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REVIEW

Dysregulation of iron and copper homeostasis in nonalcoholic fatty liver

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Abstract

Elevated iron stores as indicated by hyperferritinemia with normal or mildly elevated transferrin saturation and mostly mild hepatic iron deposition are a characteristic finding in subjects with non-alcoholic fatty liver disease (NAFLD). Excess iron is observed in approximately one third of NAFLD patients and is commonly referred to as the "dysmetabolic iron overload syndrome". Clinical evidence suggests that elevated body iron stores aggravate the clinical course of NAFLD with regard to liver-related and extrahepatic disease complications which relates to the fact that excess iron catalyses the formation of toxic hydroxyl-radicals subsequently resulting in cellular damage. Iron removal improves insulin sensitivity, delays the onset of type 2 diabetes mellitus, improves pathologic liver function tests and likewise ameliorates NAFLD histology. Several mechanisms contribute to pathologic iron accumulation in NAFLD. These include impaired iron export from hepatocytes and mesenchymal Kupffer cells as a consequence of imbalances in the concentrations of iron regulatory factors, such as hepcidin, cytokines, copper or other dietary factors. This review summarizes the knowledge about iron homeostasis in NAFLD and the rationale for its therapeutic implications.

Key words: Dysmetabolic iron overload syndrome; Hepcidin; Iron overload; Metabolic syndrome; Non-alcoholic fatty liver disease; Nonalcoholic steatohepatitis

Core tip: Hyperferritinemia with normal transferrin saturation and mostly mild hepatic iron deposition is a frequent finding in subjects with non-alcoholic fatty liver disease. Excess iron in non-alcoholic fatty liver disease (NAFLD) patients is referred to as the "dysmetabolic iron overload syndrome". Clinical evidence suggests that elevated body iron stores aggravate the clinical course of NAFLD with regard to liver-related and extrahepatic disease complications. Iron removal improves insulin sensitivity, delays the onset of type 2 diabetes mellitus,

improves pathologic liver function tests and ameliorates NAFLD histology The mechanisms contributing to iron excess in fatty liver include impaired iron export from hepatocytes and mesenchymal Kupffer.

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INTRODUCTION

Physiological regulation of iron homeostasis

Iron is essential for life of mammalian organisms due to its paradigmatic role in oxygen transport and also in being a central component of many enzymes and proteins involved in mitochondrial respiration, DNA biosynthesis and the citric acid cycle, among others. However, excess iron is detrimental and may lead to severe organ damage as it facilitates the formation of reactive oxygen species (ROS) *via* the Fenton reaction. On the other hand, iron deficiency can lead to anemia and fatigue which are among the most common disorders in the world. In order to provide enough iron for biological function and at the same time avoid iron overload and toxicity, iron trafficking and storage are diligently balanced by a mechanisms involving bone marrow, intestine, liver and the reticuloendothelial system (RES)[1,2].

Many aspects of iron metabolism have been unravelled in recent years. Dietary iron is taken up as Fe^{2+} in the duodenum by the cation transporter divalent metal transporter 1[3,4]. After transfer through the duodenal baso-lateral membrane *via* the iron exporter ferroportin (FPN)[5,6], iron is oxidized by the copper containing ferroxidase hephaestin and loaded onto transferrin for systemic distribution[7]. Most cells facilitae iron uptake by transferrin bound Fe^{3+} *via* the transferrin-receptor (TfR1). Most iron is required for erythropoiesis and the biosynthesis of other heme enzymes like cytochromes, and excess iron is stored in hepatocytes[5,8]. Most iron for physiological requirements, mainly erythropoiesis, is obtained from re-utilisation of senescent erythrocytes which are taken up and degraded in splenic macrophages. Only approximately 1-2 mg of daily body iron requirements which are used for compensation of iron losses *via* bleeding, enteric and cutaneous cell desquamation are replenished *via* duodenal iron absorption. Iron export is facilitated by FPN from hepatocytes, macrophages and all other cells[9].

Systemic iron homeostasis is equilibrated by the peptide hepcidin (hepatic bactericidal protein) mainly derived from hepatocytes and regulated by iron status, hypoxia, anemia and inflammation[10-12]. Hepcidin impacts on iron trafficking by attaching to FPN which leads to the degradation of FPN and thereby to down-regulation of iron export inducing a decline in serum iron concentrations[13]. Quantitatively hepatocytes are the most important source for hepcidin, however, expression has also been reported in adipose tissue, pancreatic islets, macrophages, and even cardiac myocytes. Hence, iron homeostasis *via* FPN mediated iron export may be regulated in an autocrine fashion in these cells[14-16].

Perturbations of iron homeostasis are frequently observed in patients suffering from non-alcoholic fatty liver disease (NAFLD)[17,18]. As the prevalence of obesity rises, NAFLD with or without associated metabolic syndrome (MetS), has become the most frequent cause of hyperferritinemia. The first report of non-hemochromatotic iron overload linked to metabolic characteristics such as insulin resistance and overweight in a French study subsequently stimulated extensive research on the potential mechanisms underlying iron accumulation in NAFLD[19]. The dysmetabolic iron overload syndrome (DIOS) commonly refers to the characteristic association of fatty liver with moderate histological iron deposition (hemosiderosis) and increased serum ferritin[17,20].

WHAT IS THE IRON PHENOTYPE OF NAFLD?

An increase in ferritin concentrations is the key feature of iron dysregulation in subjects with NAFLD. It is found in one third to half of patients with NAFLD and ranges from mild elevations to rarely 1000-1500 ng/mL[17]. Serum ferritin concentrations increase with the number of features of the MetS[21]. Transferrin saturation (TfS) is typically in the upper range of normal or mildly elevated (45%-50%) which is distinct from hereditary hemochromatosis, where hyperferritinemia is accompanied by markedly elevated TfS and usually TfS is elevated before the development of hyperferritinemia in early stages of hemochromatosis[22].

Iron deposits in NAFLD are found in Kupffer cells which are the resident liver macrophages as well as in hepatocytes[20]. Mesenchymal iron deposition is more frequent than hepatocellular iron accumulation but mostly both compartments are affected[23]. This is different from tissue iron deposition in primary genetic iron overload, hemochromatosis, where the metal is almost exclusively found in the hepatocellular compartment (with the exception of ferroportin disease) and macrophages are iron deficient as a result of uninhibited iron export from these cells[24,25]. The extent of hyperferritinemia in subjects with NAFLD and/or the MetS overestimates the degree of iron overload compared to hemochromatosis. Phlebotomy studies demonstrated that in DIOS patients the amount of iron need to be removed

for normalisation of circulating iron parameters is usually significantly less than in hemochromatosis, indicating only mild body iron excess[26,27]. Few studies have performed liver iron quantification in NAFLD subjects and these results confirm the mild degree of tissue iron excess compared to genetic iron overload disorders[19,28]. The mild degree of body iron excess compared to markedly raised serum ferritin concentrations suggests that iron overload in NAFLD subjects results from a combination of alimentary and inflammatory driven iron loading and retention[20,29,30]. This is in line with the current evidence that NAFLD is both a metabolic and an inflammatory disease[31].

WHAT IS THE CLINICAL RELEVANCE OF ELEVATED IRON STORES?

IR and associated metabolic conditions

In 1981 Sullivan[32] suggested that the postponed occurrence of cardiovascular diseases in women compared to men and the subsequent postmenopausal increase could be caused by low premenopausal iron stores. This report likely is the first report of an impact of iron stores in non-hemochromatotic metabolic disorders. An association of iron stores with type 2 diabetes mellitus (T2DM) and various manifestations of IR has been repeatedly confirmed and a detailed discussion thereof is beyond the scope of this review[33]. However, glucose metabolism and iron homeostasis appear to be functionally interconnected, due to the fact that gluconeogenetic signals regulate iron homeostasis *via* hepcidin[34] while iron loading or deficiency directly affect circulating glucose concentrations in mammals most likely *via* its effects on citric acid cycle enzyme activities[35,36], thereby also affecting lipid profiles[37]. Ferritin concentrations were associated with an increased rate of diabetes and gestational diabetes[38-43], with BMI[44], visceral fat mass[45], serum glucose levels and insulin sensitivity[46], blood pressure[47], the MetS[21,48], the polycystic ovary syndrome (PCOS)[49] and cholesterol[50]. Higher parameters of iron storage clustered with metabolic risk markers in a study of obese[51] and healthy lean adolescents[52]. These observations are epidemiologically important as patients with IR have a higher risk of developing cerebrovascular or cardiovascular disease[53,54]. However, the most convincing argument for causative involvement of iron in obesity-related conditions is derived from iron removal studies mentioned in detail below. In summary, available studies convincingly suggest a direct impact of body iron on manifestations of IR or the MetS.

NAFLD

NAFLD has been firmly established as the hepatic manifestation of the MetS/IR[55]. The disease spectrum of NAFLD ranges from simple steatosis which is generally considered benign to steatosis with various stages of inflammation, hepatocellular ballooning and fibrosis called non-alcoholic steatohepatitis (NASH). NASH is the potentially progressive manifestation leading to cirrhosis, end-stage liver disease and hepatocellular carcinoma in a minority of patients[56]. To our knowledge, there is no data available suggesting that excess iron is linked to the extent of hepatic steatosis. Although multiple associations between iron homeostasis and lipid metabolism have been reported[57], no characteristic lipid phenotype has been documented to distinguish NAFLD with iron overload from NAFLD without iron. Underlying NAFLD may explain the link between MetS features and ferritin on the population level[58].

Several studies provide evidence that iron may contribute to more advanced fibrosis and thus to progression of NAFLD[18,59-63], however, this association was not confirmed in all studies[64-66]. The to date largest study reported that iron in NAFLD liver biopsies, particularly in Kupffer cells, was linked to more fibrosis and disease severity[67]. Iron deposition particularly in the Kupffer cell compartment was associated with higher markers of hepatocellular apoptosis and oxidative stress[68]. Some studies also suggested that an increased rate of HFE mutations could account for more progressed stages of NAFLD, but this was not reported in all studies[65,69-72]. Additionally the beta-globin trait[73], TMPRSS6[74], and the alpha-1-antitrypsin genotype[75] may modify the iron phenotype of NAFLD. It appears reasonable to conclude that the contribution of the genetic background may vary according to the geographic region. Data evaluating causality of iron in disease progression is limited by the feasibility of a prospective study with serial liver biopsies in enough patients to adjust for known co-factors of disease progression[64,76]. Retrospective studies demonstrated that, hyperferritinemia was linked to mortality of patients on the transplantation waiting list and it also had an impact on post-transplant mortality[77,78]. It is important to note that particularly sinusoidal iron deposition may be linked to the development of HCC in NASH[79].

In summary, the prevailing body of evidence suggests that excess iron is a contributing factor for the progression of steatosis to NASH, liver cirrhosis and also hepatocellular carcinoma. It remains to be established to what extent different patterns of iron deposition affect outcomes such as cirrhosis, HCC or cardiovascular diseases. The data mentioned above suggest that the pattern of iron deposition may have distinct effects.

HOW DOES IRON LEAD TO DISEASE PROGRESSION IN NAFLD?

It has been well recognized that iron overload leads to diabetes in patients with hemochromatosis where

IR increases and insulin secretion decreases with the rise of body iron stores[25,80-82]. Hepatic insulin sensitivity and insulin secretion are re-established in the majority once iron is removed[83,84]. However, the prediabetic stage in hemochromatotic mice and humans displays impaired β-cell function along with increased insulin sensitivity, whereas dietary iron overload similar to the prediabetic state in humans are characterized by peripheral IR[85]. Hence, lessons drawn from hemochromatosis models are likely not fully applicable to the role of iron in human IR and NAFLD.

Iron is well-recognized as a catalyst for the production of reactive oxygen intermediates *via* the Fenton reaction, and it is generally held that an increase of oxidative stress is a central mechanism for IR although direct proof for this hypothesis has not been obtained so far. Oxidative stress is a central pathogenic factor in NAFLD, T2DM and obesity[86-88] and markers of oxidative stress were increased in NAFLD with iron loading as compared to NAFLD without iron excess[68,89,90]. Generation of ROS may induce lipid peroxidation and cellular damage which may contribute to the progression of NAFLD. Importantly, oxidative stress induced molecules such as malonyldialdehyd and 4-hydroxynonenal may induce the formation of *de-novo* antigens with subsequent activation of T-lymphocytes and development of immunoglobulin G reactive against these antigens. This response was further enhanced by previous immunization against these antigens with a stimulated M1 macrophage response[91]. Although no studies have been performed, iron may contribute to this process by further augmentation of oxidative stress.

In cell culture, iron chelation re-established insulin receptor signalling and iron inhibited insulin receptor activity[92]. Desferoxamine increased the phosphorylation of Akt/protein kinase B (Akt/PKB), forkhead transcription factor O1 (FoxO1) and glycogen synthase kinase 3β (GSK3β) reflecting insulin effects on gluconeogenesis and glycogen synthesis. Likewise, genes playing a role in glucose utilization such as GLUT1 or hypoxia-inducible factor 1α (HIF1α) were up-regulated in hepatoma cells resulting in enhanced glucose removal[92]. In summary, these molecular observations indicate that iron affects IR by modulating insulin receptor signalling as has been recently reviewed[93].

Importantly, dietary iron intake may impact on glucose metabolism by affecting circadian rhythm *via* heme mediated effects on RevErb-α. Disruption of circadian rhythms, *e.g.,* through night-shift work is an established risk factor for metabolic and cardiovascular diseases[94,95].

In cultured fat cells, iron favored an IR, characterised by impaired glucose uptake and suppression of lipolysis in response to insulin[96,97]. Ferritin was inversely associated with adiponectin concentrations in insulin resistant and sensitive patients[98,99]. Knockout of FPN1 in adipocytes increased intracellular iron and subsequently reduced adiponectin biosynthesis, thus establishing a molecular link between adipocyte iron concentration and insulin resistance[100]. Furthermore, excess iron the diet may be routed to visceral adipose tissue and change the expression of adipokines, as demonstrated for resistin[101] Adipokines represent a diverse group of hormones which mediate the metabolic effects of diseased adipose tissue to organs and tissues. Associations have been observed between retinol-binding protein 4 (RBP4) and visfatin serum concentrations and parameters of iron metabolism[102,103]. However, these reports may reflect the co-incidence of elevated iron stores with surrogate markers of IR and do not prove causality[93].

Liver macrophages named Kupffer cells, which are an important site of iron storage in NAFLD, are tightly involved in the initiation of the hepatic inflammatory cascade in response to the uptake of oxidized lipoproteins[104] or oxidized phosphatidylcholines[105]. It is well known that macrophage iron status affects their inflammatory response pattern and polarization towards a pro-inflammatory phenotype[106], however, the particular role of these potential interactions have to our knowledge not been investigated in NAFLD.

Thus, the potential mechanisms of iron-induced NAFLD disease progression are complex and involve protean effects of iron in extrahepatic tissues as well direct liver damage.

WHAT ARE THE MECHANISMS UNDERLYING IRON ACCUMULATION IN NAFLD?

Hepcidin is the key regulator of systemic iron homeostasis and plays a role for the hemochromatotic and the inflammatory driven misdistribution of iron. Whereas the lack of hepcidin in hemochromatosis leads to uncoordinated duodenal iron absorption and iron accumulation in parenchymal tissues such as the liver[107]. the inflammation driven iron retention occurs mainly in monocytes/macrophages as a consequence of increased iron accumulation and reduced FPN mediated iron export from these cells, the latter being due to increased circulating hepcidin levels along with negative effects of certain cytokines on FPN expression[108]. The histological hallmarks of hemochromatosis, *i.e.*, hepatocellular iron, and also the inflammatory phenotype iron deposition in macrophages are both observed concurrently, suggesting that iron dysregulation is multifaceted in NAFLD. Several stimuli of hepcidin regulation have been reported which may be of particular relevance in NAFLD and also be related to different iron phenotypes. These stimuli and their relation

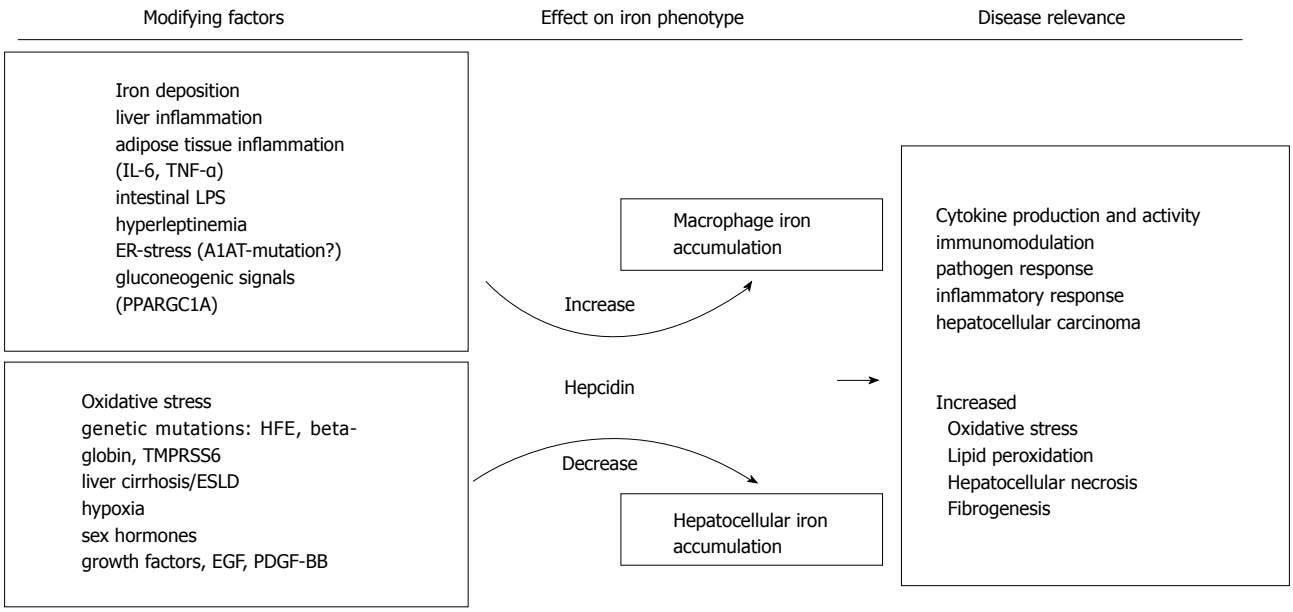

Figure 1 Summary of the potential stimuli that may affect iron homeostasis in non-alcoholic fatty liver disease. Both, increasing and decreasing stimuli have been reported in non-alcoholic fatty liver disease and it appears likely that the net balance of these frequently counteracting forces finally determines the iron phenotype in the individual. Patterns of iron deposition may also be linked to distinct clinical consequences. IL-6: Interleukin 6; TNF-α: Tumor necrosis factor-α; LPS: Lipopolysaccharide; ER: Endoplasmatic reticulum; A1AT: α-1-antitrypsin; PPARGC1A: Peroxisome proliferator-activated receptor gamma coactivator 1-α; TMPRSS6: Transmembrane protease, serine 6; ESLD: End-stage liver disease; EGF: Epidermal growth factor; PDGF-BB: Platelet derived growth factor BB.

to NAFLD iron accumulation are summarized in Figure 1. For several of these, like sex hormones, growth factors and hypoxia-induced circulating factors, the contribution to the dys-regulation of iron homeostasis in NAFLD has not been directly demonstrated but is physiologically plausible and these have therefore been included in the summary figure[109-112]. Additionally, alcohol consumption may decrease hepcidin expression and thus modify iron accumulation in NAFLD subjects[113], and although relevant alcohol consumption should be excluded in NAFLD subjects both conditions frequently co-exist. Thus, in NAFLD multiple, potentially counteracting signals impacting on hepcidin expression may be present at the same time. It is likely that the net balance of these signals finally determines the pattern of iron accumulation in the fatty liver of the individual patient.

Hepcidin levels in urin, serum and liver were elevated in NAFLD patients with iron excess compared to healthy subjects, hemochromatosis patients and NAFLD subjects without excess iron[28,114-116]. Hepcidin expression correlated directly with liver iron indicating an intact physiological response of hepcidin biosynthesis to iron in the liver[28,114]. Additionally, hepcidin is expressed in adipocytes of morbidly obese subjects[15]. Moreover, obesity is characterised by a chronic subclinical inflammation and in humans hepcidin concentrations and TNF-α were directly related, suggesting that both iron and inflammation contribute to hepcidin biosynthesis in NAFLD[28]. Furthermore, hepcidin and cytokines may be derived from both, the inflamed adipose and the liver[117,118]. Activation of gluconeogenesis *via* starvation, namely activation of peroxisome proliferator activated receptor gamma co-activator-1 α (PGC1α) increased hepcidin expression in a mouse model[34]. Likewise, iron fortification decreased gluconeogenesis *via* PGC1α in a murine model[119]. Hence, although PGC1α offers an intriguing cellular link between glucose and iron homeostasis, its relevance to human NAFLD remains to be elucidated. Leptin, was demonstrated to up-regulate hepcidin in hepatocytes in vitro by activation of the JAK2/STAT3 pathway. Hence, hyperleptinemia may directly contribute to higher hepcidin and thereby to iron deposition in NAFLD[120,121].

In NAFLD with iron overload the iron exporter FPN is lower than in controls and hemochromatosis patients in the liver and in the duodenum[28,114,122,123]. In NAFLD without liver iron accumulation, FPN levels were comparable to control subjects, but were significantly lower in NAFLD with hepatic iron on histology[28]. Along the same line of the observations, duodenal iron absorption was decreased in DIOS patients[124]. Obesity also represents a risk factor for an inadequate dietary iron fortification, linked to high hepcidin and low FPN expression[125]. Along this line mice feed a high fat diet presented with significantly reduced iron absorption which could be traced back diminished intestinal iron uptake. Mechanistically, the impaired iron absorption was independent of hepcidin but resulted from reduced metal uptake into the mucosa and transfer of iron across enterocyte membranes as a consequence of dietary induced discordant membrane-bound oxidoreductase expression[126].

An additional mechanism may be the phagocytosis of fragile erythrocytes by liver Kupffer cells. This was documented in rabbits on a high-fat diet and the phagocytosis of fragile erythrocytes was observed *in vitro*. Accumulation of erythrocytes was microscopically detected in inflamed regions in human NAFLD[127] suggesting that uptake of heme-iron *via* erythrophagocytosis may contribute to NAFLD iron accumulation, then promoting oxidative stress and inflammation.

Although cellular iron uptake *via* TfR1 is the most important route of iron uptake under physiological circumstances TfR1 appears not to be involved in excess iron uptake in NAFLD[128,129]. Hepatic TfR1 expression in NAFLD patients with low iron was increased compared to NAFLD and iron accumulation or patients with hemochromatosis suggesting physiologically intact TfR1 expression in response to iron stimuli[28] (Figure 2).

WHAT IS THE ROLE OF COPPER IN NAFLD?

Similar to iron, an adequate supply of copper is essential for proper biological function. Chronic copper deficiency can elicit anemia, leucopenia, myelopathy or skin abnormalities and excess copper may also facilitate the formation of ROS.

Copper affects lipid and glucose metabolism

There are several ways in which inadequate copper supply may be involved in the pathogenesis of NAFLD. Epidemiological studies found that copper deficiency is linked to atherogenic dyslipidemia and dietary copper supplementation improved cardiovascular risk markers in healthy adults[130] Investigations in rodent models demonstrated that dietary copper restriction induces hypertension or cardiac dysfunction, hypertriglyceridemia, hypercholesterolemia and modifies LDL and VLDL composition[131,132]. We recently reported low intrahepatic copper concentrations in human NAFLD compared to other liver diseases and that rats on a copper depleted diet developed IR and liver steatosis[133]. Increased oxidative stress is considered a key trigger in the pathogenesis of human NAFLD and one of the enzymes counteracting oxidative stress, Cu/Zn superoxide dismutase (SOD) depends on adequate copper availability, suggesting a potential link between copper availability and impaired antioxidant defense in NAFLD[134]. Sprague-Dawley rats exhibited an increased activity of the pro-inflammatory protein cyclo-oxygenase-2, when fed a diet with a low copper content[135]. Systemic copper deficiency causes mitochondrial dysfunction in mice and similar morphological and functional alterations have also been described in human NAFLD[136]. Recently, a detailed examination revealed an interaction of a high-fructose diet (which is also a culprit in the rise of obesity-related conditions) with low copper intake in triggering liver steatosis and damage as well as iron overload. Fructose acts as an inhibitor of duodenal copper absorption thereby leading to impaired oxidant defense and augmented lipid peroxidation[137]. As dietary copper content of the Western diet is rather low whereas iron and fructose are consumed in excess, this model offers attractive data to speculate that a dysbalance in micronutrient intake may have a significant role in NAFLD beyond calorie excess. Hence, animal and human data suggest that the therapeutic effect of dietary copper supplementation should be investigated as a subset of patients may potentially benefit.

Copper affects NAFLD iron homeostasis

Copper modulates iron homeostasis and is also linked to the iron perturbations of NAFLD. Hephaestin ferroxidase activity in duodenal enterocytes is critically dependent on copper as it oxidizes ferrous to ferric iron which is subsequently loaded onto Tf[7]. Similarly, copper is necessary for ceruloplasmin function to export iron from the liver or the RES and also for FPN expression[138]. Expression of a membrane-bound form of ceruloplasmin is mandatory for stable FPN expression[139,140]. Accordingly, a lack of ceruloplasmin as found in the heritable disease aceruloplasminemia leads to tissue iron accumulation and damage most notably in the brain[141].

Low liver and serum copper concentrations were reported in iron overloaded NAFLD and were linked to decreased ferroxidase activity of ceruloplasmin[122]. The expression of FPN was found to be decreased in livers of rats on a copper deficient diet. These observations provide evidence that in addition to decreased FPN expression due to low-grade systemic inflammation, low copper bioavailability contributes to iron retention in NAFLD.

WHAT IS THE THERAPEUTIC POTENTIAL OF MODULATING IRON STORES IN NAFLD?

Elimination of iron may confer a beneficial effect on IR-associated conditions. Removal of iron using phlebotomies is usually well tolerated, with the caution that DIOS patients frequently show a fast decline in TfS[142]. These clinical observations are expected due to the underlying molecular mechanisms of impaired iron export. The incidence of diabetes, postprandial serum insulin and pancreatic insulin sensitivity, *i.e.,* beta cell function were al improved in subjects with previous phlebotomy treatment[143]. Iron removal also improved coronary vascular dysfunction in patients with T2DM[144] and endothelial function in patients with known coronary artery disease and in subjects with primary iron

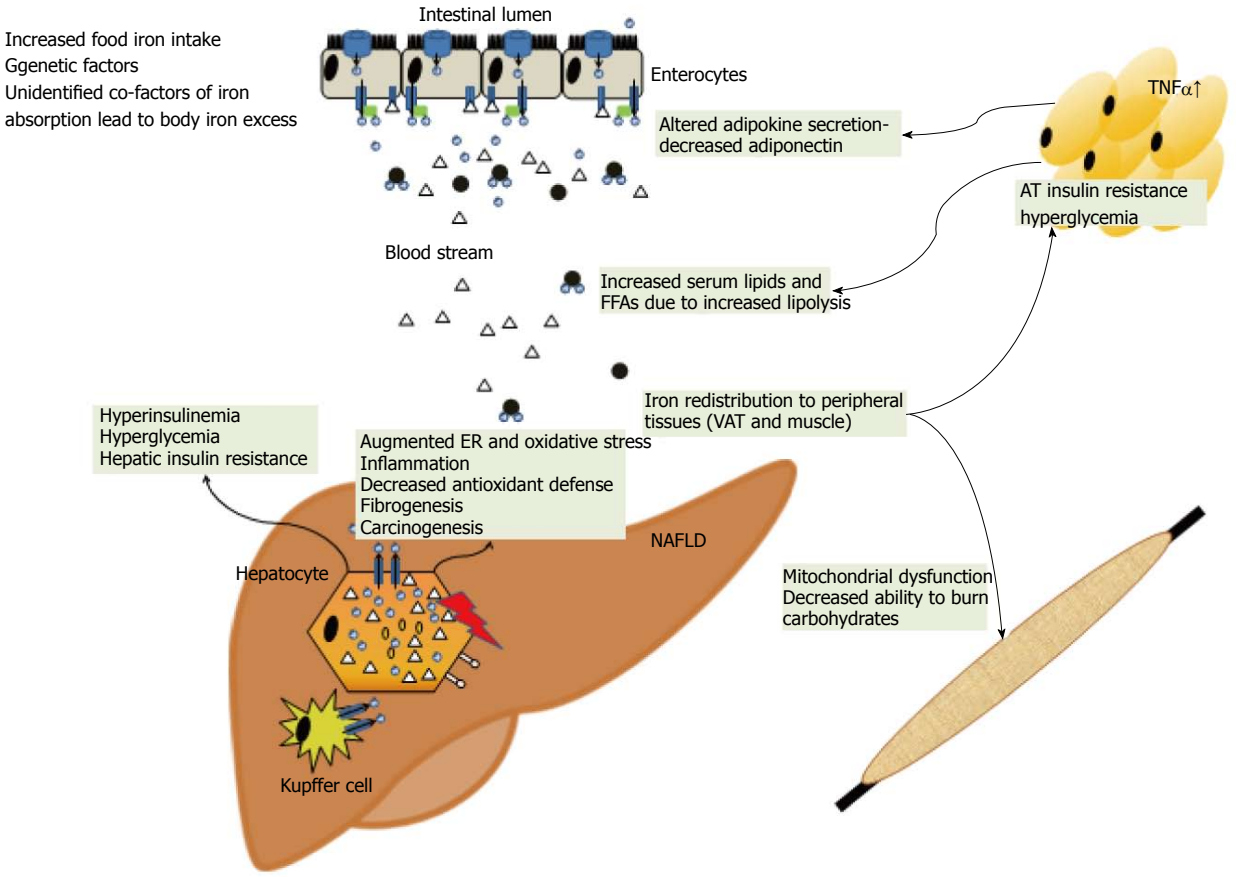

Figure 2 Summary of how iron excess and low copper availability may affect whole body glucose and lipid homeostasis. Iron excess may promote insulin resistance in the liver, muscle and adipose tissue. Iron may increase ER and oxidative stress whereas low copper is potentially associated with an impaired antioxidant defence. These factors may result in the propagation of inflammation, fibrogenesis and hepatocarcinogenesis. TNF-α: Tumor necrosis factor-α; ER: Endoplasmatic reticulum; FFA: Free fatty acid; VAT: Visceral adipose tissue; AT: Adipose tissue.

overload[145,146]. Blood donations were linked to insulin sensitivity even in healthy subjects[46]. Studies on iron depletion in NAFLD in humans have demonstrated benefits regarding systemic or hepatic insulin resistance and pancreatic insulin sensitivity[142,147,148]. A randomized trial demonstrated improved HbA1c, insulin sensitivity and secretion subjects who received phlebotomy treatment[149]. The effects of iron depletion were additive to successful lifestyle modifications[150]. Similar observations were reported the effect of iron depletion on other cardiovascular risk factors[151] and iron removal may prevent development and progression of malignancies[152].

As far as practical treatment of iron excess in NAFLD patients with elevated ferritin is concerned, available data suggest that iron removal may thus be beneficial in addition to weight loss, diet and lifestyle modification or antidiabetic medication as indicated in an individual patient. We have adopted the practice to perform biweekly phlebotomies in these subjects until serum ferritin concentrations are between 50 and 100 ng/L, however, no evidence-based recommendation for this is currently available. In contrast to hemochromatosis patients, NAFLD subjects have impaired iron mobilisation from storage sites and may therefore develop anemia in response to phlebotomy treatment. We therefore recommend close monitoring of serum ferritin, TfS and hemoglobin at each visit for the period of time while these patients are on phlebotomy treatment[26,153].

CONCLUSION

Elevated serum ferritin concentrations are a frequent finding in NAFLD. Excess iron is linked to IR, accelerated disease progression and adverse outcomes. Removing excess iron *via* phlebotomies is safe and has clinical benefits. We suggest that on the basis of available evidence it can be offered to NAFLD patients as it is linked to improvement of IR and inflammation. The mechanisms underlying iron accumulation in NAFLD are tightly linked to impaired iron export from liver cells as a consequence of low expression of the iron export molecule FPN and elevated hepcidin concentrations. Inflammation of adipose tissue as indicated by TNF-α and IL-6 and altered adipokine secretion (leptin, resistin) or hepcidin represent potent signals from diseased

adipose tissue to dysregulate iron as well as glucose or lipid homeostasis.

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