Review

EASL EUROPEAN ASSOCIATION FOR THE STUDY OF THE LIVER | JOURNAL OF HEPATOLOGY

Iron in fatty liver and in the metabolic syndrome: A promising therapeutic target

Paola Dongiovanni, Anna Ludovica Fracanzani, Silvia Fargion, Luca Valenti*

Department of Internal Medicine, Centro Malattie Metaboliche del Fegato, Università degli Studi di Milano, and Fondazione IRCCS "Ca' Granda" Ospedale Maggiore Policlinico, Milan, Italy

Summary

The dysmetabolic iron overload syndrome (DIOS) is now a frequent finding in the general population, as is detected in about one third of patients with nonalcoholic fatty liver disease (NAFLD) and the metabolic syndrome. The pathogenesis is related to altered regulation of iron transport associated with steatosis, insulin resistance, and subclinical inflammation, often in the presence of predisposing genetic factors. Evidence is accumulating that excessive body iron plays a causal role in insulin resistance through still undefined mechanisms that probably involve a reduced ability to burn carbohydrates and altered function of adipose tissue. Furthermore, DIOS may facilitate the evolution to type 2 diabetes by altering beta-cell function, the progression of cardiovascular disease by contributing to the recruitment and activation of macrophages within arterial lesions, and the natural history of liver disease by inducing oxidative stress in hepatocytes, activation of hepatic stellate cells, and malignant transformation by promotion of cell growth and DNA damage.

Based on these premises, the association among DIOS, metabolic syndrome, and NAFLD is being investigated as a new risk factor to predict the development of overt cardiovascular and hepatic diseases, and possibly hepatocellular carcinoma, but most importantly, represents also a treatable condition. Indeed, iron depletion, most frequently achieved by phlebotomy, has been shown to decrease metabolic alterations and liver enzymes in controlled studies in NAFLD. Additional studies are warranted to evaluate the potential of iron reductive therapy on hard clinical outcomes in patients with DIOS.

A strong association between iron overload unrelated to hereditary hemochromatosis (HHC) and several manifestations of the metabolic syndrome (MetS), including nonalcoholic fatty liver disease (NAFLD), has been demonstrated during the last years. Furthermore, iron stores have been linked to a heightened risk of metabolic complications, such as diabetes, and faster progression of organ damage, including hepatic and cardiovascular diseases. Although emerging evidence suggests that the association between iron, NAFLD, and MetS represents a clinically ominous condition, the mechanisms underpinning the dysmetabolic iron overload syndrome (DIOS) and the pathogenesis of organ damage are still debated, whereas the potential therapeutic role of iron depletion therapy (ID) for the prevention of clinical complications is just beginning to be evaluated in controlled trials. Here, we review the recent overall evidence on epidemiology, pathogenesis, genetics, natural history, and treatment of DIOS, and provide a hypothetical interpretation of contrasting findings, with possible lines of future research.

Association between hyperferritinemia, MetS alterations, and NAFLD: the dysmetabolic iron overload syndrome

Ferritin and increased body iron stores have been associated with insulin resistance (IR) and metabolic abnormalities defining MetS in population studies conducted both in Western and Eastern countries [1–4].

Several studies confirmed the association between hyperferritinemia and type 2 diabetes (T2D). In a case–control study in Europe, subjects with hyperferritinemia had a 2.4-fold higher risk to develop T2D [5], whereas in a cross-sectional study in 9486 US subjects, elevated ferritin was associated with T2D [6]. More recently, in a prospective nested case–control study in 32,826 healthy Chinese women, higher iron stores were associated with T2D independently of known risk factors [7], and in a case–control study nested in the EPIC-Norfolk cohort, ferritin levels were again an independent predictor of incident T2D [8]. In the HEIRS study considering 97,470 subjects belonging to six racial/ethnic groups, ferritin was independently associated with

Keywords: Iron; Insulin resistance; Nonalcoholic fatty liver disease; Metabolic syndrome; Vascular damage; Hereditary hemochromatosis; Nonalcoholic steatohepatitis; Oxidative stress.

Received 22 February 2011; received in revised form 29 May 2011; accepted 31 May 2011

* Corresponding author. Address: Department of Internal Medicine, Università degli Studi di Milano, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Padiglione Granelli, via F Sforza 35, 20122 Milan, Italy. Tel.: +39 02 503 20278; fax: +39 02 503 2096.

E-mail address: luca.valenti@unimi.it (L. Valenti).

Abbreviations: DIOS, dysmetabolic iron overload syndrome; NAFLD, nonalcoholic fatty liver disease; HHC, hereditary hemochromatosis; MetS, metabolic syndrome; ID, iron depletion; IR, insulin resistance; NASH, nonalcoholic steatohepatitis; HFD, high-fat diet; FFAs, free fatty acids; ER, endoplasmic reticulum; T2D, type 2 diabetes; PCOS, polycystic ovary syndrome; Cp, ceruloplasmin; Fp-1, ferroportin-1; HFE, hemochromatosis gene; AAT, alpha1-antitrypsin; ROS, reactive oxygen species; HO-1, heme oxygenase-1; RBP4, retinol binding protein-4; HSCs, hepatic stellate cells; 8-oxodG, 7,8-dihydro-8-oxo-2′ deoxyguanosine; MCD, methionine choline deficient diet; InsR, insulin receptor.

T2D [9]. As concerning the relationship among iron stores, MetS and IR, in a cross-sectional study in 6044 US adults, ferritin was associated with MetS and IR [1]. Other epidemiological studies showed a correlation between ferritin, MetS, and IR severity, which was independent of inflammation [2,3].

An interesting disease model to dissect the relationship between iron and MetS is also provided by the polycystic ovary syndrome (PCOS). PCOS is characterized by overweight, IR, and increased ferritin levels, which are not related to inflammation, but likely to oligomenorrhea [10]. Increased iron stores have been suggested to contribute to IR frequently found in PCOS patients [11]. In PCOS, metformin reduced ferritin in parallel with an increase in insulin sensitivity, thus suggesting that hyperinsulinemia and IR play a role on the increased iron stores in these patients [12].

Thus, overall evidence indicates that high ferritin, reflecting iron stores, is associated with MetS and T2D in ethnically diverse populations. However, the extent to which ferritin truly reflects iron stores (since inflammation and oxidative stress may complicate the picture), and whether iron overload is causally associated with IR are still a matter of debate.

Key Points

- The dysmetabolic iron overload syndrome (DIOS) is detected in about one third of patients with nonalcoholic fatty liver disease (NAFLD) and the metabolic syndrome.
- The pathogenesis is related to altered regulation of iron transport associated with steatosis, insulin resistance, and subclinical inflammation, often in the presence of predisposing genetic factors.
- Increased body iron plays a causal role in insulin resistance through still undefined mechanisms that probably involve a reduced ability to burn carbohydrates and altered function of adipose tissue.
- DIOS may facilitate the evolution to type-2 diabetes, the progression of cardiovascular disease, the natural history of liver disease, and malignant transformation by promotion of cell growth and DNA damage.
- Iron depletion by phlebotomy, has been shown to decrease metabolic alterations and liver enzymes in controlled studies in NAFLD, and possibly to reduce the progression of vascular damage.

A different perspective focused on the liver: Mendler *et al.* first described a cohort of patients with unexplained hepatic iron overload characterized by the association with IR, and coined the term "insulin resistance-associated hepatic iron-overload syndrome" [13]. On the other hand, increased ferritin is detected in about 30% of unselected patients with NAFLD [14], and in these subjects it has been associated with increased hepatic iron, as determined by histological and radiological assessment, and by quantitative phlebotomy [14–17]. The acronym NAFLD refers to a broad spectrum of liver diseases ranging from uncomplicated steatosis to nonalcoholic steatohepatitis (NASH), which may progress to cirrhosis and hepatocellular carcinoma [18], and poses a high risk of cardiovascular disease [19]. Now the leading cause of liver disease in Western countries [20], NAFLD is characterized by hepatic insulin resistance (IR) and is considered a manifestation of the MetS [21–23]. Progression of liver damage is more severe when fatty liver is complicated by NASH [23–25], which is thought to be provoked by lipid peroxidation and mitochondrial dysfunction determining oxidative stress and cytokine release [26].

The condition characterized by hepatic iron overload involving both hepatocytes and macrophages [27], absence of inflammation, normal transferrin saturation, and associated with features of MetS is now more commonly referred as DIOS [28,29]. Although the diagnostic criteria are not clearly defined, DIOS represents the most frequent iron overload condition, since, as the clinical presentation overlaps almost completely with that of hyperferritinemia associated with metabolic abnormalities, it is observed in 15% of patients with MetS [2], and it is associated in at least half of the cases with NAFLD [30]. DIOS patients have mild hepatic iron excess with a predominantly mixed sinusoidal/hepatocellular pattern [31], which presupposes macrophage iron retention and an iron recycling defect that is associated with the severity of inflammation and IR [32].

Recently, a more strict definition of DIOS has been proposed, based on the presence of two or more MetS components, steatosis, normal transferrin saturation, and mild hepatic iron overload, with typical involvement of the sinusoidal compartment [29]. However, this definition is not applicable to subjects who do not have an indication for liver biopsy.

It has been reported that in DIOS iron absorption is decreased and hepcidin, the hormone that acts by decreasing intestinal iron absorption and recycling from macrophages [33], is increased compared to healthy controls, indicating that iron compartmentalization in monocytes is likely related to a relatively preserved upregulation of hepcidin as an attempt to counteract iron excess [28,34].

Metabolic hyperferritinemia and DIOS: different faces of the same problem?

As "metabolic" hyperferritinemia associated with NAFLD and MetS and DIOS share the majority of clinical features (Table 1), we propose that they might be considered as two faces of the same health problem. In particular, both are characterized by (1) the presence of metabolic alterations typical of MetS [21,29,35]; (2) the presence of fatty liver [29]; (3) hyperferritinemia with normal or only slightly elevated transferrin saturation, reflecting physiological upregulation of hepcidin in response to increased iron stores [14,35,36]; (4) besides that in DIOS, mildly increased hepatic (as detected by histological scores after Perls' stain for iron, and determination of liver iron concentration by atomic absorption spectrometry, or superconducting quantum interference device – SQUID) and body iron stores (indirectly estimated by the association with risk factors such as transferrin saturation, age, male gender, increased alcohol intake within normal limits, and *HFE* mutations, and quantitatively assessed by quantitative phlebotomy) have been demonstrated in patients with NAFLD associated with hyperferritinemia compared to those without hyperferritinemia, and the amount of body iron has been associated with serum ferritin [14,15,37,38]. Data obtained in recent studies by our group have been summarized in Supplementary Table 1. Furthermore, serum ferritin has been associated

Table 1. Comparison of clinical features of hyperferritinemia associated with NAFLD and MetS and of DIOS. Clinical features not referenced are present by definition [29].

	Hyperferritinemia in NAFLD and MetS	DIOS
Male predominance	Yes [14]	Yes [13]
Serum ferritin	Increased	Increased
Transferrin saturation	Normal/slightly increased [14]	Normal
Hepcidin	Increased [38, 41]	Increased [28]
Hepatic iron concentration	Mild increase [15, 16, 37]	Mild increase
Hepatic iron pattern	Non-parenchymal/mixed Hepatocellular if *HFE* or *beta-globin* mutations altering hepcidin [17,41]	Non-parenchymal/mixed
Ferroportin-1 muts/SNPs	None [41]	Rare [42]
Dietary iron intake	Increased ? [154]	?
MetS components	Present	Present
Insulin resistance	Increased Correlates with ferritin [37-39, 154]	Increased
Steatosis	Present	Present
CRP levels	Within normal range [38]	Within normal range
Cytokines	Correlation with IL-6 and TNF-α, MCP-1 [39, 123]	?
Response to phlebotomy	Yes [15, 146, 152, 155]	Yes [145, 151]

NAFLD, nonalcoholic fatty liver disease; MetS, metabolic syndrome; DIOS, dysmetabolic iron overload syndrome; muts, mutations; SNPs, single nucleotide polymorphisms; CRP, C reactive protein; IL-6, interleukin-6; TNF-α, tumor necrosis factor-α; MCP-1, macrophage chemoattractant protein-1 (CCL-2).

with the severity of insulin resistance [38–40]. (5) Ferroportin-1 (*Fp-1*) mutations and polymorphisms have been excluded as a common cause of iron overload in both DIOS and NAFLD/MetS [41,42]. Unfortunately, the role of acquired factors, cytokines, and other genetic factors have been reported in details only for patients with a diagnosis of NAFLD and not in DIOS. Thus, comparative studies are clearly required to verify the aforementioned hypothesis.

Molecular mechanism underlying body iron accumulation in DIOS

Iron accumulation in DIOS likely involves altered regulation of molecules involved in cellular iron export, such as ceruloplasmin (Cp) and Fp-1 induced by inflammation and micronutrients imbalance. A striking down-regulation of the cellular iron exporter Fp-1 has been observed in NASH, whereas hepcidin was physiologically increased in DIOS [43,44], confirming that altered iron trafficking underlies iron accumulation in NAFLD, whereas preserved hepcidin regulation inhibits Fp-1 protein activity, thus limiting further iron absorption and transferrin saturation. Since the Cu^{2+}-dependent ferroxidase Cp, the physiological plasma Cu^{2+} transporter, is required for the mobilization of iron by Fp-1, it was hypothesized that low Cp may be a cause of iron accumulation in DIOS. Indeed, NAFLD patients with the lowest liver and serum Cu^{2+} and Cp levels were more likely to have iron overload [45]. Moreover, *Fp-1* mRNA was lower in patients with low hepatic Cu^{2+}, and associated with steatosis and IR. As mentioned above, unbalanced oxidative stress is considered a trigger of NAFLD [46], and SOD1, one of the enzymes counteracting oxidative stress, depends on adequate Cu^{2+} availability [47]. Systemic Cu^{2+} deficiency causes mitochondrial dysfunction in mice [48], with similar morphological and functional alterations to that described in NAFLD [49], and patients with NASH had lower Cu^{2+} than those with simple steatosis [50], suggesting a possible involvement of altered Cu^{2+}metabolism in the pathophysiology of NASH by favoring both iron accumulation and reduced antioxidant activity.

Other sources of increased serum ferritin in NAFLD are represented by subclinical inflammation and ferritin release by activated leukocytes, and hepatocellular necrosis. Hepatic iron accumulation is an early event in the natural history of NAFLD, and increased ferritin levels in pediatric patients correlate with IR and increased levels of cytokines [39]. An interesting hypothesis based on data obtained in the HFD model is that activated Kupffer cells may accumulate iron and release ferritin because of increased erythrophagocytosis, which would cause cytokines release and fibrogenesis [51].

Genetics of iron overload in NAFLD and the MetS

In the attempt to explain the reason behind DIOS development in only a proportion of patients with NAFLD, several studies analyzed whether mutations in the HHC gene (*HFE*) may be involved, with conflicting results [40,52–54]. In a multicenter study in 587 Italian patients, we recently investigated whether the C282Y and H63D mutations predispose to iron overload in NAFLD [17]. Both hepatocellular and non-parenchymal siderosis were associated with *HFE* mutations, but the penetrance of *HFE* mutations was relatively low, so that only one third of carriers had the hepatocellular iron accumulation typical of HHC, explaining less than half of the variability of this phenotype. Interestingly, we had previously shown that carriers of the C282Y mutation have lower insulin release, and develop NAFLD in the presence of less severe

metabolic abnormalities (in particular the degree of adiposity), suggesting that heterozygosity for C282Y *HFE* mutation, responsible for mild iron overload, may increase the susceptibility to clinically overt NAFLD [14]. It could be hypothesized that the mechanism linking *HFE* mutations to iron accumulation in non-parenchymal cells may involve a relative hampering in hepcidin upregulation and facilitation of intestinal iron absorption, thus allowing the increase of body iron stores that will possibly localize in non-parenchymal cells because of defective iron export.

Other genetic factors influencing hepatocellular damage, inflammation and iron handling might be involved. Alpha1-antitrypsin (AAT), the principal serum protease inhibitor synthesized by the liver, potentially represents one of such factors. The most common variants are the PiZ and PiS alleles, where amino acid substitutions lead to abnormal folding and spontaneous protein polymerization, determining endoplasmic reticulum (ER) stress and hepatocellular damage. Heterozygosity for the PiZ, and to a lesser extent for the PiS allele has been associated with cirrhosis and hepatocarcinoma [55–57]. We found that the *AAT* mutations were highly prevalent in NAFLD, and associated with hyperferritinemia in the presence of normal transferrin saturation, and sinusoidal hepatic siderosis [16], the typical abnormalities of DIOS. It is thus possible that the coexistence of multiple genetic variants contributes to DIOS [13,14,27,30], and it could be speculated that *AAT* modulate iron metabolism by inducing ER stress [58].

Since *HFE* and *AAT* mutations did not fully explain the variability of the phenotype, we next evaluated whether a wider panel of genetic variants reported to influence hepatic iron, including *Fp-1* and *beta-globin*, might better predict DIOS and fibrosis progression [41]. The beta-thalassemia trait, commonly observed in the Mediterranean area, was more frequent in subjects with hyperferritinemia, and specifically associated with low hepcidin and parenchymal siderosis, leading to increased fibrosis. In Italian patients with NAFLD with predominantly parenchymal, non-parenchymal/mixed, or no hepatic siderosis, we observed a prevalence of H63D+/+ *HFE* genotype of 15%, 6%, and 3%; of the C282Y+/− *HFE* genotype of 25%, 12%, and 3%; of the PiS and PiZ AAT mutations of 22%, 15%, and 6%; and of beta-thalassemia trait of 32%, 8%, and 5%, respectively [41]. The prevalence of these genetic factors in patients without hepatic iron staining was superimposable to that of the general population. These differences were statistically significant, and 65% of patients with parenchymal iron accumulation carried at least one of these genetic factors *vs*. 9% of the control population [41]. Thus, these results support a preponderant effect of genetic factors, such as *HFE* and *beta-globin* mutations in the development of hepatocellular iron overload in NAFLD, suggesting it could represent a distinct and genetically determined sub-phenotype of DIOS at high risk of liver damage.

Possible mechanisms of IR associated with metabolic hyperferritinemia/DIOS

Clinical evidence suggests that iron might play a role in pathogenesis of IR [59]. This hypothesis has been addressed in experimental studies including those determining an association of increased ferritin with iron stores [37] and IR and amelioration of IR after ID [15].

As observed in HHC, in experimental models of obesity, iron accumulation within beta-cells alters insulin secretion, [60], whereas dietary iron restriction or chelation protects from diabetes [61]. However, the relative deficit in insulin secretion is not sufficient to explain the metabolic alterations observed in DIOS.

Supporting a causal role of iron overload in inducing IR, recent data indicate that manipulation of body iron stores by means of diet, genetic manipulation, or iron chelators is able to influence IR in different models of metabolic disease [61–63], but the molecular mechanisms underlying the association between iron accumulation and IR and the tissues primarily involved are far from being clear.

Iron overload has been hypothesized to induce IR by catalyzing oxidative stress [64,65]. Reactive oxygen species (ROS) have been implicated in IR pathogenesis on the basis of two types of indirect evidence: (1) an association of oxidative stress markers with obesity and T2D [66,67] and (2) evidence that factors that increase ROS in adipocytes induce IR [65,68]. Inhibition of mitochondrial superoxide dismutase (SOD2) has been hypothesized to mediate iron dependent oxidative damage and metabolic dysfunction [69,70].

Activation of NFκB in macrophages and Kupffer cells, and consequent release of TNFα [71–73], a major player in IR in MetS and NAFLD by means of its ability to downregulate insulin signaling and decrease adiponectin levels [39,74–76], may also be implicated in the pathogenesis of IR associated with DIOS, which is typically characterized by iron accumulation in this cellular compartment.

Supporting a role for iron in the induction of IR and a possible involvement of adipose tissue, Green *et al.* [77] demonstrated that isolated adipocytes treated with iron become insulin resistant, as detected by decreased insulin-stimulated glucose transport and increased lipolysis. If confirmed *in vivo*, these metabolic alterations would promote IR, and raise the risk of T2D and steatosis [77], but the effect of body iron overload on adipose tissue *in vivo*, except for a few data on adipokines (see below), is still under definition. Further work is required to determine whether iron may directly accumulate in adipose tissue and alter its function.

Despite the model does not reflect the typical pattern of iron overload of DIOS, novel insights into the pathogenesis of iron induced IR have been provided *in vivo* by the detailed metabolic characterization of iron overloaded mice due to the deletion of the *Hfe* gene of HHC. Despite higher glucose uptake, these mice had lower glucose oxidation in skeletal muscle, which was linked to Ampk- and Pdk4-mediated [78] decrease in pyruvate dehydrogenase activity, and higher hepatic glucose output and metabolic inflexibility (i.e. a decreased ability to transition between utilization of carbohydrate and lipid fuel sources), both of which are characteristics of T2D [62]. Contrary to what expected, the metabolic alterations described in this model did not depend on mitochondrial oxidative damage. As iron sufficiency and deletion of *Hfe* facilitate erythropoiesis [79], it would seem advantageous for an iron-loaded mouse to shift to the more energy-efficient but oxygen-inefficient fuel source of fatty acids to make use of that full capacity for oxygen transport [62]. Preliminary data from our group also confirm that dietary iron overload induces IR in mice, and the mechanism might be related to iron accumulation within visceral adipose tissue resulting in altered release of adipocytokines [80]. A working model of the mechanisms underlying iron associated IR is proposed in Fig. 1.

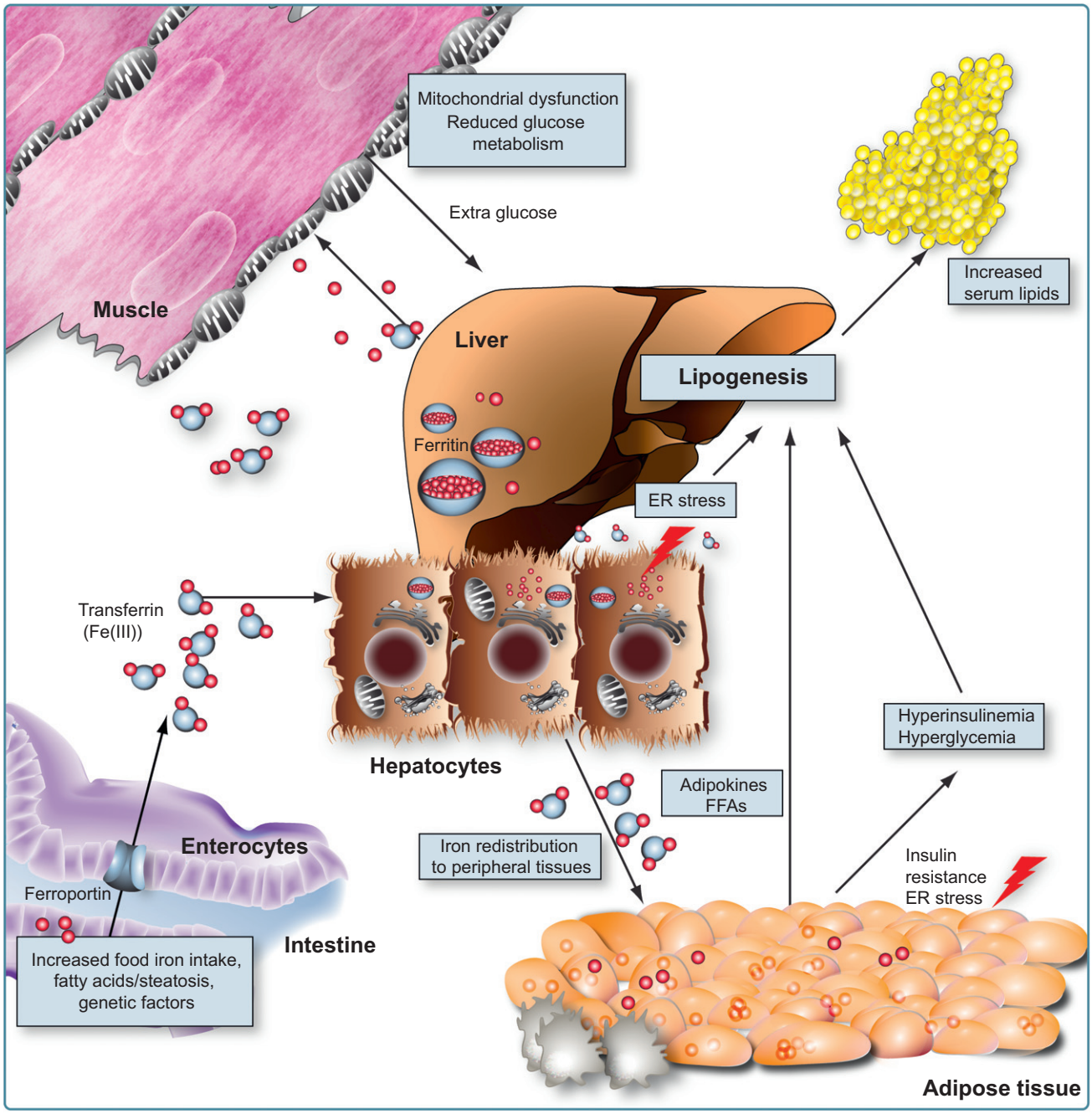

Fig. 1. Proposed mechanisms explaining iron induced insulin resistance and metabolic alterations. FFAs, free fatty acids; ER, endoplasmic reticulum.

Iron, adipose tissue, and adipokines

NAFLD is highly prevalent in obesity, associated with chronic inflammation in adipose tissue, and with abnormal release of adipocytokines that play an endocrine role in the progression to NASH, T2D, and cardiovascular disease. Thus, it is likely that the effect of iron on IR involves altered regulation of adipose tissue and of adipokines.

Serum levels of adiponectin, the major anti-steatotic and anti-inflammatory adipocyte-derived mediator, are reduced in obesity, T2D, and IR, whereas weight loss and PPARγ activation by glitazones induce adiponectin [81]. In obese mice, deletion of adiponectin receptors induces inflammation, oxidative stress, and IR, whereas adiponectin overexpression improves IR and reverts the diabetic phenotype [82]. Patients with NAFLD have decreased adiponectin [83], and hypo-adiponectinemia predicts the severity of inflammation and fibrosis in NASH [84]. Interestingly, a negative correlation between adiponectin and ferritin levels has been reported in patients with type 2 diabetes and in the general population [85,86], although no data are available

in NAFLD. Induction of heme oxygenase-1 (HO-1) by adiponectin, mediated by AMPK-mediated PPARα activation, elicited an antiapoptotic effect by decreasing iron in hepatocytes [87], thus linking adiponectin to iron related liver damage. Adiponectin also induced cyclooxygenase-2 expression in mouse hepatocytes, conferring further protection against iron injury [88].

Leptin is another well-studied adipokine, which plays an important role in the regulation of body weight through inhibition of food intake and stimulation of energy expenditure [89]. High levels of leptin have been observed in obesity, indicating the development of leptin resistance [90]. Indeed, both leptin-mutant (*ob/ob*) and leptin receptor-deficient (*db/db*) mice are severely obese and insulin resistant, due to increased food intake and decreased energy expenditure [91]. Leptin rapidly reverses steatosis induced by high sucrose diet in rats [92], promotes the proliferation and migration of hepatoma cells *in vitro* [93], and is thought to be involved to the progression from NASH to fibrosis and hepatocellular carcinoma [94]. Hepatoma cells exposure to leptin directly up-regulates hepcidin, resulting in decreased iron absorption and impaired iron recycling, possibly contributing to DIOS pathogenesis [95]. Thus, increased hepcidin, partially related to hyper-leptinemia, may represent the missing link between obesity and DIOS [96]. However, there are no data on the correlation between leptin and iron stores in patients with NAFLD and MetS.

Resistin is a recently discovered adipokine secreted by adipose tissue and macrophages that circulates at increased levels in obesity [97]. Treatment of mice with recombinant resistin impairs glucose tolerance, and anti-resistin antibodies improve IR in obese mice. Incubation of 3T3-L1 adipocytes with resistin inhibits insulin-stimulated glucose uptake [97], whereas in skeletal muscle resistin reduces the uptake and metabolism of FFAs [98]. Moreover, it seems that resistin significantly induces the gene expression of suppressor of cytokine signaling 3 (*SOCS3*), a known inhibitor of insulin signaling [99]. So far there are no data supporting a relationship between resistin and iron overload, but an interaction between a polymorphism in the promoter of human resistin and oxidative stress has been reported [100], and antioxidants inhibited the expression of resistin in mice [101]. In a randomized trial, short-term vitamin C supplementation reduced resistin levels independently of inflammation [102].

Visfatin is another novel adipokine predominantly secreted by visceral adipose tissue and increased in T2D [103] that exerts adipogenic effects *in vitro* and is, therefore, a good candidate to explain the accumulation of visceral adipose tissue that is associated with IR. In men with hyperglycemia, serum prohepcidin was strongly associated with visfatin, suggesting that circulating visfatin is perhaps upregulated by increasing iron stores, but no data are available specifically in patients with NAFLD, although most of the subjects with impaired fasting glucose or diabetes have increased liver fat. Moreover, visfatin correlated negatively with serum transferrin receptor, a marker of iron deficient erythropoiesis [104]. Finally, retinol binding protein-4 (RBP4), an adipokine associated with IR, was correlated with ferritin levels in middle aged men and in subjects with type 2 diabetes, and iron increased RBP4 release by adipocytes *in vitro* [105].

These data suggest that DIOS is associated with abnormal endocrine function of adipose tissue and adipokines signaling, potentially contributing to metabolic abnormalities, liver damage, and cardiovascular disease.

Association between iron overload and vascular damage in MetS and NAFLD

NAFLD has been associated both with increased susceptibility to develop increased iron stores (DIOS), and with heightened risk of vascular damage, independently of classic risk factors [19,106], and cardiovascular disease represents the first cause of death in patients with NAFLD [107]. Iron deposition in arterial wall macrophages is increased in atherosclerotic lesions [108] and, although evidence is controversial [109–111], increased iron stores have been suggested as a marker of cardiovascular risk [112]. Indirect confirmation of the "iron hypothesis" comes from studies of atherosclerosis treatment. Indeed, ID decreased atherogenesis in experimental models [108,113], blood donation was associated with lower risk of myocardial infarction [5] and phlebotomy slowed the progression of vascular disease [114,115], whereas the lack of association between *HFE* mutations with vascular damage might be explained by the decrease in hepcidin levels, which would paradoxically facilitate iron export from macrophages [33,116], determining more rapid clearance of iron from arterial lesions. Indeed, besides iron overload, hepcidin is also induced by inflammation and obesity, and local production determines iron trapping into macrophages [115,117]. Thus, excessive iron in macrophages would increase oxidative stress and transformation into foam cells and hepcidin may be responsible for iron induced atherogenesis [108].

The still unexplained association between the C282Y hemochromatosis mutation and low LDL cholesterol [118,119], which was confirmed in a meta-analysis of genome-wide association studies [120], may also contribute to explain atherosclerosis protection in individuals carrying *HFE* mutations. Interestingly, also the beta-thalassemia trait is strongly associated with reduced cholesterol levels and lower cardiovascular risk [121,122], but the elucidation of the relationship between iron and cholesterol metabolism requires, therefore, further studies.

Recently, our group has shown that serum ferritin and hepcidin levels predicted vascular damage in NAFLD, but only in patients negative for *HFE* genotypes or *beta-globin* mutations associated with low hepcidin [38]. The mechanism seems to involve upregulation of macrophage chemoattractant protein-1 (MCP-1/CCL2), a chemokine involved in the recruitment of leukocytes to plaques and correlated with the atherosclerotic burden, by intracellular iron in monocytes [123]. However, whether the presence of iron overload is associated with an increased rate of cardiovascular events in NAFLD and the MetS is presently unproven.

Association between iron overload and liver damage

We have shown [17] that, in NAFLD, hepatocellular iron accumulation was associated with a higher risk of fibrosis compared to the absence of siderosis or the non-parenchymal iron accumulation, which is more commonly observed and typical of DIOS. However, evidence that only parenchymal iron carries a higher risk of progressive liver disease is still conflicting, since in a large US cohort, non-parenchymal iron, related to more severe metabolic alterations, was associated with histological inflammation and more advanced fibrosis [32]. Furthermore, non-parenchymal iron overload has been associated with hepatocellular carcinoma in Italian patients with NASH-related cirrhosis [124]. It is,

therefore, likely that the genetic background underlying iron accumulation influences the outcome in ethnically different populations. Due to incomplete association with iron overload, *HFE* mutations were not associated with liver fibrosis in Italian patients with NAFLD [17], although they predicted liver damage in US Caucasian patients with NASH [125], whereas *beta-globin* mutations, the best predictor of parenchymal iron overload in the Mediterranean area, were associated with an almost double risk of severe fibrosis [41].

Nevertheless, longitudinal studies with follow-up liver biopsies are needed to investigate the relationship between iron overload, ID, and the progression of hepatic disease.

Mechanism of iron induced liver damage

Evidence is accumulating that mild hepatic iron overload promotes the progression of liver damage associated with fatty liver also independently of IR, and once again the mechanism involves increased oxidative stress. Iron is a potent catalyst of oxidative stress via the Fenton reaction and can directly cause lipid peroxidation generating malonyldialdehyde, which is capable to activate hepatic stellate cells (HSCs), a major player of fibrogenesis in NAFLD [126,127]. ROS cause peroxidation of polyunsaturated fatty acids and nucleic acids [128,129], and a lipophilic antioxidant, such as vitamin E reduced liver enzymes and hepatocellular damage in a large randomized controlled trial in NASH [130]. Iron overload may thus play a role in NASH by generating oxidative DNA damage; supporting this hypothesis, hepatic 7,8-dihydro-8-oxo-2′ deoxyguanosine (8-oxodG), a DNA base-modified product generated by hydroxyl radicals, was increased in NASH, and correlated with iron overload, IR, and severity of hepatic steatosis. Moreover, ID decreased oxidative stress and HSCs activation in experimental models of liver injury [131], and after phlebotomy hepatic 8-oxodG levels decreased with concomitant reduction of serum transaminases in NASH patients [132].

Iron can also directly induce fibrogenesis, as HSCs can be activated by the generation of ROS with ascorbate/$FeSO_4$ and by malonyldialdehyde. In addition, HSCs activation by collagen type I and TGFα was blocked by antioxidants [126].

A specific receptor for ferritin has been demonstrated on activated HSCs, and it has been proposed that ferritin acts as a cytokine with pro-inflammatory activity regulating fibrogenesis via NFκB-regulated signaling in HSCs [133].

Is iron overload sufficient to trigger oxidative damage? In rats, iron accumulation is associated with induction of HO-1, a sensitive indicator of oxidative stress, but not with fibrosis [134], highlighting the difference between oxidative stress and damage, and suggesting that the former is not sufficient to elicit overt fibrosis, at least in rodents. While hepatic iron overload leads to oxidative stress, there is an associated up-regulation of antioxidant defenses that may be a critical factor limiting the accumulation of oxidative damage. Probably, co-existing liver injury or nutritional/genetic factors, and in particular the coexistence of steatosis [135], may compromise the ability to mount an effective antioxidant defense, and thus predispose to fibrogenesis. In a rat model of T2D, lipid peroxidation and hepatic superoxide production decreased in rats fed an iron-deficient diet or treated with phlebotomy [63]. In the methionine choline-deficient (MCD) model of NASH, hepatic iron overload was associated with necroinflammation and a trend toward increased perivenular fibrosis [136], whereas in the same model, a single injection of iron induced fibrosis development and worsening of steatosis, thereby emphasizing the role of iron in the progression of nutritional NASH to the fibrotic stage [137], which is possibly mediated by the facilitation of apoptosis [138]. Apoptotic hepatocytes may indeed stimulate HSCs either directly or indirectly via TGF-β production [139].

Thus, iron could represent a second hit in the progression of liver damage from simple uncomplicated steatosis to fibrotic NASH, but since fibrosis is not a constant feature of DIOS, other genetic or acquired conditions are necessary to trigger this process. In addition, iron may favor malignant transformation and hepatocellular carcinoma by promotion of cell growth and oxidative dependent DNA damage [140,141]. A model depicting the proposed mechanisms underlying liver damage associated with iron overload in steatosis and DIOS is shown in Fig. 2.

Iron depletion therapy in DIOS: experimental studies

Experimental evidence suggests that ID not only is able to counteract the negative effect of iron overload, but that mild iron deficiency itself may further positively impact on IR. Recently, our group has investigated the effect of iron depletion on glucose metabolism in hepatocytes *in vitro* and in an *in vivo* model. The data obtained indicate that cellular ID induced by chelators induces glucose uptake and utilization, increasing insulin receptor (InsR) binding activity and signaling, and that the mechanism is probably associated with the hypoxia inducible factor-1α HIF-1α stabilization by reduced iron availability [142]. In line with these findings, as skeletal muscles play a major part in glucose utilization, it has been shown that L6 myocytes adapt to ID by increasing glucose utilization through enhanced expression of the main basal glucose transporter Glut-1 [143]. Furthermore, increased insulin sensitivity in peripheral tissues has been shown in a rat model of iron deficiency anemia [144]. This response may represent a metabolic adaptation (which is specular to that observed during iron overload) to decreased oxygen availability secondary to a deficiency in hemoglobin, myoglobin, and cytochromes due to the scarcity of iron [62], which forces tissues to depend more heavily on the anaerobic catabolism of glucose for their energy supply. A model of the proposed mechanisms underlying improved glucose clearance and insulin sensitivity under ID is shown in Fig. 3.

Iron depletion therapy in patients with NAFLD, MetS, and DIOS

Several reports indicate that ID may be beneficial in patients with DIOS. ID has been first reported to be well tolerated in patients with DIOS [145], and to improve insulin sensitivity in the short term (without changes in body weight) in patients with NAFLD with and without increased ferritin levels, in two uncontrolled studies conducted in 17 patients with impaired glucose tolerance [146] and in 12 patients with normal glucose tolerance [147]. Phlebotomy led to decreased HbA1c levels, heightened insulin secretion and insulin sensitivity in a randomized controlled study in 28 patients with T2D and increased ferritin levels and stable body weight [148]. In addition, ID improved insulin release in an uncontrolled study in 17 carriers of *HFE* mutations with steatosis [149]. Regular blood donation was also associated with

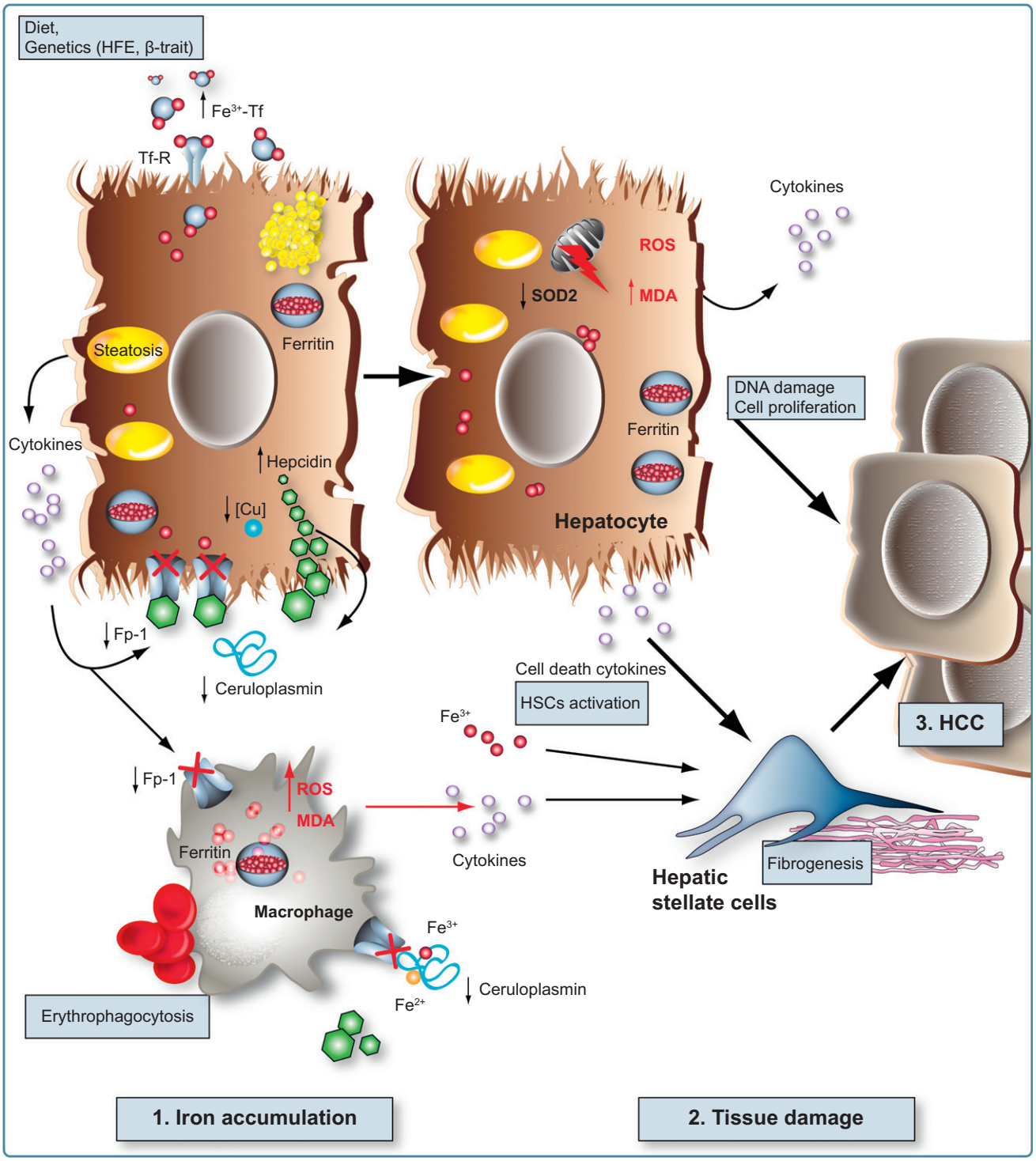

Fig. 2. Proposed mechanisms explaining iron induced liver damage associated with steatosis and DIOS in hepatocytes (brown), macrophages (gray), and hepatic stellate cells (yellow). *Cp*, ceruloplasmin; Cu, copper; *Fe-Tf*, ferric-transferrin; *Fp-1*, ferroportin-1; HCC, hepatocellular carcinoma; *HFE*, hemochromatosis gene; HSCs, hepatic stellate cells; MDA, malonyl-dialdehyde; ROS, reactive oxygen species; *SOD2*, Mn superoxide dismutase; *Tf-R*, transferrin receptor.

increased insulin sensitivity in 21 frequent donors compared to 66 healthy subjects, suggesting that stored iron impacts negatively on insulin action even in healthy people [150]. Both venesection therapy (in the absence of weight loss) and dietary treatment have been reported to improve serum ferritin, metabolic parameters, and liver function tests in 59 patients with DIOS in a controlled unmatched study [151]. However, in a case–control study in 128 patients (matched for age, sex, ferritin, and

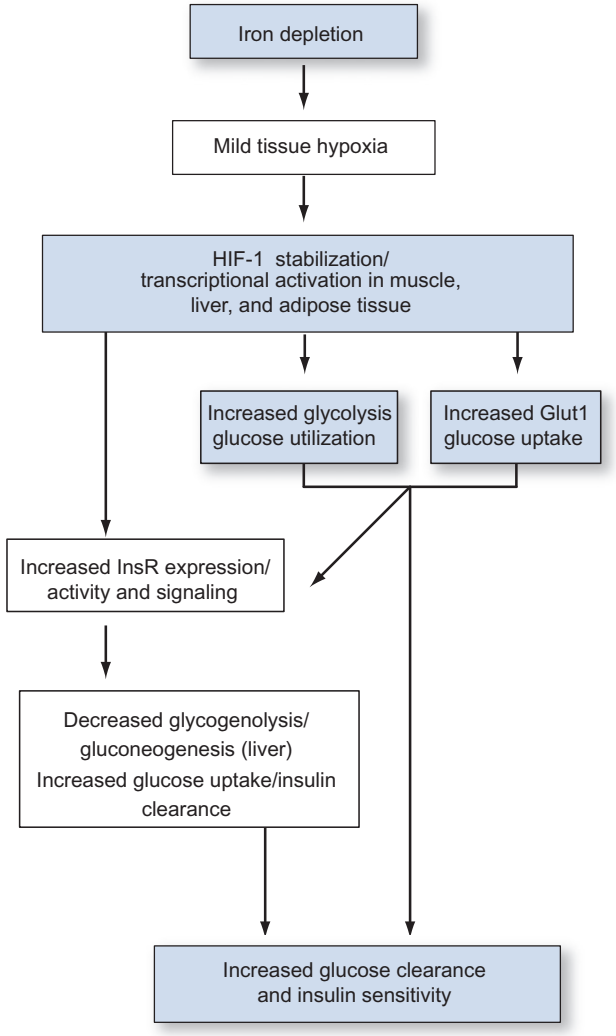

Fig. 3. Proposed additional mechanisms by which iron depletion improves glucose disposal and insulin sensitivity. *InsR*, insulin receptor; *HIF-1α*, hypoxia inducible factor-1α; *Glut1*, glucose transporter 1.

ALT levels) with diet-resistant NAFLD followed for 12 months, which took into account changes in body weight during the study, it was shown that ID reduced IR more than lifestyle modifications alone, independently of confounding factors [15]. Lifestyle modifications were modestly effective on ferritin and liver enzymes, but did not improve IR, and the effect of ID was independent of changes in body weight and metabolic parameters [15]. Of note, the advantage of ID by phlebotomy was more marked in patients with higher baseline iron stores (ferritin >320 ng/ml) [15].

Concerning the direct effect of ID on liver damage, in a relatively large multi-center prospectively enrolled observational study in 198 NAFLD patients without diabetes, after adjustment for propensity score (which is used to simulate the effect of randomization on treatment choice in observational studies), ID was associated with a higher probability of normalization not only of insulin resistance, but also of liver enzymes compared to lifestyle modifications alone during follow-up [152]. Furthermore, the analysis of a cardiovascular trial suggests that ID may also prevent cancer development and progression [153], indicating that in patients with liver disease, it might protect from hepatocellular carcinoma independently of fibrosis progression [124,140].

Thus, ID associated with lifestyle modifications may represent an eligible therapy for patients with NAFLD in the presence of iron overload. However, while it is almost well established that ID may improve metabolic and biochemical parameters in patients with NAFLD, whether it also prevents progression to cirrhosis and hepatocellular carcinoma is not demonstrated. A randomized controlled trial is ongoing to evaluate the effect of ID on the progression of histologically evaluated liver damage in patients with NAFLD and increased iron stores (NCT00658164).

Conclusions

DIOS is now a frequent finding in the general population, and hyperferritinemia, which reflects fatty liver and hyperinsulinemia, but also mildly increased body iron stores, is also detected in about 20–30% of patients with NAFLD and the MetS. Excessive body iron may play a causal role in IR through mechanisms that involve a reduced ability to burn carbohydrates and altered function of adipose tissue and release of adipokines. Furthermore, DIOS may facilitate the evolution to T2D by altering beta-cell function, the progression of cardiovascular disease by contributing to the recruitment and activation of macrophages within arterial lesions, and the natural history of liver disease by inducing oxidative stress in hepatocytes, activation of HSCs, and malignant transformation by promotion of cell growth and DNA damage.

Based on these premises, the association among DIOS, MetS, and NAFLD is being investigated as a new risk factor to predict the development of overt cardiovascular, and hepatic diseases, but most importantly represents also a treatable condition. Indeed, ID, most frequently achieved by phlebotomy, has already been reported to decrease IR, metabolic alterations, and liver enzymes in controlled studies in NAFLD. Additional, randomized controlled studies are warranted to evaluate the potential of ID on hard clinical outcomes in patients with hyperferritinemia, and results are awaited before iron depletion therapy can be recommended for the treatment of hyperferritinemia associated with NAFLD, MetS, and DIOS.

Conflict of interest

The authors declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

Financial support

Bando Giovani Ricercatori 2007, Ministero della Salute e delle Politiche Sociali (GR-2007-683265), PUR 10% 2009 (2009-ATE0087) Università degli Studi di Milano to L.V.

Acknowledgments

We thank all the clinical and laboratory members of the Metabolic Liver Diseases Research Center, and our collaborators.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jhep.2011.05.008.

References

[1] Jehn M, Clark JM, Guallar E. Serum ferritin and risk of the metabolic syndrome in US adults. Diabetes Care 2004;27:2422–2428.
[2] Bozzini C, Girelli D, Olivieri O, Martinelli N, Bassi A, De Matteis G, et al. Prevalence of body iron excess in the metabolic syndrome. Diabetes Care 2005;28:2061–2063.
[3] Wrede CE, Buettner R, Bollheimer LC, Scholmerich J, Palitzsch KD, Hellerbrand C. Association between serum ferritin and the insulin resistance syndrome in a representative population. Eur J Endocrinol 2006;154:333–340.
[4] Kim CH, Kim HK, Bae SJ, Park JY, Lee KU. Association of elevated serum ferritin concentration with insulin resistance and impaired glucose metabolism in Korean men and women. Metabolism 2010;60:414–420.
[5] Salonen JT, Tuomainen TP, Salonen R, Lakka TA, Nyyssonen K. Donation of blood is associated with reduced risk of myocardial infarction. The Kuopio Ischaemic Heart Disease Risk Factor Study. Am J Epidemiol 1998;148:445–451.
[6] Ford ES, Cogswell ME. Diabetes and serum ferritin concentration among US adults. Diabetes Care 1999;22:1978–1983.
[7] Jiang R, Manson JE, Meigs JB, Ma J, Rifai N, Hu FB. Body iron stores in relation to risk of type 2 diabetes in apparently healthy women. JAMA 2004;291:711–717.
[8] Forouhi NG, Harding AH, Allison M, Sandhu MS, Welch A, Luben R, et al. Elevated serum ferritin levels predict new-onset type 2 diabetes: results from the EPIC-Norfolk prospective study. Diabetologia 2007;50:949–956.
[9] Acton RT, Barton JC, Passmore LV, Adams PC, Speechley MR, Dawkins FW, et al. Relationships of serum ferritin, transferrin saturation, and HFE mutations and self-reported diabetes in the Hemochromatosis and Iron Overload Screening (HEIRS) study. Diabetes Care 2006;29:2084–2089.
[10] Escobar-Morreale HF, Luque-Ramirez M, Alvarez-Blasco F, Botella-Carretero JI, Sancho J, San Millan JL. Body iron stores are increased in overweight and obese women with polycystic ovary syndrome. Diabetes Care 2005;28:2042–2044.
[11] Ehrmann DA. Polycystic ovary syndrome. N Engl J Med 2005;352:1223–1236.
[12] Luque-Ramirez M, Alvarez-Blasco F, Botella-Carretero JI, Sanchon R, San Millan JL, Escobar-Morreale HF. Increased body iron stores of obese women with polycystic ovary syndrome are a consequence of insulin resistance and hyperinsulinism and are not a result of reduced menstrual losses. Diabetes Care 2007;30:2309–2313.
[13] Mendler MH, Turlin B, Moirand R, Jouanolle AM, Sapey T, Guyader D, et al. Insulin resistance-associated hepatic iron overload. Gastroenterology 1999;117:1155–1163.
[14] Valenti L, Dongiovanni P, Fracanzani AL, Santorelli G, Fatta E, Bertelli C, et al. Increased susceptibility to nonalcoholic fatty liver disease in heterozygotes for the mutation responsible for hereditary hemochromatosis. Dig Liver Dis 2003;35:172–178.
[15] Valenti L, Fracanzani AL, Dongiovanni P, Bugianesi E, Marchesini G, Manzini P, et al. Iron depletion by phlebotomy improves insulin resistance in patients with nonalcoholic fatty liver disease and hyperferritinemia: evidence from a case–control study. Am J Gastroenterol 2007;102:1251–1258.
[16] Valenti L, Dongiovanni P, Piperno A, Fracanzani AL, Maggioni M, Rametta R, et al. Alpha1-antitrypsin mutations in NAFLD: high prevalence and association with altered iron metabolism but not with liver damage. Hepatology 2006;44:857–864.
[17] Valenti L, Fracanzani AL, Bugianesi E, Dongiovanni P, Galmozzi E, Vanni E, et al. HFE genotype, parenchymal iron accumulation, and liver fibrosis in patients with nonalcoholic fatty liver disease. Gastroenterology 2010;138:905–912.
[18] Bugianesi E, Leone N, Vanni E, Marchesini G, Brunello F, Carucci P, et al. Expanding the natural history of nonalcoholic steatohepatitis: from cryptogenic cirrhosis to hepatocellular carcinoma. Gastroenterology 2002;123:134–140.
[19] Fracanzani AL, Burdick L, Raselli S, Pedotti P, Grigore L, Santorelli G, et al. Carotid artery intima-media thickness in nonalcoholic fatty liver disease. Am J Med 2008;121:72–78.
[20] Browning JD, Szczepaniak LS, Dobbins R, Nuremberg P, Horton JD, Cohen JC, et al. Prevalence of hepatic steatosis in an urban population in the United States: impact of ethnicity. Hepatology 2004;40:1387–1395.
[21] Marchesini G, Brizi M, Bianchi G, Tomassetti S, Bugianesi E, Lenzi M, et al. Nonalcoholic fatty liver disease: a feature of the metabolic syndrome. Diabetes 2001;50:1844–1850.
[22] Angulo P. Nonalcoholic fatty liver disease. N Engl J Med 2002;346:1221–1231.
[23] Marchesini G, Bugianesi E, Forlani G, Cerrelli F, Lenzi M, Manini R, et al. Nonalcoholic fatty liver, steatohepatitis, and the metabolic syndrome. Hepatology 2003;37:917–923.
[24] Villanova N, Moscatiello S, Ramilli S, Bugianesi E, Magalotti D, Vanni E, et al. Endothelial dysfunction and cardiovascular risk profile in nonalcoholic fatty liver disease. Hepatology 2005;42:473–480.
[25] Ekstedt M, Franzen LE, Mathiesen UL, Thorelius L, Holmqvist M, Bodemar G, et al. Long-term follow-up of patients with NAFLD and elevated liver enzymes. Hepatology 2006;44:865–873.
[26] Day CP. From fat to inflammation. Gastroenterology 2006;130:207–210.
[27] Turlin B, Mendler MH, Moirand R, Guyader D, Guillygomarc'h A, Deugnier Y. Histologic features of the liver in insulin resistance-associated iron overload. A study of 139 patients. Am J Clin Pathol 2001;116:263–270.
[28] Barisani D, Pelucchi S, Mariani R, Galimberti S, Trombini P, Fumagalli D, et al. Hepcidin and iron-related gene expression in subjects with dysmetabolic hepatic iron overload. J Hepatol 2008;49:123–133.
[29] Riva A, Trombini P, Mariani R, Salvioni A, Coletti S, Bonfadini S, et al. Revaluation of clinical and histological criteria for diagnosis of dysmetabolic iron overload syndrome. World J Gastroenterol 2008;14:4745–4752.
[30] Moirand R, Mendler MH, Guillygomarc'h A, Brissot P, Deugnier Y. Nonalcoholic steatohepatitis with iron: part of insulin resistance-associated hepatic iron overload? J Hepatol 2000;33:1024–1026.
[31] Pietrangelo A. Hereditary hemochromatosis – a new look at an old disease. N Engl J Med 2004;350:2383–2397.
[32] Nelson JE, Wilson L, Brunt EM, Yeh MM, Kleiner DE, Unalp-Arida A, et al. Relationship between the pattern of hepatic iron deposition and histological severity in nonalcoholic fatty liver disease. Hepatology 2011;53:448–457.
[33] Nemeth E, Ganz T. Regulation of iron metabolism by hepcidin. Annu Rev Nutr 2006;26:323–342.
[34] Ruivard M, Laine F, Ganz T, Olbina G, Westerman M, Nemeth E, et al. Iron absorption in dysmetabolic iron overload syndrome is decreased and correlates with increased plasma hepcidin. J Hepatol 2009;50:1219–1225.
[35] Fernandez-Real JM, Ricart-Engel W, Arroyo E, Balanca R, Casamitjana-Abella R, Cabrero D, et al. Serum ferritin as a component of the insulin resistance syndrome. Diabetes Care 1998;21:62–68.
[36] Adams PC, Reboussin DM, Barton JC, McLaren CE, Eckfeldt JH, McLaren GD, et al. Hemochromatosis and iron-overload screening in a racially diverse population. N Engl J Med 2005;352:1769–1778.
[37] Haap M, Machann J, von Friedeburg C, Schick F, Stefan N, Schwenzer NF, et al. Insulin sensitivity and liver fat: role of iron load. J Clin Endocrinol Metab 2011;96:E958–E961.
[38] Valenti L, Swinkels DW, Burdick L, Dongiovanni P, Tjalsma H, Motta BM, et al. Serum ferritin levels are associated with vascular damage in patients with nonalcoholic fatty liver disease. Nutr Metab Cardiovasc Dis 2011;31:683–690.
[39] Manco M, Alisi A, Real JM, Equitani F, Devito R, Valenti L, et al. Early interplay of intra-hepatic iron and insulin resistance in children with non-alcoholic fatty liver disease. J Hepatol 2011. in press [PMID: 21168460].
[40] Bugianesi E, Manzini P, D'Antico S, Vanni E, Longo F, Leone N, et al. Relative contribution of iron burden, HFE mutations, and insulin resistance to fibrosis in nonalcoholic fatty liver. Hepatology 2004;39:179–187.
[41] Valenti L, Canavesi E, Galmozzi E, Dongiovanni P, Rametta R, Maggioni P, et al. Beta-globin mutations are associated with parenchymal siderosis and fibrosis in patients with non-alcoholic fatty liver disease. J Hepatol 2010;53:927–933.
[42] Pelucchi S, Mariani R, Salvioni A, Bonfadini S, Riva A, Bertola F, et al. Novel mutations of the ferroportin gene (SLC40A1): analysis of 56 consecutive patients with unexplained iron overload. Clin Genet 2008;73:171–178.
[43] Aigner E, Theurl I, Theurl M, Lederer D, Haufe H, Dietze O, et al. Pathways underlying iron accumulation in human nonalcoholic fatty liver disease. Am J Clin Nutr 2008;87:1374–1383.
[44] Pigeon C, Ilyin G, Courselaud B, Leroyer P, Turlin B, Brissot P, et al. A new mouse liver-specific gene, encoding a protein homologous to human antimicrobial peptide hepcidin, is overexpressed during iron overload. J Biol Chem 2001;276:7811–7819.

[45] Aigner E, Theurl I, Haufe H, Seifert M, Hohla F, Scharinger L, et al. Copper availability contributes to iron perturbations in human nonalcoholic fatty liver disease. Gastroenterology 2008;135:680–688.
[46] Sanyal AJ. Mechanisms of disease: pathogenesis of nonalcoholic fatty liver disease. Nat Clin Pract Gastroenterol Hepatol 2005;2:46–53.
[47] Prohaska JR, Geissler J, Brokate B, Broderius M. Copper, zinc-superoxide dismutase protein but not mRNA is lower in copper-deficient mice and mice lacking the copper chaperone for superoxide dismutase. Exp Biol Med (Maywood) 2003;228:959–966.
[48] Nose Y, Kim BE, Thiele DJ. Ctr1 drives intestinal copper absorption and is essential for growth, iron metabolism, and neonatal cardiac function. Cell Metab 2006;4:235–244.
[49] Wei Y, Rector RS, Thyfault JP, Ibdah JA. Nonalcoholic fatty liver disease and mitochondrial dysfunction. World J Gastroenterol 2008;14:193–199.
[50] Aigner E, Strasser M, Haufe H, Sonnweber T, Hohla F, Stadlmayr A, et al. A role for low hepatic copper concentrations in nonalcoholic fatty liver disease. Am J Gastroenterol 2010;105:1978–1985.
[51] Otogawa K, Kinoshita K, Fujii H, Sakabe M, Shiga R, Nakatani K, et al. Erythrophagocytosis by liver macrophages (Kupffer cells) promotes oxidative stress, inflammation, and fibrosis in a rabbit model of steatohepatitis: implications for the pathogenesis of human nonalcoholic steatohepatitis. Am J Pathol 2007;170:967–980.
[52] Valenti L, Dongiovanni P, Fracanzani AL, Fargion S. HFE mutations in nonalcoholic fatty liver disease. Hepatology 2008;47:1794–1795, [author reply 1795–1796].
[53] Chitturi S, Weltman M, Farrell GC, McDonald D, Kench J, Liddle C, et al. HFE mutations, hepatic iron, and fibrosis: ethnic-specific association of NASH with C282Y but not with fibrotic severity. Hepatology 2002;36:142–149.
[54] Neri S, Pulvirenti D, Signorelli S, Ignaccolo L, Tsami A, Mauceri B, et al. The HFE gene heterozygosis H63D: a cofactor for liver damage in patients with steatohepatitis? Epidemiological and clinical considerations. Intern Med J 2008;38:254–258.
[55] Eigenbrodt ML, McCashland TM, Dy RM, Clark J, Galati J. Heterozygous alpha 1-antitrypsin phenotypes in patients with end stage liver disease. Am J Gastroenterol 1997;92:602–607.
[56] Serfaty L, Chazouilleres O, Poujol-Robert A, Morand-Joubert L, Dubois C, Chretien Y, et al. Risk factors for cirrhosis in patients with chronic hepatitis C virus infection: results of a case–control study. Hepatology 1997;26:776–779.
[57] Graziadei IW, Joseph JJ, Wiesner RH, Therneau TM, Batts KP, Porayko MK. Increased risk of chronic liver failure in adults with heterozygous alpha1-antitrypsin deficiency. Hepatology 1998;28:1058–1063.
[58] Vecchi C, Montosi G, Zhang K, Lamberti I, Duncan SA, Kaufman RJ, et al. ER stress controls iron metabolism through induction of hepcidin. Science 2009;325:877–880.
[59] Fargion S, Dongiovanni P, Guzzo A, Colombo S, Valenti L, Fracanzani AL. Iron and insulin resistance. Aliment Pharmacol Ther 2005;22:61–63.
[60] Cooksey RC, Jouihan HA, Ajioka RS, Hazel MW, Jones DL, Kushner JP, et al. Oxidative stress, beta-cell apoptosis, and decreased insulin secretory capacity in mouse models of hemochromatosis. Endocrinology 2004;145:5305–5312.
[61] Cooksey RC, Jones D, Gabrielsen S, Huang J, Simcox JA, Luo B, et al. Dietary iron restriction or iron chelation protects from diabetes and loss of beta-cell function in the obese (ob/ob lep−/−) mouse. Am J Physiol Endocrinol Metab 2010;298:E1236–E1243.
[62] Huang J, Jones D, Luo B, Sanderson M, Soto J, Abel ED, et al. Iron overload and diabetes risk: a shift from glucose to fatty acid oxidation and increased hepatic glucose production in a mouse model of hereditary hemochromatosis. Diabetes 2010;60:80–87.
[63] Minamiyama Y, Takemura S, Kodai S, Shinkawa H, Tsukioka T, Ichikawa H, et al. Iron restriction improves type 2 diabetes mellitus in Otsuka Long-Evans Tokushima fatty rats. Am J Physiol Endocrinol Metab 2010;298:E1140–E1149.
[64] Fernandez-Real JM, Lopez-Bermejo A, Ricart W. Cross-talk between iron metabolism and diabetes. Diabetes 2002;51:2348–2354.
[65] Houstis N, Rosen ED, Lander ES. Reactive oxygen species have a causal role in multiple forms of insulin resistance. Nature 2006;440:944–948.
[66] Furukawa S, Fujita T, Shimabukuro M, Iwaki M, Yamada Y, Nakajima Y, et al. Increased oxidative stress in obesity and its impact on metabolic syndrome. J Clin Invest 2004;114:1752–1761.
[67] Urakawa H, Katsuki A, Sumida Y, Gabazza EC, Murashima S, Morioka K, et al. Oxidative stress is associated with adiposity and insulin resistance in men. J Clin Endocrinol Metab 2003;88:4673–4676.
[68] Lin J, Yang R, Tarr PT, Wu PH, Handschin C, Li S, et al. Hyperlipidemic effects of dietary saturated fats mediated through PGC-1beta coactivation of SREBP. Cell 2005;120:261–273.
[69] Valenti L, Conte D, Piperno A, Dongiovanni P, Fracanzani AL, Fraquelli M, et al. The mitochondrial superoxide dismutase A16V polymorphism in the cardiomyopathy associated with hereditary haemochromatosis. J Med Genet 2004;41:946–950.
[70] Jouihan HA, Cobine PA, Cooksey RC, Hoagland EA, Boudina S, Abel ED, et al. Iron-mediated inhibition of mitochondrial manganese uptake mediates mitochondrial dysfunction in a mouse model of hemochromatosis. Mol Med 2008;14:98–108.
[71] Scaccabarozzi A, Arosio P, Weiss G, Valenti L, Dongiovanni P, Fracanzani AL, et al. Relationship between TNF-alpha and iron metabolism in differentiating human monocytic THP-1 cells. Br J Haematol 2000;110:978–984.
[72] Fargion S, Valenti L, Dongiovanni P, Scaccabarozzi A, Fracanzani AL, Taioli E, et al. Tumor necrosis factor alpha promoter polymorphisms influence the phenotypic expression of hereditary hemochromatosis. Blood 2001;97:3707–3712.
[73] She H, Xiong S, Lin M, Zandi E, Giulivi C, Tsukamoto H. Iron activates NF-κB in Kupffer cells. Am J Physiol Gastrointest Liver Physiol 2002;283:G719–G726.
[74] Valenti L, Fracanzani AL, Dongiovanni P, Santorelli G, Branchi A, Taioli E, et al. Tumor necrosis factor alpha promoter polymorphisms and insulin resistance in nonalcoholic fatty liver disease. Gastroenterology 2002;122:274–280.
[75] Kern PA, Di Gregorio GB, Lu T, Rassouli N, Ranganathan G. Adiponectin expression from human adipose tissue: relation to obesity, insulin resistance, and tumor necrosis factor-alpha expression. Diabetes 2003;52:1779–1785.
[76] Hotamisligil GS, Peraldi P, Budavari A, Ellis R, White MF, Spiegelman BM. IRS-1-mediated inhibition of insulin receptor tyrosine kinase activity in TNF-alpha- and obesity-induced insulin resistance. Science 1996;271:665–668.
[77] Green A, Basile R, Rumberger JM. Transferrin and iron induce insulin resistance of glucose transport in adipocytes. Metabolism 2006;55:1042–1045.
[78] Huang J, Gabrielsen JS, Cooksey RC, Luo B, Boros LG, Jones DL, et al. Increased glucose disposal and AMP-dependent kinase signaling in a mouse model of hemochromatosis. J Biol Chem 2007;282:37501–37507.
[79] Ramos P, Guy E, Chen N, Proenca CC, Gardenghi S, Casu C, et al. Enhanced erythropoiesis in Hfe-KO mice indicates a role for Hfe in the modulation of erythroid iron homeostasis. Blood 2011;117:1379–1389.
[80] Dongiovanni P, Ruscica M, Benedan L, Borroni V, Recalcati S, Steffani L, et al. Dietary iron overload induces visceral adipose tissue insulin resistance associated with hyper-resistinemia, and synergizes with obesity and fatty liver in inducing systemic insulin resistance. J Hepatol 2011;54:S505.
[81] Lutchman G, Modi A, Kleiner DE, Promrat K, Heller T, Ghany M, et al. The effects of discontinuing pioglitazone in patients with nonalcoholic steatohepatitis. Hepatology 2007;46:424–429.
[82] Kim JY, van de Wall E, Laplante M, Azzara A, Trujillo ME, Hofmann SM, et al. Obesity-associated improvements in metabolic profile through expansion of adipose tissue. J Clin Invest 2007;117:2621–2637.
[83] Musso G, Gambino R, Biroli G, Carello M, Faga E, Pacini G, et al. Hypoadiponectinemia predicts the severity of hepatic fibrosis and pancreatic beta-cell dysfunction in nondiabetic nonobese patients with nonalcoholic steatohepatitis. Am J Gastroenterol 2005;100:2438–2446.
[84] Polyzos SA, Toulis KA, Goulis DG, Zavos C, Kountouras J. Serum total adiponectin in nonalcoholic fatty liver disease: a systematic review and meta-analysis. Metabolism 2011;60:313–326.
[85] Aso Y, Takebayashi K, Wakabayashi S, Momobayashi A, Sugawara N, Terasawa T, et al. Relation between serum high molecular weight adiponectin and serum ferritin or prohepcidin in patients with type 2 diabetes. Diabetes Res Clin Pract 2010;90:250–255.
[86] Ku BJ, Kim SY, Lee TY, Park KS. Serum ferritin is inversely correlated with serum adiponectin level: population-based cross-sectional study. Dis Markers 2009;27:303–310.
[87] Lin H, Yu CH, Jen CY, Cheng CF, Chou Y, Chang CC, et al. Adiponectin-mediated heme oxygenase-1 induction protects against iron-induced liver injury via a PPAR{alpha}-dependent mechanism. Am J Pathol 2010;177:1697–1709.
[88] Lee FP, Jen CY, Chang CC, Chou Y, Lin H, Chou CM, et al. Mechanisms of adiponectin-mediated COX-2 induction and protection against iron injury in mouse hepatocytes. J Cell Physiol 2010;224:837–847.

[89] Halaas JL, Boozer C, Blair-West J, Fidahusein N, Denton DA, Friedman JM. Physiological response to long-term peripheral and central leptin infusion in lean and obese mice. Proc Natl Acad Sci USA 1997;94:8878–8883.
[90] Maffei M, Halaas J, Ravussin E, Pratley RE, Lee GH, Zhang Y, et al. Leptin levels in human and rodent: measurement of plasma leptin and ob RNA in obese and weight-reduced subjects. Nat Med 1995;1:1155–1161.
[91] Friedman JM, Halaas JL. Leptin and the regulation of body weight in mammals. Nature 1998;395:763–770.
[92] Huang W, Dedousis N, O'Doherty RM. Hepatic steatosis and plasma dyslipidemia induced by a high-sucrose diet are corrected by an acute leptin infusion. J Appl Physiol 2007;102:2260–2265.
[93] Saxena NK, Sharma D, Ding X, Lin S, Marra F, Merlin D, et al. Concomitant activation of the JAK/STAT, PI3K/AKT, and ERK signaling is involved in leptin-mediated promotion of invasion and migration of hepatocellular carcinoma cells. Cancer Res 2007;67:2497–2507.
[94] Kitade M, Yoshiji H, Kojima H, Ikenaka Y, Noguchi R, Kaji K, et al. Leptin-mediated neovascularization is a prerequisite for progression of nonalcoholic steatohepatitis in rats. Hepatology 2006;44:983–991.
[95] Chung B, Matak P, McKie AT, Sharp P. Leptin increases the expression of the iron regulatory hormone hepcidin in HuH7 human hepatoma cells. J Nutr 2007;137:2366–2370.
[96] del Giudice EM, Santoro N, Amato A, Brienza C, Calabro P, Wiegerinck ET, et al. Hepcidin in obese children as a potential mediator of the association between obesity and iron deficiency. J Clin Endocrinol Metab 2009;94:5102–5107.
[97] Steppan CM, Bailey ST, Bhat S, Brown EJ, Banerjee RR, Wright CM, et al. The hormone resistin links obesity to diabetes. Nature 2001;409:307–312.
[98] Palanivel R, Sweeney G. Regulation of fatty acid uptake and metabolism in L6 skeletal muscle cells by resistin. FEBS Lett 2005;579:5049–5054.
[99] Steppan CM, Wang J, Whiteman EL, Birnbaum MJ, Lazar MA. Activation of SOCS-3 by resistin. Mol Cell Biol 2005;25:1569–1575.
[100] Smith SR, Bai F, Charbonneau C, Janderova L, Argyropoulos G. A promoter genotype and oxidative stress potentially link resistin to human insulin resistance. Diabetes 2003;52:1611–1618.
[101] Felipe F, Bonet ML, Ribot J, Palou A. Modulation of resistin expression by retinoic acid and vitamin A status. Diabetes 2004;53:882–889.
[102] Bo S, Ciccone G, Durazzo M, Gambino R, Massarenti P, Baldi I, et al. Efficacy of antioxidant treatment in reducing resistin serum levels: a randomized study. PLoS Clin Trials 2007;2:e17.
[103] Fukuhara A, Matsuda M, Nishizawa M, Segawa K, Tanaka M, Kishimoto K, et al. Visfatin: a protein secreted by visceral fat that mimics the effects of insulin. Science 2005;307:426–430.
[104] Fernandez-Real JM, Moreno JM, Chico B, Lopez-Bermejo A, Ricart W. Circulating visfatin is associated with parameters of iron metabolism in subjects with altered glucose tolerance. Diabetes Care 2007;30:616–621.
[105] Fernandez-Real JM, Moreno JM, Ricart W. Circulating RBP4 concentration might reflect insulin resistance-associated iron overload. Diabetes 2008;57:1918–1925.
[106] Targher G, Bertolini L, Padovani R, Rodella S, Zoppini G, Zenari L, et al. Relations between carotid artery wall thickness and liver histology in subjects with nonalcoholic fatty liver disease. Diabetes Care 2006;29:1325–1330.
[107] Jepsen P, Vilstrup H, Mellemkjaer L, Thulstrup AM, Olsen JH, Baron JA, et al. Prognosis of patients with a diagnosis of fatty liver – a registry-based cohort study. Hepatogastroenterology 2003;50:2101–2104.
[108] Lapenna D, Pierdomenico SD, Ciofani G, Ucchino S, Neri M, Giamberardino MA, et al. Association of body iron stores with low molecular weight iron and oxidant damage of human atherosclerotic plaques. Free Radic Biol Med 2007;42:492–498.
[109] van der AD, Peeters PH, Grobbee DE, Roest M, Marx JJ, Voorbij HM, et al. HFE mutations and risk of coronary heart disease in middle-aged women. Eur J Clin Invest 2006;36:682–690.
[110] Pardo Silva MC, Njajou OT, Alizadeh BZ, Hofman A, Witteman JC, van Duijn CM, et al. HFE gene mutations increase the risk of coronary heart disease in women. Eur J Epidemiol 2010;25:643–649.
[111] Danesh J, Appleby P. Coronary heart disease and iron status: meta-analyses of prospective studies. Circulation 1999;99:852–854.
[112] Sullivan JL. Macrophage iron, hepcidin, and atherosclerotic plaque stability. Exp Biol Med (Maywood) 2007;232:1014–1020.
[113] Ganz T, Nemeth E. Iron imports. IV. Hepcidin and regulation of body iron metabolism. Am J Physiol Gastrointest Liver Physiol 2006;290:G199–G203.
[114] Lee TS, Shiao MS, Pan CC, Chau LY. Iron-deficient diet reduces atherosclerotic lesions in apoE-deficient mice. Circulation 1999;99:1222–1229.
[115] Zacharski LR, Chow BK, Howes PS, Shamayeva G, Baron JA, Dalman RL, et al. Reduction of iron stores and cardiovascular outcomes in patients with peripheral arterial disease: a randomized controlled trial. JAMA 2007;297:603–610.
[116] Chen YH, Chau LY, Chen JW, Lin SJ. Serum bilirubin and ferritin levels link heme oxygenase-1 gene promoter polymorphism and susceptibility to coronary artery disease in diabetic patients. Diabetes Care 2008;31:1615–1620.
[117] Theurl I, Theurl M, Seifert M, Mair S, Nairz M, Rumpold H, et al. Autocrine formation of hepcidin induces iron retention in human monocytes. Blood 2008;111:2392–2399.
[118] Adams PC, Pankow JS, Barton JC, Acton RT, Leiendecker-Foster C, McLaren GD, et al. HFE C282Y homozygosity is associated with lower total and low-density lipoprotein cholesterol: the hemochromatosis and iron overload screening study. Circ Cardiovasc Genet 2009;2:34–37.
[119] Pankow JS, Boerwinkle E, Adams PC, Guallar E, Leiendecker-Foster C, Rogowski J, et al. HFE C282Y homozygotes have reduced low-density lipoprotein cholesterol: the Atherosclerosis Risk in Communities (ARIC) Study. Transl Res 2008;152:3–10.
[120] Teslovich TM, Musunuru K, Smith AV, Edmondson AC, Stylianou IM, Koseki M, et al. Biological, clinical and population relevance of 95 loci for blood lipids. Nature 2010;466:707–713.
[121] Deiana L, Garuti R, Pes GM, Carru C, Errigo A, Rolleri M, et al. Influence of beta(0)-thalassemia on the phenotypic expression of heterozygous familial hypercholesterolemia: a study of patients with familial hypercholesterolemia from Sardinia. Arterioscler Thromb Vasc Biol 2000;20:236–243.
[122] Maioli M, Vigna GB, Tonolo G, Brizzi P, Ciccarese M, Donega P, et al. Plasma lipoprotein composition, apolipoprotein(a) concentration and isoforms in beta-thalassemia. Atherosclerosis 1997;131:127–133.
[123] Valenti L, Dongiovanni P, Motta BM, Swinkels DW, Bonara P, Rametta R, et al. Serum hepcidin and macrophage iron correlate with MCP-1 release and vascular damage in patients with metabolic syndrome alterations. Arterioscler Thromb Vasc Biol 2011;31:683–690.
[124] Sorrentino P, D'Angelo S, Ferbo U, Micheli P, Bracigliano A, Vecchione R. Liver iron excess in patients with hepatocellular carcinoma developed on non-alcoholic steato-hepatitis. J Hepatol 2009;50:351–357.
[125] Nelson JE, Bhattacharya R, Lindor KD, Chalasani N, Raaka S, Heathcote EJ, et al. HFE C282Y mutations are associated with advanced hepatic fibrosis in Caucasians with nonalcoholic steatohepatitis. Hepatology 2007;46:723–729.
[126] Lee KS, Buck M, Houglum K, Chojkier M. Activation of hepatic stellate cells by TGF alpha and collagen type I is mediated by oxidative stress through c-myb expression. J Clin Invest 1995;96:2461–2468.
[127] Marra F, Bertolani C. Adipokines in liver diseases. Hepatology 2009;50:957–969.
[128] Sumida Y, Yoshikawa T, Okanoue T. Role of hepatic iron in non-alcoholic steatohepatitis. Hepatol Res 2009;39:213–222.
[129] Day CP. Steatohepatitis: a tale of two "hits"? Gastroenterology 1998;114:842–845.
[130] Sanyal AJ, Chalasani N, Kowdley KV, McCullough A, Diehl AM, Bass NM, et al. Pioglitazone, vitamin E, or placebo for nonalcoholic steatohepatitis. N Engl J Med 2010;362:1675–1685.
[131] Otogawa K, Ogawa T, Shiga R, Nakatani K, Ikeda K, Nakajima Y, et al. Attenuation of acute and chronic liver injury in rats by iron-deficient diet. Am J Physiol Regul Integr Comp Physiol 2008;294:R311–R320.
[132] Fujita N, Miyachi H, Tanaka H, Takeo M, Nakagawa N, Kobayashi Y, et al. Iron overload is associated with hepatic oxidative damage to DNA in nonalcoholic steatohepatitis. Cancer Epidemiol Biomarkers Prev 2009;18:424–432.
[133] Ruddell RG, Hoang-Le D, Barwood JM, Rutherford PS, Piva TJ, Watters DJ, et al. Ferritin functions as a proinflammatory cytokine via iron-independent protein kinase C zeta/nuclear factor kappaB-regulated signaling in rat hepatic stellate cells. Hepatology 2009;49:887–900.
[134] Brown KE, Dennery PA, Ridnour LA, Fimmel CJ, Kladney RD, Brunt EM, et al. Effect of iron overload and dietary fat on indices of oxidative stress and hepatic fibrogenesis in rats. Liver Int 2003;23:232–242.
[135] Powell EE, Ali A, Clouston AD, Dixon JL, Lincoln DJ, Purdie DM, et al. Steatosis is a cofactor in liver injury in hemochromatosis. Gastroenterology 2005;129:1937–1943.
[136] Kirsch R, Sijtsema HP, Tlali M, Marais AD, Hall Pde L. Effects of iron overload in a rat nutritional model of non-alcoholic fatty liver disease. Liver Int 2006;26:1258–1267.
[137] Imeryuz N, Tahan V, Sonsuz A, Eren F, Uraz S, Yuksel M, et al. Iron preloading aggravates nutritional steatohepatitis in rats by increasing apoptotic cell death. J Hepatol 2007;47:851–859.
[138] Takehara T, Tatsumi T, Suzuki T, Rucker 3rd EB, Hennighausen L, Jinushi M, et al. Hepatocyte-specific disruption of Bcl-xL leads to continuous hepa-

tocyte apoptosis and liver fibrotic responses. Gastroenterology 2004;127:1189–1197.
[139] Feldstein AE, Canbay A, Angulo P, Taniai M, Burgart LJ, Lindor KD, et al. Hepatocyte apoptosis and fas expression are prominent features of human nonalcoholic steatohepatitis. Gastroenterology 2003;125: 437–443.
[140] Fargion S, Valenti L, Fracanzani AL. Hemochromatosis gene (HFE) mutations and cancer risk: expanding the clinical manifestations of hereditary iron overload. Hepatology 2010;51:1119–1121.
[141] Dongiovanni P, Fracanzani A, Cairo G, Megazzini CP, Gatti S, Rametta R, et al. Iron dependent regulation of MDM2 influences p53 activity and hepatic carcinogenesis. Am J Pathol 2010;176. doi:10.2353/amjpath.2010.090249.
[142] Dongiovanni P, Valenti L, Ludovica Fracanzani A, Gatti S, Cairo G, Fargion S. Iron depletion by deferoxamine up-regulates glucose uptake and insulin signaling in hepatoma cells and in rat liver. Am J Pathol 2008;172:738–747.
[143] Potashnik R, Kozlovsky N, Ben-Ezra S, Rudich A, Bashan N. Regulation of glucose transport and GLUT-1 expression by iron chelators in muscle cells in culture. Am J Physiol 1995;269:E1052–E1058.
[144] Borel MJ, Beard JL, Farrell PA. Hepatic glucose production and insulin sensitivity and responsiveness in iron-deficient anemic rats. Am J Physiol 1993;264:E380–E390.
[145] Guillygomarc'h A, Mendler MH, Moirand R, Laine F, Quentin V, David V, et al. Venesection therapy of insulin resistance-associated hepatic iron overload. J Hepatol 2001;35:344–349.
[146] Facchini FS, Hua NW, Stoohs RA. Effect of iron depletion in carbohydrate-intolerant patients with clinical evidence of nonalcoholic fatty liver disease. Gastroenterology 2002;122:931–939.
[147] Valenti L, Fracanzani AL, Fargion S. Effect of iron depletion in patients with nonalcoholic fatty liver disease without carbohydrate intolerance. Gastroenterology 2003;124:866, [author reply 866–867].
[148] Fernandez-Real JM, Penarroja G, Castro A, Garcia-Bragado F, Hernandez-Aguado I, Ricart W. Blood letting in high-ferritin type 2 diabetes: effects on insulin sensitivity and beta-cell function. Diabetes 2002;51:1000–1004.
[149] Equitani F, Fernandez-Real JM, Menichella G, Koch M, Calvani Menotti, Nobili V, et al. Blood letting ameliorates insulin sensitivity and secretion in parallel to reduce iron overload in carriers of HFE mutations. Diabetes Care 2008;31:3–8.
[150] Fernandez-Real JM, Lopez-Bermejo A, Ricart W. Iron stores, blood donation, and insulin sensitivity and secretion. Clin Chem 2005;51:1201–1205.
[151] Piperno A, Vergani A, Salvioni A, Trombini P, Vigano M, Riva A, et al. Effects of venesections and restricted diet in patients with the insulin-resistance hepatic iron overload syndrome. Liver Int 2004;24:471–476.
[152] Valenti L, Moscatiello S, Vanni E, Fracanzani AL, Bugianesi E, Fargion S, et al. Venesection for non-alcoholic fatty liver disease unresponsive to lifestyle counselling – a propensity score-adjusted observational study. QJM 2011;104:141–149.
[153] Zacharski LR, Chow BK, Howes PS, Shamayeva G, Baron JA, Dalman RL, et al. Decreased cancer risk after iron reduction in patients with peripheral arterial disease: results from a randomized trial. J Natl Cancer Inst 2008;100:996–1002.