Copper Physiology
Copper is an important co-factor for many redox enzymes. It has important roles in several bodily functions. Copper’s natural flow through the body is as follows: copper is absorbed within the GIT → enters portal circulation → hepatic uptake → re-circulation via the peripheral bloodstream → distribution to other organs → excretion via the biliary or urinary system.

In order to better understand copper physiology, there are 3 locations that must be described in more detail: the GIT, liver, and bloodstream. 1) The majority of copper (40-60%) is absorbed within the small intestines. Copper is taken up by enterocytes via the di-cation metal transporter (DMT1) and cation transporter (CTR1) receptors. 2) Once the copper reaches the portal circulation, it is taken up by hepatocytes via the same receptors (DMT1 and CTR1). Within the cytosol, copper is either free or bound to metallothionein (MT). Prior to exiting the hepatocyte, the copper must travel to the Golgi apparatus. From there, it either binds to ceruloplasmin and enters the peripheral bloodstream or binds to ATP7B and MURR1 (also known as COMMD1) and enters the biliary system. 3) Within the bloodstream, copper is bound to carrier proteins which distribute it to other organs. In dogs, copper is bound to albumin (5%), ceruloplasmin (45%), and transcuprein (50%). Copper bound to albumin and transcuprein is freely dissociable and comprises the biologically active pool of copper. Copper bound to ceruloplasmin is not freely dissociable.

Copper can accumulate within the body secondary to physiologic or pathologic causes. Physiologic causes include excessive dietary copper and cholestasis (intrahepatic or extrahepatic). Pathologic causes include defects in copper metabolism which result in decreased copper excretion. When copper accumulation is excessive, it results in oxidative damage and apoptosis of cells. In dogs, this primarily manifests as hepatitis since the liver is the main storage organ. Intracellular copper that is bound to MT is not considered harmful to the cells; whereas, intracellular copper that is not bound to MT is considered harmful to the cells.

Canine Copper-Associated Hepatitis
Pathophysiology
In Bedlington Terriers, a deletion of the exon 2 in the MURR1 (COMMD1) gene exists. This deletion prevents those affected from being able to properly excrete copper via the biliary system. Mutations in other breeds have not been identified thus far.
Signalment/Clinical Signs
Reported breeds include Bedlington terriers, west Highland white terriers, Labrador retrievers, Doberman pinschers, skye terriers, and dalmatians. Clinical signs are not usually apparent until dogs are middle-aged since it takes years for the copper to accumulate. Clinical signs are mostly associated with hepatitis. These can include icterus, polyuria, polydipsia, vomiting, anorexia, lethargy, diarrhea, and ascites. Hemolysis is uncommon and has only been reported in Bedlington terriers.

Diagnosis
Clinicopathologic abnormalities include increased ALT, increased ALP, hyperbilirubinemia, bilirubinuria, hypercholesterolemia, leukocytosis, mild anemia, and hypoalbuminemia. On ultrasound, the liver echogenicity may appear hyperechoic to heterogeneous. Hepatic lymph nodes may be enlarged. Hepatic fine needle aspirates are not diagnostic for copper-associated hepatitis (CAH). Instead, hepatic biopsies are necessary for diagnosis. Biopsies can be obtained via ultrasound-guided percutaneous Tru-Cut (14 gauge), laparoscopy, or laparotomy. When submitting samples, at least 2 biopsies are recommended for histopathology and at least 1 biopsy is recommended for mineral analysis (to quantify the amount of copper). For mineral analysis, the tissue must be submitted within a royal blue top vacutainer; it is the only vacutainer that will not chelate the minerals within the sample. If possible, liver biopsies and bile should also be submitted for aerobic and anaerobic cultures.

Histopathology may reveal fibrosis, copper-laden macrophages, and inflammation. Rhodanine or rubeanic acid stains can be applied to the samples to highlight cells containing copper. Location of copper accumulation is essential to determining if the copper accumulation is primary (CAH) or secondary (cholestasis). Centrilobular accumulation is consistent with CAH, and periportal accumulation is consistent with cholestasis. Once CAH is diagnosed, quantification of copper within the tissues is important for guiding therapy.

Treatment
If the underlying cause is CAH, chelation or maintenance therapy is warranted. If the underlying cause is cholestasis, treatment of the underlying cause should resolve the copper accumulation. Therapy for CAH includes anti-inflammatory, chelation, and maintenance therapy.

Anti-inflammatory therapy
Use and duration of anti-inflammatory medications is dictated by the degree of inflammation present on histopathology. Prednisolone (1-2 mg/kg/day) is the drug of choice. Therapy is generally started close to 1 mg/kg/day and adjusted according to ALT concentrations (goal of <400 U/L) on rechecks. Once inflammation is controlled, the dose can be tapered until it is discontinued. CAH does not usually require long-term anti-inflammatory medications.

Chelation therapy
Chelation therapy is generally used when the amount of copper is >1000 ug/g. The goal is to quickly remove copper from the liver. Ideally, a follow-up liver biopsy and mineral analysis (goal of <400 ug/g) should be performed at 3 months to guide duration of therapy. If owners will not allow follow-up biopsies, chelators should be administered for ≥3 months, but the
duration is dependent upon the initial concentration of copper. Once chelation is complete, maintenance therapy should be started. Chelators include D-penicillamine or trientine.

D-penicillamine (aka Depen and Cuprimine) is considered the treatment of choice (mainly by default). D-penicillamine works by making copper more water soluble and increasing urinary excretion of it. It also increases the synthesis of MT; this decreases cellular damage by decreasing the amount of free intracellular copper. It can be scripted out from a human pharmacy. The target dose is 10-15 mg/kg PO Q12 hours. Common side effects include nausea, vomiting, and anorexia. Rare side effects include fever, lymphadenopathy, skin hypersensitivity reactions, and immune-complex glomerulonephropathy. With long-term use, deficiencies of zinc, iron, and calcium have also been reported. Unfortunately, therapeutic efficacy is variable among dogs, and D-penicillamine cannot directly remove intracellular or MT-bound copper. Currently, this drug is expensive and costs ~$1000/month for a Labrador retriever (35 kg). The nausea, vomiting, and anorexia can be significant enough for owners to discontinue therapy. D-penicillamine MUST be given on an empty stomach and without food. Although some resources state that it can be given with food (to reduce GI side effects), a pharmacokinetic study performed by Langlois DK, et. al. (2013) demonstrates otherwise. They administered D-penicillamine to fasted and fed dogs and then measured serum drug concentrations afterwards. The mean maximum concentration was significantly higher in the fasted dogs (8.7 ± 3.1 ug/mL) versus the fed dogs (1.7 ± 1.6 ug/mL). The mean area under the curve (relative bioavailability) was significantly higher in the fasted dogs (17.6 ± 6.0 ug/mL.hr) versus the fed dogs (5.5 ± 3.5 ug/mL.hr). To increase success of therapy, it is recommended to administer the drug on an empty stomach and without food. If the GI side effects are significant, therapy can be started at 25% of the target dose and increased by 25% each week until the target dose is reached. Anti-emetics can also be used.

Trientine (aka Syprine) is not commonly used in dogs. Its mechanism of action is similar to that of D-penicillamine. It can be scripted out from a human pharmacy. The target dose is 10-15 mg/kg PO Q12 hours. Side effects include vomiting, nausea, anorexia, acute kidney injury, proteinuria, bone marrow suppression, and autoimmune disorders. Unfortunately, therapeutic efficacy is variable among dogs, and trientine cannot directly remove intracellular or MT-bound copper. Currently, this drug is prohibitively expensive and costs ~$20,000/month for a Labrador retriever (35 kg). The nausea, vomiting, and anorexia can be significant enough for owners to discontinue therapy. Trientine must also be given on an empty stomach and without food.

**Maintenance Therapy**

Maintenance therapy is generally used when the amount of copper is <1000 ug/g or after chelation therapy. The goal is to prevent accumulation of copper within the liver. This is continued for life. Maintenance therapy includes zinc salts and a copper-restricted diet.

Zinc salts compete with copper for binding to DMT1 and CTR1 receptors on the enterocytes thus decreasing intestinal copper absorption. It also induces the synthesis of MT, similar to D-penicillamine. This can be purchased from human pharmacies or health food stores. The starting dose is 10-15 mg/kg (based upon elemental zinc) PO Q12 hours. Zinc gluconate is the best zinc salt to use. Side effects include vomiting, nausea, anorexia, and hemolysis (secondary
to overdose). It must be administered on an empty stomach. It will bind to phytates in food which reduces intestinal absorption; therefore, it is only safe to administer this drug with tuna juice or pure meat. To monitor therapy, serum zinc concentrations should be measured monthly. It is important to draw the sample 4-6 hours after the morning dose. The dose should be adjusted to maintain a serum zinc concentration around 20 ug/mL.

Feeding a copper-restricted diet is also important. The best commercial foods include Science Diet l/d and Royal Canin Hepatic. For treats, avoid eggs, shellfish, organ meats, beans, nuts, legumes, mushrooms, and cereals.

**Prognosis**

The prognosis generally depends on how much damage the liver has sustained by the time of diagnosis. However, the prognosis is generally good if owners can afford therapy.

**Future Directions**

Given the limitations associated with D-penicillamine and trientine, additional therapies need to be investigated. Ammonium tetrathiomolybdate (TTM) is currently being used for humans with Wilson’s disease (copper storage disease), especially those with neurologic manifestations. Its mechanism of action is complex and not fully understood. Basically, TTM is able to chelate copper from 3 main locations (GIT, bloodstream, and liver) and increase biliary excretion of copper. Side effects include vomiting, nausea, anorexia, and transiently increased ALT, AST, cholesterol, and triglycerides. When copper deficiency is induced, anemia, leukopenia, and thickened epiphyseal growth plates can occur.

TTM has several advantages. It is a specific chelator that does not remove iron, cadmium, or zinc from the body. It can be administered IV, PO, and SQ. The bulk chemical is currently inexpensive and would cost <$100 per month for a Labrador Retriever (35 kg). When given with food, TTM will prevent dietary copper from being absorbed. When given without food, TTM will be absorbed and will chelate copper from the bloodstream and liver. Thus it can be used as both chelation and maintenance therapy. It is the only chelator that can directly removes intracellular copper from MT.

TTM does have limitations though. It is unstable in aerobic environments and must be stored in airtight containers. It is not commercially available and must be compounded into a clinically useful form. Nausea, vomiting, and anorexia can be significant enough for owners to discontinue therapy. No pharmacokinetic data has been published on TTM to date; however, the author and colleagues have recently completed a pharmacokinetic study. Our manuscript is currently under review. No efficacy studies in dogs have been published to date, which means that a therapeutic dose has not been established yet.


