogs	Age	Gender	Signs	Liver Enzymes	Copper (ppm dw)	Copper Location	Histology	Therapy and Outcome	Reference
Bedlington terrier	21	8mo–14y	female = male	Partial anorexia, depression, weight loss, vomiting	ALT + ALP elevation	Assessed in wet weight	No assessment in intact lobuli	Chronic hepatitis, cirrhosis, acute hepatocytic necrosis, liver failure	Not assessed	Hardy et al.[48]
	149	1mo–17y	female = male	No signs, family of high copper dog	N/A	N/A	Begin centrolobular, later all zones	Hepatitis	N/A	Thornburg et al.[34]
	68	6mo–15y	female = male	19 dogs: 3 clinical syndromes: 1. acute (6y): anorexia, vomiting, weakness, 2. chronic: (5–12y) 13 dogs: anorexia, weight loss, intermittent vomiting, diarrhea, unthriftiness, 3. Hemolytic/jaundice	ALT increased	850–10,600	Begin centrolobular (stage 1) later all zones	Focal hepatitis – cytologic	d-penicillamine = > improvement	Twedt et al.[31]
	24	1–14y	female = male	No signs	N/A	Numbers not given	N/A	Study compared cytologic versus histologic staining results	N/A	Taske et al.[54]
	18	1.7–11y	female = male	No signs, anorexia, vomiting, weight loss, hemolytic crisis	ALT > AST elevation	2638 (1443–3373)	Periacinar	Necrosis, inflammation, fibrosis, extramedullary hematopoiesis	Preventative feeding of low-copper diet	Hyun et al.[52]

	5	3–10y	female = male	No signs, 1 dog hemolysis	ALT increased	3000–11,000		Necrosis, chronic hepatitis, cirrhosis	2,3,2-tetramine = > effective chelating drug	Twedt et al.[55]
	4	N/A	N/A	N/A	N/A	>471	N/A	N/A	N/A	Hoff et al.[51]
	2	3 + 5y	female = male	anorexia, weight loss > vomiting, PU/PD	ALT × 10, AST × 10	1027 + 10,728	N/A	Chronic hepatitis/ cirrhosis	Penicillamine = > died	Kelly et al.[100]
Doberman pinscher	30	N/A	female ≫ male	no signs, routine blood screen, ascites, weight loss, jaundice	N/A	650–4700	centrolobular	Chronic hepatitis in zone 3	N/A	Thornburg.[101]
	26	1.5–10y	female ≫ male	Anorexia, weight loss, PU/PD, icterus, ascites, bleeding, seizures vomiting	ALP × 10, ALT × 11, high billirubin	509 (88–722)	N/A	Chronic hepatitis	Prednisolone = > moderate – poor response	Crawford et al.[102]
	22	3y	female ≫ male	No signs	ALT > ALP elevation bile acids elevated	419 ± 414	Centrolobular	Hepatitis	N/A	Mandigers et al.[29]
	20	1mo–17y	N/A	no signs, family of high copper dog		140–1500	Begin centro-lobular	Hepatitis	N/A	Thromburg et al.[34]
	18	2.5–7y	female ≫ male	no signs	ALT elevated in 2 dogs	Histology: elevated	Multifocal & portal	Inflammation, necrosis, fibrosis	N/A	Speeti et al.[103]
	11	2.5–11y	female ≫ male	PU/PD, weight loss, decreased activity, poor appetite, vomiting, diarrhea	ALT + ALP > billirubin elevated	404–1700	Centrolobular	Degeneration, inflammation, necrosis, fibrosis, cirrhosis	Diuretics, antibiotics, penicillamine = > 6 dogs died within 9 months	Johnson et al.[104]

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Table 4
(continued)

Breed	No. Dogs	Age	Gender	Signs	Liver Enzymes	Copper (ppm dw)	Copper Location	Histology	Therapy and Outcome	Reference
	8	2–8y	female	Anorexia, weight loss, apathy, exercise intolerance, vomiting, PD	ALT × 20, AST × 7, ALP × 4.5	Histology: 3 +	Periphery of hyperplastic nodules	Cirrhosis/cholestasis	N/A	van den Ingh et al.[46]
	5	6–8y	female	No signs	ALT × 5, ALP × 2–3	1036 (630–1330)	Centrolobular	Subclinical hepatitis	200 mg d-penicillamine PO q12 h for 4 months = > improvement	Mandigers et al.[59]
	3	N/A	N/A	N/A	N/A	>471	N/A	N/A	N/A	Hoff et al.[51]
	2	3 + 4y	female	N/A	N/A	600 + 804	Juxtaseptal hepatocytes of pseudolobule	Cirrhosis	N/A	Thornburg et al.[105]
	2	3y (f) + 6y (m)	male = female	Partial anorexia, weight loss, vomiting	ALT × 10–20, ALP normal	1465 + 2500	Centrolobular and in macrophages	Focal hepatitis	Died	Thornburg et al.[106]
Dalmatian	10	2–10y	male = female	Inappetence, vomiting	ALT × 6 (2–12x), AST x 7 (2–22x), ALP × 2,7 (07–10x)	3197 (754–8390)	Centrilobular	Necrosis, fibrosis, inflammation	Penicillamine, trientine, zinc = > died/ euthanized	Webb et al.[32]
	1	2y	female	Vomiting, PU/PD, diarrhea, seizures	AST, ALT, ALP elevated	1916	N/A	Hepatic necrosis/ cirrhosis	Antibiotics, fluid, lactulose, penicillamine = > died	Napier[107]

	1	1.5y	male	Vomiting, anorexia, weight loss, lethargy	ALT × 10 + AST × 4, ALP × 1.3	2356 up/g wet weight	Centrolobular	Hepatocellular necrosis & inflammation	Manifold = > died	Noaker et al.[108]
	1	2y	female	Lethargy vomiting, paleness, icterus	ALT × 25, ALP ×3, bili × 15	7940	Centrolobular – midzonal	Hepatocellular necrosis, inflammation & fibrosis	N/A	Cooper et al.[109]
Skye terrier	9	18 mo–15y	male = female	Intermittent anorexia, vomiting, ascites ≫ terminal jaundice	—	358–2257	Centrolobular	Cirrhosis, chronic hepatitis	N/A	Haywood et al. [27]
	1	1y	female	Anorexia, vomiting, melaena, seizures, aggression	Bile acids × 36 fasted, bili × 15 alb (-30%), glop-6% ALP × 1.5, target cells	462	N/A	Micronodular cirrhosis, uneven distribution of inflammation	Antibiotics, lactulose, ursodeoxycholic acid, colchicine, zinc, Waltham hepatic support diet for 12 months, symptom free 2 years post diagnosis	McGrotty et al.[110]
West Highland white terrier	44	3–7y	female> male	N/A	N/A	Normal–3500	24 dogs related	29 dogs: high Cu + normal histology, 15× high copper and hepatitis or cirrhosis	N/A	Thornburg et al.[111]
	395	1mo–17y	female = male	No signs	N/A	20–6800	Begin centrolobular, later all zones	Hepatitis	N/A	Thornburg et al.[34]
	7	N/A	N/A	N/A	N/A	>1100	Copper excretion study	N/A	N/A	Brewer et al.[112]
	2	N/A	N/A	N/A	N/A	>471	N/A	N/A	N/A	Hoff et al.[51]
	2	—	—	—	—	—				Thornburg et al.[113]
Labrador retriever	23	7y (2–10)	female> male	anorexia> vomiting	ALT × 10, ALP × 4.5	1317 (402–2576)	Centrolobular	Chronic hepatitis, cirrhosis	Penicillamine and prednisolone = > improvement	Hoffmann et al.[28]

(*continued on next page*)

Table 4
(*continued*)

Breed	No. Dogs	Age	Gender	Signs	Liver Enzymes	Copper (ppm dw)	Copper Location	Histology	Therapy and Outcome	Reference
	17	9.3y (3.9–14y)	female = male	Decreased appetite, vomiting, lethargy, weight loss diarrhea, PU/PD	mean ALT > ALP	N/A	8 dogs: all 3 zones, 3 dogs: centrolobular, 5 dogs: portal	Inflammation, degeneration (hydropic and necrosis), fibrosis	Ursodeoxycholic acid, prednisone, antibiotics, azathioprin, SAMe	Shih et al.[114]
	1	N/A	N/A	N/A	N/A	>471	N/A	N/A	N/A	Hoff et al. [51]
Other Breeds and Cats:										
German shepherd	3	1.5–3y	male = female	Ascites, icterus	ALP 4× elevated (1–6×), ALT 4× elevated (2–12×)	1441-2921	N/A	Macronodular cirrhosis and high Cu	N/A	Zentek et al.[10]
	3	4mo, 8 + 9y	male = female	N/A	N/A	570, 1352, 2202	Juxtaseptal hepatocytes of pseudolobule	Cirrhosis	N/A	Thornburg et al.[115]
Anatolian shepherd	1	7y	male	Intermittent inappetence, weight loss, decreased endurance, vomiting	ALT × 3, ALP × 1.5	4+	Centrolobular > all zones	Chronic hepatitis	Penicillamine + prednisolone, improvement	Bosje et al.[116]
Keeshond	11	1mo–17y	female = male	No signs, family or high copper dog	N/A	90–2400	Begin centrolobular, later all zones	Hepatitis	N/A	Thornburg et al.[34]
Boxer	1	6y	female	PU/PD	ALT and ALP increased	1101	Centrolobular (zone 3 + 2)	Pigment granulomas, normal architecture	N/A	van den Ingh et al.[117]
European Shorthair cat	1	2y	male	Inappetence, vomiting, fever	—	4170	Centrolobular	Cirrhosis, chronic hepatitis	N/A	Meertens et al.[118]
Siamese cat	1	2y	female	Anorexia, depression	ALT × 15, AST × 6	4074	Centrolobular	Hepatocellular necrosis & inflammation	Died	Heynes et al.[119]

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; dw, dry weight liver; N/A, not assessed; PU/PD, polyuria/polydipsia.

a cysteine-rich protein, which acts as an endogenous chelator of metals with high affinity for copper. Metallothionein binds copper from the diet, preventing its transport into the circulation. Most of the bound copper is lost in the feces when intestinal cells are shed from the villi. Zinc might also induce hepatic metallothionein for nontoxic storage of copper. Because the rate of removal of hepatic copper is relatively slow, dogs with severe or fulminant copper-induced hepatitis should not be treated with zinc alone. Theoretically, zinc given orally together with penicillamine may decrease the effectiveness of both drugs.

The type of zinc salt used does not influence efficacy of the drug in people, but may affect tolerability. Acetate and gluconate salts may be more tolerable than sulfate. Theoretically, zinc should be given apart from feeding, because some food constituents (such as phytates) can bind zinc and diminish its efficacy. However, the salts might be an irritant to the gastric mucosa and lead to nausea and vomiting; therefore, mixing of the drug with small amounts of food has been recommended. The plasma zinc concentration of dogs normally ranges from about 90 to 120 μg/dL. As plasma zinc concentration increases above 200 μg/dL, copper uptake may be suppressed. Zinc is a relatively safe drug, but large doses may cause gastrointestinal disturbances. At plasma zinc concentrations above 1000 μg/dL, hemolysis may occur. In a study of three Bedlington terriers and three West Highland white terriers with copper toxicosis, 200 mg of elemental zinc was given daily to each dog to achieve therapeutic plasma concentrations of zinc above 200 μg/dL. The effectiveness of zinc in the prevention of copper uptake from the intestine was assessed by measurement of peak plasma concentrations of radioactive copper after oral application. A minimum of 3 months of zinc treatment was necessary before copper uptake from the intestine was blocked.[61] Although zinc is currently reserved for maintenance treatment, is has been used as first-line therapy in people, most commonly for asymptomatic or presymptomatic patients. For this indication, the drug appears to be equally effective to penicillamine and is much better tolerated.[61,63–65]

TRIENTINE (2-2-2-TETRAMINE TETRAHYDROCHLORIDE)

Recommended dosage: *10 to 15 mg/kg every 12 hours*[28]

Trientine is a chelator, which enhances the urinary excretion of copper. Trientine is poorly absorbed from the gastrointestinal tract. The drug is described for treatment of Wilson's disease in people, where it is used in patients who are intolerant to penicillamine. Symptoms of toxicity in people include bone marrow suppression, proteinuria, and autoimmune disorders, such as systemic lupus erythematosus. In addition trientine has teratogenic effects.[55,61,64–66]

Another tetramine salt, 2,3,2-tetramine (= tetramine) was studied in five Bedlington terriers with copper toxicosis. The drug was very potent and patients remained without adverse effects. Hepatic copper concentrations decreased more than 50% during treatment with tetramine for 6 months, and histologic changes were improved (150 mg trientine salt in capsules twice a day orally per dog, 10 kg average weight, range 6.8 to 13.6 kg). The authors of the study recommended serial copper assessment during long-term treatment with the drug to avoid copper depletion of liver tissue and blood.[55]

TETRATHIOMOLYBDATE

Ammonium tetrathiomolybdate forms a tripartite complex with copper, which is stable. Given with food, tetrathiomolybdate can form complexes between copper and food

proteins, and therefore prevents the absorption of copper. When given between meals, tetrathiomolybdate forms complexes with available serum copper (free copper) and albumin, rendering cellular uptake of copper ineffective. The drug is described for intravenous use in sheep with copper toxicosis, as well as a possible emergency approach in patients with acute hemolytic crisis from hepatic copper release. No studies have been performed in dogs. Tetrathiomolybdate is toxic, and copper deficiency can occur with use of this drug, which can lead to anemia because of copper depletion of bone marrow. Tetrathiomolybdate is not commercially available.[15,22,63,64]

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