Liver Research 6 (2022) 203–213

Contents lists available at ScienceDirect

Liver Research

journal homepage: http://www.keaipublishing.com/en/journals/liver-research

Review Article

Iron metabolism in non-alcoholic fatty liver disease: A promising therapeutic target☆

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A R T I C L E I N F O

Article history:
Received 28 March 2022
Received in revised form
5 June 2022
Accepted 12 September 2022

Keywords:
Non-alcoholic fatty liver disease (NAFLD)
Hepatic iron overload
Phlebotomy
Iron chelators
Nanomedicine
Ferroptosis

A B S T R A C T

Non-alcoholic fatty liver disease (NAFLD) has become the most common cause of chronic liver disease worldwide, and is closely associated with the increased risk of the prevalence of obesity and diabetes. NAFLD begins with the presence of >5% excessive lipid accumulation in the liver, and potentially develops into non-alcoholic steatohepatitis, fibrosis, cirrhosis and hepatocellular carcinoma. Therefore, insight into the pathogenesis of NAFLD is of key importance to its effective treatment. Iron is an essential element in the life of all mammalian organisms. However, the free iron deposition is positively associated with histological severity in NAFLD patients due to the production of reactive oxygen species via the Fenton reaction. Recently, several iron metabolism-targeted therapies, such as phlebotomy, iron chelators, nanotherapeutics. and ferroptosis, have shown their potential as a therapeutic option in the treatment of NAFLD and as a clinical strategy to intervene in the progression of NAFLD. Herein, we review the recent overall evidence on iron metabolism and provide the mechanism of hepatic iron overload-induced liver pathologies and the recent advances in iron metabolism-targeted therapeutics in the treatment of NAFLD.

1. Introduction

Non-alcoholic fatty liver disease (NAFLD) has become the most common cause of chronic liver disease worldwide with an increased risk of liver-related morbidity and mortality and is associated with the increased risk of development of type 2 diabetes (T2D), obesity, and cardiovascular disease.[1–3] Abnormalities in NAFLD begin with the presence of excessive lipid accumulation (>5%) in the liver without drug abuse and excess alcohol intake, and encompass a wide array of the hepatic clinicopathologic spectrum of natural history ranging from simple steatosis to non-alcoholic steatohepatitis (NASH), liver fibrosis, cirrhosis and ultimately hepatocellular carcinoma (HCC).[3,4] The global prevalence of NAFLD has grown tremendously in recent decades due to dramatic lifestyle changes, and NAFLD affects approximately 1.7 billion individuals worldwide with approximately 30–40% in males and 15–20% in females.[5] A recent Meta-analysis exhibited a rapid increase in the national prevalence of NAFLD from 18% to 29% from 1999 to 2018 in China, indicating that NAFLD has become the most prevalent liver disease in China.[6] In 2016, the prevalence and severity of NAFLD varied by geographic region, ethnicity, age, and socio-economic status, with the highest incidences in the Middle East (31.79%) and South America (30.45%), followed by Asia (27.37%), North America (24.13%), Europe (23.71%), and Africa (<13.00%) in 2016.[7] Additionally, metabolic dysfunction-associated fatty liver disease (MAFLD) is considered the hepatic manifestation of fatty liver, and has been proposed as a new nomenclature to replace NAFLD in 2020 to further encapsulate the pathophysiology of the disease.[8,9] In a word, insights into the pathogenesis of NAFLD are of key importance to its effective treatment.

Iron is an essential element in the life of all mammalian organisms and acts as a component of several metalloproteins and enzymes involved in crucial metabolic progress and systemic energy homeostases, such as mitochondrial respiration, oxygen sensing and transport, citric acid cycle, and deoxyribonucleic acid (DNA) biosynthesis.[10,11] Although the role of hepatic iron in the initiation and progression of NAFLD remains controversial, iron may have a role in the pathogenesis of NAFLD in some patients. Increased hepatic iron deposition in parenchymal and/or non-

☆ Edited by Peiling Zhu.

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https://doi.org/10.1016/j.livres.2022.09.003

parenchymal cells of the reticuloendothelial systems was observed in approximately one-third of adult patients with NAFLD.[12] Recent studies have demonstrated that hepatic iron deposition is positively associated with histological severity in NAFLD patients,[13] addressing the direct evidence of the role of hepatic iron overload involved in the pathogenesis of NAFLD. In mammalian cells, iron exists mainly in the form of heme to be a subunit of hemoglobin, whereas excessive free iron can catalyze the Fenton reaction to generating reactive oxygen species (ROS), such as hydroxyl radical.[14] ROS can initiate oxidative damage in the liver by attacking cellular membranes, proteins and nucleic acid, which results in the disruption of the lipid metabolism, loss of mitochondrial membrane potential (MMP), and cell death.[15] Therefore, it is imperative to understand the role and mechanism of hepatic iron metabolism in the pathogenesis and progression of NALFD, and to review the recent advances in iron metabolism-targeted therapeutics in the treatment of NAFLD.

2. Summary of the molecular mechanism of iron homeostasis in the liver

2.1. Systemic iron metabolism

As our knowledge of iron homeostasis has increased, it has become evident that the liver is the center of the regulation of iron storage and hepcidin signaling. In addition to hepatocytes, other three major cell types, i.e., duodenal enterocytes for dietary iron absorption, erythroid precursors for iron utilization, and reticuloendothelial macrophages for iron storage and recycling,[10] are essential for the body to regulate iron homeostasis, which determines the systemic iron metabolism (Fig. 1).

To maintain the homeostatic balance, 1–3 mg of absorbed inorganic iron or heme from dietary sources are required per day, and 20–25 mg of iron were recycled by specialized tissue macrophages, found mostly in the bone marrow, liver, and spleen, from senescent erythrocytes per day.[16–18] Dietary non-heme iron is firstly reduced from ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}) by the ferrireductase duodenal cytochrome b (Dcytb) in the epithelial side of the duodenum.[10] Then the Fe^{2+} is absorbed and transported across the apical membrane of enterocytes by divalent metal transporter 1 (DMT1).[19] After being incorporated into villous epithelial cells, the Fe^{2+} is exported into the blood by the iron exporter ferroportin 1 (FPN), and oxidized to Fe^{3+} by the membrane-bound copper-containing ferroxidase hephaestin.[20,21] The Fe^{3+} is then bonded with transferrin for circulation transport. Transferrin-bound iron is primarily used for the synthesis of heme by erythroid precursors and is the physiologic source of the reticuloendothelial macrophages.[22] Reticuloendothelial macrophages clear senescent erythrocytes to release approximately 25 mg of iron from heme to export into the circulation.[10] Moreover, the released iron can be stored in the reticuloendothelial macrophages as ferritin, indicating that reticuloendothelial macrophages represent the mainly dynamic iron storage in the iron cycle.[22] Recent study has reported that transferrin-conjugated Fe^{3+} can be transported into the liver via the portal vein.[23] An adult organism has 3–5 g iron, more than 60% of which is incorporated into hemoglobin in erythrocytes, and other is conjugated to ferritin and hemosiderin for storage in hepatocytes and macrophages.[19]

2.2. Hepatic iron homeostasis

In addition to acting as an iron storage organ, the liver serves a central role in iron homeostasis by regulating the production of the hormone hepcidin in response to the signals reflecting iron status, inflammation, erythropoietic activity, and oxygen tension.[24,25] Hepcidin is a 25 amino acid circulating peptide secreted from hepatocytes to maintain iron homeostasis via a hormone-like negative feedback mechanism.[26] Hepcidin restricts iron absorption by enterocytes, and reduces iron efflux from macrophages and hepatocytes by binding to ferroportin to induce its internalization and degradation.[18] The increased production of hepcidin is positively associated with the elevated levels of circulating iron through the bone morphogenetic protein and hemojuvelin (BMP/HJV)-small mothers against decapentaplegic (SMAD) signaling pathway, and in sensing plasma transferrin levels through the hemochromatosis proteins (HFE) and transferrin receptor 2 (TFR2) complex,[27,28] indicating an intact physiological response to full iron stores. The potential mediators to produce hepcidin are due to interleukin (IL) -6 and IL-1-mediated inflammation and infection through activation of the Janus kinase/signal transducer and activator of the transcription 3 (JAK/STAT3) signaling pathway.[29–31]

2.3. Hepatic iron overload

Iron from the senescence or damaged red blood cells is recycled for erythropoiesis, and the excess iron stores in the parenchymal organs for later use, which is detrimental and exacerbates the pathogenesis of some iron overload disorders.[26,32] Excess iron in the blood saturates the buffering capacity of serum transferrin and results in increased non-transferrin-bound iron, which can be imported into hepatocytes via SLC39A14.[33,34] More recent studies have demonstrated that hepatic iron deposition is linked to an increased incidence of chronic metabolic diseases including T2D, obesity, and NAFLD.[17,35] The content of iron in the liver is about 300 mg to 1 g and reaches up to more than 25 g in patients with hereditary hemochromatosis.[10,36] In NAFLD, serum iron is often increased (53%), followed by a decrease in serum hepcidin (47%) and elevated ferritin (42%), and about 2 times less frequently elevated transferrin saturation (18%), which indicated that an increase in the ferritin levels is a crucial key feature of iron dysregulation in patients with NAFLD.[37] To date, the majority of studies have demonstrated that a mild or modest degree of hepatic iron overload is positively associated with the development of NAFLD,[37] and progression of advanced liver injury including NASH,[38] fibrosis,[39] cirrhosis,[40] and HCC.[41] The distribution of iron in the liver has been found in three different patterns, such as hepatocellular iron deposition only, reticuloendothelial system (RES) cells only, or a mixed pattern of both hepatocellular and RES.[42] A study examining the degree and distribution of hepatic iron contents in the United States (US) has confirmed that stainable iron in liver biopsy of patients with NAFLD was present in hepatocellular only (63/293, 21.5%), RES only (91/293, 31.1%) or a mixed pattern of hepatocellular/RES (139/293, 47.4%).[43] Moreover, the pattern of hepatic iron deposition has been revealed to be associated with the severity of NALFD.[43] Advanced NAFLD, including increased histologic features, a higher mean NAFLD Activity Score (NAS), elevated serum aminotransferases, and decreased platelets, has been observed in the patients with RES iron accumulation. RES iron deposition has also shown to be more prevalent and in greater amounts in patients with HCC than the patients without HCC,[44] indicating that hepatic iron overload plays an important role in the pathogenesis and progression of NAFLD.

3. Molecular mechanism of hepatic iron overload in the pathogenesis and progression of NAFLD

3.1. Oxidative stress

NAFLD is the most common cause of chronic liver disease, and approximately one-third of adult patients with NAFLD show signs of iron abnormality, which is termed “dysmetabolic iron overload

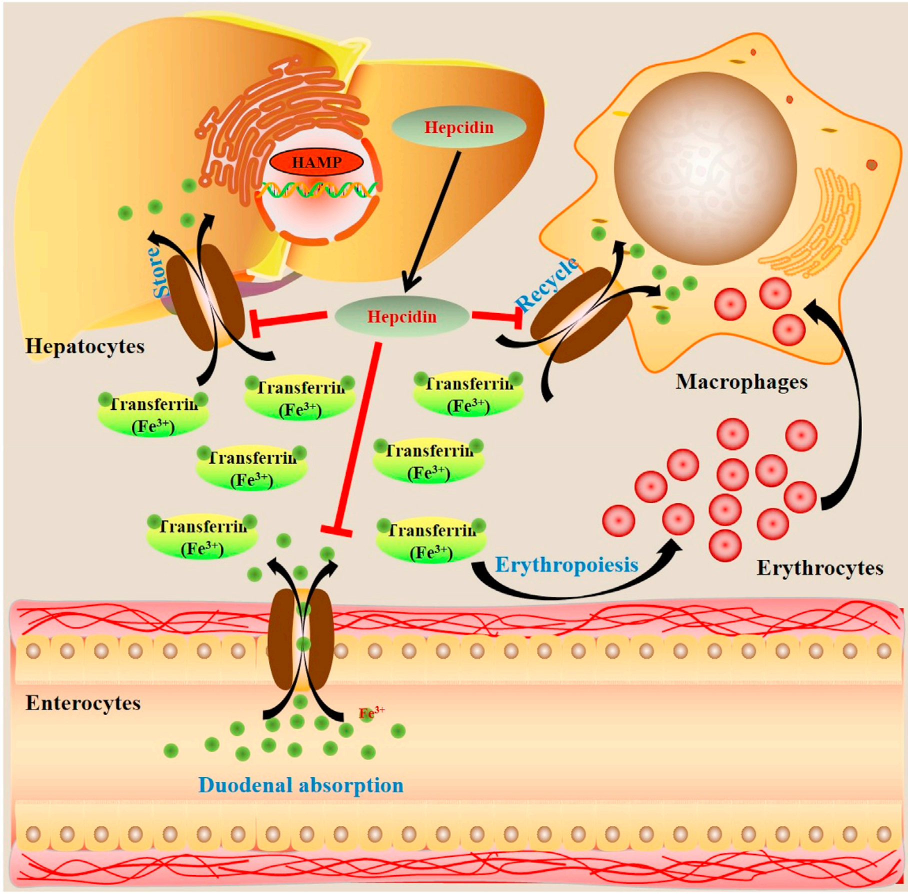

Fig. 1. Systemic iron metabolism. Four major cell types, i.e., duodenal enterocytes for dietary iron absorption, erythroid precursors for iron utilization, reticuloendothelial macrophages for iron storage and recycling, and hepatocytes for iron storage and endocrine regulation, play the important role in the regulation of iron homeostasis, which determines the distribution and content of iron in the body. Hepcidin restricts iron absorption by enterocytes and reduces iron efflux from macrophages and hepatocytes by binding to ferroportin to induce its internalization and degradation. Abbreviations: Fe^{3+}, ferric iron; Fe^{2+}, ferrous iron; Dcytb, duodenal cytochrome b; DMT1, divalent metal transporter 1; FPN, ferroportin 1; HAMP, hepcidin antimicrobial peptide.

syndrome".[14,45] Excessive hepatic iron-induced Fenton reaction aggravates the deleterious characteristics of hepatocyte injury and hepatic dysfunctions by promoting the formation of reactive oxygen intermediates.[46] Reactive oxygen intermediates, such as peroxides and free radicals, can damage cellular proteins, lipids, and DNA.[47–49] Iron overload in the liver increased the markers of oxidative stress and promoted histological change to NAFLD pathogenesis and progression in experimental animals and patients,[24,50] which is similar to the patients with alcoholic liver disease, chronic hepatitis B and C.[51–54] Moreover, increased hepatic iron accumulation is associated with the severity of hepatic histology in NAFLD, such as in NASH and hepatic fibrosis. Iron oxide nanoparticle (IONP) treatment increased the iron deposition in the liver of high-fat diet-fed NAFLD mice, and then aggravated liver inflammation and sterol regulatory element binding proteins-1c (SREBP-1c)-mediated *de novo* lipogenesis (DNL) through disruption of BMP-SMAD pathway,[55] suggesting that hepatic iron overload is associated with the severity and progression of NAFLD. Mild or moderate mesenchymal or hepatocellular iron deposition in liver biopsies is encountered in about 30–50% of patients with NASH and NAFLD, and serum ferritin concentration in patients with NASH increased to more than 1.5 times compared with the normal subjects.[25,42]

3.2. Hepatic steatosis

In addition to catalyzing the production of ROS, excessive iron may participate in the initiation and progression of NAFLD by promoting the lipid accumulation.[14,56,57] NAFLD is a clinical pathological disease characterized by triglyceride accumulation in the cytoplasm of hepatocytes and will develop into NASH by the presence of hepatocellular inflammation, ballooning, and Mallory-Denk bodies.[4,58,59] The liver is the main organ in the regulation of lipid metabolism to maintain the homeostasis of major hepatocellular events, including membrane structure, energy storage, and metabolic pathways.[4] Dysregulation of lipid homeostasis, such as importing free fatty acid from the plasma into the liver, and manufacturing, storing and exporting lipids, may promote the occurrence and progression of NAFLD.[60] Iron-induced oxidative stress is known to increase cellular damages and organelle membrane injury through lipid peroxidation-induced altered membrane integrity and function.[61] Iron overload in *Caenorhabditis elegans* induced the expression of serum/glucocorticoid regulated kinase 1 (SGK1), the homologs of mammalian fatty acid transport proteins 1 and 4 (FATP1/4), and promoted the synthesis of ferritin, which favored cellular lipid uptake and translocation of lipids into lipid droplets.[62] There is growing evidence confirming that iron may disrupt lipid homeostasis by altering the expression of hepcidin, indicating a positive association between hepatic iron overload and the levels of lipid in the serum in NAFLD subjects.[63] A significant number of patients with NAFLD display increased iron deposition in the hepatic macrophages through SREBP-1a/c-mediated expression of the iron regulator hepcidin.[64] An ongoing pilot study has reported that phlebotomy therapy can improve hepatic and peripheral insulin sensitivity, and reduce triglyceride biosynthesis in the plasma.[65] Rats with an iron overload hyperlipidemic diet exhibited excessive iron overload in the liver, which significantly increased serum triglyceride and glucose levels but did not alter the concentrations of serum cholesterol.[66,67] The molecular mechanism of iron in the regulation of lipid metabolism is that iron-mediated ferritin can inhibit the secretion of apolipoprotein B through endoplasmic reticulum-associated degradation (ERAD) of the apolipoprotein.[68]

3.3. Insulin resistance

NAFLD/NASH is accompanied by insulin resistance, which plays a pivotal role in the onset and progression of its pathophysiology.[42,69] Insulin resistance primarily results in increased circulating free fatty acids along with hepatic steatosis, which is common in patients with NAFLD and contributes to the progression of NASH and hepatic fibrosis.[70,71] Recent studies from the *in vitro* and *in vivo* models of NAFLD progression have shown that increased iron overload in the liver generated oxidative stress and led to worsened insulin sensitivity.[72,73] However, their mechanisms remain unknown. Iron-induced oxidative stress and inflammation have been implicated in the down-regulation of insulin signaling by reducing expression of glucose transporter 4 (GLUT4) and phosphorylation of insulin receptor substrate-1 (IRS-1).[14,72] A recent study determined that iron overload and increased expression of transferrin increased adipocyte lipolysis and reduced glucose uptake, which contributed to insulin resistance, the key features of NAFLD pathogenesis.[74,75] In summary, it is urgent to need more research to further address the relationship of hepatic iron overload between excessive lipid accumulation and insulin resistance in the pathogenesis of NAFLD.

3.4. NASH and fibrosis

Hepatic iron overload has been reported to be an important predictor and risk factor in NAFLD progression. Liver fibrosis is characterized by excessive deposition of extracellular matrix, which is mediated by a complex network of interrelated and regulated signaling interactions between the resident parenchymal cells (termed hepatocytes), non-parenchymal cells including hepatic stellate cells (HSCs), liver sinusoidal endothelial cells, Kupffer cells (KCs), liver associated lymphocytes, and the non-resident infiltrating immune cells.[39] KCs play a central role in the progression of hepatic steatosis to liver fibrosis. HSCs sense iron disturbances in maintaining the homeostasis of the liver via phagocytosis of red blood cells and recycling of iron, which maintains iron homeostasis and prevents iron toxicity.[76] Iron accumulation in the liver is found within the KCs and hepatocytes of mice or humans with NAFLD.[76]Iron deposition in the KCs is associated with the initiation of the inflammatory cascade and catalyzes the formation of toxic hydroxyl radicals, which resulted in cellular damage and liver injury.[77,78] Prolonged liver injury in response to chronic inflammation, infection, or oxidative stress contributes to recruiting inflammatory cells and secreting inflammatory cytokines, which promote the persistent activation of HSCs.[79,80] HSCs play an important role in the development and regeneration of the liver by fibrogenesis.[81] Iron overload-induced oxidative stress and lipid peroxidation could exacerbate the activation of HSCs *in vitro* and increase the production of collagen in primary HSCs.[82,83] Liver iron concentration exceeding 250 μmol/g has been reported to increase the potential risk for enhanced progression of NAFLD through elevating gene expression of collagen in HSCs, increasing the

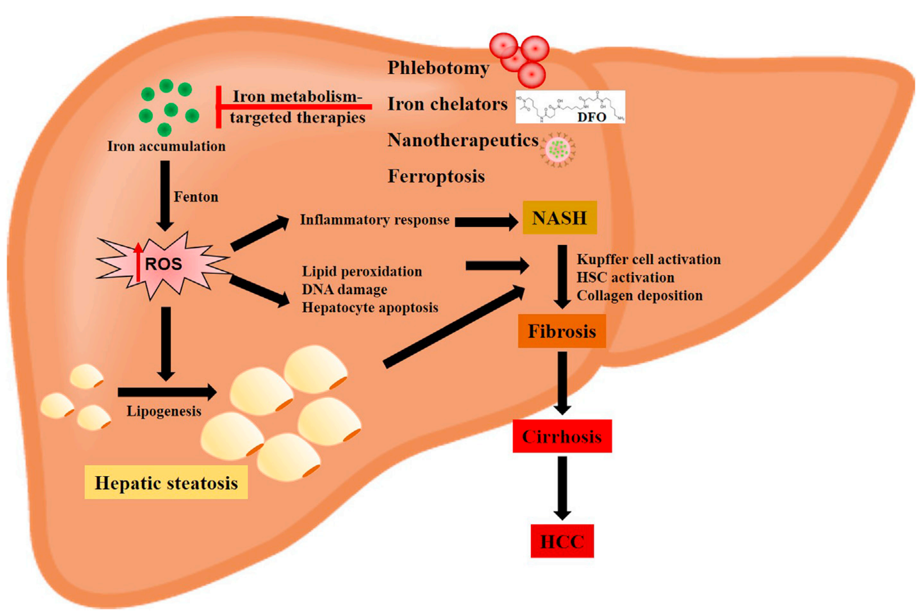

Fig. 2. Target iron metabolism to intervene in the pathogenesis and progression of NAFLD. Excess iron in the liver exacerbates deleterious characteristics in cellular injury and organ dysfunctions due to the formation of ROS through catalyzing the Fenton reaction. The excessive production of iron-induced reactive oxygen intermediates, such as peroxides and free radicals, can damage cellular proteins, lipids, and DNA, which increased the disease severity of NAFLD and elevated histological progression to NASH. Increased iron deposition in the liver is the "second hit" for contributing to NAFLD progression through increased liver inflammation and oxidative stress in the liver. In addition to catalyzing the production of ROS, excess iron may participate in the initiation of NAFLD by promoting the development of lipid peroxidation and insulin resistance, and will develop into NASH by the presence of hepatocellular inflammation, ballooning, and Mallory-Denk bodies. Iron metabolism-targeted therapies, such as phlebotomy, iron chelators, and nanotechnology, showed their potential as therapeutic options and as clinical strategies to intervene in the pathogenesis and progression of NAFLD. Abbreviations: NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; HCC, hepatocellular carcinoma; ROS, reactive oxygen species; DNA, deoxyribonucleic acid; HSC, hepatic stellate cell; DFO, deferoxamine.

expression of transforming growth factor beta (TGF-β) mRNA in rats, inducing the deposition of collagen in gerbils, and promoting cirrhosis in mice.[39,84,85] Exposure to environmental pollutants, such as 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) promotes liver fibrosis accompanied by liver iron deposition in hepcidin knockout mice through disordering systemic and hepatic iron homeostasis.[86] Iron deposition in KCs activates MiT/TFE transcription factors to increase liver inflammation and induce fibrosis-enhancing effects in a murine NASH model and human NASH.[87] Treatment with iron led to hepatic overload and disrupted the balance between M1 and M2 hepatic macrophage polarization through activation of M1 and inhibition of M2, which promoted macrophage-driven inflammation and fibrogenesis as drivers of NASH progression and fibrosis.[88] Iron chelation by deferasirox (DFX) attenuated the progression of concanavalin A-induced liver fibrosis in rats by regulating its antioxidant effect, offering an antifibrotic effect, and preventing the immunological stimulation of the liver.[89]

3.5. *Liver cirrhosis and HCC*

Recent studies have demonstrated that excessive iron overload in the liver is associated with increased severity of NAFLD and favors the progression of NAFLD to liver cirrhosis and HCC.[90] The oncogenic potential of iron is yet an unsolved dilemma. Iron deposition in the hepatic RES cells is an independent predictor of worsening fibrosis and chronic liver disease in patients with NAFLD.[91] Deferoxamine (DFO), as an iron chelator to remove iron from the liver, exhibited significant improvement in bone mineralization alongside its significant effect on liver function test in a rat model of liver cirrhosis-induced osteoporosis.[92] The possibility that hepatic iron overload exerts an oncogenic potential is related to ROS overproduction in the liver, which exceeded the intracellular antioxidant defense mechanisms. A direct role of iron in mutagenesis and hepatocarcinogenesis has been reported that patients with hereditary hemochromatosis (HH) increase 200-fold risk of developing HCC over the general population.[93,94] Hepcidin expression is inhibited in response to hepatic overload and is the cause of hepatocarcinogenesis in HH patients.[95] Muto *et al.*,[96] found that hepatic iron overload is a risk factor for the progression of HCC through disruption of F-box and leucine-rich repeat protein 5 (FBXL5)-mediated cellular iron homeostasis. Therefore, clinical and animal experiments have demonstrated that hepatic iron overload is considered a potential risk factor and diagnosis predictor in the initiation of liver cirrhosis and HCC, which provides a theoretical and experimental basis for targeting iron metabolism as a novel treatment for NAFLD.

4. Targeting iron metabolism in NAFLD: a promising new therapeutic strategy

As discussed above, iron removal from the liver by iron metabolism-based therapeutics and the corresponding combination therapy could provide a novel paradigm for NAFLD treatment. Below, we will summarize the recent progress in iron metabolism-based therapeutic strategies with a focus on phlebotomy, iron chelators, nanotherapeutics, and ferroptosis as the promising treatments for NAFLD (Fig. 2 and Table 1).

4.1. *Phlebotomy*

An ongoing pilot study has reported that phlebotomy therapy can improve hepatic and peripheral insulin sensitivity, and reduce triglyceride biosynthesis in the plasma.[97] Phlebotomy, withdrawing 500 mL of whole blood once-weekly per single clinical

Table 1
Summary of the clinical trials on iron metabolisme-based therapeutics in patients with NAFLD.

NCT Number[a]	Start date	Liver disease	Clinical status	Interventions	Age (Enrollment)	Location	Study purpose
NCT00658164	Oct 2007	NAFLD	Phase III	Phlebotomy	18–65 years (150)	Italy	Interventional/Treatment
NCT00641524	Jan 2009	NAFLD	Completed	Phlebotomy	18–75 years (45)	Canada	Interventional/Treatment
NCT01342705	May 2011	Cirrhosis	Terminated in phase III	Phlebotomy	≥18 years (17)	France	Interventional/Prevention
NCT02477462	May 2015	Cirrhosis	Completed	Phlebotomy	≥18 years (124)	US	Observational/Prospective
NCT03652467	Sep 2018	HCC	Recruiting in phase I	Deferoxamine	≥18 years and older (100)	China	Interventional/Treatment
NCT01033747	Feb 2003	Liver iron overload	Completed in phase II and III	Deferasirox	≥18 years (70)	Italy	Interventional/Treatment
NCT00432627	Dec 2006	Hepatic impairment	Completed in phase I	Deferasirox	18–75 years (24)	Germany	Interventional/Treatment
NCT01278056	Mar 2010	NASH	Completed in phase Iand II	Deferasirox	≥18 years (5)	Germany	Interventional/Treatment
NCT01767103	Jan 2013	Hepatic impairment	Completed in phase IV	Ferriprox®	18–75 years (21)	US	Interventional/Treatment
NCT02449109	May 2015	HCC	Completed in phase I and II	Nano drug encapsulated with Gemzar® and Compound Glycyrrhizin	18–80 years (60)	China	Interventional/Treatment
NCT01650181	Nov 2011	NAFLD, NASH	Completed in phase IV	Metformin + Siliphos + Selenium - Methionine + Alpha Lipoic Acid	18–65 years (50)	Mexico	Interventional/Treatment

[a] Data were obtained from ClinicalTrials: https://www.clinicaltrials.gov.
Abbreviations: NAFLD, non-alcoholic fatty liver disease; HCC, hepatocellular carcinoma; NASH, non-alcoholic steatohepatitis; US: United States.

treatment procedure, is a common therapeutic strategy used as a treatment in patients with T2D, hyperserotonemia, and HH.[98] Recently, reduction of hepatic iron stores has been reported to slow the progression of NAFLD. Treatment with phlebotomy in patients with NAFLD significantly decreased the concentrations of serum ferritin from (299 ± 41) μg/L to (15 ± 1) μg/L, and then improved insulin resistance, hepatic steatosis, and liver injury measured by fasting glucose, insulin, homeostatic model assessment-insulin resistance (HOMA-IR), and serum alanine aminotransferase (ALT) levels.[99] In phase II clinical trial of iron reduction therapy in the US (clinicaltrials.gov, Identifier NCT 00641524) showed that phlebotomy significantly improved the NAFLD score and reduced histological features of lobular inflammation, steatosis, and hepatocyte ballooning in thirty-one patients with NAFLD.[100] Khodadoostan *et al.*,[101] also showed that phlebotomy improved liver enzymes and histology of liver significantly and induced reduction of ferritin in thirty-two eligible patients with NAFLD, indicating phlebotomy is effective for the therapeutic strategy in the managing of NAFLD and hyperserotonemia. Similarly, iron reduction by phlebotomy not only improved serum ALT levels but also significantly reduced the staining for 7,8-dihydro-8-oxo-2′-deoxyguanosine (8-oxoG) in eleven patients with NASH.[102,103] A few studies have investigated the therapeutic efficacy of phlebotomy in patients with liver fibrosis, cirrhosis, and HCC, finding that liver function, hepatic fibrosis, and cirrhosis may be improved and reversed after long-term phlebotomy, while this data needs to be supported by randomized trials.[39,104–106]

4.2. Iron chelators

Iron chelators form complexes with iron and allow their removal from the body in urine or bile. Recent studies in various cell lines and animal models revealed that iron chelators successfully removed hepatic excess iron,[16,26,42] indicating the potential benefits of iron chelation therapy in the clinical management of NAFLD. Iron chelators have been sorted into three main groups depending on the mode of binding to the metal (Table 2), including hexadentate compounds (*e.g.,* DFO), tridentate (*e.g.,* DFX), and bidentate compounds (*e.g.,* deferiprone (DFP)).[107] As a polar molecule with low membrane permeability, DFO was the first chelator in the treatment of iron overload by reacting with Fe^{3+} in the form of methane-sulfonate and removing iron via fecal and urinary excretion.[108] Additionally, DFO, a membrane-permeable iron chelator, is the first oral medication approved by the US Food and Drug Administration (FDA) for chronic iron overload through formation of the DFO nanochelators and excreting through the kidney.[108] Furthermore, DFP is selective for hepatic iron and is used as a second-line drug for hepatic iron overload by combining with Fe^{3+} in a ratio of 3:1 and eliminating iron in the urine.[109] Mice with genetically obesity treated with DFO exhibited a decrease in hepatic steatosis and an improvement in hepatic lipid accumulation along with an increased expression of proteins, such as uncoupling protein 1 (UCP-1), peroxisome proliferator-activated receptor gamma (PPARγ), and PPARγ coactivator-1alpha (PGC-1α)[109] Meanwhile, Xue *et al.*,[109] found that DFO treatment significantly reduced hepatic cell apoptosis, liver inflammation, and oxidative stress in *ob/ob* mice. In other studies, DFO plays a critical role in decreasing the stability of procollagen mRNA in human fetal fibroblasts,[39]

Table 2
Comparision of the general properties of iron chelators.

Properties	DFO	DFX	DFP
Brand name	Desferal	Exjade Jadenu	Ferriprox
Year of FDA approval	1968	2005	2011
Administration routes	Subcutaneous intravenous	Oral	Oral
Usual doses	20–50 mg/kg/d	20–40 mg/kg/d	75–100 mg/kg/d
Usual schedules	8–24 h, 5 d per week	Once a day	Three times per day
Iron-binding affinity	26.6 pM	22.5 pM	19.9 pM
Indications[a]	Iron overload Thalassemia Sickle cell disease Hemosiderosis Transfusional iron overload Diabetes mellitus Hepatitis C	Iron overload Thalassemia Sickle cell disease Hemosiderosis Hepatic impairment	Iron overload Transfusional iron overload Sickle cell disease Hepatic impairment Thalassemia Hemochromatosis
Efficacy in liver	Good	Good	Moderate
Elimination $t_{1/2}$	6 h	8–16 h	1.9 h
Metabolism	Plasma enzymes	Liver UGT 1A1	Liver UGT 1A6
Clearance	Renal Hepatic	Hepatic	Renal
Excretion	Urine Bile Feces	Feces Urine	Urine
Adverse effects	Abdominal discomfort Nausea Vomiting Diarrhea Hypotension Anaphylaxis Local reactions Bone abnormalities Allergic reaction Bacterial infections	Nausea Vomiting Diarrhea Abdominal pain Rash Cytopenia Hepatic and renal dysfunction Gastric intolerance Increased transaminases and creatinine	Nausea Vomiting Diarrhea Abdominal pain Arthralgia Neutropenia Increased ALT Agranulocytosis

[a] Data were obtained from ClinicalTrials: https://www.clinicaltrials.gov.
Abbreviations: FDA, Food and Drug Administration; DFO, deferoxamine; DFP, deferiprone; UGT, UDP glucuronosyltransferases; ALT, alanine aminotransferase.

reducing elastin mRNA and elastin deposition in human skin fibroblasts,[110] and inhibiting the activation of rat HSCs by decreasing the expression of α-smooth muscle actin (α-SMA), procollagen, and tissue inhibitor of metalloproteinases (TIMPs).[111] Repeated injection of the iron chelator 2,2′-dipyridyl (2-DP) significantly attenuated proinflammatory and profibrotic changes in NASH-like liver phenotypes in the inducible NASH model.[88] DFO also exhibited obvious antifibrotic and antioxidant potential in carbon tetrachloride (CCl_4)-treated mice through reducing lipid peroxidation, fibrosis markers including hydroxyproline, and HSC-activation, and increasing superoxide dismutase (SOD) and glutathione peroxidase (GPx).[112]

4.3. Nanotherapeutics

In addition to phlebotomy and iron chelation, nanotechnology has been used to overcome cellular barriers and improve iron-related drug delivery in the treatment of systemic iron overload.[113,114] Due to their unique physicochemical properties and highly tunable natures, nanomaterials have been explored in biomedical fields, such as drug delivery, diagnosis, and disease therapy.[115–117] Although DFO has been shown as a highly effective iron chelator in chelating iron, it has a poor oral bio-availability with a short half-life (20–30 min in humans) to limit its routine use (subcutaneous or intravenous administrations at a dose of 20–50 mg/kg/day for 8–24 h, 5 days a week).[118,119] Iron metabolism-targeted nanotherapeutics can reduce the effective dose of iron chelators and mitigate their toxicity to achieve biological treatment standards. A liposomal formulation significantly extended the half-life of DFO and improved its urinary iron excretion.[119] Additionally, liposomes accumulated in the key iron storage organs (*e.g.,* liver and spleen), which increased DFO exposure in these organs.[119] To prolong the chelator's half-life, reduce administration frequency, enhance the safety profile, and minimize side effects, nanochelators have been developed through both conjugation and controlled release approaches.[119] PEGylated DFO was able to reduce iron accumulation, and exhibited significantly higher stability, longer half-life, lower cytotoxicity, and better hemocompatibility compared to DFO.[120] Marzban *et al.,*[121] developed DFO nanoliposomes by encapsulating DFO in a nonionic surfactant-based vesicle, which significantly reduced cytotoxicity of DFO and enhanced iron chelation in hepatocytes. Guo *et al.,*[118] have demonstrated that the encapsulation of DFO within polymeric nanoparticles is an effective and safe way to deliver the iron chelator for the clinical treatment of human iron overload disorders. ROS-responsive polyrotaxane nanoplatform encapsulated with DFO significantly enhanced the elimination of excess systemic and hepatic iron *in vivo*, indicating the promising alternative for safety prolonging the circulation of DFO in the treatment of iron overload disorders.[122] Liu *et al.,*[122] designed a nanochelator through the incorporation of DFO and ROS-sensitive thioketal groups into an α-cyclodextrin-based polyrotaxane platform (rPR-DFO), which served as a promising alternative for safely prolonging the circulation of DFO and more rapidly eliminating iron chelates from the liver.

4.4. Ferroptosis

Ferroptosis is a novel form of programmed cell death caused by iron-dependent lipid peroxidation.[11] Dysregulated iron metabolism (including increased iron uptake and reduced iron storage), lipid peroxidation, and accumulation of polyunsaturated fatty acid phospholipids (PUFA-PLs) may be the main factors that cause ferroptosis.[123] Emerging evidence indicates that ferroptosis plays a critical role in the pathological progression of NAFLD.[124] Ferroptosis can trigger the inflammatory response of simple fatty liver degeneration, and it promotes the occurrence and development of NASH.[125] Ferroptosis can aggravate the inflammatory response, oxidative stress, and cell damage in the early stages of NAFLD/NASH.[125,126] The ferroptosis inhibitors, including but not limited to β-mercaptoethanol, selenium, cycloheximide, thymosin β 4, dopamine, and glutaminolysis inhibitors, can almost completely reverse the death of liver cells, inflammation, and lipid peroxidation in the initial disease model of NAFLD.[123,127] Thus, targeting ferroptosis may provide a promising new therapeutic strategy for treating patients with NAFLD, NASH, liver fibrosis, and even HCC.[123,124]

5. Iron metabolism-targeted therapies in metabolic syndrome

NAFLD is the most prevalent chronic liver disease with a prevalence of 20–40% in the general population and up to 95% in subjects with metabolic syndrome including overweight or obesity and T2D.[8,128] Obesity is characterized by the increased size of adipocyte and the raised amounts of ectopic fat in the liver, which contributes to disabilities, reduced life expectancy, and impaired quality of life.[129] Iron overload in the body exacerbates adipose tissue dysfunction, which results in decreased adipogenesis, enhanced adipocyte inflammation, and adipose tissue macrophage infiltration.[130] Excess iron in adipose tissue stimulates the growth of adipocytes, which has detrimental effects on adipocyte differentiation and contributes to obesity.[131,132] Diabetes mellitus is a common and ever-increasing global health problem,[133,134] and is characterized by the impaired glucose metabolism. Its main symptom is hyperglycemia caused by impaired insulin secretion or impaired insulin action, or both.[135] Body iron stores and hepatic iron accumulation might be responsible for pathological conditions of glucose intolerance and diabetes through inducing oxidative stress and ROS.[17,135] Clinical studies have reported that elevated ferritin levels in serum are observed in most patients with T2D. The prevalence of diabetes in hemochromatosis is 13–22%, and impaired glucose tolerance is 18–30%,[45,135] indicated that hepatic iron overload increases the risk of developing T2D. Increased iron body accumulation is related to developing obesity and diabetes, both of which are ameliorated by iron reduction. Iron chelators, such as DFO, DFX, and DFP, can ameliorate oxidative stress and inflammation in obesity and T2D.[16,135] Hence, clinical studies and experimental data could be designed to evaluate the potential of iron chelators as therapeutic options in the management of obesity and T2D.[16,130,136] Body iron accumulation contributes to the pathophysiology of obesity and insulin resistance in adipose tissues of *ob/ob* mice, and iron depletion by DFO ameliorates adipocyte dysfunction in the epididymal adipose tissues of obese mice.[130]

6. Conclusions and perspectives

Here, we primarily reviewed and summarized recent advances in iron metabolism-targeted therapies in the treatment of NAFLD, namely, phlebotomy, iron chelators, nanotechnology, and ferroptosis, which showed their potential as therapeutic options and as clinical strategies for intervention in the pathogenesis and progression of NAFLD. Iron depletion via phlebotomy or iron chelation is a safe and effective treatment for NAFLD in several studies with small sample. However, many issues (*e.g.,* side effects) remain to be addressed. Although phlebotomy is a very effective method in the clinical treatment of NAFLD and T2D through reducing serum ferritin concentrations, ALT activity, and improving both hepatic and peripheral insulin sensitivity, its side effects are still common, including fatigue, fainting, pain at the venous access site, hematomas, and anemia.[98] Despite the success of DFO and DFX as effective strategies for mobilizing and removing iron in patients with NAFLD, both have been reported to induce liver dysfunction, renal dysfunction, hypersensitivity reactions, and neuronal hearing loss, which limited their clinical application prospects. The adverse reactions of orally active iron chelator have been shown in patients who reported gastric discomfort, zinc depletion, leukopenia, transient agranulocytosis or transient musculoskeletal, and joint pain.[137] Therefore, further *in vivo* studies must be conducted to clarify the molecular mechanism and mode of action of these iron chelators. There are still challenges in developing a candidate iron chelator with relatively low toxicity and high efficiency to remove hepatic iron in the treatment of iron overload. With the rapid development of nanotechnology, iron metabolism-based nanotherapeutics have been increasingly exploited as attractive treatment modalities in the treatment of NAFLD in recent years. Nanomaterials deposited in the liver do not change the liver function in healthy mice, but significantly induced worsened liver injury and increased lipid accumulation in mice with NAFLD due to impaired BMP-SMAD-mediated hepcidin expression and elevated hepatic iron deposition[138,139] The majority of iron-regulatory nanoparticles are still in the preclinical stage, and many tasks are lining up to be completed such as reducing the adverse effects. This will provide the theoretical basis and guide future research. Further in-depth understanding of the biological mechanisms of iron in the pathogenesis of NAFLD and the emerging advanced strategies for clinical iron metabolism-targeted therapy to intervene in the progression of NAFLD are still needed to be unraveled and implemented in field studies. More importantly, the molecular mechanism of the development of NAFLD is revealed and characterized by the "two-hit" mechanism. NAFLD is initiated with disruption of the lipid metabolism homeostasis in the liver, accompanied by hepatic steatosis, which further accelerates the vulnerability of the liver to a "second hit" in the form of inflammation. Therefore, it will be of particular interest to target on regulating lipid metabolism and inflammatory responses for the prevention of the progression of NAFLD. Recently, the therapeutic recommendations for NAFLD clinically start with lifestyle management, including weight loss, alcohol consumption restriction, and exercise.

Author' s contributions

H. Chen conceived the project, wrote, critically reviewed, and edited the manuscript.

Declaration of competing interest

The author declares that he has no conflict of interest.

Acknowledgements

This study was supported by the National Natural Science Foundation of China (Grant Nos. 32171370 and 11505193), the Natural Science Foundation of Guangdong Province (Grant No. 2022A1515010415), and the Research Foundation of Guangzhou First People's Hospital (Grant No. KY09040029).

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