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Authors
Mazi, Tagreed A
Shibata, Noreene M
Medici, Valentina

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Lipid and energy metabolism in Wilson disease⋆

Tagreed A. Mazi[a,b], **Noreene M. Shibata**[c], **Valentina Medici**[c,*]

[a]Department of Nutrition, University of California Davis, Davis, CA, USA

[b]Department of Community Health Sciences, College of Applied Medical Sciences, King Saud University, Riyadh, Saudi Arabia

[c]Department of Internal Medicine, Division of Gastroenterology and Hepatology, University of California Davis, Sacramento, CA, USA

Abstract

Copper accumulation and deficiency are reciprocally connected to lipid metabolism. In Wilson disease (WD), which is caused by a genetic loss of function of the copper-transporting P-type ATPase beta, copper accumulates mainly in the liver and lipid metabolism is dysregulated. The underlying mechanisms linking copper and lipid metabolism in WD are not clear. Copper may impair metabolic machinery by direct binding to protein and lipid structures or by generating reactive oxygen species with consequent damage to cellular organelles vital to energy metabolism. In the liver, copper overload results in mitochondrial impairment, down-regulation of lipid metabolism, and the development of steatosis with an etiology not fully elucidated. Little is known regarding the effect of copper overload on extrahepatic energy homeostasis. This review aims to discuss alterations in hepatic energy metabolism associated with WD, highlights potential mechanisms involved in the development of hepatic and systemic dysregulation of lipid metabolism, and reviews current knowledge on the effects of copper overload on extrahepatic energy metabolism.

Keywords

Copper; Copper-transporting P-type ATPase B; (ATP7B); Metabolism; Steatosis; Lipid; Carbohydrate

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*Corresponding author. vmedici@ucdavis.edu (V. Medici).

1. Introduction

Current evidence indicates a reciprocal connection between copper and lipid metabolism. In both animals and humans, the dietary and genetic modulation of copper levels affect cellular and systemic lipid homeostasis.[1–7] Furthermore, many chronic diseases featuring dysregulated lipid metabolism are associated with alterations in copper levels.[8–10] In Wilson disease (WD), a genetic copper overload condition, lipid metabolism is dysregulated and hepatic steatosis is a common early presentation. Despite the evidence, the underlying mechanism connecting copper and lipid metabolism in WD is not clear and the etiology of hepatic steatosis is not understood. In addition, the effects of copper overload on extrahepatic energy metabolism are understudied. Recently, dietary lipid composition was shown to play a role in WD liver injury,[11] further highlighting a potential interaction between copper and lipids in the progression of WD.

This review aims to discuss copper homeostasis and alterations in hepatic energy metabolism in WD. It examines the interplay between copper metabolism and hepatic steatosis. It also highlights potential mechanisms underlying the observed dysregulation in hepatic and systemic lipid metabolism. Last, it reviews the current state of knowledge regarding the effects of copper overload on extrahepatic lipid metabolism.

2. Copper metabolic dysregulation, a common feature of metabolic disorders

Copper homeostasis is altered in many chronic conditions featuring dysregulation in lipid metabolism, including metabolic syndrome, obesity, and non-alcoholic fatty liver disease (NAFLD).[8–10] In NAFLD, both adults and children present with relative hepatic copper deficiency. Aigner *et al.*[12] showed patients with NAFLD have 50% less hepatic copper content compared to healthy subjects or patients with other liver diseases. In the same study, there was an inverse correlation between hepatic copper content and severity of steatosis, fasting glucose levels, insulin resistance, and the diagnosis of diabetes and metabolic syndrome.[12] Similarly, pediatric patients with NAFLD presented with lower serum ceruloplasmin and copper levels in association with severe fatty liver activity scores.[13] In liver biopsies from children with non-alcoholic steatohepatitis (NASH), there was evidence of lower copper levels in association with severe steatosis.[14] Interestingly, obese patients with no or minimal hepatic steatosis presented with higher hepatic copper content compared to lean subjects and a positive correlation was reported between serum copper and body mass index, leptin, and insulin.[15]

NAFLD patients with lower serum and hepatic copper concentrations also presented higher serum ferritin levels and increased hepatic iron concentrations.[16] Transcript levels of ferroportin, a transporter responsible for hepatic iron export, were reduced in NAFLD patients and associated with reduced hepatic copper levels.[16]

Extensive studies in animal models from the McClain group[17] indicated features of dietary-induced NASH are exacerbated by concurrent dietary copper deficiency. When receiving high amounts of fructose, a major Western diet component involved in the development of

NAFLD and NASH, diet-induced copper-deficient rats presented marked hepatocyte apoptosis, hepatic steatosis, and elevated plasma ferritin and hepatic iron when compared to rats receiving the same diet but with adequate copper status.[17] In addition, alterations in dietary copper and fructose content were associated with changes in gut microbiome and fecal metabolome,[18,19] which could act as possible mediators of liver injury.

Epidemiological data indicate the Western diet is relatively copper deficient.[20,21] Though it is difficult to make a direct association between population studies, copper intake, and NAFLD incidence, NAFLD is coincidentally becoming an epidemic, a major cause of morbidity, and a frequent indicator for liver transplant.[22,23]

The mechanisms connecting lower copper levels with severe NAFLD histological features are largely unknown and multiple factors are potentially involved. Hepatic lipid metabolism is regulated by multi-level interactions between nutrients, hormones, nuclear receptors, transcription factors, and post-translational modifications.[24,25] Hepatocellular lipid accumulation is a reflection of imbalanced hepatic uptake of circulating free fatty acids (FFA), *de novo* lipogenesis (DNL), fatty acid β-oxidation, and triglyceride (TAG) export as very low-density lipoproteins (VLDL).[24]

3. Copper metabolism and copper homeostasis

Copper is an essential micronutrient and the third most abundant transition metal in humans. [26] It is estimated close to 1% of the total eukaryotic proteome contains recognized copper-binding proteins.[27] The capacity of copper to shift between oxidation states, copper (I) and (II), enables it to function as a cofactor for enzymes involved in numerous vital biological functions, including cytochrome *c* oxidase (COX), or complex IV of the electron transport chain, which is central in mitochondrial energy generation[28]; copper-zinc superoxide dismutase 1 (SOD1), which is involved in antioxidant defense[29]; tyrosinase, which is involved in pigmentation[30]; and peptidylglycyl α-amidating monooxygenase, which is part of neuropeptide processing machinery.[31] In addition, copper is involved in iron transport and is a mediator in various cellular signaling processes.[32–36]

Under a normal physiological state, copper rarely exists in a free and unbound form,[37] and copper homeostasis is tightly controlled by regulating gastrointestinal uptake, transport, targeted tissue and intracellular shuttling, and hepatic biliary excretion.[38] Free copper is detrimental to many cellular sub-structures by favoring the production of reactive oxygen species (ROS) which, ultimately, damage cellular proteins, lipids, and nucleic acids.[26]

Following dietary acquisition and intestinal absorption, copper is loosely bound to albumin. It is then delivered to the liver where about 75% of circulating copper is cleared and the remaining is distributed to other organs.[39] In the hepatocyte, copper is incorporated into ceruloplasmin and exported to systemic circulation, whereas surplus copper is eliminated with bile secretion.

At the cellular level, hepatic copper uptake is mediated by copper transporter 1 (CTR1), which is responsible for copper trafficking across the plasma membrane.[40] CTR1 is also thought to be involved in intestinal copper absorption as it is highly expressed on the apical

membrane located on the luminal surface of enterocytes.[41] With normal copper status, CTR1 functions by creating a channel which allows copper uptake into the cell, whereas with elevated copper, it is internalized and distributed in intracellular vesicles.[42]

In the cytosol, copper can be either stored in a complex with metallothioneins or distributed to various intracellular targets via a system of copper "chaperone" proteins.[43] In particular, the copper chaperone for superoxide dismutase (CCS) mediates the delivery of copper to SOD1, located in the cytosol and mitochondrial intermembrane space; chaperones COX11, COX17, and COX19 deliver copper to COX in the mitochondria.[43–45] Therefore, the mitochondria play an important role in intracellular copper metabolism as these organelles have a high content of COX and SOD1, both with high copper-binding affinity. COX is the terminal electron-accepting complex of OXPHOS and it requires multiple copper-binding assembly factors to have copper delivered, with mitochondrial phosphate carrier SLC25A3 playing a recently discovered central role in these mechanisms.[46]

The copper chaperone antioxidant protein 1 delivers copper for biliary excretion via activity of the copper-transporting P-type ATPases (ATP7A and ATP7B) in the trans-Golgi network. [43–45,47,48] The homeostatic mechanism of biliary copper secretion is activated in response to excess copper and includes the delivery of copper to vesicles which, by merging with the hepatocyte's canalicular membrane, allow copper excretion into the biliary tract. Beside copper excretion, ATP7B is also responsible for the supply of copper to ceruloplasmin and the maturation of apo-ceruloplasmin into holo-ceruloplasmin.[49] The ATP7A copper transporter is universally expressed, whereas ATP7B is predominantly expressed in the liver. However, ATP7B is also expressed in the intestinal epithelial cells and regulates copper vesicular storage, with potential implications for intestinal lipid metabolism.[50]

4. Wilson disease and associated metabolic alterations

4.1. Definition, clinical manifestations, and treatment

WD is an autosomal recessive genetic condition due to a mutation in the *ATP7B* gene.[51] The disease-causing mutations affecting *ATP7B* are responsible for varying degrees of impairment of copper transporter functions with consequent copper accumulation mainly in the liver, brain, and cornea. Traditionally, patients with WD are classified according to their prevalent phenotype as hepatic and/or neurologic (or psychiatric). However, WD is a systemic disease and multiple organs are thought to be involved simultaneously; clinical manifestations are often diversely combined and can present with variable severity. In addition, organ involvement is often not correlated with the amount of accumulated copper, *e.g.*, severe neurologic involvement is not always associated with advanced liver disease, making the prediction of disease progression and outcome very challenging.[52,53]

The most common neurologic presentations are tremors, parkinsonism, and dystonia. Patients can also present with gait abnormalities, ataxia, choreoathetosis, dysarthria, and dysphagia. Virtually all patients with neurologic symptoms have corneal copper depositions, or Kayser-Fleischer rings.[54] Psychiatric symptoms are very common and often undiagnosed. Cognitive impairment, depression, anxiety, psychosis, and sleep disturbances are frequently observed.[55] Cardiac involvement is an emerging clinical issue and consists mainly of

arrhythmias and cardiac myopathy. Recent data indicate patients with WD are frequently hospitalized for signs and symptoms related to cardiac complications.[56,57] Other organs affected by copper accumulation include the kidney and patients can present with renal tubular acidosis, proteinuria, aminoaciduria, and hematuria.

The hepatic manifestations in WD include an elevation of transaminases, steatosis, cirrhosis with portal hypertension, and acute liver failure. WD is not commonly associated with the development of hepatocellular carcinoma or other types of liver cancer, even in the presence of cirrhosis.[58] Interestingly, hepatic steatosis is a common histological feature in WD. In a large study of patients with WD and biopsy-confirmed liver pathology, the patatin-like phospholipase domain-containing protein 3 (*PNPLA3)* G allele, known to be strongly associated with increased hepatic fat content, was found to be associated with moderate to severe steatosis.[59] Intriguingly, WD and NASH histological features partially overlap. Similar to NASH, WD can present with both micro- and macro-vesicular steatosis and glycogenated hepatocyte nuclei, ballooning of hepatocytes, and Mallory bodies.[60]

Evidence from animal models of WD indicates copper does not accumulate in hepatocytes continuously during WD progression and is redirected to other cells when hepatocytes are saturated with copper to facilitate hepatic regeneration. In *Atp7b$^{-/-}$* mice, *in situ* imaging indicates that in early stages, copper is sequestered by metallothioneins in the cytosol and, with further accumulation, it enters the nucleus and triggers transcriptome remodeling to up-regulate cell-cycle machinery and down-regulate lipid metabolism.[61] After an excess threshold of 250- to 800-fold above the normal level, copper uptake is inhibited, possibly by a down-regulation in CTR1. The authors also suggested the presence and activation of alternative copper-export mechanisms, yet to be characterized, to prevent copper accumulation and favor the uptake of copper by inflammatory cells, extracellular deposition, or urinary excretion.

If left undiagnosed or untreated, WD can be fatal. Once diagnosis is established, adherence to treatment is required for life.[62] Treatment options include copper-chelating agents (D-penicillamine, trientine, or tetrathiomolybdate) and zinc salts (zinc sulphate, gluconate, and acetate).[51] These agents aim to favor copper urinary excretion and/or to block its intestinal absorption, promoting copper elimination with feces.[51,63,64] Patients with liver failure or with end-stage liver disease are considered for liver transplantation, which ultimately corrects hepatic metabolic defects in WD.[51] Dietary interventions include restricting foods with high copper content, such as beef liver, nuts, chocolate, shellfish, mushrooms, and soy.[65]

4.2. Hepatic metabolic dysregulation in Wilson disease

4.2.1. Mitochondria structural and functional defects—The mitochondrion is the cellular hub for energy metabolism and production. It houses β-oxidation, the tricarboxylic acid cycle (TCA), and adenosine triphosphate (ATP) synthesis through oxidative phosphorylation (OXPHOS) complexes, related to the electron transport chain.[66] The process of ATP production is dependent on a steady copper flux to the OXPHOS enzyme complexes.[37] Although copper is essential for mitochondrial function, excess copper causes mitochondria morphological and functional changes. Signs of mitochondrial impairment

appear to be independent from liver function as they are evident at an early age, before signs of hepatic dysfunction manifest in humans and animal models of WD.[67–72] Furthermore, a gradual mitochondrial copper overload was shown to cause a steady decline in mitochondrial function, as defined by decreased ATP production.[73] Copper-chelation therapy, particularly with methanobactin, was shown to restore these alterations.[71,73]

Described morphological changes of hepatic mitochondria include elongations and enlarged cristae and intermembrane space,[70,71,73,74] as well as cardiolipin fragmentation,[75] and are linearly correlated to the amount of accumulated copper.

Functional mitochondrial alterations are also reported in both WD patients and animal models. These include abnormalities in the OXPHOS system, with decreased activities of complex IV and complexes I, II, and III.[70,76] Mitochondrial fatty acid β-oxidation is also impaired in WD. Work from our group has previously shown the transcript and protein levels of peroxisome proliferator-activated receptor α (PPARα) and carnitine palmitoyl transferase 1A were lower in an untreated Jackson Laboratory toxic milk mouse model of WD (tx-j) compared to control mice.[77] A similar finding of decreased hepatic *Ppar*a transcript levels was reported from *Atp7b*$^{-/-}$ knockout mice.[5] Hepatic *PPAR*a expression was also decreased in WD patients and the magnitude of this change was related to the progression of liver damage.[78] In many genetic defects with mitochondrial β-oxidation impairment, alternative rescue pathways are induced, including ω-oxidation of fatty acids that may take place in mitochondria and microsomes.[79] Nevertheless, the effects of mitochondrial β-oxidation impairment and compensating mechanisms to maintain energy homeostasis in WD are not understood. Metabolomic analysis of serum and hepatic metabolites also suggest an impaired TCA cycle. In *Atp7b*$^{-/-}$ mice, there was an accumulation of TCA intermediates (oxaloacetate, citrate, fumarate, and malate) and a decrease in acetyl-CoA and α-ketoglutarate.[80] In patients with WD, we have previously reported alterations in TCA cycle intermediates.[81]

The mechanism by which copper overload results in mitochondrial defects can be direct and indirect. At initial stages of copper overload, alterations in mitochondrial labile proteins originate via copper-mediated thiol modifications.[37] At later stages, multiple lines of evidence indicate a major role of oxidative stress in the pathogenesis of WD and in inducing mitochondrial dysfunction. These include reduced aconitase activity,[76,82] and an increase in transcript levels of the antioxidants thioredoxin-2 and peroxiredoxin-3 in tx-j mice.[70] Moreover, patients with WD presented single or multiple hepatic mitochondrial DNA deletions, a common response to oxidative stress,[83] and patients and animal models of WD presented increased mitochondrial lipid peroxidation.[84,85] Among mitochondrial phospholipids, cardiolipin is a copper-sensitive target which fragments upon copper accumulation, likely via free-radicals.[75] Therefore, the generation of ROS is thought to contribute to copper-mediated mitochondrial damage.[84,86] Of note, compensatory mechanisms for copper-induced mitochondrial abnormalities are not fully investigated.

4.2.2.   Hepatic bioenergetic defects—Adenosine monophosphate-activated protein kinase (AMPK) is a key responder to both metabolic and oxidative stimuli and its downstream signaling cascade is a chief regulator of cellular energy metabolism in hepatic

and extrahepatic tissue.[87] During metabolic stress and energy deprivation states, the intracellular AMP to ATP ratio is elevated and the binding of AMP to the AMPK regulatory subunit results in AMPK phosphorylation and activation.[87,88] The activated AMPK initiates signal cascades aiming to restore intracellular energy by which multiple ATP-consuming anabolic pathways, *e.g.*, isoprenoid, cholesterol, lipogenesis, TAG, phospholipid, glycogen, and protein syntheses, are down-regulated, whereas multiple ATP-producing pathways, *e.g.*, glucose uptake and glycolysis, fatty acid uptake and oxidation, and enhanced mitochondrial biogenesis, are up-regulated.[88]

Beside ATP deprivation, AMPK activity is affected by pro-oxidant conditions as it is induced by ROS to initiate apoptosis and auto-phagy.[89] The direct effect of copper excess on AMPK activation has been demonstrated *in vitro* in human neuroblastoma cells. Compared to untreated cells, treatment with the synthetic isatin-Schiff base copper (II) complexes Cu(isapn) and Cu(isaepy)$_2$ at a concentration of 50 μM resulted in increased ROS production, apoptosis induction, and subsequent varying degrees of oxidative damage to proteins and lipids.[90] Further work from the same group has shown impairment of complex I of the OXPHOS chain and enhanced apoptosis via an AMPK-dependent mechanism.[91]

In animals, dietary copper modulation can alter tissue AMPK activity[1]; the effect of progressive copper accumulation on hepatic AMPK activity and signaling was recently demonstrated in *Atp7b$^{-/-}$* mice. While on a chow diet, *Atp7b$^{-/-}$* mice exhibited increased hepatic phosphorylated-AMPKα with reduced gluconeogenic and lipogenic gene expression, including phosphoenolpyruvate carboxykinase, *pyruvate carboxylase*, fructose bisphosphatase 1, fatty acid synthase (*Fasn*), acetyl-CoA carboxylase, and sterol regulatory element-binding protein 1c (Srebp1c).[80]

4.2.3. Alterations in lipid metabolism

4.2.3.1. Hepatic steatosis.: Beside hepatocyte mitochondrial insufficiency and energy deprivation, dysregulation in hepatic lipid metabolism is another hallmark alteration of WD, with hepatic steatosis being a frequent feature at diagnosis.[59,67,92–94] The molecular mechanisms underlying steatosis in WD remains poorly understood. However, mitochondrial dysfunction and the marked inhibition of hepatic β-oxidation are likely contributing factors.[95] Interestingly, evidence from animal models indicates hepatic DNL is impaired in WD. At the transcriptional level, lipogenesis is regulated by multiple signaling proteins including liver X receptor (LXR), SREBP1c, and carbohydrate-responsive element-binding protein.[24] Work from our group has shown SREBP1c transcript and protein levels were reduced in untreated tx-j mice compared to control mice with normal copper metabolism.[77] Similar findings were observed in *Atp7b$^{-/-}$*–knockout mice, showing an alteration in SREBP-2 function rather than maturation and re-localization.[5] There was also a down-regulation in many genes involved in lipid metabolism, including ATP citrate lyase, *Fasn*, elongation of very long chain fatty acids protein 6, and lipin-1. Remarkably, when fed an obesogenic Western diet, Atp7b$^{-/-}$ mice exhibited lower body weight and adiposity and lower hepatic steatosis compared to wild-type control mice. When comparing chow-fed and Western diet-fed mice, no difference was observed in body weight, adiposity, and hepatic

steatosis, suggesting the lack of a diet-specific effect. This reduced susceptibility to hepatic steatosis was attributed to the blunted weight gain while on the Western diet.[80]

Hepatic steatosis is also associated with impaired synthesis and export of VLDL, both understudied pathways in WD. The association of the *PNPLA3* G allele with hepatic steatosis in WD suggests impaired lipid remodeling and involvement of VLDL lipidation and export in the etiology of WD hepatic steatosis.[59,96,97] The process of VLDL synthesis and export is mediated by the microsomal triglycerides transfer protein that combines TAG and apolipoprotein B to create neutral lipid masses, which are further packaged with phospholipid monolayers enriched with phosphatidylcholine (PC) before export to circulation.[98] Relevant to VLDL metabolism, we and others have described a dysregulated methionine cycle and altered choline levels in humans and animal models of WD.[77,99–101] These dysregulations in methionine and choline metabolism may impact the assembly or export of VLDL in WD. Methionine is a precursor to S-adenosylmethionine (SAM), a universal methyl-group donor, which is required for transmethylation reactions.[102,103] An elevated methylation potential, expressed as high SAM levels in relation to its product S-adenosylhomocysteine, is required for activation of the hepatic phosphatidylethanolamine methyltransferase (PEMT) pathway and the synthesis of PC.[104–106] Choline is also a substrate for the synthesis of PC via the choline dependent cytidine-diphosphate-choline (CDP-choline) pathway, the major contributor to the PC pool.[107] We recently reported a dysregulated serum phospholipid profile in WD patients with profound alterations in PC levels compared to healthy controls as well as a down-regulation of PC synthesis genes in tx-j mice, including *Pemt*, [phosphate cytidylyltransferase 1 choline-alpha], and choline phosphotransferase 1.[101] In general, alterations in phospholipids are implicated in hepatic steatosis,[108–111] and evidence indicates both pathways, hepatic PC synthesis via CDP-choline or PEMT, are critical for VLDL production.[104,112]

4.2.3.2.   Cholesterol synthesis.: Along with copper accumulation, defects of lipid utilization in isoprenoid and cholesterol syntheses are shown in various animal models and in humans with WD. The transcript and activity levels of 3-hydroxy-3-methyl-glutaryl-CoA reductase (*Hmgcr*) were down-regulated in Long-Evans Cinnamon rats.[113] Similar findings were described in *Atp7b*$^{-/-}$ mice at an early stage and prior to the appearance of histopathological changes.[5,114] Concomitantly, there was a significant reduction in hepatic VLDL-cholesterol and dysregulation of enzymes involved in bile acid biosynthesis, including a down-regulation in cholesterol 7 α-hydroxylase and up-regulation in microsomal oxysterol 7 α-hydroxylase. *In vitro*, it was shown copper does not continuously accumulate in *Atp7b*$^{-/-}$ hepatocytes throughout the course of WD. However, there was a down-regulation in cholesterol metabolism and a reduction in the VLDL-cholesterol fraction that remained significantly lower throughout the course of the disease.[61]

Copper was shown to impair the transcriptional function of several nuclear receptors by assimilating into the DNA-binding domain and inducing conformational changes that prevent DNA binding.[115] An *in silico* gene expression analysis indicated LXR, retinoid X receptor (RXR), and the LXR/RXR-mediated regulation of lipid metabolism are impaired in patients with WD and *Atp7b*$^{-/-}$ mice.[116] In the same study, the hepatic mRNA levels of *Rxr*, *Fasn*, and *Hmgcr* were significantly down-regulated in *Atp7b*$^{-/-}$ livers. Treatment with an

LXR agonist significantly increased *Fasn* expression as well as plasma total cholesterol, low-density lipoproteins (LDL), and high-density lipoproteins compared to untreated *Atp7b* −/−mice, suggesting alterations in nuclear receptor-mediated lipid metabolism are major contributors to dysregulated lipid metabolism and liver damage pathogenesis in WD.[116] Similar findings were reported in patients with WD, with a significant reduction in hepatic transcript levels of *HMGCR* and LDL receptor compared to control subjects.[5] Another study investigated serum lipid profiles in WD patients treated with a copper chelator compared to healthy subjects, describing significantly lower levels of circulating total cholesterol and LDL cholesterol in WD patients, with no difference in TAG levels between groups.[117] Comparing preand post-treatment WD patients, copper-chelation treatment was associated with lower plasma cholesterol and no TAG change.[117,118] In a different study, a significant reduction in cholesterol was reported only in WD with hepatic manifestations compared to asymptomatic, neurologic, and combined phenotype with both neurologic and hepatic manifestations.[114] This reduction was restored to healthy control levels after two years of copper-chelation therapy.[114] Together, these defects may reflect impaired lipid utilization as a possible contributor to the development of steatosis. A proposed mechanism for the development of hepatic steatosis in WD is depicted in Fig. 1.

4.2.4. Dysregulation in carbohydrate metabolism—The effect of copper overload on hepatic glucose metabolism is understudied. However, dysregulation in hepatic glucose metabolism is reported in subjects with WD. Hypoglycemia, a typical complication of advanced liver disease, is also present as an early clinical sign in WD. In a case report, two siblings with WD and abnormal liver histology in the absence of cirrhosis, exhibited fasting hypoglycemia, mild glucose intolerance, and hyper-insulinemia that were normalized with penicillamine treatment.[119]

In another case report of a young female with normal liver morphology as confirmed by imaging, hypoglycemia was reported and resolved with copper-chelation treatment.[120] The described hypoglycemia could be due to impaired glycolysis or gluconeogenesis. In light of mitochondrial defects, particularly impairment in mitochondrial β-oxidation, copper overload may be associated with impairment in the hepatic metabolic capacity to transition between fat and glucose metabolism. During the fasting state, the liver maintains glucose homeostasis by glycogenolysis from glycogen and by gluconeogenesis using lactate, glycerol, and amino acids as substrates for glucose production.[121] In a rat model of WD, the circulating level of glucose is decreased and lactate is increased,[122] possibly indicating the glycolysis by-product, pyruvate, accumulates and is further converted to lactate and exported to circulation.[123] However, elevated lactate may be due to defects in gluconeogenesis. In fasted *Atp7b*−/−mice, an elevation in several hepatic glycolysis intermediates and decreased hepatic glucose, pyruvate, and lactate levels are reported. Furthermore, insulin administration resulted in hypoglycemia, indicating an impaired glucose counterregulatory response, a finding supported with marked down-regulation of hepatic genes involved in gluconeogenesis.[80] Interestingly, *Atp7b*−/−mice were more glucose-tolerant and insulin-sensitive compared to their wild-type counterpart.[80]

In *Atp7b*−/− mice and WD patients, there is evidence of decreased nuclear receptor binding to promoter response elements, including farnesoid X receptor (FXR), RXR, hepatic nuclear

factor 4 alpha, and liver receptor homolog-1, and decreased transcript levels of nuclear receptor target genes, indicating impaired nuclear receptor signaling.[115] FXR signaling is a modulator of glucose homeostasis and insulin sensitivity.[124–126] Hepatic *Fxr* expression is regulated by insulin and glucose in rodent hepatocytes *in vitro*.[127] Moreover, *Fxr*^{−/−}mice display an accelerated hepatic response to high carbohydrate re-feeding, mainly by induction of glycolytic and lipogenic genes and a repression of gluconeogenic genes.[128] Therefore, impaired nuclear receptor signaling in copper overload can have implications in carbohydrate metabolism and homeostasis.

In a metabolomic analysis of WD patients, we reported elevated serum sorbitol levels,[81] further corroborating the dysregulation in WD carbohydrate metabolism. Sorbitol is an intermediate of the polyol pathway by which glucose is converted to sorbitol by aldose reductase, followed by the conversion of sorbitol to fructose by sorbitol dehydrogenase.[129] The hepatic polyol pathway is activated under hyperglycemic conditions. However, further work is needed to understand the relevance of elevated sorbitol with regard to carbohydrate metabolism in WD. Of note, in type 2 diabetes, a state in which alternating use of carbohydrates and lipids as metabolic fuel is impaired, fasting serum sorbitol is reported to be significantly elevated compared to healthy controls, with a significant elevation in sorbitol persisting in the post-feeding state.[130]

4.3.   Metabolic dysregulation in extrahepatic tissues

The substantial role of ATP7B-mediated copper homeostasis is well-recognized in the liver. However, it is poorly studied in extrahepatic tissues, including adipose tissue, muscle, and intestine.

4.3.1.   Adipose tissue—White adipose tissue (WAT) is an active endocrine organ regulating body fat mass and energy homeostasis. Lipogenesis and lipolysis are central to these functions and are tightly regulated.[131] Lipogenesis takes place mainly in adipose tissue, liver, and muscle, where esterification of FFA results in the formation of TAG. In contrast, lipolysis mobilizes metabolic fuel to peripheral tissues, mainly from adipose tissue, in response to energy needs.

The role of copper-mediated lipid metabolism in adipocytes and its contribution to whole-body energy metabolism are not well-understood. We observed tx-j mice to be lean and protected from obesity, despite dysregulated lipid metabolism. While on the same chow diet, tx-j mice gained less weight and fat mass compared to their wild-type littermates.[77,100] Similar observations were reported in *Atp7b*^{−/−}mice. Compared to wild-type littermates, chow-fed *Atp7b*^{−/−}mice had lean body weight and less total body weight gain, fat mass, and epididymal WAT weight, with markedly reduced weight gain when fed the Western diet. Although this variation was attributed to decreased food intake and, to an extent, increased activity, a significant increase in respiratory exchange ratio was only observed in the active (dark) cycle. In addition, the total energy expenditure was comparable between the two genotypes in both active (dark) and inactive (light) cycles, suggesting the involvement of a metabolic derangement component.[80]

On the other hand, the hepatocyte-specific deletion of *Atp7b* in mice resulted in steatosis before evident signs of liver damage and, interestingly, increased adiposity with an up-regulation in *Hmgcr* attributed to nonparenchymal liver cell signaling.[132] It is worth mentioning *Atp7b*^{−/−}rats exhibited increased visceral fat and hepatic fat content when fed the Western diet, contradicting the obesity-protected phenotype.[11]

Evidence demonstrates copper has a signaling role in the regulation of lipolysis, by which TAG are broken down to FFA and glycerol via the $3',5'$-cyclic AMP (cAMP) pathway. In rabbits, dietary copper supplementation inhibited lipid accumulation in adipose tissue via increased lipolysis.[1] In *Atp7b*^{−/−}mice compared to wild-type, hepatic copper overload was associated with a significant decrease in WAT copper levels and lipolytic activity.[33] When lipolysis was induced by a β-adrenergic receptor agonist, cAMP levels were found to be lower and less glycerol was released from WAT, indicating impaired lipolysis. In the same study, using 3T3-L1 white adipocytes, cellular copper content increased lipolysis in a dose-dependent manner, and treatment with a copper chelator decreased FFA and glycerol release upon inducing lipolysis. Furthermore, the cAMP-degrading phosphodiesterase 3B (PDE3B) was identified as a target of copper accumulation; PDE3B activity is inhibited when bound to copper resulting in decreased cAMP degradation and increased lipolysis. These findings were confirmed in mice with an adipocyte-specific deletion of *Atp7a* that showed increased adipose copper levels compared to control mice and progressive loss of WAT due to increased lipolysis.[48] This lipolytic state was associated with decreased levels of serum leptin and adiponectin and increased levels of TAG and insulin, suggesting adipose dysfunction, in addition to pronounced glucose intolerance, insulin resistance, and hepatic steatosis, indicating systemic metabolic impairment. Collectively, these data provide *in vivo* evidence that alteration in adipose tissue copper levels influence WAT lipolytic activity and lipid homeostasis. Yet, the mechanism by which tx-j and *Atp7b*^{−/−}mice are protected from obesity remains unclear and multiple factors need to be considered, including lipogenesis, energy expenditure, and physical activity.

It is possible that the impaired lipolytic activity in *Atp7b*^{−/−}mice could reflect adipose dysfunction. Recently, the role of copper in adipocyte morphology, metabolism, and fat storage was identified through the activity of the copper-dependent enzyme, semicarbazide-sensitive amine oxidase (SSAO).[133] SSAO synchronizes changes in metabolic fuel availability via proteome-remodeling to regulate the uptake of glucose and long-chain fattyacids. Inparticular, copper-activated SSAO increases glucose uptake and glycolysis; in states of copper deficiency, SSAO is inactivated and fatty acid uptake is up-regulated, leading to adipocyte hypertrophy and lipid deposition. Therefore, this evidence, together with decreased WAT copper levels and lipolytic activity,[33] highlights potential impairment in adipose function and metabolic adaptability in *Atp7b*^{−/−}mice.

4.3.2. uscle—Data examining the effect of *ATP7B* mutations on extrahepatic copper levels and lipid metabolism are limited, and discrepancies in muscle copper levels have been reported in WD. In one small study, patients with WD had a significant increase in muscle copper level compared to healthy subjects.[134] On the other hand, using functional imaging aided with administration of ^{64}CuCl$_2$ as a tracer, there was no significant difference in ^{64}Cu levels between *Atp7b*^{−/−}and control mice in multiple extrahepatic tissues, including the

muscle.[135] Interestingly, in rabbits, dietary copper supplementation resulted in muscle growth and inhibited lipid accumulation in skeletal muscle via elevated β-oxidation.[1] More studies are needed to investigate the effect of hepatic copper overload on adipose tissue energy metabolism.

4.3.3.   Intestine—Recent evidence suggests copper modulates processing of chylomicrons in the intestine. In enterocytes, copper-transporter ATP7A facilitates dietary copper export into portal circulation.[47,136–138] The intestine also expresses ATP7B, which appears to modulate enterocyte vesicular copper storage and regulate chylomicron formation and dietary fat absorption.[139] The *Atp7b*$^{-/-}$ mouse model presents duodenal copper deficiency and dysregulated fat-processing in enterocytes.[50] Intriguingly, *Atp7b*$^{-/-}$ mice also presented duodenal villi with increased microvilli length and enterocytes with mitochondrial ballooning and lipid accumulation, indicating impaired dietary fat absorption and aberrant chylomicron maturation which most likely require both adequate copper levels and ATP7B function. These alterations were not observed in mice with the hepatocyte-specific deletion of *Atp7b*, which showed normal intestinal morphology with no enterocyte lipid droplet accumulation.

Alterations in the intestinal microbiome are reported in many diseases, including obesity and type 2 diabetes.[140,141] A great body of evidence supports a role of gut microbiota in modulating host metabolism. This was demonstrated in germ-free mice, which are more glucose-tolerant, insulin-sensitive, and have reduced adiposity compared to conventional-colonized mice.[142–144] When fed the Western-diet, germ-free mice are protected from obesity.[145] Diet, among other environmental factors, can modulate the gut microbiota. [146–148] In WD, copper intestinal content is significantly altered as the genetic mutation in *ATP7B* leads to impaired biliary copper excretion into the intestine and subsequent fecal elimination. In addition, WD therapy with zinc salts may modulate dietary copper absorption. Studies of the intestinal microbiome composition in WD patients are limited. One study reported a significant difference in the diversity and composition of WD patients' intestinal flora. Compared to healthy subjects, Bacteroidetes was significantly more abundant in WD patients, while *Firmicutes*, *Proteobacteria*, and *Fusobacteria* were less abundant.[149] However, the relation between the observed microbiome alterations with energy metabolism in WD is not clear and worth further investigation. It is thought the gut microbiome can affect host lipid metabolism by modulating the production of secondary bile acids which, after being absorbed into systemic circulation, target multiple nuclear receptors to regulate hepatic and/or systemic lipid and glucose metabolism.[150,151] Another potential mechanism involves the production of short-chain fatty acids, such as acetate, propionate, and butyrate, derived from the fermentation of non-digestible carbohydrates. Short-chain fatty acids affect energy expenditure and insulin sensitivity via mechanisms involving G protein-coupled receptors.[152,153] Also, the hepatic production of trimethylamine *N*-oxide, derived from the oxidation of the gut-produced trimethylamine, can exert direct effects on reverse cholesterol sterol and bile acids metabolism.[152,153]

5. Conclusion

The mechanisms linking copper to dysregulated lipid and energy metabolism in WD are not clear and may include direct copper binding and modifications to labile proteins that impair enzymes and nuclear receptor function. Copper, via the generation of ROS, can also damage subcellular protein and lipid structures of vital organelles, including the mitochondria.

Hepatic copper accumulation is accompanied by mitochondrial bioenergetic defects, with evident mitochondria structural and functional abnormalities that appear to be independent from liver function. However, the hepatic response to this energetic defect is not clear.

Dysregulation of hepatic lipid metabolism and the development of steatosis is an early feature of copper accumulation in WD. The molecular mechanisms underlying hepatic steatosis in WD remains poorly understood. However, hepatic DNL is down-regulated and steatosis appears to be a net result of impaired mitochondrial β-oxidation, reduced VLDL assembly or export, decreased utilization of lipids, and a marked down-regulation in cholesterol synthesis. Altered carbohydrate metabolism is also suggested, mainly via defects in the alternating use of carbohydrates and fat as metabolic fuel. This was evident with hypoglycemia and impaired gluconeogenesis in animal models of WD. These metabolic alterations may be explained by the impaired function of nuclear transcription factors, a feature reported in both animal models and human subjects with WD.

There is a gap in current knowledge regarding the effects of *ATP7B* mutations and copper accumulation on metabolic dysregulation and energy homeostasis in extrahepatic tissue. It is plausible the metabolic effects of copper overload in WD are tissue-dependent, as copper accumulation is uneven across different tissues. In the liver, where copper selectively accumulates, copper overload is profound and is reflected by down-regulated lipid metabolism and lipid droplet accumulation. In enterocytes, copper is excessively stored in vesicles and lipid droplets accumulate in the cytosol, suggesting impaired chylomicron formation and dietary lipid absorption. In adipose tissue, copper is reduced, lipolysis is impaired, and lipogenesis and hypertrophy are down-regulated.

Ultimately, the systemic dysregulation of energy metabolism in WD may reflect a process of metabolic adaptation to synchronize metabolic fuel availability and tissue energy demands.

Acknowledgements

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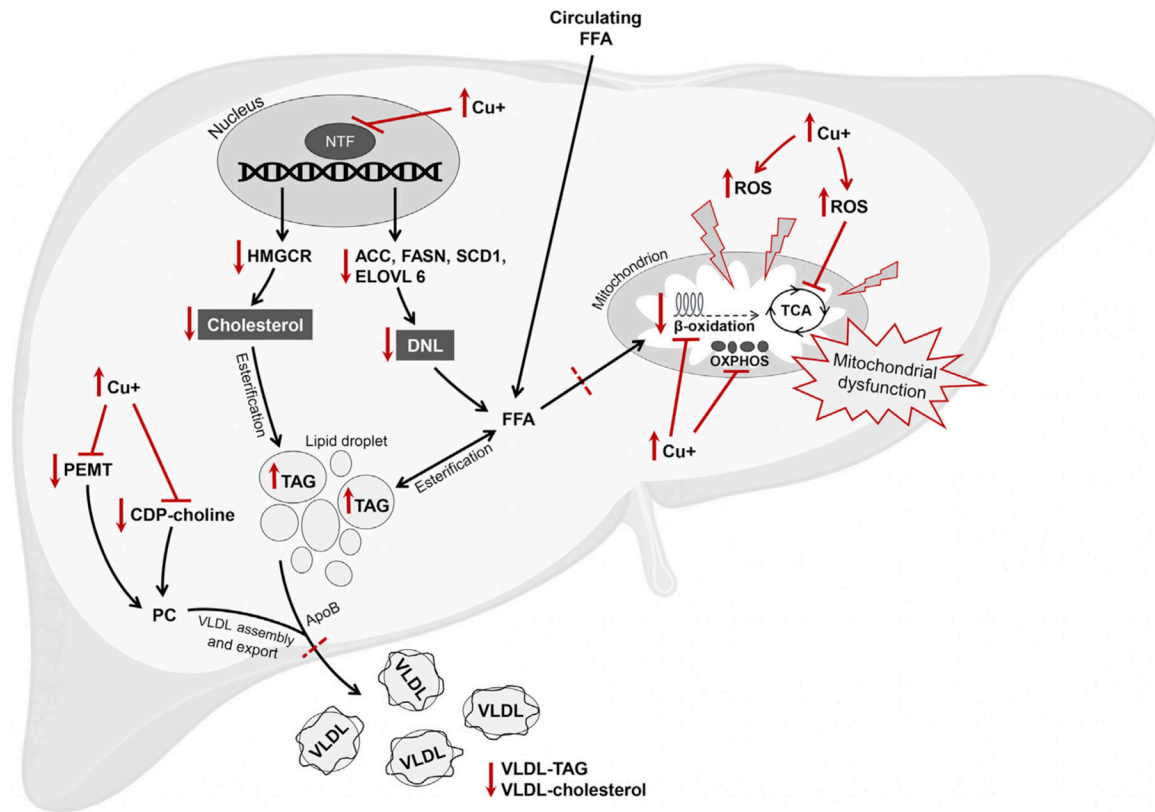
Fig. 1. Hypothetical framework for the development of hepatic steatosis in Wilson disease.
Copper overload induces the production of ROS which interacts with subcellular protein
structures, including the mitochondria, to cause structural and functional mitochondrial
alteration and bioenergetic defects. Copper may also directly impair enzymes involved in the
TCA cycle, fatty acid β-oxidation, and OXPHOS chain. Copper accumulation impairs the
function of multiple nuclear transcription factors including retinoid X receptor, liver X
receptor, carbohydrate-responsive element-binding protein, and sterol regulatory element-
binding protein 1c. The transcript and protein levels of genes involved in cholesterol
synthesis and lipogenesis are down-regulated, including HMGCR, ACC, FASN, SCD1, and
ELOVL6. The impairment of PC synthesis pathways results in impaired assembly and/or
export of VLDL. Together, the uptake of FFA and subsequent esterification into TAG,
combined with impaired utilization of FFA and impaired assembly and/or export of VLDL,
result in the net effect of hepatic lipid accumulation, or steatosis. ACC, acetyl-CoA
carboxylase; ApoB, apolipoprotein B; CDP-choline, cytidine-diphosphate-choline; Cu^+, free
copper; DNL, *de novo* lipogenesis; ELOVL6, elongation of very long chain fatty acids
protein 6; FASN, fatty acid synthase; FFA, free fatty acids; HMGCR, 3-hydroxy-3-methyl-
glutaryl-CoA reductase; NTF, nuclear transcription factor; OXPHOS, oxidative
phosphorylation; PC, phosphatidylcholine; PEMT, phosphatidylethanolamine
methyltransferase; ROS, reactive oxygen species; SCD1: stearoyl-CoA desaturase; TAG,
triglycerides; TCA, tricarboxylic acid; VLDL, very low-density lipoproteins.