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Review

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# Mineral metabolism and Ferroptosis in Non-Alcoholic Fatty Liver Diseases

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Chenhui Ma [a,b,c], Li Han [b,c], Zheying Zhu [a,*], Cheng Heng Pang [a,*], Guoyu Pan [b,c,*]

[a] Department of Chemical and Environmental Engineering, University of Nottingham Ningbo China, Ningbo 315100, China

[b] Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai 201203, China

[c] University of Chinese Academy of Sciences, Beijing 100049, China

[d] Division of Molecular Therapeutics & Formulation, School of Pharmacy, The University of Nottingham, University Park Campus, Nottingham NG7 2RD, U.K

* Zheying Zhu

Room C04 Boots Science Building

The University of Nottingham

Nottingham NG7 2RD

Nottingham, UK

Email: Zheying.Zhu@nottingham.ac.uk

*Cheng Heng Pang

Room 332, Sir Peter Mansfield Building

University of Nottingham Ningbo China

199 Taikang East Road

Ningbo, China

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Email: chengheng.pang@nottingham.edu.cn

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*Guoyu Pan

Room 1715

Shanghai Institute of Materia Medica, Chinese Academy of Sciences

501 Haike Road

Shanghai, China

**Email:** gypan@simm.ac.cn

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**Abstract**

Nonalcoholic fatty liver disease (NAFLD) has become the most prevalent chronic liver disease worldwide. Minerals including iron, copper, zinc, and selenium, fulfil an essential role in various biochemical processes. Moreover, the identification of ferroptosis and cuproptosis further underscores the importance of intracellular mineral homeostasis. However, perturbation of minerals has been frequently reported in patients with NAFLD and related diseases. Interestingly, studies have attempted to establish an association between mineral disorders and NAFLD pathological features, including oxidative stress, mitochondrial dysfunction, inflammatory response, and fibrogenesis. In this review, we aim to provide an overview of the current understanding of mineral metabolism (i.e., absorption, utilization, and transport) and mineral interactions in the pathogenesis of NAFLD. More importantly, this review highlights potential therapeutic strategies, challenges, future directions for targeting mineral metabolism in the treatment of NAFLD.

**Keywords**

Mineral, iron, ferroptosis, copper, non-alcoholic fatty liver disease, non-alcoholic steatohepatitis

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## Abbreviations

Non-alcoholic steatohepatitis (NASH)，Nonalcoholic fatty liver disease, NAFLD; non-alcoholic steatohepatitis, NASH; Reactive oxygen species, ROS; Superoxide dismutase, SOD; glutathione peroxidase, GSH-Px; Tumor growth factor-β1, TGF-β1; High-fat diet, HFD; Choline-deficient and methionine-supplemented diet, CDE; Dysmetabolic iron overload syndrome, DIOS; Heme Oxygenase-1, HO-1; Ferroportin, FPN; Transferrin, Tf; Hephaestin, HEPH; Ceruloplasmin, Cp; Transferrin receptor 1, Tfr1; Transferrin receptor 1, Tfr2; Hepcidin, HAMP; Matriptase-2, MT-2; Hemojuvelin, HJV; Non-transferrin-bound iron, NBTI; Zrt- and Irt-like protein 8, ZIP8; Zrt- and Irt-like protein 14, ZIP14; Poly r(C) binding protein 1/2, PCBP1/2; labile iron pool, LIP; Iron-regulatory proteins, IRP; iron-responsive elements, IRE; Untranslated region, UTR; Nuclear receptor coactivator 4, NCOA4; BMP-binding endothelial regulator, BMPER; Liver sinusoidal endothelial cells, LSECS; Methionine/choline-deficient diet, MCD; Deferoxamine, DFO; Deferiprone, DFP; deferasirox, DFX; microRNAs, miRNAs; Cytochrome c oxidase, COX; Copper transporter 1, Cu chaperone for Cu/Zn superoxide dismutase, CCS; Antioxidant 1 Copper Chaperone, Atox1; CTR1; ATPase copper transporting alpha, ATP7A; Cu-transporting ATPases beta, ATP7B; trans-Golgi network, TGN; Metallothionein, MT; Epigallocatechin gallate, EGCG; Peroxisome proliferator-activated receptor alpha, PPAR-α; Insulin-like growth factor-1, IGF-1;

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## 1. Introduction

Nonalcoholic fatty liver disease (NAFLD) is recognized as the liver manifestation of metabolic syndrome. Its main characteristic is excessive lipid accumulation (≥ 5%) in hepatocytes in the absence of excessive alcohol consumption [1]. It has been estimated that 24% of the global adult population might develop NAFLD [2]. NAFLD encompasses a broad spectrum of disease severity, from simple steatosis to a more aggressive form, nonalcoholic steatohepatitis (NASH), with three histological hallmarks of steatosis, lobular inflammation and hepatocyte ballooning [3]. Unfortunately, NAFLD and NASH might soon replace hepatic viruses as the dominant contributors to end-stage liver diseases and hepatocellular carcinoma (HCC) [4].

The pathogenesis mechanism of NAFLD is complex. Oxidative stress is considered to be the main contributor to the progression from steatosis to NASH. Oxidative stress reflects an imbalance between reactive oxygen species (ROS) production and antioxidant defense. ROS are highly reactive molecules, and excessive ROS can damage cell structures such as DNA, lipids, and proteins. Antioxidant defense includes enzymatic antioxidants such as superoxide dismutase (SOD), catalase, and glutathione peroxidase (GSH-Px) and small molecules such as vitamin c and GSH [5]. However, a highly oxidative environment aggravates inflammation, fibrogenesis, hepatocyte death, and the pathologic progression of NAFLD [6].

Emerging studies have found a deterioration of mineral homeostasis in NAFLD or NASH patients [7]. Minerals, including iron, copper, zinc, and selenium, are essential

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for the oxidative/redox balance in the body since they are indispensable cofactors for multiple oxidant or antioxidant enzymes. Although the contribution of mineral imbalance to oxidative stress is well understood, few studies have reported mineral metabolism-targeted therapeutic agents in NAFLD. In this review, we will highlight the contribution of minerals to NAFLD progression, with particular regard to iron, copper, zinc, selenium, and ferroptosis (iron-dependent regulatory cell death). The key players in mineral homeostasis and the potential pharmacokinetic applications will also be discussed. Overall, this may provide new insights into developing a treatment strategy for this liver disease, which is often regarded as difficult to treat.

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## 2. Iron

### 2.1 Disturbance of iron homeostasis in NAFLD

Iron is the most abundant transition metal required for erythropoiesis and other fundamental cellular processes, such as mitochondrial respiration, DNA replication, and host defense [8]. Thus, iron homeostasis is critical, and its dysregulation is found in many diseases. For example, hepatic iron deposition may be detected in patients with chronic hepatitis C, alcoholic fatty liver disease, NAFLD, and end-stage liver diseases [9]. In contrast, iron deficiency anaemia is a common complication of severe illnesses due to chronic haemorrhage[10].

Ferroptosis is a recently defined form of programmed cell death driven by iron-dependent lipid peroxidation that causes damage to mitochondrial membrane and ultimately leads to cell death [11]. Intracellular iron overload and uncontrollable lipid

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peroxidation are two critical events in ferroptosis [8]. Notably, it has been increasingly recognized that ferroptosis plays an essential role in the pathogenesis of liver diseases, including NAFLD and NASH [12].

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Hepatic iron accumulation may cause hepatocyte injury and mitochondrial stress. Excessive iron contributes to free radical and ROS generation via Fenton's reaction. In addition, iron deposits in the liver and adipose tissue promote insulin resistance, the most common abnormality in metabolic disorders [13] Iron also contributes to inflammatory responses, alters the macrophage polarization, directly activates hepatic stellate cells, and induces fibrogenesis via tumour growth factor-β1 (TGF-β1) [14]. Thus, iron metabolism is closely associated with hepatic inflammation, oxidative stress, and fibrogenesis.

In fact, many studies have found iron disorder and ferroptosis in the early stage of experimental NAFLD. For example, Tsuchiya et al. showed that hepatic iron content and oxidative stress significantly increased even two weeks earlier than typical NAFLD biomarkers such as hepatic fatty acid accumulation and insulin resistance [15]. Another study reported the harmful free-iron accumulation and altered iron homeostasis after only five weeks of feeding a high-fat diet (HFD) [16]. In addition, the amounts of oxidized phosphatidylethanolamines (PEs), one class of phospholipids that drives the execution of ferroptosis, increased as early as 18 h post-choline-deficient and methionine-supplemented diet (CDE) feeding [17]. Furthermore, ferroptosis inhibitors could almost reverse hepatic death, inflammation, and oxidized PE [17]. Hence, in NASH, ferroptosis may trigger hepatocyte death and initiate hepatic inflammation and

steatohepatitis [17]. In addition, some critical regulators of iron metabolism, such as ferritin and hepcidin, also serve as acute phase reactants in response to inflammatory cytokines [18]. Overall, the concept of "multiple hits" in the progression of NAFLD might consider the contribution of impaired iron metabolism.

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### 2.2 The impacts of NAFLD and NASH on iron metabolism

Disturbed iron homeostasis has also been reported in patients. More than 30% of patients with NAFLD and metabolic syndrome were estimated to develop hyperferritinemia with mostly mild hepatic iron deposits (50~150 μmol/g). These symptoms could be described by the term "dysmetabolic iron overload syndrome (DIOS)" [19]. Other manifestations of iron dysfunction include dysregulated hepcidin expression, high transferrin saturation, and altered systemic iron levels. However, there are also some debates about iron status and the alteration of iron regulators in NAFLD. In the following sections, we will summarize the systemic and intracellular iron metabolism under normal physiological conditions and in NAFLD and NASH and explore the potential origin of the iron disorder.

#### 2.2.1 Iron metabolism under physiological conditions

##### 2.2.1.1 Systemic iron metabolism

Iron homeostasis is maintained at both the cellular and organ levels (Figure 1). Systemic metabolism is a closed system, including absorption (mainly in the duodenum), utilization (erythropoiesis), storage (mainly in the liver), recycling (in the spleen and reticuloendothelial system, macrophages), and regulation (hepcidin-ferroportin axis).

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Approximately 1-2 mg of iron is lost through bleeding and by shedding of skin cells [20]. A typical cycle of iron metabolism is as follows:

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Dietary nonheme iron, typically in the ferric form ($Fe^{3+}$, 90%), is reduced to ferrous ($Fe^{2+}$) by reductases such as duodenal cytochrome b (Dcytb) or six-transmembrane epithelial antigen of the prostate 2 (STEAP2) and transported across the apical membrane mainly via divalent metal transporter 1 (DMT1) [21, 22]. Heme-bound iron is absorbed via the vesicular transport system and haem transporters, and free iron ($Fe^{2+}$) is released by degradation via heme oxygenase-1 (HO-1) [23]. Once entering the enterocyte, $Fe^{2+}$ can be stored or exported on the basolateral side by ferroportin (FPN).

Iron is transported through the circulation by binding to transferrin (Tf). However, transferrin has a higher affinity for $Fe^{3+}$, so oxidation is required before its exporting. Ferroxidases such as hephaestin (HEPH, a multicopper enzyme) and ceruloplasmin (Cp, also a multicopper enzyme) are involved in this step. Tf has two iron-binding sites, and Tf saturation reflects the amount of transferrin iron-binding sites occupied, a helpful indicator of iron availability. Transferrin receptor 1 (TfR1, in most cells) and transferrin receptor (TfR2, mainly in hepatocytes) mediate the uptake of Tf-bound iron [24]. Iron is utilized in the body in several ways, mainly for the production of haem heme proteins (haemoglobin, myoglobin) and for the formation of iron-containing enzymes. Iron in senescent erythrocytes is recycled by the phagocytosis of specialized macrophages in the liver and spleen [25].

Hepcidin (HAMP) is the master hormone controlling systemic iron homeostasis,

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similar to insulin in glucose metabolism. Hepcidin is synthesized mainly by hepatocytes. FPN is currently the known mammalian iron exporter [26]. Hepcidin modulates the systemic flux by inducing the intestinal duodenum to degrade FPN and limit absorption, hepatocytes to limit stored iron release, and iron-recycling macrophages to limit recycling [25]. Two signaling pathways mainly regulate the expression of hepcidin, the BMP/SMAD pathway in response to iron status and the IL-6/JAK/STAT3 pathway in response to inflammation [27].

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**2.2.1.2 Cellular iron metabolism**

At the cellular level, iron homeostasis is achieved by various transporters and regulatory proteins to ensure that iron is transported to the appropriate site while minimize oxidative toxicity (Figure 1). Cells acquire iron in four ways, and the major mechanism depends on Tf. As discussed above, Tf-bound $Fe^{3+}$ is accepted by TfR, followed by the internalization of the whole complex. $Fe^{3+}$ is released under acidic conditions in the endosome and reduced to $Fe^{2+}$ to be exported by DMT1 or ZIP14. Tf and TfR are recycled back to the cell surface. In addition to Tf-mediated transport, the uptake of non-transferrin-bound iron (NBTI) or “free iron” is a substantial alternative pathway, especially when Tf is overwhelmed. Several transporters could facilitate NTBI uptake, including DMT1, Zrt- and Irt-like protein 8 (ZIP8), or Zrt- and Irt-like protein 14 (ZIP14) [28]. The third and fourth pathways involve the formation of iron-containing ferritin or haem and haemoglobin [29]. Released iron, either from ferritin degradation or heme, is imported into the cytosol for further usage.

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Cytosolic iron is routed for utilization, storage, or export with the help of an iron chaperone, poly r(C) binding protein 1/2 (PCBP1/2). PCBP1 coordinates and delivers iron to several iron-containing enzymes or ferritin, while PCBP2 can interact with DMT1 and FPN to regulate the iron distribution [30]. Iron is also destined for mitochondria via mitoferrin 1 and 2, where iron incorporates the synthesis of haem and Fe-S clusters [31]. In addition, there are two major forms of iron storage, ferritin (stable form) and the labile iron pool (LIP, unbound iron, active form). Since excessive LIP is the primary source of intracellular oxidative stress, most intracellular iron is stored as ferritin [29]. In some cases of severe iron overload, ferritin is saturated, and iron storage in haemosiderin is increased [32]. Haemosiderin-bound ferric iron is insoluble and can be stained by Prussian blue, an indicator of iron deposition [33]. Iron export is mediated by FPN, as discussed above.

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The iron-regulatory protein (IRP)/iron-responsive element (IRE) system controls the posttranscription of crucial players such as DMT1, TFR1, ferritin, and FPN [34]. For example, IRP1 could bind to the IRE located in the 5' untranslated region (UTR) of mRNA to inhibit translation initiation, whereas binding to the 3' UTR promotes translation [35]. The binding of IRPs to IRE is, in turn, regulated by the intracellular iron level. For instance, in iron-sufficient or overloaded cells, IRP1 binds to the 3'UTR of ferritin and FPN and thus contributes to iron exportation and storage [36].

Some novel regulatory pathways have been defined recently. For example, nuclear receptor coactivator 4 (NCOA4) mediates the autophagic degradation of ferritin [37]. However, iron regulation is delicate and complex. Fully understanding the mechanism,

especially at the level of cells and cellular organelles such as mitochondria, remains a further challenge.

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### 2.2.2 Dysregulated iron metabolism in NAFLD and NASH

#### 2.2.2.1 Intestinal iron absorption

Increased intestinal iron absorption is observed in some NAFLD patients. One study [38] suggested that the potential explanation was associated with elevated DMT1 expression by IRP1 activation in the duodenum. Meanwhile, the increased absorption was correlated with the grade of hepatic iron deposition and liver damage [38]. Higuchi et al. further indicated that intestinal DMT1 and TfR1 mRNA expression was enhanced by chronic inflammatory attack in NASH [39]. However, the duodenal expression of the iron exporter, FPN, may remain unchanged [38] or be suppressed [40] in response to increased hepcidin in NAFLD patients with iron overload.

#### 2.2.2.2 Iron traffic

Tf is approximately 30% saturated with iron in healthy individuals, but the saturation may increase with a reduced Tf level in liver diseases [33]. Low serum Tf levels are related to a poor prognosis in severe clinical conditions, such as liver cirrhosis [41]. Accordingly, Tf saturation is unchanged or mildly elevated to 45–50% in NAFLD [42]. Elevated hepatic iron content could be attributed to upregulation of the influx transporters TfRs and DMT1 or downregulation of the efflux transporter FPN in NAFLD [16, 43-45]. One explanation is the time-dependent activation of IRP1 in the liver [16]. Once Tf saturation exceeds 75%, NTBI accumulates [46]. A previous study

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demonstrated that most NTBI was assimilated by the liver [47]. Therefore, toxic NTBI accumulates in hepatocytes, leading to oxidative stress and dysfunction. One recent study reported that the hepatic expression of ZIP14 is significantly higher in liver cirrhosis patients than in controls [48]. Furthermore, conditional depletion of hepatic SLC39A14 (the encode gene for ZIP14) can reduce hepatic iron levels and rescue livers from ferroptosis-mediated liver fibrosis by either a high-iron diet or $CCl_4$ injection [48].

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The iron chaperones PCBP 1 and 2 are critical for restricting iron toxicity and bridging the interaction between iron transporters and regulators. A study has shown that the hepatocyte-specific depletion of PCBP1 resulted in defects in iron homeostasis [49]: mice spontaneously developed hepatic steatosis, inflammation, and degeneration, probably due to excessive lipid peroxidation and ferroptosis [49]. Indeed, other studies also found that PCBP-knockout suppressed iron delivery to ferritin and increased LIP in human cells [50]. In addition, PCBP2 may act as a "gate-keeper" for intracellular iron trafficking. PCBP2 silencing suppressed DMT1-mediated iron uptake and FPN-dependent iron export [30]. Some publications revealed that PBCP 1/2 proteins were associated with tumour invasion and a poor prognosis [51] but the role of PCBP1/2 in the pathogenesis of chronic liver diseases remains largely unexplored.

Ferrireductases (such as Dcytb and the Steap family members) and ferroxidase (HEPH and Cp) orchestrate to efficiently direct flexible valence conversion. However, their activities are likely to be altered in NAFLD. For example, the hepatic expression of STEAP4 protein was significantly impaired in NAFLD patients and HFD-induced mice [52]. Meanwhile, recombinant FGF21 might ameliorate hepatic iron and lipid

accumulation through STEAP4-mediated upregulation of FPN [53]. In addition, one study reported that mice lacking HEPH and Cp accumulated excessive iron in various tissues, including the duodenum, liver, and heart [54]. Similarly, both paediatric and adult NAFLD patients with lower Cp concentrations had an increased incidence of more severe liver damage and hepatic iron deposits [55]. One clinical study also demonstrated that Cp was most easily -affected among the non-HFE genes (HFE is the most common risk gene for inherited iron overload), and Cp variants were associated with hyperferritinemia and iron deposition in NAFLD [56].

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**2.2.2.3 Iron recycling and usage**

NAFLD is also linked with disrupted iron recycling and utilization. For example, the phagocytosis of erythrocytes was increased in HFD-fed rabbits due to a higher proportion of fragile erythrocytes and elevated HO-1 expression [57]. Erythrocyte aggregates could be observed in patient specimens [57]. However, there are contradictions regarding the role of HO-1 in iron metabolism. The Nrf2/HO-1 antioxidant pathway is one of the most important pathways to defend cells against oxidative stress. Studies have reported decreased expression of downstream genes of the Nrf2 pathway, including HO-1 and glutathione peroxidase 4 (GPX4), in an HFD-induced NAFLD model [58, 59]. In addition, since mitochondria are the primary consumers of intracellular iron, mitochondrial dysfunction is a critical risk factor for iron imbalance and ferroptosis. Mitoferrin 1/2 can be a potential drug target [60]. However, few studies have linked mitoferrin 1/2 with NAFLD and chronic liver diseases.

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#### 2.2.2.4 Iron Storage

Increased ferritin levels are often detected in preclinical studies and NAFLD patients [43, 53]. Therefore, researchers applied the serum ferritin level to diagnose and predict NAFLD and fibrosis [61]. In addition to its contribution to storage, recent studies proposed that ferritin degradation (or ferritinophagy) led to the release of iron into the LIP [62]. The process requires NCOA4 as a cargo receptor. Thus, suppressing ferroptosis, such as restricting NCOA4, may prevent hepatocytes from iron overload or ferroptosis.

#### 2.2.2.5 Iron Regulation

Multiple studies have reported hepcidin disturbance in NAFLD [63]. However, its serum level and hepatic expression varied across studies. According to some studies, NAFLD patients with iron overload had a higher serum hepcidin level [38]. In addition, chronic inflammation in severe obesity and metabolic syndrome is also associated with elevated hepcidin levels [64]. Hormones such as leptin and insulin [45], ER stress signaling [65], and lipid dysmetabolism [63] contribute to the increased hepcidin level in NAFLD. Therefore, hepcidin is either activated in response to increased serum iron levels or metabolic stress in NAFLD [66].

However, the elevated hepcidin might still be unable to restrain iron in response to an iron load, namely, the hepcidin resistance state. Hepcidin resistance describes impaired hepcidin activity more than disabled hepcidin production [19]. Some papers deduced that the underlying mechanism was decreased sensitivity to cellular iron change and the

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subsequently delayed hepcidin response, or that the elevated hepcidin levels were still insufficient to deal with massive amounts of iron [67]. Moreover, the downregulation of hepatic hepcidin was also found in some cases. Hasebe et al. found that in HFD-fed mice, BMP-binding endothelial regulator (BMPER) secreted by the liver sinusoidal endothelial cells (LSECs) decreased the mRNA expression of HAMP by suppressing SMAD phosphorylation [68]. In addition, impaired hepatocytes may not be able to secrete sufficient hepcidin.

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Other signaling molecules usually converge on the regulation of hepcidin. In addition to BMPER, other BMP isoforms are also involved [69]. Arndt et al. showed that a methionine/choline-deficient diet (MCD) caused a more obvious hepatic inflammation and steatosis in BMP6-deficient mice and even more severe hepatic iron accumulation and lower hepcidin expression [70]. Haemojuvelin (HJV) is a BMP coreceptor implicated in the modulation of hepcidin expression. The BMP/SAMD pathway, in concert with HJV, is an important part of the iron-sensing mechanism in the liver [71]. Therefore, some studies have suggested that insufficient HJV is responsible for hepatic iron accumulation in NAFLD due to ineffective iron sensing [72]. Matriptase-2 (MT-2) mediates the cleavage of HJV [73]. To explore the implications of MT-2 in NAFLD, one study established MT-2-KO mice and found that the depletion of MT-2 could rescue obesity and typical NAFLD symptoms [74]. However, this beneficial effect was blocked by the downregulation of hepcidin and restored iron levels after administering an anti-HJV antibody [74].

In conclusion, perturbations in different iron homeostasis stages have been reported in

NAFLD and NASH (Figure 2), which may lead to iron overload and hepatocyte ferroptosis, and eventually aggregate the transition from steatosis to NASH. The potential mechanism is complex and deserves further exploration since the regulation of iron metabolism would greatly benefit NAFLD therapy based on the above discussion.

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### 2.3 Targeting iron metabolism provides therapeutic approaches for NAFLD/NASH

#### 2.3.3 Targeting iron availability

Intestinal iron absorption is critical for maintaining homeostasis, but increased iron uptake has been reported in NAFLD patients. Therefore, restricting dietary iron supplements or inhibiting iron absorption could be a potential therapeutic option for iron overload diseases[75]. Hence, several DMT1 inhibitors have been designed [76] , but their efficacy is still awaiting elucidation, especially in NAFLD-related liver diseases. In addition, protecting intestinal epithelial cells from inflammatory injury [39] and maintaining gut microbiome homeostasis [77] are potential therapeutic strategies.

Iron chelators restrict systemic iron availability and diminish iron toxicity. Currently, available iron chelators include deferoxamine (DFO), deferiprone (DFP), and deferasirox (DFX). Furthermore, some researchers have investigated the potential of iron chelators in treating NAFLD, NASH, and other liver diseases. Xue et al. reported that the intraperitoneal injection of DFO can reduce hepatic iron concentrations and improve proteins related to lipid metabolism [78]. They found that DFO could protect

hepatocytes from apoptosis and inhibit hepatic steatosis in ob/ob mice [78]. Additionally, iron chelating agents are ferroptosis inhibitors. In the MCD-induced NAFLD rat model, researchers found that the administration of DFO or ferroptosis inhibitors could suppress liver inflammation, lipid ROS accumulation, and lipid droplet formation. In contrast, an injection of RSL-3 (a ferroptosis inducer) exerted the opposite effects [79, 80]. Similar conclusions could be obtained from another CDE diet-induced NASH model [17]. In addition, iron loading may polarize hepatic macrophages to a proinflammatory phenotype [81], but DFO could attenuate iron-mediated M1 activation and help to maintain a steady-state, according to an *in vitro* study [14].

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### 2.3.4 Targeting iron transport and utilization

Lactoferrin belongs to the transferrin family and is responsible for iron transport. Some studies have reported its modulatory effects on lipid metabolism and the oxidative response in NAFLD animals [82]. Furthermore, one recent study demonstrated that an improvement of iron balance could explain the protective effect of lactoferrin by targeting the hepcidin-FPN axis [44]. Iron egress from hepatocytes could be accelerated by promoting FPN expression while suppressing DMT1 and TfR. The mechanism is similar to FGF21 therapy in the NAFLD model [53]. In addition, restoring the function of ceruloplasmin and hephaestin in enterocytes might reduce iron availability.

ZIP14 and ZIP8, the central players mediating the transport of NTBI, represent appealing targets in iron disorder. One study on skeletal muscle showed a decreased Tfr1 level and an elevated ZIP14 protein level in aged mice compared to young

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mice[83]. They further revealed that disrupted Tfr1 and ZIP14 contributed to labile iron accumulation and ferroptosis and impaired skeletal muscle cell regeneration [83]. However, there is still insufficient information about the role of ZIP8 and ZIP14 in liver diseases.

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HO-1 has received much attention in NAFLD research. For example, chemicals such as ginkgolide B [59], dehydroabietic acid [58], and aucubin [84] can attenuate lipid peroxidation and hepatocyte ferroptosis through the Nrf2/HO-1 pathway [85]. However, overactivation of HO-1 expression induces extra iron release and ferroptosis [86]. Therefore, the dual role of HO-1 should be considered when developing HO-1-targeted treatment in NAFLD.

**2.3.5 Targeting hepcidin**

Approaches that interfere with hepcidin and the regulatory pathway may help modulate iron disorders. Hepcidin agonists, such as small molecule hepcidin agonists, minihepcidin, and FPN inhibitors, could theoretically reduce iron overload or redistribute iron to a "safe" state [87]. These compounds are of particular interest in restricting iron damage in thalassemia [87]. However, unlike the multiorgan iron overload and hepcidin deficiency in these inherited iron diseases [88, 89], how the liver acquires excessive iron is complex and was only partially elucidated in secondary iron overload syndromes [90]. The same was true for NAFLD and NASH. For example, directly targeting FPN may limit the amount of dietary absorbed iron but could prevent excessive iron release from hepatocytes and macrophages. The specificity of drug

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action is a critical issue. Another challenge is that the effect of hepcidin analogues may be offset by the underlying hepcidin and iron feedback loop. In other words, reduced circulating iron, in turn, suppresses hepcidin production [91].

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Therefore, restoring the sensitivity of hepatic iron sensing and regulating the upstream pathways could be more flexible strategies to improve iron homeostasis as NAFLD/NASH therapy. For example, a study revealed the benefits of antisteatotic and hepcidin activation by depleting MT-2 in mice[74]. Some MT-2 inhibitors have been developed as new hepcidin agonists in the treatment of β-thalassemia and iron overload diseases [92]. Moreover, one peptidomimetic inhibitor has successfully blocked MT-2-mediated hepcidin repression in HepG2 cells and human primary hepatocytes [93] However, whether MT-2 antagonists are also beneficial for NAFLD patients remains to be evaluated. Meanwhile, several preclinical studies have reported similar results by using recombinant BMP6 protein [70], a compound that activates the BMP6/SMAD4 pathway [94], and a BMPER inhibitor [68] in NAFLD and NASH.

### 2.3.6 Targeting Ferroptosis

In addition to the use of the ferroptosis inhibitors discussed above, inhibition of ferritnophagy could potentially suppress hepatic iron overload and ferroptosis [95, 96]. Curcumol protected hepatocytes against ferroptosis by reducing the expression of the NCOA4 receptor [95]. Therefore, targeting ferroptosis represents a novel and promising salutary approach to NAFLD, NASH, and other liver diseases.

### 2.3.7 Noncoding RNAs involved in iron metabolism for NAFLD therapy

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Noncoding RNAs, such as microRNAs (miRNAs) and long noncoding RNAs (LncRNAs), play a regulatory role in gene expression and biological processes, and iron homeostasis is no exception [97]. Shpyleva et al. suggested that iron disturbance in a mouse model of NAFLD could be partially explained by the upregulation of miR-200a and miR-223 [98]. In turn, iron overload could affect the expression of miRNAs, as evidenced by the fact that fructose-induced ferroptosis might induce hepatic-specific miR-33 spillover into serum [99]. Some studies have shown that miRNA-34a or LncRNA MAYA might be a promising target to improve iron overload, mitochondrial damage, and hepatocyte senescence in NAFLD [100, 101].

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**2.4 Challenges of targeting iron metabolism in NAFLD/NASH**

Because the pathogenesis and manifestations of iron disturbance in NAFLD and NASH patients vary, it is challenging to develop a universal remedy to rectify the iron imbalance in all patients [102]. For instance, increased iron absorption, hepatic iron accumulation, lipid peroxidation, and increased iron and ferritin levels are more prevalent in severe NAFLD and NASH than in obese and simple steatosis patients. Similarly, a low hepcidin/ferritin ratio could mainly be observed in NASH and NAFLD with DIOS but not in NAFLD patients with simple steatosis [63]. Moreover, paediatric NAFLD exhibits unique features in its liver pathophysiology compared with adult patients [103]. Children with obesity and NAFLD exhibit serum iron deficiency with no or low-mild iron accumulation in the liver [104]. Young patients will usually not develop dysmetabolic iron overload syndrome, unlike their adult counterparts [103]. These variations are of particular importance when conducting preclinical research,

since each animal model may not fully represent all clinical NAFLD phenotypes. For instance, the MCD diet model is known to increase hepatic iron uptake and is a validated ferroptosis model [80, 105], but db/db mice may have a special inability to accumulate iron in their tissues [45]. The evaluation criteria for iron status are critical since the total amount of hepatic iron may not change obviously while the $Fe^{2+}$ or $Fe^{3+}$ concentration changes dramatically in injured mice, according to Yang's results [59] and our unpublished data.

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In conclusion, this section discusses the alterations of iron metabolism in NAFLD and NASH. Emerging evidence has shown that iron disorder is not only a collateral manifestation of chronic liver diseases but may also promote the progression of NAFLD and related metabolic derangements. Meanwhile, efforts to target hepatic iron absorption, transport, utilization, regulation, and ferroptosis have already yielded positive results in treating NAFLD and NASH diseases, as summarized in Table 1.

## 3. Copper

### 3.1 Copper dysregulation in NAFLD/NASH

Copper is another essential mineral required for physiological function. A typical example is Wilson's disease, an inherited copper dyshomeostasis disease with excessive copper accumulation in the liver and insufficient Cp levels [106]. Conversely, copper deficiency is correlated with dyslipidaemia, such as obesity and NAFLD [107, 108].

Hepatic copper deficiency in NAFLD patients results in more pronounced steatosis,

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NASH, and metabolic symptoms [109]. A large cohort-based case–control study revealed the relationship between NAFLD severity and reduced blood copper concentrations in men [110]. Direct experimental evidence indicated that rats would develop spontaneous hepatic steatosis if fed a restricted copper diet [109, 111]. These reports highlighted that low copper availability might contribute to the risk of developing NAFLD [112]. Meanwhile, studies have demonstrated that heavy fructose intake exacerbates the development of NAFLD and other metabolic disorders, probably due to impaired copper homeostasis [113, 114].

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Since copper is fundamental to mitochondrial function and peroxisomal beta-oxidation of fatty acids, copper deficiency causes mitochondrial dysfunction and oxidative stress [115, 116]. Mitochondria underwent abnormal morphological enlargement in hepatocytes in copper-deficient rats [117]. Another explanation is an antioxidative defence defect because copper is an indispensable element for antioxidant enzymes, including cytochrome c oxidase (COX), SOD1 (Cu/Zn SOD), ceruloplasmin, and hephaestin [117]. Accordingly, NAFLD patients or animals with insufficient copper are accompanied by hepatic iron accumulation. A possible explanation is reduced FPN and ceruloplasmin expression and decreased iron export from the liver [111, 118, 119]. However, studies have also revealed copper overload in end-stage NAFLD patients, including those with hepatic cirrhosis and HCC [120, 121]. Overall, copper perturbation in NAFLD and NASH is far more complex than often thought.

**3.2 NAFLD and NASH influence key regulators involved in copper metabolism**

### 3.2.1 Physiological copper metabolism

Copper metabolism is quite similar to iron metabolism (Figure 3). Copper is mainly

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absorbed from the diet ($Cu^{2+}$ form, cuprous copper) in the duodenum, which needs to be reduced to the $Cu^{+}$ form (cupric copper) by the metalloreductase Dcytb1 or the Steap family proteins [122, 123]. Copper transporter 1 (CTR1) is the principal copper transporter in mammals [124]. Copper released from enterocytes is driven by ATPase copper transporting alpha (ATP7A) [125]. Unlike $Fe^{2+}$, the oxidation of $Cu^{+}$ is a spontaneous process because of the oxygen tension in the interstitial fluids [126]. There are two phases for copper distribution in the body [108, 127]. First, absorbed copper binds transcuprein and albumin and is transported to the liver and kidney [128]. Subsequently, copper is redistributed from the liver to other tissues, such as the heart and lungs, primarily in the form of ceruloplasmin. In this case, the liver is the central organ controlling systemic copper turnover.

At the cellular level, CTR1 mediates the uptake of copper together with reductase. After being transported into cells, copper interacts with various chaperones to further synthesize copper-containing enzymes. For example, CCS is the copper chaperone for SOD1 in the cytoplasm and intermitochondrial space, while COX17 is another chaperone delivering copper to mitochondrial COX [129]. In addition, the chaperone ATOX1 transfers copper to ATP7A or Cu-transporting ATPase beta (ATP7B) [130]. The transporter ATP7A is expressed in most tissues except the liver, while ATP7B is expressed primarily in the liver [131]. Under normal conditions, ATP7A and ATP7B are located in the trans-Golgi network (TGN), where copper is incorporated into protein

synthesis, similar to ceruloplasmin, in hepatocytes. However, ATP7A and ATP7B relocate to the plasma membrane to facilitate extra copper efflux [132]. Of note, hepatic ATP7B pumps excessive copper into the bile [133]. Excessive copper is stored by metallothionein (MT) via GSH [119].

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Similarly, the regulation of copper homeostasis consists of two aspects. Cellular homeostasis is achieved by copper influx, export, and storage cooperation: the expression of CTR1 is negatively correlated with the cellular copper concentration through a feedback mechanism [134]. In addition, an elevated copper level could facilitate the transcription of metallothionein genes to scavenge excessive copper [135]. A higher copper concentration in the liver enhances ATP7B-mediated copper excretion into the bile duct, the primary regulatory mechanism for systemic copper balance.

### 3.2.2 Copper metabolism in NAFLD/NASH

Emerging evidence suggests that inadequate dietary copper intake is associated with impaired copper status in NAFLD [111]. Of note, the expression of duodenal CTR1 was increased in rats fed a marginal copper-deficient diet, but this upregulation could be abrogated by high fructose feeding [111]. Among all four groups, rats fed a marginal copper -deficient diet plus fructose developed the most severe liver damage with the lowest concentration of copper and ceruloplasmin and a robust high hepatic iron accumulation [111]. Hepatic CTR1 abundance remained unchanged in response to dietary fructose, insufficient copper in the diet [136], or a high-fat diet [137], suggesting hepatic copper deficiency is unlikely a simple case of impaired cellular uptake by

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NAFLD, unlike iron.

Instead, the hepatic expression of ATP7B in high fructose diet-fed rats [136] and HFD-

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treated mice [137] was increased compared with that in normal mice. The upregulation could also be found in NASH patients' gene expression profiling [138]. In addition, Wilson's disease is predominantly caused by ATP7B gene defects [139]. Therefore, we could infer that altered copper exporter protein ATP7B in hepatocytes may explain copper disturbances under the diseased state. Consistently, Norheim et al. showed that the induction of Atox1, a chaperone of ATP7B, had a pronounced correlation with hepatic triglyceride accumulation in high-fat and high-sucrose diet-fed mice [140].

Mitochondria mainly utilize copper to synthesize complex IV (COX) and SOD1. Because the decreased antioxidant defence and functional abnormalities of mitochondria are closely related to the worsening hepatic pathology of NAFLD, hepatic COX activity and SOD1 expression were either unchanged or reduced [117, 141-143]. The level of a copper chaperone, CCS, which was induced in copper deficiency - deficient rats [144], was elevated in HFD mice [137], suggesting that CCS may serve as a negative marker of copper availability.

The above section has discussed the alterations of copper-binding protein ceruloplasmin in NAFLD patients. Although there have not been extensive reports regarding the role of transcuprein in NAFLD, the synthesis of transcuperin, ceruloplasmin, and albumin should be influenced by NAFLD since the liver is the major source of those proteins. In the liver, MT families mediate the detoxification of copper

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and zinc [145]. Decreased hepatic levels of metallothionein might result in copper and zinc deficiency in NAFLD In addition. In addition, metallothionein protects hepatocytes against oxidative damage[146] . Hence, the downregulation of metallothionein might be an indicator of enhanced oxidative stress in NAFLD and NASH disorders [141, 147, 148].

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### 3.3 Drug discovery and mechanistic studies focusing on copper metabolism

Given that copper deficiency may exacerbate hepatic steatosis and lipid peroxidation, improving copper levels could be beneficial for NAFLD patients, especially those with heavy fructose consumption. According to several studies, many compounds possess the ability to scavenge ROS and improve mitochondrial homeostasis in NAFLD and NASH [116]. Their actions partially depend on the capacity to modulate copper dysfunction. For example, studies indicated that epigallocatechin gallate (EGCG), a phenolic antioxidant, could alleviate fibrosis and hepatic inflammation in NAFLD induced by a HFD or MCD diet [149, 150]. EGCG was also described as a potent CTR1 regulator [151] . In addition to antioxidants, bezafibrate and captopril can modify the hepatic oxidative milieu by restoring hepatic copper homeostasis, metallothionein, and SOD1 expression[141] However, additional copper supply should be more careful. One clinic case series described an improvement in copper and ceruloplasmin levels, along with the recovery of liver function in two of three patients with liver diseases after receiving copper supplementation [152]. However, improper copper supplementation has a detrimental effect on the liver, while copper overdose may lead to the disruption of mitochondrial metabolism, as suggested by the newly discovered cuproptosis (a

copper-dependent regulated cell death) [153]. Finally, the role of copper dyshomeostasis in NAFLD and NASH needs further exploration and the utility of targeting copper metabolism as a therapeutic strategy needs to be validated further.

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**4. Zinc in NAFLD and NASH**

Zinc is the most abundant mineral, second only to iron in living organisms. Unlike iron and copper, zinc is redox neutral and is also a structural component in various proteins, known as the "zinc finger" motif [154]. In addition, zinc modulates cellular signaling pathways involving peroxisome proliferator-activated receptor alpha (PPAR-α) and lipid metabolism [155], insulin-like growth factor-1 (IGF-1), and insulin activity [156]. Zinc possesses antioxidant properties by antagonizing iron and copper and protecting antioxidant proteins. Moreover, zinc deficiency may exacerbate endoplasmic reticulum stress and the unfolded protein response [157]. Adequate zinc is necessary for hepatic regeneration and hepatocyte proliferation [158]. Therefore, various molecular processes involving zinc underscore the critical importance of controlling zinc homeostasis.

Systemic zinc homeostasis is primarily maintained by the balance between absorption and excretion in the GI tract (Figure 3). To date, 14 ZIP transporter members from the SLC39 family and ten members of zinc ZnT transporters from the SLC30 family have been identified in humans [159]. Generally, ZIP proteins increase cytosolic Zn concentrations by transporting zinc from the extracellular space and the lumen of the organelle to the cytoplasm, while ZnT reduces zinc availability by exporting zinc from

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cells [154]. Zinc binds to albumin or in the free state to be delivered to other tissues. ZIP14, ZnT1, and ZIP10 are necessary transporters for zinc homeostasis in hepatocytes [160]. Cytosolic zinc could bind to metallothionein and metalloenzymes, or be compartmentalized into intracellular organelles and vesicles for storage or utilization [161].

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Studies have revealed systemic zinc deficiency in chronic liver diseases, obesity, and diabetes [162]. A low level of serum zinc was found to be an independent risk factor for the progression of hepatic fibrosis [163, 164] and the severity of NAFLD [165]. Inadequate intake might partially explain this finding [166]. The urinary excretion of Zn was increased in patients with hepatic cirrhosis and in animals treated with ethanol [167], which may also lead to insufficient zinc supply in NAFLD. Finally, because of its implications for hepatic cellular adaptation to ER stress, hepatic ZIP14 was increased; thus, more serum zinc was mobilized to attenuate ER stress in HFD-induced mice [157]. However, the potential mechanism remains to be explored in detail.

**5. Selenium in NAFLD and NASH**

Dietary selenium exists in an inorganic form such as selenate and selenite, or an organic form containing selenocysteine and selenomethionine [168]. Various forms of selenium undergo several steps to be converted into selenoprotein in the liver and then be transported to other tissues. In humans, 25 selenoproteins have been identified [169]. Among them, GSH-Px (or GPXs) and thioredoxin reductases (TXNRDs) participate in antioxidation while selenoprotein P is involved in selenium transport and storage [170].

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Abnormal metabolism of selenium has been suggested in NAFLD [171, 172]. However, no uniform conclusion has been drawn about the relationship between selenium and NAFLD severity. Day et al. found that the NASH liver may have a lower selenium concentration [171]. Interestingly, the selenotranscriptome network revealed a distinct selenoprotein profile that might be associated with disturbed iron metabolism and ferroptosis in NASH [171]. Likewise, some studies reported favourable effects of selenium and zinc cosupplementation on lipid accumulation and insulin resistance [173, 174]. However, high serum selenium levels may contribute to disease development, and some studies have shown a positive association of serum selenium with the NAFLD prevalence in U.S. adults [172] and Chinese adults [175]. Selenoprotein P was proposed as a novel marker for NAFLD because it would induce insulin resistance and metabolic dysfunction in hepatocytes and myocytes [176]. The serum level of selenoprotein P was significantly higher in subjects with NAFLD, obesity, and diabetes [177-179]. Furthermore, another selenoprotein, GPX4, which is also the main negative regulator in the ferroptotic process, is implicated in the pathophysiology of NAFLD [59, 79, 180]. Therefore, further investigation is warranted to determine the role of selenium metabolism in NAFLD and the potential mechanism.

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## 6. Interactions among trace elements

The interactions among trace elements appear to play an essential role in mineral homeostasis and is related to the presence of multiple trace element perturbations in NAFLD. According to the description above, the critical player modulating interactions of iron-copper, iron-zinc, zinc-copper, and iron-selenium are summarized in this

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section.

### 6.1 Interactions between iron and copper

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Iron and copper have similar biological properties and metabolism processes, and their interplay is predictable. Ferrireductases, including Dcytb1 and STEAP family members, regulate dietary absorption of two metals, suggesting a potential target of crosstalk. Moreover, one study showed that intestinal DMT1 might enhance copper absorption by iron-deprived rats [181]. Another link is multicopper oxidase ceruloplasmin which is required for iron and copper delivery through the blood. Meanwhile, the ceruloplasmin-FPN system is the major intracellular iron exporter. Decreased copper availability and increased hepatic iron storage in NAFLD patients could probably be attributed to impaired ceruloplasmin activity [56].

Of note, no hormonal mediator like hepcidin has been characterized for copper and zinc metabolism. However, some literature has implied that copper might be involved in regulating hepatic hepcidin expression. Under the condition of dietary copper deficiency, hepcidin expression was decreased while the level of FPN was enhanced [182]. In addition, hepcidin can bind copper with a high affinity [183]. Therefore, hepcidin potentially links the copper disorders and iron disturbances in liver diseases.

### 6.2 Interaction between iron and zinc

Excessive iron exacerbates its progression, but zinc may diminish the severity of NAFLD and NASH. Iron and zinc also interact with each other. The iron transporter DMT1 has a low affinity for zinc, while the zinc transporters, ZIP8 and ZIP14 are

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involved in NTBI and intracellular iron trafficking. Hence, iron and zinc may compete for access to shared transporters [184]. Zinc status might modulate hepcidin expression by MT-2 (one zinc-dependent enzyme) [160]. Mice fed zinc depletion diets had increased intestinal and hepatic iron accumulation as well as hepcidin production [185]. It appears that an insufficient zinc supply may lead to the inhibition of MT-2 and increase hepcidin production which subsequently induces iron accumulation in tissues [186].

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### 6.3 Interaction between copper and zinc

The crosstalk between copper and zinc is less well investigated. Zinc and copper are indispensable elements for SOD1 function, which is ubiquitously expressed in cells as a critical antioxidative defensor. Metallothionein is also involved in zinc and copper interactions. A good example is the introduction of zinc salt supplements in the clinical treatment of Wilson's disease since high-dose zinc impedes intestinal absorption of copper by inducing metallothionein synthesis in intestinal cells and the shedding of these cells [187].

### 6.4 Interaction between iron and selenium

Iron homeostasis and selenium metabolism converge in the study of ferroptosis. Adequate selenium is required for the biosynthesis of ROS-scavenging selenoproteins, especially the key inhibitor GPX4. Some studies have already confirmed the protective effect of selenium supplementation on GPX4 production and subsequent resistance towards ferroptosis [188]. Selenium and selenoprotein offer promising opportunities to

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control diseases related to iron disorder and ferroptosis, such as NAFLD.

## 7. Conclusion and outlook

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Mineral dysregulation is evident in liver diseases. In turn, disease progression suffers from mineral disturbance. Despite what has been discussed above, the mechanistic basis of mineral imbalance in NAFLD and related diseases still remains largely unexplored. It is an important agenda of research to understand the molecular basis of mineral transport and utilization within cells and organelles. Among these minerals we discussed, iron is the most studied one and would inspire others. For example, mice lacking iron chaperone PCBP1 spontaneously developed steatosis [49] while increased copper chaperone CCS was found in the HFD mice [137]. Thus, questions are arisen naturally towards the role of copper chaperones in the pathogenesis of liver diseases, so was the recently defined zinc chaperone [189]. In addition, sex differences in the metabolic effects of iron and copper have been noted in animals and humans [110, 136]. The investigation of sex-variant mineral metabolism may help to decipher the mechanism underlying the sex variances in the prevalence of NAFLD and NASH. Next, although NAFLD patients and animals may be prone to developing iron overload with copper, zinc or selenium deficiency, levels of these minerals may change in the context of animal models used and the cause of liver dysfunction. For instance, mice fed a high-fat diet for 16 weeks showed systemic iron accumulation, but intrahepatic iron overload was not evident until longer exposure (48 weeks) [68]. Last, more research on these mineral interactions will provide us with comprehensive insights into mineral disorder and potential NAFLD treatment, as the application of zinc salt in Wilson's disease.

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Overall, it has become clear that mineral imbalance is associated with the manifestation of NAFLD. Most importantly, targeting mineral metabolism, especially iron disorder and ferroptosis, has made progress in NAFLD and NASH management, inspiring novel mechanistic therapies for these chronic liver diseases.

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**Author Contributions**

All the authors contributed to the present form of the manuscript. C.H.M. and L.H. collected and analyzed the literature sources; C.H.M. drafted the manuscript and created figures; Z.Y.Z, C.-H.P, G.-Y.P reviewed and revised the final version of the manuscript. All authors have read and agreed to the published version of the manuscript.

**Conflicts of Interest**

The authors declare no conflict of interest.

**CRediT authorship contribution statement**

**Chenhui Ma:** Conceptualization, Investigation, Writing – original draft, Writing – review & editing. **Li Han:** Conceptualization, Investigation, Writing – review & editing. **Zheying Zhu:** Conceptualization, Writing – review & editing, Project administration. **Cheng Heng Pang:** Conceptualization, Writing – review & editing, Project administration. **Guoyu Pan:** Conceptualization, Writing – review & editing, Project administration.

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**Figure legend**

**Figure 1. Systemic and cellular iron metabolism.** Dietary iron is taken up in nonheme and heme forms. Most iron ($Fe^{3+}$) is mobilized across the apical membrane

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of enterocytes by the DMT1, a process accompanied by the prior reduction of ferric iron by Dcytb. The mechanism of heme intake is poorly understood, while the iron is released from heme by the degradation of HO-1. Once in enterocytes, iron is either stored in ferritin, utilized by mitochondria, or exported by FPN. Cp or HEPH must oxidate fe2+ before binding to Tf to be further transported into the bloodstream. Specialized macrophages lyse senescent red blood cells to liberate and recycle iron. The liver is the main organ for iron storage and regulation. Tf-bound iron binds to its receptors (TfR1 and TfR2) in hepatocytes and is endocytosed. Subsequently, iron is released from Tf through endosomal ferrireductase and DMT1. Besides, under a high level of plasma iron, NTBI rapidly enters into cells via DMT1, ZIP14, and ZIP8 after reduction. Absorbed iron enters the labile iron pool where iron is destined to ferritin or mitochondria to synthesize heme or Fe-S cluster via mitoferrin. Iron chaperone PCBP is involved in those processes. NCOA4 is a cargo receptor that delivers ferritin to autophagic degradation. The cooperation between FPN and ferroxidases like Cp and HEPH mediates iron export. Hepcidin is produced in the liver as the master of systemic iron regulation. HJV, MT-2, BMPR, and BMP/SMAD pathways mainly modulate the expression of hepcidin. Meanwhile, IRP/IRE system maintains intracellular iron homeostasis by regulating the translation of iron transporters and ferritin. DMT1, divalent metal transporter 1; Dcytb, duodenal cytochrome B; Steap, six-transmembrane epithelial antigen of the prostate; PCBP, poly (rC) binding protein; HO-1, heme oxygenase 1; FPN, ferroportin; Cp, ceruloplasmin; HEPH, hephaestin; FPN, ferroportin; TfR1/2, transferrin receptor 1/2; ZIP 14/8, Zrt–Irt-like protein 14 and 8; LIP, labile iron pool; NCOA4, nuclear receptor coactivator 4; HAMP, hepcidin; IRP, iron-regulatory proteins; IRE, iron-responsive elements; BMP, bone morphogenetic protein; BMPER, BMP binding endothelial regulator; HJV, Hemojuvelin; MT-2, matriptase-2.

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**Figure 2. Iron dyshomeostasis in NAFLD and NASH**. Duodenal iron absorption might increase as evidently by the increased expression of DMT1due to the activation of IRP1. FPN at the basal side is decreased or unchanged. Multi-copper protein Cp

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experiences a pronounced decrease in NAFLD patients. In the circulation, the amount of ferritin and transferrin saturation might increase. In hepatocyte, the transferrin receptor and DMT1 expression may increase while FPN decrease. Toxic NTBI accumulates in the liver, and the increased expression of ZIP14 in NAFLD-related diseases. These changes might be related to the IRP1 activation. Hepcidin resistance or insufficient hepcidin production may contribute to excess iron deposition in livers, probably due to the downregulation of the BMP/SAMD signaling pathway, like the inhibition of HJV or the elevation of MT-2 activity. Consequently, plenty of iron is accumulated in the liver and would aggregate oxidative balance and lipid peroxidation, driving the progression of NAFLD and NASH. Finally, the animal model result showed increased phagocytosis of erythrocytes. DMT1, divalent metal transporter 1; Dcytb, duodenal cytochrome B; Steap, six-transmembrane epithelial antigen of the prostate; PCBP, poly (rC) binding protein; FPN1, ferroportin 1; Cp, ceruloplasmin; HEPH, hephaestin; FPN, ferroportin; TS, transferrin saturation; TfR1/2, transferrin receptor 1/2; ZIP 14, Zrt–Irt-like protein 14; LIP, labile iron pool; HAMP, hepcidin; IRP, iron-regulatory proteins; BMP, bone morphogenetic protein; BMPER, BMP binding endothelial regulator; HJV, Hemojuvelin; MT-2, matriptase-2.

**Figure 3. Systemic and cellular Copper and Zinc metabolism.** Copper transport is shown with green arrows and lines while zinc is indicated by blue color. For copper metabolism, dietary copper (Cu2+) is absorbed in the small intestine after being reduced to Cu+ by the metalloreductases Dcytb1 or the Steap family protein. Once reduced, copper enters enterocytes via CTR1 and is then distributed to various copper chaperones like CCS, COX17 and ATOX1 to specific proteins or organelles. Excess copper is stored by MT. ATOX1 ferry copper to ATP7A which deliver it to the TGN or traffics to the basolateral membrane to pump copper into the blood via endosome or vesicles. Exported copper binds albumin and transcuperin and is destined to the liver

and kidney. In hepatocyte, ATOX1 guides copper to ATP7B for incorporation into ceruloplasmin (mainly), or excretion into the bile duct. For zinc metabolism, there are main two zinc transporter families, the ZIP transporters and the ZnT transporters which

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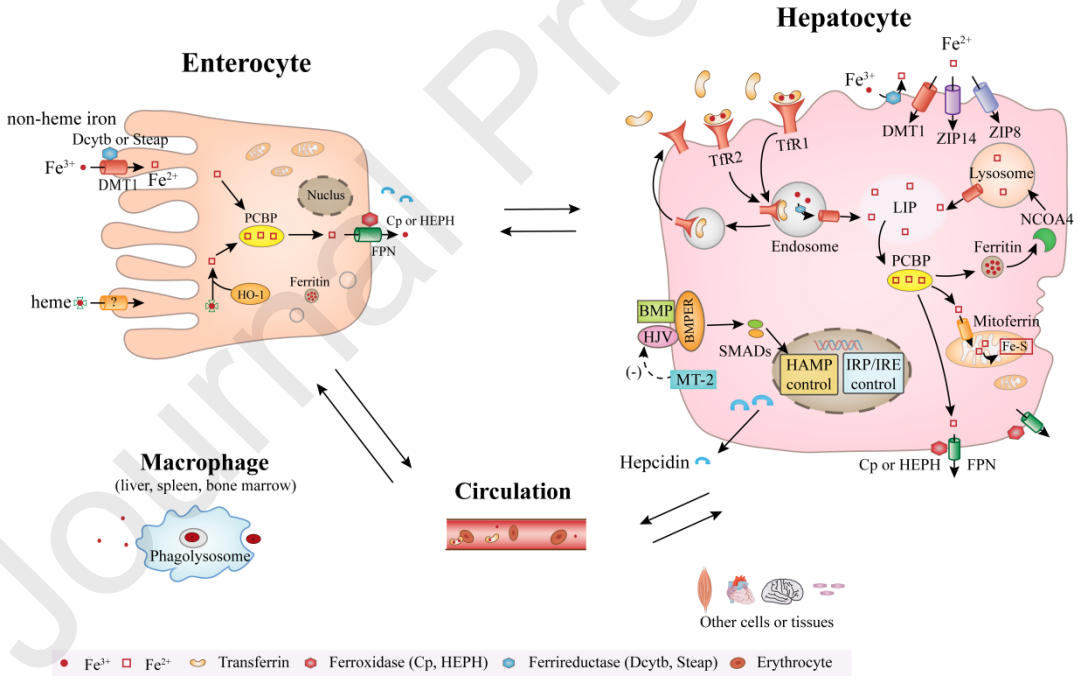
increase or decrease cytosolic zinc levels, responsively. Dietary zinc (Zn2+) is absorbed via ZIP4 at enterocytes while is exported to the circulation via ZnT1. ZIP5 is expressed in the basolateral surface of enterocytes to monitor zinc status by sequestering zinc from the blood. Zinc is incorporated into protein like SOD1, translocated into organelles, or stored by MT. Zinc binds to albumin (main) or in the free form to be delivered to other tissues. In hepatocyte, ZIP14, ZIP10 and ZnT1 are important transporters to maintain zinc homeostasis. DMT1, divalent metal transporter 1; Dcytb, duodenal cytochrome B; Steap, six-transmembrane epithelial antigen of the prostate; CTR1, Copper Transporter 1; ATP7A, ATPase copper transporting alpha; ATP7B, Cu-transporting ATPases beta; Cp, ceruloplasmin; ZIP 4, Zrt–Irt-like protein 4; ZIP 5, Zrt–Irt-like protein 5; ZnT1, Zinc transporter 1; MT-2, matriptase-2; CCS, Cu chaperone for Cu/Zn superoxide dismutase; COX17, Cytochrome c oxidase 17; ATOX1, Antioxidant 1 Copper Chaperone; TGN, trans-Golgi network; SOD, Superoxide dismutase.



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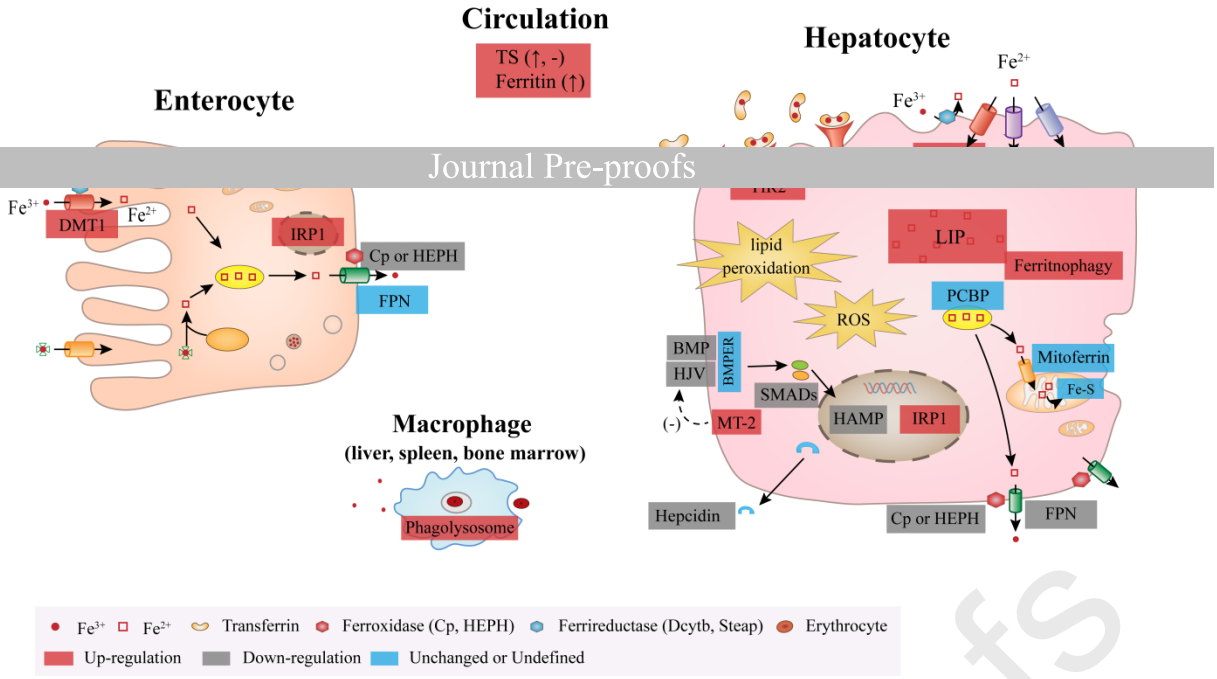


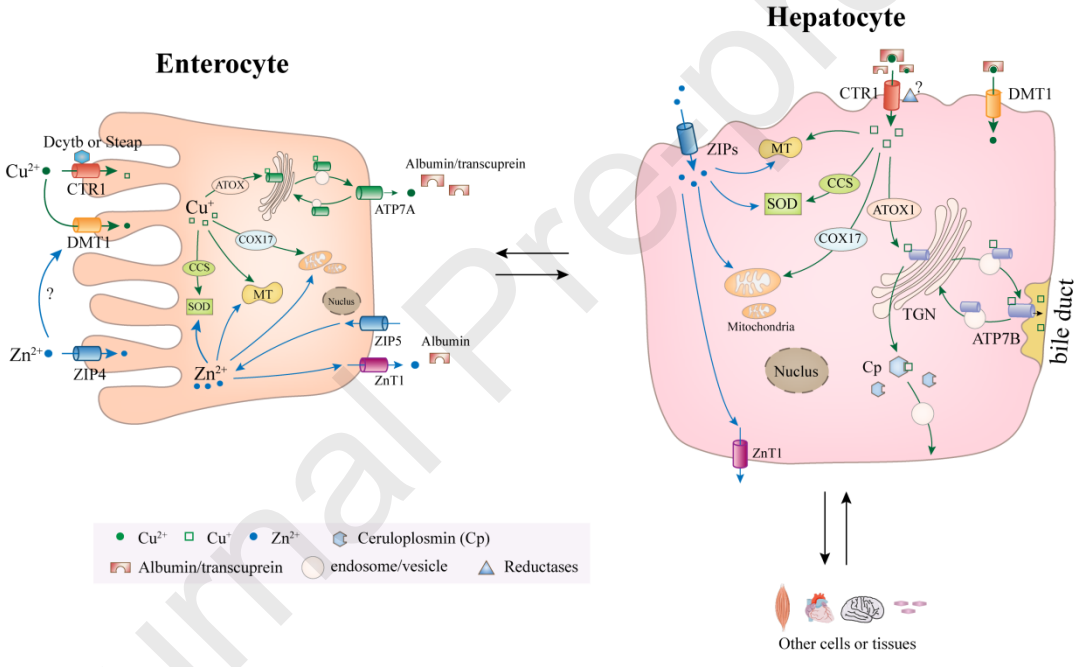


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# Mineral metabolism and Ferroptosis in Non-Alcoholic Fatty Liver Diseases

Chenhui Ma [a,b,c], Li Han [b,c], Zheying Zhu [d,*], Cheng Heng Pang [a,*], Guoyu Pan [b,c,*]

**Table 1 Potential targets involved with iron metabolism in NAFLD/NASH treatment**

| | | | |
|---|---|---|---|
| | Restrict dietary iron | DMT1 | [75] |
| | Inhibit intestinal iron uptake | | [76] |
| | Inhibit intestinal inflammation | | [39] |
| | Maintain gut microbiome homeostasis | | [77] |

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| | | | |
|---|---|---|---|
| | Limit hepatic iron influx | TfR, DMT1, ZIP14 | [76, 83] |
| | | Lactoferrin | [44, 82] |
| | | FGF21 | [53] |
| | efflux and ferroxidase function | Cp, HEPH | [54-56] |
| | Decrease heme-iron | HO-1 | [57, 86] |
| | | PCBP | [49, 50] |
| | Regulate intracellular iron transport | Mitoferrin | [60] |
| | Accelerate iron excretion | DFO, DFX, DFP | [17, 78-80] |
| | Inhibit ferroptosis | Fer-1, Lip-1, Trolox, Rosiglitazone | [17, 95, 96] |
| | Regulate ferritnophagy | Curcumol | [95] |
| | Iron chelator | DFO, DFX, DFP | [17, 78-80] |
| | Target the Nrf2/HO-1 pathway | Ginkgolide B, Aucubin, Dehydroabietic acid | [58, 59, 84, 85] |
| | Target the Hepcidin-FPN pathway | Hepcidin mimics, FPN inhibitor | [87] |
| | Restore the sensitivity of cellular iron sensing | Matriptase-2 inhibitor, Recombinant BMP6 protein, BMPER inhibitor | [68, 70, 74, 94] |

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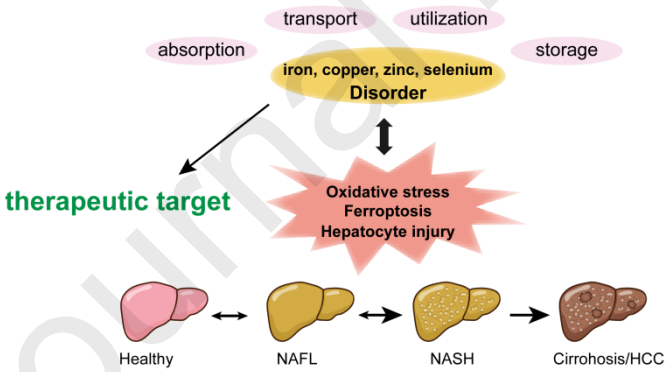
| | | | |
|---|---|---|---|
| | | miR-34a, miR-33, miR223, miR-220a | [98-100] |
| | | | [101] |
| | Modulate key players in iron homeostasis | LncRNA MAYA | |

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