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Review

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Copper and liver fibrosis in MASLD: the two-edged sword of copper deficiency and toxicity

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Abstract

Copper is a trace metal whose absence or deficiency can cause structural and functional alterations that can be corrected by copper administration. Copper excess is associated with significant liver toxicity, such as that seen in Wilson's disease, which often exhibits liver steatosis and can be managed by copper sequestrants. Copper, due to its ability to either accept or donate electrons, is a cofactor in many physiological redox reactions, playing an essential role in cell energy homeostasis, detoxification of reactive oxygen species, and hepatic immunometabolism. Given these facts, it is reasonable to speculate that copper might be involved in the pathogenesis of liver fibrosis in the setting of metabolic dysfunction-associated fatty liver disease (MASLD). To address this research question, a narrative review of published studies was conducted, spanning from the needs, sources, and toxicity of copper to Menkes and Wilson's disease. Most epidemiological studies have demonstrated that MASLD is associated with copper deficiency. However, several studies show that MASLD is associated with copper excess and very few conclude that copper is not associated with MASLD. Therefore, the putative pathomechanisms associating both copper excess and deficiency with MASLD development and progression are reviewed. In conclusion, epidemiological and pathogenic data support the notion that well-balanced copper homeostasis is a prerequisite for liver health. Accordingly, both copper excess and deficiency may potentially predispose to liver fibrosis via the development of MASLD. Therefore, studies aimed at restoring normal bodily stores of copper should be tailored according to precision medicine approaches based on the specific features of copper metabolism in individual MASLD patients.

Keywords: Copper, epidemiology, fructose, iron, liver histology, pathomechanisms, sex differences, steatohepatitis

INTRODUCTION

Metals play crucial roles in the liver as essential cofactors, catalysts, or regulators of biochemical reactions, contributing to various metabolic activities[1]. These activities include maintaining energy balance, synthesizing hormones, producing and storing proteins, lipids, sugars, and fats, as well as metabolizing xenobiotics, excreting unwanted substances through bile, and performing various immunological functions[2]. Hemochromatosis is a well-known example of liver disease caused by the accumulation of metals in liver tissue[3].

Disturbed homeostasis of copper is typically associated with the generation of reactive oxygen species (ROS) and metabolic imbalance, leading to DNA damage and apoptosis[4], as well as hepatic and neurological diseases such as Wilson's and Alzheimer's diseases[5]. Additionally, it is linked to various cardio-metabolic disorders, including arterial hypertension, hypertriglyceridemia, aortic calcifications, and stroke[6-9]. Given the strong association between steatotic liver disease and cardiometabolic disorders[10], this research suggests the possibility of linking deranged copper metabolism with metabolic dysfunction-associated steatotic liver disease (MASLD).

The first investigation incidentally highlighting the role of copper in what we now call MASLD was published in 2003 by Loria *et al.*[11]. These authors found that, compared to MASLD individuals who were negative for non-organ-specific autoantibodies (NOSA), serum values of copper and ceruloplasmin levels were more elevated among NOSA-positive MASLD patients, none of whom had any evidence supporting the diagnosis of Wilson's disease. These authors argued that raised copper levels could result from hepatocytolysis, that high serum copper concentrations are associated with an increased risk of vascular mortality, and ceruloplasmin is an acute-phase reactant. On these grounds, Loria *et al.* suggested that elevated copper and ceruloplasmin levels in NOSA-positive subjects might indicate increased cardiovascular risk[11]. Studies conducted in animal models have yielded conflicting results regarding copper's role in experimentally induced liver fibrosis[12,13].

A recent conversation among the authors (https://www.oaepublish.com/interviews/mtod.250) inspired the current narrative review article dedicated specifically to the underappreciated and controversial role of copper excess and deficiency in the development of liver fibrosis associated with MASLD. More than 20 years after the initial clinical observations, we decided to update our understanding of the role of copper in liver fibrosis among individuals with MASLD. To achieve this, we conducted a bibliographic search in the PubMed database using key terms such as nonalcoholic fatty liver disease (NAFLD), metabolic dysfunction-associated fatty liver disease (MAFLD), MASLD, steatohepatitis, fibrosis, and copper. This search spanned from inception to May 31, 2024. Relevant articles identified by both authors were included, along with cross-references and any additional publications from the authors' personal archives.

Needs and sources of copper, toxicity, and deficiency

Needs and sources

Copper is one of eleven essential trace elements, the absence of which can lead to biochemical, structural, and functional alterations that can be corrected by administering the missing element[14].

Healthy adults require daily oral intake of copper ranging from 1.1 to 2 mg per day, a requirement typically met through Western diets[14]. Dietary sources of copper include potable water, liver, meats, shellfish, seeds, beans, cereals, nuts, potatoes, whole grains, vegetables, mushrooms, crustaceans, and chocolate[14,15].

Copper toxicity and Wilson's disease

The potential toxicity of copper is exemplified by Wilson's disease (WD)[16,17]. WD is an autosomal recessive disorder caused by pathogenic variants of the *ATP7B* gene, which encodes a P-type copper transport ATPase, leading to the accumulation of toxic copper concentrations in various organs, particularly the liver and brain[18]. As a result, WD may manifest as hepatic, neurologic, or psychiatric symptoms, or a combination of these [Table 1][17,19,20].

The clinical diagnosis of WD disease remains challenging due to the wide-ranging spectrum of manifestations in the individual patient[18]. Interestingly, an estimated subset of 30% of WD patients, who are primarily detected through family screening, are asymptomatic and considered to be in the "pre-destructive phase of copper accumulation"[21]. In symptomatic individuals, the diagnosis of WD relies on a high level of suspicion determined by a compatible medical history, physical examination, laboratory tests, liver biopsy, and imaging of the central nervous system[17]. Studies indicate that WD may be prevalent among individuals with unexplained (cryptogenic) chronic liver disease in specific geographical areas[22].

Clinical chemistry includes liver tests, serum ceruloplasmin levels, and basal 24-hour Cupruria. While it is uncommon, ceruloplasmin levels can fall within the normal range in WD. However, ceruloplasmin levels alone are not sufficient for diagnosis unless they are extremely low (< 5 mg per deciliter), which strongly indicates the presence of the disease. Alternatively, levels between < 11.5-14 mg/dL may also suggest WD[23,24]. Basal 24-hour urinary copper excretion is typically > 40 μg.

Liver biopsy permits establishing the severity of liver damage, ruling out competing etiologies of liver disease (e.g., autoimmune hepatitis, MASLD, aceruloplasminemia, and other rare genetic disorders), and enables copper quantification[17]. Liver histology in proven WD may mimic NAFLD[25] and NASH[26], and intrahepatic accumulation of copper is strongly associated with the extent of steatosis[27]. Therefore, electron microscopy is considered essential in the diagnostic work-up of pediatric liver biopsies, given that ultrastructural mitochondrial abnormalities help to distinguish WD from NAFLD and autoimmune hepatitis[28].

Additional investigations include brain magnetic resonance imaging, and the identification of *ATP7B* mutations[17] based on which the prevalence rate of WD is now estimated to be 1 in 7,026 people compared to 1 in 35,000-45,000 people before the advent of this molecular investigation[18]. Emerging diagnostic approaches include determining the relative exchangeable copper, proteomics-based methods, and positron emission tomography[17,29,30]. WD with acute onset is clinically indistinguishable from other acute liver diseases. Fulminant onset is a strong indication for liver transplantation[31]. Accepted innovative management options in WD are displayed in Table 2[17,21,30].

It is postulated that it is impossible to remove any amount of copper from the liver tissue. Therefore, the aim of therapy is to target albumin-bound non-ceruloplasmin free copper, which is toxic. This goal can be achieved by copper chelators, especially zinc[19].

Open issues in the treatment of WD

Further research is needed to differentiate the natural course of WD from treatment-related early deterioration[32]. During treatment, monitoring of copper metabolism is essential[33].

Copper deficiency and Menkes disease

Causes of copper deficiency

Table 1. Clinical manifestations of Wilson's Disease

Hepatic	**Hepatomegaly, steatotic liver disease, hepatitis and cirrhosis. Wilson's disease rarely manifests as acute liver failure**
Neurologic	Early manifestations in adolescents may include clumsiness, decline in athletic skills, or decreased academic performance, tremors, incoordination, and difficulty with fine motor skills. Tremors, dystonia, athetosis, problems with writing, dysarthria, ataxic gait, and Parkinson's disease-like symptoms can also be present. Drooling and difficulty swallowing suggest pseudobulbar involvement. Sleep disorders and restless legs syndrome are being increasingly identified. While cognitive functions are typically preserved, there may be subtle impairments in executive function or integrative abilities
Psychiatric	Depressive disorders are common and bipolar disorder and psychosis may develop. Phobias and compulsive behaviors, as well as aggressive and antisocial behaviors, have been described
Ocular	Kayser-Fleischer rings, sunflower cataracts
Other manifestations	Renal*: tubular dysfunction can range from mild hydroelectrolytic and acid-base disorders to complete Fanconi syndrome and include proximal and distal renal tubular acidosis, nephrolithiasis and nephrocalcinosis. Additionally, the development of tubular casts may occur during acute hemolysis, rhabdomyolysis, or bile cast nephropathy. Other symptoms may include aminoaciduria and acute kidney failure
	Cardiac: arrhythmias or cardiomyopathy
	Musculoskeletal: arthritis, osteopenia, osteoporosis, deformity, rhabdomyolysis, and Duchenne-like muscular weakness
	Endocrine: panhypopituitarism; hypoparathyroidism; amenorrhea, infertility, and testicular dysfunction
	Other potential complications: acute hemolysis and pancytopenia

*Membranous nephropathy, minimal-change disease, and, rarely, severe glomerulonephritis may result from the long-term administration of D-penicillamine.

Table 2. Management options in Wilson's disease

	Treatment	Remark
Medical	Diet avoiding copper-rich food	WD cannot be successfully treated with diet alone
	D-penicillamine	Primary treatment
	Trientine dihydrochloride	Primary treatment
	Trientine tetrahydrochloride	Noninferior to penicillamine
	Zinc salts	Maintenance therapy
Liver transplant	Reserved for individuals with acute liver failure or decompensated chronic liver disease who are unresponsive to medical treatment	Neurologic WD remains a controversial primary indication for liver transplantation, but it is a promising option for individuals with severe neurological manifestations who do not respond to medical treatment
Emerging treatments	TTM	Very high-affinity copper-specific chelator
	Methanobactin	This compound halts oxidative stress and hepatic mitochondrial injury by increasing the biliary excretion of copper
	Curcumin	Able to rescue the subcellular localization of misfolded gene products of *ATP7B* variants *in vitro*
	Inhibitors of stress kinases p38 and JNK	Under development
	Reduction of cell injury caused by copper	The LXR agonist T0901317 and zinc treatment reverse copper-induced functional downregulation of FXR and RXR
	Gene therapy	Viral vectors containing modified *ATP7B* constructs have been shown to be effective in restoring copper homeostasis and preventing liver injury in rodent models of WD. Although experience with lentiviral gene delivery is limited, adenovirus-associated delivery of extrachromosomal DNA can be overcome by using smaller *ATP7B* constructs. This approach has successfully transfected hepatocytes and corrected metabolic defects in rodent models and is currently being evaluated in Phase 1 and 2 studies
	Gene repair	Somatic gene modification can be achieved using CRISPR-Cas to correct *ATP7B* mutations and restore proper copper transport in hepatocytes, like that in an unaffected simple heterozygote
	Hepatocyte transplantation	Tested in WD rodent models, this technique requires immunosuppression to prevent rejection of the transplanted cells in humans. Innovative cell transplantation strategies such as autologous liver progenitor cells or non-hepatic stem cells are being evaluated to avoid the need for immunosuppression. A concern is that, unlike liver transplantation, hepatocyte transplantation may not correct portal hypertension and its complications

CRISPR-Cas: Clustered regularly interspaced short palindromic repeats associated with a Cas endonuclease; FXR: farnesoid X receptor; LXR: liver X receptor; RXR: retinoid X receptor; TTM: tetrathiomolybdate; WD: Wilson's disease.

Copper deficiency can arise from rare hereditary causes or, more commonly, acquired origins. Examples include insufficient stores (found in preterm newborns and infants), inadequate intakes (a significant portion of the North American population consumes insufficient amounts of dietary copper, which is considered a risk factor for the development of MASLD), increased demands (such as during pregnancy, lactation, and wound healing), increased losses and malabsorption (which often occurs as a result of Roux-en-Y gastric bypass bariatric surgery)[14,15,34].

Menkes disease

First described in 1962, Menkes disease is caused by approximately 360 different mutations in the *ATP7A* gene, located on Xq21.1, which encodes the *ATP7A* transmembrane protein[35]. The incidence of Menkes disease varies among different countries, with the highest rates found in Australia, possibly due to the founder effect[35]. Menkes disease is an X-linked recessive trait, leading to the majority of patients being male, while females are usually carriers and only a few female patients have been reported. Menkes disease typically manifests between six to eight weeks after birth with seizures or growth failure[35]. Skin hypopigmentation, hair abnormalities, joint tissues, bone, and vascular complaints are also common. Renal complications may arise due to copper accumulation in the proximal renal tubule and various eye disorders have also been observed in Menkes disease[35].

Typically, death occurs by the age of three years, often due to vascular complications or respiratory infections[35]. In adults, copper deficiency can manifest with anemia, altered immunity, and manifestations of the cardiovasculature and the skin[14].

Diagnosis of copper deficiency

Copper deficiency may be identified by cupremia < 0.8 μg/mL (12.6 μmol/L). Cupremia and ceruloplasminemia increase in the presence of active inflammation proportionally to the intensity of the inflammatory process, therefore masking partial copper deficiency[14,36]. Ceruloplasmin < 20 mg/L associated with hypocupremia and raised C-reactive Protein strongly support copper deficiency[14]. Finally, genetic variants of ceruloplasmin have been associated with hypocupremia[37], hyperferritinemia, increased hepatic iron stores, and more severe liver fibrosis among MASLD patients[38].

Management of copper deficiency

The parenteral route of copper administration should be used if intestinal absorption is compromised or if copper deficiency is severe[39]. However, intravenous copper administration can induce severe hemolysis and potentially lethal hepatic necrosis[40].

Metabolic fate and physiological functions of copper

The absorption of Cu^{+} in the gut occurs when dietary Cu^{++} is reduced due to the activity of cytochrome B reductase 1 and the 6-transmembrane epithelial antigen of the prostate family proteins. This process is highly regulated and saturable, primarily mediated by copper transport protein 1 located in the apical portion of intestinal epithelial cells[14,41,42]. Cu^{+} is taken up by a high-affinity transporter on the hepatocyte cell membrane, incorporated into ceruloplasmin, released into the bloodstream, delivered to all organs, and eventually excreted via the biliary route[14]. Figure 1 summarizes the metabolic pathways of copper from intake to excretion.

Figure 1. Metabolic fate of copper. The magnification of the duodenum illustrates the notion that this segment of the digestive system is mainly responsible for the absorption of copper, together with the stomach and jejunum. The transport of copper in the blood is mediated by copper-binding proteins, among which ceruloplasmin and albumin play a prominent role. In addition to bile and the urinary system, a fraction of the copper contained in food is directly excreted in stool[14,41,42].

Due to its ability to transfer electrons, copper plays a crucial role in cell energy homeostasis by generating an electrical gradient utilized by mitochondria to synthesize adenosine triphosphate[43,44]. Additional physiological roles of copper include detoxification of reactive oxygen species, synthesis of neurotransmitters, control of epigenetic modifications, and modulation of immune-metabolic phenomena[44-47].

Copper and MASLD

Conflicting epidemiological evidence

Table 3 demonstrates that most studies support an association between altered copper homeostasis and MASLD, while only a few studies have found copper to be neutral. Among the former group of published studies, the number of studies revealing that copper decreased in MASLD outweighs those showing that copper increased. While thorough assessment with meta-analytic reviews is necessary, it is important to note that a meta-analytic review of six published studies[68] discovered low hepatic copper concentration in NAFLD, while serum copper and ceruloplasmin were not linked to NAFLD. To date, only one Mendelian Randomization analysis has been published on this topic[72], which reported negative results by revealing no causal association between copper and NAFLD.

Putative pathomechanisms involved

Copper deficiency

Copper deficiency may potentially increase the risk of MASLD and fibrosing MASH through a variety of pathomechanisms that result from altered energy homeostasis, dysfunctional lipid metabolism, a pro-inflammatory prostaglandin profile, increased lipid peroxidation, and decreased antioxidant defense. Additionally, these physiological effects of copper deficiency may also be potentiated by iron- and fructose-rich diets [Figure 2].

Marginal copper deficiency is a risk factor for the development of conditions that exhibit mitochondrial dysfunction and deregulated lipid metabolism, including MASLD[15,73]. Oxidative stress plays a key role in the multi-layered pathogenesis of MASLD, and antioxidants have the potential to combat this condition[74]. Superoxide dismutase (SOD), one of the antioxidant enzymes, depends on adequate copper availability, suggesting that reduced copper availability may eventually result in impaired antioxidant defense, increasing the risk of MASLD and its vascular complications[75].

Western diets exhibit low copper content and excess iron and fructose, suggesting that an unbalanced intake of micronutrients could exert a synergistic role in MASLD[76]. Both fructose and iron inhibit duodenal copper absorption, leading to impaired oxidant defense and increased lipid peroxidation[77,78]. Studies on copper-deficient mice fed a high fructose diet have shown increased lipogenesis[79], biochemical profile, hepatic gene expression, and liver histology consistent with MASLD and MASH independent of obesity[80]. Additionally, low copper bioavailability contributes to iron retention in MASLD[75], supporting investigations assessing the therapeutic effect of dietary copper supplementation among MASLD individuals with proven copper deficiency.

Table 3. Epidemiological evidence linking disrupted Copper homeostasis with MASLD

Author, year[Ref]	Method	Findings	Conclusion
Copper is increased			
Liggi *et al.*, 2013[27]	Comparative analysis of 35 WD patients and 44 NASH patients	Patients with severe steatosis had higher liver copper concentrations compared to those with mild and moderate steatosis. Liver copper content was positively and significantly correlated with steatosis scores	Hepatic steatosis in WD is strongly associated with copper accumulation in the liver tissue rather than with metabolic comorbidities
Guo *et al.*, 2013[48]	Blood chemistry was assessed in 30 HC, 30 NAFLD-free CHC patients, and 32 with HCV-NAFLD	Compared to individuals with CHC, those with HCV-NAFLD had higher Cu plasma concentrations of Cu	The deterioration of Cu homeostasis may reflect increased oxidative stress and inflammation
Porcu *et al.*, 2018[49]	NAFLD cirrhosis (n = 20); NAFLD-HCC (n = 9); and HC (n = 20)	Individuals with NAFLD cirrhosis had higher serum Cu concentrations than HC. Higher cupremia was found among NAFLD-HCC than in NAFLD-cirrhosis. Cu levels could be used as a valuable biomarker in differentiating HCC from cirrhosis	Cu may be involved in the progression from NAFLD cirrhosis to NAFLD-HCC
Chen *et al.*, 2021[50]	Cross-sectional survey of 3,211 subjects was conducted using data from the NHANES database. NAFLD was defined using HSI and USFLI criteria	Being in the highest quartile of Cu levels was associated with a 97% increased risk of NAFLD at LRA. The risk of Cu on NAFLD was more pronounced among middle-aged women and those with lower HOMA-IR and non-MetS compared to controls. Circulating Cu was correlated with the severity of NAFLD only in men	High serum Cu levels are significantly associated with NAFLD (particularly among women, middle-aged individuals, and those with low insulin resistance) and severe NAFLD
Zhang *et al.*, 2023[51]	A cohort-based case-control study enrolled 648 men with NAFLD and 648 NAFLD-free control men	A single metal analysis revealed significant relationships between Cu and NAFLD	Compared to single metals, exposure to mixtures of metals is associated with a higher risk of NAFLD
Hou *et al.*, 2023[52]	This cross-sectional study enrolled 5,976 subjects (of whom 3,437 had MAFLD) from the NHANES database	After adjusting for potential confounding factors, Cu intake at quartile 3 and quartile 4 was found to be associated with a decreased risk of MAFLD	High Cu intake is associated with MAFLD in the general US population
Li *et al.*, 2024[53]	This study included 1,377 participants from the NHANES 2011-2016 database. The diagnosis of NAFLD and its fibrotic progression were determined with serum biomarkers	Higher serum Cu levels were associated with an increased prevalence of NAFLD and advanced liver fibrosis. Additionally, serum Cu levels were positively associated with hypertension and overweight. Moreover, women under 60 years and those with a BMI > 24.9 kg/m^2 were the most vulnerable to the Cu-and-NAFLD association	In the U.S. population, elevated serum Cu levels due to metabolic dysfunction are associated with an increased risk of development and fibrotic progression of NAFLD
Copper is decreased			
Aigner *et al.*, 2008[54]	Retrospective study of 140 patients with NAFLD and 25 controls	Compared to controls, NAFLD patients had lower hepatic copper concentrations. NAFLD patients with low serum and liver Cu concentrations also had higher serum ferritin levels, increased histological prevalence of hepatic siderosis, and elevated hepatic iron concentrations. Additionally, these patients had lower serum concentrations of the copper-dependent ferroxidase ceruloplasmin and decreased liver FP-1 mRNA expression compared to NAFLD patients with high liver or serum Cu concentrations. In rats, a reduced dietary copper intake was associated with decreased hepatic FP-1 protein expression	NAFLD patients are often copper deficient, which is associated with increased stores of hepatic iron. This is due to impaired export of iron from the liver, caused by decreased expression of FP-1 and ceruloplasmin ferroxidase activity
Aigner *et al.*, 2010[55]	Liver and serum Cu concentrations were determined in 124 subjects with NAFLD (31 with NASH) and compared to 50 individuals with CHC, 35 with hemochromatosis, 13 with ALD, 11 with AIH, and 27 HC	Subjects with NAFLD had lower hepatic Cu concentrations compared to healthy controls and patients with other liver diseases. In NAFLD, lower liver Cu levels were correlated with steatosis, fasting glycemia, and components of MetS. Subjects with NASH had lower hepatic Cu concentrations than individuals with simple steatosis	In humans, decreased hepatic Cu concentrations are associated with NAFLD, more severe steatosis, NASH, and characteristics of the MetS
Nobili *et al.*, 2013[56]	100 children with biopsy-proven NAFLD	Decreased serum ceruloplasmin and Cu levels are associated with more severe liver	The serum antioxidant capacity is strongly associated with the

		histology	histological severity of NAFLD in children
Church *et al.*, 2015[57]	Experimental study was conducted in defatted-dried liver samples from ob/ob mice	Metal measurements in the livers showed a specific decrease in Cu levels in ob/ob mice, which is consistent with hepatic isolated Cu deficiency	Analysis of the ob/ob mouse model shows consistent signs of hepatic selective Cu deficiency
Stättermayer *et al.*, 2017[58]	174 cases of NAFLD that have been confirmed through biopsy	An inverse correlation was found between the mean% of steatotic hepatocytes and hepatic Cu content, which remained significant only in patients without MetS	In Mets-free NAFLD patients, the hepatic Cu content is associated with steatosis, while the presence of MetS seems to obscure the effects of hepatic Cu
Mendoza *et al.*, 2017[59]	An analysis was conducted on 102 individuals with biopsy-proven NAFLD compared to 48 non-NAFLD controls, all within the pediatric age group	Compared to controls, individuals with NAFLD exhibited lower levels of hepatic Cu ($P = 0.005$) and tissue Cu concentration decreased in parallel with increasing severity of steatosis ($P < 0.001$). However, Cu levels were not associated with other histological features such as fibrosis stages, lobular/portal inflammation, or hepatocyte ballooning	In pediatric liver tissue samples, Cu levels are lower in patients with NAFLD than in non-NAFLD controls and the levels are even more reduced in patients with NASH compared to simple steatosis
Fujii *et al.*, 2017[60]	A total of 196 subjects underwent pancreatoduodenectomy and computed tomography scans at 1 month, 6 months and 1 year after surgery	At the multivariate analysis, decreased serum Cu levels and female sex were identified as independent predictors of NAFLD at 1 and 6 months	NAFLD after pancreatoduodenectomy frequently occurs among women with decreased serum Cu levels
El-Rayah *et al.*, 2018[61]	This study involved 231 patients undergoing elective liver transplantation	Compared to individuals with liver disease caused by viral and autoimmune etiology, those with ALD or NAFLD had significantly lower serum ceruloplasmin and serum Cu levels	In liver transplant recipients, SLD of metabolic and alcoholic origin was associated with lower serum ceruloplasmin and Cu levels
Lee *et al.*, 2018[62]	Clinical and laboratory parameters were analyzed in 751 Korean adults who were divided into quintiles based on their hair Cu concentration	Lower hair Cu concentrations were associated with an increased risk of NAFLD and were significantly correlated with the components of metabolic syndrome. However, individuals in the lower hair Cu quintile groups exhibited a significantly higher risk of NAFLD regardless of the components of metabolic syndrome	NAFLD is associated with lower hair Cu concentration independent of metabolic syndrome
Nasr *et al.*, 2021[63]	Ultra-trace elemental analysis was utilized to determine (manganese, iron, and) Cu using ICP-SFMS in liver tissue samples from 76 subjects with chronically elevated liver tests	Patients with steatosis grade ≥ 1 had significantly lower Cu content compared to patients with G0 and hepatic Cu content was inversely correlated with steatosis grade and stereological point counting. Post hoc analysis also demonstrated significant differences in Cu levels between patients with steatosis grade ≥ 2 and those without steatosis	Patients with steatosis due to NAFLD or other CLD had reduced levels of Cu in their liver tissues, which were proportional to the increasing grade of steatosis
Lan *et al.*, 2021[64]	Case-control study including 1,816 subjects with NAFLD and 1,111 sex- and age-matched controls	After adjusting for confounding factors, the risk of NAFLD in the highest quartile of Cu compared to the lowest quartile was 0.57 (95%CI: 0.41-0.80). The protective effect of higher blood Cu increased with the severity of NAFLD. In stratified analysis, a lower Cu concentration was a significant additional factor contributing to a higher risk of NAFLD in male subjects with MetS. However, no significant association was observed between Cu and NAFLD in women with different characteristics, except for an NAFLD fibrosis score < -1.455 and moderate hepatic steatosis	Higher blood Cu levels provided significant protection against NAFLD in men but not in women, suggesting sex-specific interventions for disease prevention
Zhang *et al.*, 2022[65]	102 biopsy-proven NASH cases were compared to 102 age-, sex-, and residential city-matched controls with NAFL	Compared to matched NAFL controls, patients with NASH had significantly lower concentrations of serum Cu. This difference was more pronounced in men than in women. After accounting for confounding factors, each unit increase, standard deviation increase, and doubling of serum Cu levels were associated with approximately a 20%, 40%, and 90% decreased risk of NASH, respectively, even after adjusting for potential	Lower serum Cu concentrations are significantly associated with biopsy-proven NASH, especially in men

		confounders	
Kamada *et al.*, 2022[66]	The nutritional intake of 37 biopsy-proven NAFLD subjects was compared to the nutritional data of 5,074 controls from the NIHN	Compared to individuals with a low BMI, Cu (and vitamin E) intake was lower in NAFLD patients with a high BMI	BMI influences the dietary Cu intake in individuals with NAFLD
Xie *et al.*, 2022[67]	Experimental study in mice	Hepatic Cu deficiency mediated by ceruloplasmin upregulation is a cause of NAFLD. Deletion of ceruloplasmin restores Cu levels and alleviates hepatic steatosis through AMPK activation, promotion of mitochondrial biogenesis, and increased FA oxidation	Cu acts as a signaling molecule to improve the hepatic catabolism of lipids
Chen *et al.*, 2023[68]	Meta-analysis of six published articles	The hepatic concentration of Cu was significantly decreased in NAFLD patients. However, serum Cu and ceruloplasmin were not associated with NAFLD	NAFLD has a low hepatic Cu concentration, while serum Cu and ceruloplasmin levels are not associated with NAFLD
Tinkov *et al.*, 2024[69]	Case-control study of 140 women with NAFLD and 140 age-matched HC	Compared to controls, patients with NAFLD exhibited lower hair Cu content, which was significantly associated with circulating levels of amino acid regardless of confounders. However, no significant differences were found in cupremia between cases and HC	An upset metabolism of trace elements may play a role in the pathogenesis of NAFLD through changes in the levels of circulating amino acids
Jiang *et al.*, 2024[70]	This study was conducted on 17 individuals with NAFL, 12 with NASH, and 14 healthy controls. Additionally, a NASH mouse model was included in this study	Compared to those with NAFL or the HC, individuals with NASH had significantly higher ceruloplasmin levels. Consistently, hepatic ceruloplasmin was markedly upregulated in a mouse NASH model. Hepatocyte-specific ablation of ceruloplasmin, leading to the restoration of BA metabolism, mitigates dietary-induced NASH	Ceruloplasmin plays a key role in NASH and could potentially serve as an effective therapeutic target
Copper is neutral			
Arefhosseini *et al.*, 2022[71]	141 subjects with NAFLD diagnosed with USG	After adjusting for confounders, NAFLD was not associated with serum Cu levels or Ceruloplasmin levels	Serum Cu and ceruloplasmin levels are not associated with NAFLD
Liu *et al.*, 2024[72]	This MRA aims to clarify the causal relationships between SNPs associated with 14 circulating micronutrients, including Cu, and NAFLD. The data were obtained from a GWAS meta-analysis of 8,434 cases and 770,180 controls in the discovery stage, as well as two additional datasets: 1,483 NAFLD cases and 17,781 controls in replication stage 1, and 2,134 NAFLD cases and 33,433 controls in replication stages 2	Various genetically predicted micronutrients were significantly associated with an increased risk of NAFLD. However, Cu was not	Cu is not causally associated with NAFLD in this MRA

AIH: Autoimmune hepatitis; ALD: alcohol-related liver disease; AMPK: adenosine monophosphate-activated protein kinase; BA: bile acid; BMI: body mass index; CHC: chronic hepatitis C; CLD: chronic liver disease; Cu: copper; FA: fatty acid; FPN-1: ferroportin 1; HC: healthy controls; HCV: hepatitis C virus; HOMA-IR: homeostasis model assessment of insulin resistance; ICP-SFMS: inductively coupled plasma sector field mass spectrometry; GWAS: genome-wide association study; IR: insulin resistance; HCC: hepatocellular carcinoma; HSI: hepatic steatosis index; MAFLD: metabolic dysfunction-associated fatty liver disease; mRNA: messenger RNA; MetS: metabolic syndrome; MRA: Mendelian randomization analysis; NAFL: nonalcoholic fatty liver; NAFLD: nonalcoholic fatty liver disease; NAFL: nonalcoholic fatty liver; NASH: nonalcoholic steatohepatitis; NHANES: National Health and Nutrition Examination Survey; NIHN: National Institute of Health and Nutrition; SLD: steatotic liver disease; SMD: standardized mean differences; SNPs: single nucleotide polymorphisms; US: United States; USG: ultrasonography; USFLI: US fatty liver index; WD: Wilson's disease.

Figure 2. Schematic representation of the different pathomechanisms involved in the development and progression of in individuals with copper deficiency. MASH: Metabolic dysfunction-associated steatohepatitis.

Copper excess

A mutual relationship links excess copper with liver fibrosis in the context of MASLD. On one hand, advanced fibrosis may obscure the relationship between MASLD and copper, compromising the interpretation of study results[59]. On the other hand, excess copper may directly damage hepatocytes via the formation of ROS, severe mitochondrial dysfunction, impaired molecular and metabolic energy production, and activation of macrophages, and eventually result in the fibrotic wound-healing response that follows long-lasting liver injury irrespective of the etiology of chronic liver disease[81,82]. Excess copper induces cell death with a unique pathomechanism, named "cuproptosis"[83].

Cuproptosis features direct binding of excess copper to some lipoylated proteins within the tricarboxylic acid cycle, and this interaction induces the aggregation of copper-lipoylated mitochondrial proteins, reduced levels of iron-sulfur (Fe-S) cluster proteins, proteotoxic stress and culminates in a novel form of cell death, owing to protein toxicity stress[84]. Cuproptosis can be prevented with glutathione, which diminishes copper levels intracellularly and inhibits the aggregation of lipoylated proteins[83,85]. Therefore, the recently described cuproptosis involves an evolutionary ancient mechanism, protein lipoylation: notably, few mammalian proteins are lipoylated, and these are concentrated in the tricarboxylic acid cycle, where lipoylation is required for enzymatic function[83]. Importantly, additional studies should specifically highlight copper's role in the activation of macrophages[81]. Li *et al.* have recently identified two cuproptosis-related genes that were closely associated with MASLD[86]. However, the full spectrum of mechanisms, effector molecules, and clinical outcomes associated with cuproptosis in humans remains incompletely understood[87,88].

Recent investigations pinpoint complex cross-talks and potential associations between ferroptosis and cuproptosis, both significantly associated with mitochondrial metabolism[89]. Additionally, intermittent hypoxia, a strong risk factor for MASLD, can deplete hepatic copper reserves, inducing secondary iron deposition, ferroptosis, and progression of MASLD[90].

Ferroptosis, proposed in 2012, involves (often genetically) dysregulated iron homeostasis, unbalanced redox state (associated with the mitochondrial generation of reactive oxygen species), and eventually, iron-dependent peroxidation of phospholipids. The three major pathways of ferroptosis include iron storage, peroxidation of lipids, and depletion of cystine[91]. In humans, the main outcomes of ferroptosis include inflammation, neurodegenerative diseases, cardiometabolic and liver diseases, sepsis, and cancer[89]. Clinically, excess iron can be treated with iron chelators, which are the mainstay for treating ferroptosis-related disorders[92]. Enzymes involved in lipid synthesis, degradation, and β-oxidation are promising targets for the treatment of ferroptosis-related conditions[93] and cysteine supplementation, by increasing the availability of the antioxidant glutathione, may partially protect from ferroptosis[94].

Further to cuproptosis, another mechanism of excess copper toxicity is of potential interest to the MASLD arena [Figure 3]. It involves decreased binding of various nuclear receptors (FXR, RXR, HNF4α, and LRH-1) to their respective promoter response elements and decreased mRNA expression of nuclear receptor target genes in engineered mouse models and in WD patients of various ages[95]. These findings collectively demonstrate that copper-mediated nuclear receptor dysfunction disrupts liver function in WD and potentially in other disorders associated with increased hepatic copper levels[95]. Among the above-mentioned nuclear receptors, FXR, a bile acid sensor, modulates bile acid and energy homeostasis. It is also a master regulator of lipoprotein and glucose metabolism[96]. FXR agonists such as obeticholic acid and vonafexor play a major role in the treatment of MASLD and MASLD-associated chronic kidney disease[97].

Figure 3. Schematic representation of the potential connectors associated with MASH development and progression in the context of copper toxicity. In addition to cuproptosis, a novel pathway of cell death linked to excess copper, other pathomechanisms may be involved. These include excess oxidative stress, impaired energy homeostasis due to mitochondrial dysfunction, and macrophage activation with mechanisms that are not well understood. MASH: Metabolic dysfunction-associated steatohepatitis; FXR: farnesoid X receptor; ROS: reactive oxygen species.

CONCLUSION

Copper is a double-edged sword. It is a vital cofactor for enzymes involved in various biochemical processes and oxidative/redox balance in the bodies of all animals. However, it can also be toxic, leading to cell death even at modest intracellular concentrations[83]. An excess of copper can result in tissue injury due to oxidative stress mediated by a free-radical pathway. On the other hand, copper deficiency can impair the antioxidant defense system, leading to increased levels of ROS and oxidative damage to lipids, DNA, and proteins[98]. Therefore, a finely orchestrated balance of copper is necessary to maintain human health and prevent oxidative stress and free radical damage. Without this balance, there is a risk of developing metabolic disorders, neurodegenerative diseases, and cancer[44].

This notion is illustrated by MASLD and MASH pathobiology[99] pointing to the investigation of copper's role as a potential avenue to uncover important sex-specific pathogenic mechanisms of MASLD initiation, and fibrotic progression[49,58,79,100-102] and the development of targeted therapies that, moving beyond symptomatic treatment, address the underlying root causes of MASLD in individual patients[86].

DECLARATIONS

Authors' contributions

Made substantial contributions to the conception, design and writing of the study; performed data analysis and interpretation: Lonardo A, Weiskirchen R

Availability of data and materials

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Conflicts of interest

Lonardo A is the Editor-in-Chief of *Metabolism and Target Organ Damage*. However, he was not involved in any of the distinct phases in the editorial handling of the manuscript. Weiskirchen R declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

REFERENCES

1. Neuschwander-Tetri BA. Trace elements and the liver. In: Rodes J, Benhamou JP, Rizzetto M, Reichen J, Blei A, editors. Textbook in hepatology: from basic science to clinical practice, 3rd edition, 2007 Oxford: Blackwell. pp. 233-240. Available from: https://books.google.com/books?hl=zh-CN&lr=&id=xfpS5XMb9mgC&oi=fnd&pg=PP2&dq=Textbook+in+hepatology:+from+basic+science+to+clinical+practice,+3rd+edition,+2007+Oxford:+Blackwell&ots=DlMU6WA1eH&sig=xhvwuv-BfPRCEE9RxmNHGoaz_5Y#v=onepage&q=Textbook%20in%20hepatology%3A%20from%20basic%20science%20to%20clinical%20practice%2C%203rd%20edition%2C%202007%20Oxford%3A%20Blackwell&f=false.
2. Susnea I, Weiskirchen R. Trace metal imaging in diagnostic of hepatic metal disease. *Mass Spectrom Rev* 2016;35:666-86. DOI PubMed
3. Anderson GJ, Bardou-Jacquet E. Revisiting hemochromatosis: genetic vs. phenotypic manifestations. *Ann Transl Med* 2021;9:731. DOI PubMed PMC
4. Yang J, Hirai Y, Iida K, et al. Integrated-gut-liver-on-a-chip platform as an in vitro human model of non-alcoholic fatty liver disease. *Commun Biol* 2023;6:310. DOI PubMed PMC
5. Witt B, Schaumlöffel D, Schwerdtle T. Subcellular localization of copper-cellular bioimaging with focus on neurological disorders. *Int J Mol Sci* 2020;21:2341. DOI PubMed PMC
6. He P, Li H, Liu C, et al. U-shaped association between dietary copper intake and new-onset hypertension. *Clin Nutr* 2022;41:536-42. DOI
7. Shi Y, Hu H, Wu Z, et al. Associations between dietary copper intake and hypertriglyceridemia among children and adolescents in the US. *Nutr Metab Cardiovasc Dis* 2023;33:809-16. DOI
8. Liu Y, Tan L, Kuang Y, et al. A national cross-sectional analysis of dietary copper intake and abdominal aortic calcification in the US adults: NHANES 2013-2014. *Nutr Metab Cardiovasc Dis* 2023;33:1941-50. DOI
9. Yang L, Chen X, Cheng H, Zhang L. Dietary copper intake and risk of stroke in adults: a case-control study based on national health and nutrition examination survey 2013-2018. *Nutrients* 2022;14:409. DOI PubMed PMC
10. Stefan N, Lonardo A, Targher G. Role of steatotic liver disease in prediction and prevention of cardiometabolic diseases. *Nat Rev Gastroenterol Hepatol* 2024;21:136-7. DOI PubMed
11. Loria P, Lonardo A, Leonardi F, et al. Non-organ-specific autoantibodies in nonalcoholic fatty liver disease: prevalence and correlates. *Dig Dis Sci* 2003;48:2173-81. DOI
12. Song M, Zhou Z, Chen T, Zhang J, McClain CJ. Copper deficiency exacerbates bile duct ligation-induced liver injury and fibrosis in rats. *J Pharmacol Exp Ther* 2011;339:298-306. DOI PubMed PMC
13. Dhanraj P, Venter C, Bester MJ, Oberholzer HM. Induction of hepatic portal fibrosis, mitochondria damage, and extracellular vesicle formation in Sprague-Dawley rats exposed to copper, manganese, and mercury, alone and in combination. *Ultrastruct Pathol* 2020;44:182-92. DOI PubMed
14. Altarelli M, Ben-Hamouda N, Schneider A, Berger MM. Copper deficiency: causes, manifestations, and treatment. *Nutr Clin Pract* 2019;34:504-13. DOI PubMed
15. Morrell A, Tallino S, Yu L, Burkhead JL. The role of insufficient copper in lipid synthesis and fatty-liver disease. *IUBMB Life* 2017;69:263-70. DOI PubMed PMC
16. Członkowska A, Litwin T, Dusek P, et al. Wilson disease. *Nat Rev Dis Primers* 2018;4:21. DOI PubMed PMC
17. Roberts EA, Schilsky ML. Current and Emerging Issues in Wilson's Disease. *N Engl J Med* 2023;389:922-38. DOI PubMed
18. Ovchinnikova EV, Garbuz MM, Ovchinnikova AA, Kumeiko VV. Epidemiology of wilson's disease and pathogenic variants of the ATP7B gene leading to diversified protein disfunctions. *Int J Mol Sci* 2024;25:2402. DOI PubMed PMC
19. Stremmel W, Weiskirchen R. Therapeutic strategies in Wilson disease: pathophysiology and mode of action. *Ann Transl Med* 2021;9:732. DOI PubMed PMC
20. Dang J, Chevalier K, Letavernier E, et al. Kidney involvement in Wilson's disease: a review of the literature. *Clin Kidney J* 2024;17:sfae058. DOI PubMed PMC
21. Stremmel W, Merle U, Weiskirchen R. Clinical features of Wilson disease. *Ann Transl Med* 2019;7:S61. DOI PubMed PMC
22. Choudhury N, Quraishi SB, Atiqullah A, Khan MSI, Al Mahtab M, Akbar SM. High prevalence of wilson's diseases with low prevalence of kayser-fleischer rings among patients with cryptogenic chronic liver diseases in bangladesh. *Euroasian J Hepatogastroenterol* 2019;9:67-70. DOI PubMed PMC
23. Mak CM, Lam CW, Tam S. Diagnostic accuracy of serum ceruloplasmin in Wilson disease: determination of sensitivity and specificity by ROC curve analysis among ATP7B-genotyped subjects. *Clin Chem* 2008;54:1356-62. DOI PubMed
24. García-Villarreal L, Hernández-Ortega A, Sánchez-Monteagudo A, et al. Wilson disease: revision of diagnostic criteria in a clinical series with great genetic homogeneity. *J Gastroenterol* 2021;56:78-89. DOI
25. Fanni D, Guido M, Gerosa C, et al. Liver changes in Wilson's disease: the full spectrum. A report of 127 biopsies from 43 patients. *Eur Rev Med Pharmacol Sci* 2021;25:4336-44. DOI
26. Mahmood S, Inada N, Izumi A, Kawanaka M, Kobashi H, Yamada G. Wilson's disease masquerading as nonalcoholic steatohepatitis. *N Am J Med Sci* 2009;1:74-6. PubMed PMC
27. Liggi M, Murgia D, Civolani A, Demelia E, Sorbello O, Demelia L. The relationship between copper and steatosis in Wilson's disease. *Clin Res Hepatol Gastroenterol* 2013;37:36-40. DOI PubMed
28. Alqahtani SA, Chami R, Abuqteish D, et al. Hepatic ultrastructural features distinguish paediatric Wilson disease from NAFLD and

autoimmune hepatitis. *Liver Int* 2022;42:2482-91. DOI

29. Sobesky R, Guillaud O, Bouzbib C, et al. Non-invasive diagnosis and follow-up of rare genetic liver diseases. *Clin Res Hepatol Gastroenterol* 2022;46:101768. DOI
30. Litwin T, Bembenek J, Antos A, et al. Liver transplantation as a treatment for Wilson's disease with neurological presentation: a systematic literature review. *Acta Neurol Belg* 2022;122:505-18. DOI PubMed PMC
31. Cave V, Di Dato F, Iorio R. Wilson's disease with acute hepatic onset: how to diagnose and treat it. *Children (Basel)* 2024;11:68. DOI PubMed PMC
32. Antos A, Członkowska A, Smolinski L, et al. Early neurological deterioration in Wilson's disease: a systematic literature review and meta-analysis. *Neurol Sci* 2023;44:3443-55. DOI PubMed PMC
33. Litwin T, Antos A, Bembenek J, et al. Copper deficiency as wilson's disease overtreatment: a systematic review. *Diagnostics (Basel)* 2023;13:2424. DOI PubMed PMC
34. Kumar P, Hamza N, Madhok B, et al. Copper deficiency after gastric bypass for morbid obesity: a systematic review. *Obes Surg* 2016;26:1335-42. DOI
35. Ramani PK, Parayil Sankaran B. Menkes disease. Available from: https://europepmc.org/books/n/statpearls/article-24982/?extid=29262003&src=med.
36. Duncan A, Talwar D, McMillan DC, Stefanowicz F, O'Reilly DS. Quantitative data on the magnitude of the systemic inflammatory response and its effect on micronutrient status based on plasma measurements. *Am J Clin Nutr* 2012;95:64-71. DOI PubMed
37. Kharel Z, Kharel H, Phatak PD. Diagnosing aceruloplasminemia: navigating through red herrings. *Ann Hematol* 2024;103:2173-6. DOI PubMed
38. Corradini E, Buzzetti E, Dongiovanni P, et al. Ceruloplasmin gene variants are associated with hyperferritinemia and increased liver iron in patients with NAFLD. *J Hepatol* 2021;75:506-13. DOI
39. King D, Siau K, Senthil L, Kane KF, Cooper SC. Copper deficiency myelopathy after upper gastrointestinal surgery. *Nutr Clin Pract* 2018;33:515-9. DOI PubMed
40. Griffith DP, Liff DA, Ziegler TR, Esper GJ, Winton EF. Acquired copper deficiency: a potentially serious and preventable complication following gastric bypass surgery. *Obesity (Silver Spring)* 2009;17:827-31. DOI PubMed PMC
41. Kiela PR, Ghishan FK. Physiology of intestinal absorption and secretion. *Best Pract Res Clin Gastroenterol* 2016;30:145-59. DOI PubMed PMC
42. Wang Y, Pei P, Yang K, Guo L, Li Y. Copper in colorectal cancer: from copper-related mechanisms to clinical cancer therapies. *Clin Transl Med* 2024;14:e1724. DOI PubMed PMC
43. Horn D, Barrientos A. Mitochondrial copper metabolism and delivery to cytochrome c oxidase. *IUBMB Life* 2008;60:421-9. DOI PubMed PMC
44. Antonucci L, Porcu C, Iannucci G, Balsano C, Barbaro B. Non-alcoholic fatty liver disease and nutritional implications: special focus on copper. *Nutrients* 2017;9:1137. DOI PubMed PMC
45. Gale J, Aizenman E. The physiological and pathophysiological roles of copper in the nervous system. *Eur J Neurosci* 2024;60:3505-43. DOI PubMed
46. Cheng F, Peng G, Lu Y, et al. Relationship between copper and immunity: the potential role of copper in tumor immunity. *Front Oncol* 2022;12:1019153. DOI PubMed PMC
47. Xue Q, Kang R, Klionsky DJ, Tang D, Liu J, Chen X. Copper metabolism in cell death and autophagy. *Autophagy* 2023;19:2175-95. DOI PubMed PMC
48. Guo CH, Chen PC, Ko WS. Status of essential trace minerals and oxidative stress in viral hepatitis C patients with nonalcoholic fatty liver disease. *Int J Med Sci* 2013;10:730-7. DOI PubMed PMC
49. Porcu C, Antonucci L, Barbaro B, et al. Copper/MYC/CTR1 interplay: a dangerous relationship in hepatocellular carcinoma. *Oncotarget* 2018;9:9325-43. DOI PubMed PMC
50. Chen C, Zhou Q, Yang R, et al. Copper exposure association with prevalence of non-alcoholic fatty liver disease and insulin resistance among US adults (NHANES 2011-2014). *Ecotoxicol Environ Saf* 2021;218:112295. DOI
51. Zhang D, Wu S, Lan Y, et al. Essential metal mixtures exposure and NAFLD: A cohort-based case-control study in northern Chinese male adults. *Chemosphere* 2023;339:139598. DOI
52. Hou JZ, Wu QW, Zhang L. Association between micronutrients intake and metabolic-associated fatty liver disease: a cross-sectional study based on the National Health and Nutrition Examination Survey. *J Nutr Sci* 2023;12:e117. DOI PubMed PMC
53. Li L, Yi Y, Shu X, Li J, Kang H, Chang Y. The correlation between serum copper and non-alcoholic fatty liver disease in american adults: an analysis based on NHANES 2011 to 2016. *Biol Trace Elem Res* 2024;202:4398-409. DOI
54. Aigner E, Theurl I, Haufe H, et al. Copper availability contributes to iron perturbations in human nonalcoholic fatty liver disease. *Gastroenterology* 2008;135:680-8. DOI
55. Aigner E, Strasser M, Haufe H, et al. A role for low hepatic copper concentrations in nonalcoholic Fatty liver disease. *Am J Gastroenterol* 2010;105:1978-85. DOI
56. Nobili V, Siotto M, Bedogni G, et al. Levels of serum ceruloplasmin associate with pediatric nonalcoholic fatty liver disease. *J Pediatr Gastroenterol Nutr* 2013;56:370-5. DOI
57. Church SJ, Begley P, Kureishy N, et al. Deficient copper concentrations in dried-defatted hepatic tissue from ob/ob mice: a potential model for study of defective copper regulation in metabolic liver disease. *Biochem Biophys Res Commun* 2015;460:549-54. DOI

PubMed PMC

58. Stättermayer AF, Traussnigg S, Aigner E, et al. Low hepatic copper content and PNPLA3 polymorphism in non-alcoholic fatty liver disease in patients without metabolic syndrome. *J Trace Elem Med Biol* 2017;39:100-7. DOI
59. Mendoza M, Caltharp S, Song M, et al. Low hepatic tissue copper in pediatric nonalcoholic fatty liver disease. *J Pediatr Gastroenterol Nutr* 2017;65:89-92. DOI PubMed PMC
60. Fujii Y, Nanashima A, Hiyoshi M, Imamura N, Yano K, Hamada T. Risk factors for development of nonalcoholic fatty liver disease after pancreatoduodenectomy. *Ann Gastroenterol Surg* 2017;1:226-31. DOI PubMed PMC
61. El-Rayah EA, Twomey PJ, Wallace EM, McCormick PA. Both α-1-antitrypsin Z phenotypes and low caeruloplasmin levels are over-represented in alcohol and nonalcoholic fatty liver disease cirrhotic patients undergoing liver transplant in Ireland. *Eur J Gastroenterol Hepatol* 2018;30:364-7. DOI PubMed
62. Lee SH, Kim MJ, Kim YS, et al. Low hair copper concentration is related to a high risk of nonalcoholic fatty liver disease in adults. *J Trace Elem Med Biol* 2018;50:28-33. DOI
63. Nasr P, Ignatova S, Lundberg P, Kechagias S, Ekstedt M. Low hepatic manganese concentrations in patients with hepatic steatosis - a cohort study of copper, iron and manganese in liver biopsies. *J Trace Elem Med Biol* 2021;67:126772. DOI PubMed
64. Lan Y, Wu S, Wang Y, et al. Association between blood copper and nonalcoholic fatty liver disease according to sex. *Clin Nutr* 2021;40:2045-52. DOI
65. Zhang H, Zheng KI, Zhu PW, et al. Lower serum copper concentrations are associated with higher prevalence of nonalcoholic steatohepatitis: a matched case-control study. *Eur J Gastroenterol Hepatol* 2022;34:838-43. DOI
66. Kamada Y, Takahashi H, Ogawa Y, et al; Japan study group of NAFLD (JSG-NAFLD). characterization of nutrient intake in biopsy-confirmed NAFLD patients. *Nutrients* 2022;14:3453. DOI PubMed PMC
67. Xie L, Yuan Y, Xu S, et al. Downregulation of hepatic ceruloplasmin ameliorates NAFLD via SCO1-AMPK-LKB1 complex. *Cell Rep* 2022;41:111498. DOI PubMed PMC
68. Chen Y, Wu C, Li G, Wang W, Tang S. Comparison of copper concentration between non-alcoholic fatty liver disease patients and normal individuals: a meta-analysis. *Front Public Health* 2023;11:1095916. DOI PubMed PMC
69. Tinkov AA, Korobeinikova TV, Morozova GD, et al. Association between serum trace element, mineral, and amino acid levels with non-alcoholic fatty liver disease (NAFLD) in adult women. *J Trace Elem Med Biol* 2024;83:127397. DOI
70. Jiang Q, Wang N, Lu S, et al. Targeting hepatic ceruloplasmin mitigates nonalcoholic steatohepatitis by modulating bile acid metabolism. *J Mol Cell Biol* 2024:15. DOI PubMed PMC
71. Arefhosseini S, Pouretedal Z, Tutunchi H, Ebrahimi-Mameghani M. Serum copper, ceruloplasmin, and their relations to metabolic factors in nonalcoholic fatty liver disease: a cross-sectional study. *Eur J Gastroenterol Hepatol* 2022;34:443-8. DOI PubMed
72. Liu K, Chen Y, Chen J, et al. Genetically determined circulating micronutrients and the risk of nonalcoholic fatty liver disease. *Sci Rep* 2024;14:1105. DOI PubMed PMC
73. Wei Y, Rector RS, Thyfault JP, Ibdah JA. Nonalcoholic fatty liver disease and mitochondrial dysfunction. *World J Gastroenterol* 2008;14:193-9. DOI PubMed PMC
74. Ma Y, Lee G, Heo SY, Roh YS. Oxidative stress is a key modulator in the development of nonalcoholic fatty liver disease. *Antioxidants (Basel)* 2021;11:91. DOI PubMed PMC
75. Aigner E, Weiss G, Datz C. Dysregulation of iron and copper homeostasis in nonalcoholic fatty liver. *World J Hepatol* 2015;7:177-88. DOI PubMed PMC
76. Harder NHO, Hieronimus B, Stanhope KL, et al. Effects of dietary glucose and fructose on copper, iron, and zinc metabolism parameters in humans. *Nutrients* 2020;12:2581. DOI PubMed PMC
77. Song M, Schuschke DA, Zhou Z, et al. High fructose feeding induces copper deficiency in Sprague-Dawley rats: a novel mechanism for obesity related fatty liver. *J Hepatol* 2012;56:433-40. DOI PubMed PMC
78. Troost FJ, Brummer RJ, Dainty JR, Hoogewerff JA, Bull VJ, Saris WH. Iron supplements inhibit zinc but not copper absorption in vivo in ileostomy subjects. *Am J Clin Nutr* 2003;78:1018-23. DOI
79. Wei X, Song M, Yin X, et al. Effects of dietary different doses of copper and high fructose feeding on rat fecal metabolome. *J Proteome Res* 2015;14:4050-8. DOI PubMed PMC
80. Tallino S, Duffy M, Ralle M, Cortés MP, Latorre M, Burkhead JL. Nutrigenomics analysis reveals that copper deficiency and dietary sucrose up-regulate inflammation, fibrosis and lipogenic pathways in a mature rat model of nonalcoholic fatty liver disease. *J Nutr Biochem* 2015;26:996-1006. DOI PubMed PMC
81. Weiskirchen R. Targeting copper to combat macrophage-driven inflammation: a potential advanced therapeutic strategy. *Signal Transduct Target Ther* 2023;8:339. DOI PubMed PMC
82. Ballestri S, Mantovani A, Girolamo MD, Baldelli E, Capitelli M, Lonardo A. Liver fibrosis in nonalcoholic fatty liver disease patients: noninvasive evaluation and correlation with cardiovascular disease and mortality. *Metab Target Organ Damage* 2023;3:1. DOI
83. Tsvetkov P, Coy S, Petrova B, et al. Copper induces cell death by targeting lipoylated TCA cycle proteins. *Science* 2022;375:1254-61. DOI PubMed PMC
84. Li SR, Bu LL, Cai L. Cuproptosis: lipoylated TCA cycle proteins-mediated novel cell death pathway. *Signal Transduct Target Ther* 2022;7:158. DOI PubMed PMC
85. Xiong C, Ling H, Hao Q, Zhou X. Cuproptosis: p53-regulated metabolic cell death? *Cell Death Differ* 2023;30:876-84. DOI

PubMed PMC

86. Li J, Zhang Y, Ma X, Liu R, Xu C, He Q, Dong M. Identification and validation of cuproptosis-related genes for diagnosis and therapy in nonalcoholic fatty liver disease. *Mol Cell Biochem* 2024. DOI PubMed
87. Li Y, Qi P, Song SY, et al. Elucidating cuproptosis in metabolic dysfunction-associated steatotic liver disease. *Biomed Pharmacother* 2024;174:116585. DOI
88. Qu J, Wang Y, Wang Q. Cuproptosis: potential new direction in diabetes research and treatment. *Front Endocrinol (Lausanne)* 2024;15:1344729. DOI PubMed PMC
89. Liu N, Chen M. Crosstalk between ferroptosis and cuproptosis: From mechanism to potential clinical application. *Biomed Pharmacother* 2024;171:116115. DOI PubMed
90. Wang R, Lv Y, Ni Z, et al. Intermittent hypoxia exacerbates metabolic dysfunction-associated fatty liver disease by aggravating hepatic copper deficiency-induced ferroptosis. *FASEB J* 2024;38:e23788. DOI
91. Feng S, Tang D, Wang Y, et al. The mechanism of ferroptosis and its related diseases. *Mol Biomed* 2023;4:33. DOI PubMed PMC
92. Chen Y, Li X, Wang S, Miao R, Zhong J. Targeting iron metabolism and ferroptosis as novel therapeutic approaches in cardiovascular diseases. *Nutrients* 2023;15:591. DOI PubMed PMC
93. Zechner R, Zimmermann R, Eichmann TO, et al. FAT SIGNALS--lipases and lipolysis in lipid metabolism and signaling. *Cell Metab* 2012;15:279-91. DOI PubMed PMC
94. Homma T, Osaki T, Kobayashi S, Sato H, Fujii J. d-Cysteine supplementation partially protects against ferroptosis induced by xCT dysfunction via increasing the availability of glutathione. *J Clin Biochem Nutr* 2022;71:48-54. DOI PubMed PMC
95. Wooton-Kee CR, Jain AK, Wagner M, et al. Elevated copper impairs hepatic nuclear receptor function in Wilson's disease. *J Clin Invest* 2015;125:3449-60. DOI PubMed PMC
96. Ballestri S, Nascimbeni F, Romagnoli D, Baldelli E, Lonardo A. The role of nuclear receptors in the pathophysiology, natural course, and drug treatment of NAFLD in humans. *Adv Ther* 2016;33:291-319. DOI PubMed
97. Lonardo A. Association of NAFLD/NASH, and MAFLD/MASLD with chronic kidney disease: an updated narrative review. *Metab Target Organ Damage* 2024;4:16. DOI
98. Hordyjewska A, Popiołek Ł, Kocot J. The many "faces" of copper in medicine and treatment. *Biometals* 2014;27:611-21. DOI PubMed PMC
99. Ma C, Han L, Zhu Z, Heng Pang C, Pan G. Mineral metabolism and ferroptosis in non-alcoholic fatty liver diseases. *Biochem Pharmacol* 2022;205:115242. DOI
100. Song M, Li X, Zhang X, et al. Dietary copper-fructose interactions alter gut microbial activity in male rats. *Am J Physiol Gastrointest Liver Physiol* 2018;314:G119-30. DOI PubMed PMC
101. Song M, Vos MB, McClain CJ. Copper-fructose interactions: a novel mechanism in the pathogenesis of NAFLD. *Nutrients* 2018;10:1815. DOI PubMed PMC
102. Song M, Yuan F, Li X, et al. Analysis of sex differences in dietary copper-fructose interaction-induced alterations of gut microbial activity in relation to hepatic steatosis. *Biol Sex Differ* 2021;12:3. DOI PubMed PMC