Chapter 6

Iron Status Biomarkers and Cardiovascular Risk

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Abstract

Both iron excess and deficiency may be related to oxidative stress. Serum ferritin, the main marker of iron status, and hepcidin, the key regulator of iron metabolism, are increased in inflammation states and their links with insulin resistance are emerging topics. We have reviewed the role of iron deficiency/overload in cardiovascular risk, including our own results. Most studies deal with the association between iron deposition in tissues and cardiovascular risk, while decreased iron status is predominantly related to protection against atherosclerosis and coronary heart disease. Less information is available on the role of iron status in type 2 diabetes mellitus (T2DM). Serum ferritin is positively correlated with several indicators of cardiovascular risk in healthy adults and diabetics, thus excess body iron is related to cardiometabolic alterations including vascular and heart damage, central obesity, and metabolic syndrome. Our data in an ample sample of T2DM adults suggest that body iron stores, evaluated as ferritin, are clearly related with some key markers of the so-called lipidic triad (high triglyceride and low high-density lipoprotein (HDL) cholesterol) levels together with the presence of small and dense low-density lipoprotein particles which also is in the frame of the dysmetabolic iron overload syndrome.

Keywords: iron, cardiovascular diseases, iron overload, iron deficiency, oxidative stress, hepcidin, ferritin, insulin, Type 2 diabetes Mellitus, lipidic triad, biomarker, dysmetabolic iron overload syndrome, human

1. Introduction

1.1. Iron metabolism and regulation

Iron is essential for life as it plays a central role in many biological processes that involve oxygen transport and storage and oxidative metabolism. This essential metal participates in

many enzymatic systems such as those involved in DNA, RNA, and protein syntheses and in the regulation of gene expression, electron transport in the mitochondria, neurotransmitter metabolism, vitamin D activation, and cholesterol catabolism through the 7α-hydroxylase linked to isoenzyme P450 cytochrome (CYP7A1c) that depends on iron and converts cholesterol to colic acid [1, 2].

Most of the functional iron in the body is present in the form of hemoglobin and myoglobin, and minor levels are part of a variety of heme and non-heme enzymes; the remainder is stored and mobilized when physiological demands are increased. The existence of two ionic forms, Fe^{2+} and Fe^{3+}, means that this nutrient is capable to serve both as an electron donor and as an acceptor, which makes iron essential but also a potential toxic. In order to limit the amount of free ions that can induce free radical formation, iron is transported, bound to proteins, and stored intracellularly within a macro-protein structure, ferritin.

Iron in food is present in two forms, inorganic iron and heme iron. These forms are absorbed by different mechanisms; the heme route is highly efficient but contributes only to about 10–15% of total dietary iron. Non-heme iron bioavailability is enhanced principally by animal tissue and ascorbic acid, whereas phytic acid and polyphenols are the main inhibitors [1, 3–6]. Solubility is an important factor for iron uptake; soluble ferrous iron is transported by the divalent metal transporter (DMT1) located at the luminal side of the duodenal membrane. However, this is not as simple; on the one hand, this carrier is not iron specific and there is competition from other divalent metals, such as calcium [7] and zinc, and on the other hand, ferric ion can be also transported either after reduction to ferrous by the duodenal cytochrome B or by interaction to mucins and subsequent association with β3-integrin and mobilferrin that cross the membrane and internalize iron in the cytosol [8, 9].

It is important to emphasize that iron absorption is tightly controlled, but once absorbed there are no excretion mechanisms. In contrast, iron recycling in the body is highly efficient; senescent erythrocytes are phagocytosed by macrophages in the liver, spleen, and bone marrow. Under normal conditions, only about 10% of the 10–18 mg/day-ingested iron is absorbed. However, during late pregnancy (from 6 to 9 months), in order to cover fetal demands for growth and erythropoiesis, iron absorption increases to 25% [10]. The main serum transporter is transferrin, a protein capable of binding 1 or 2 ferric ions that are released into cells by the transferrin receptor (TfR1). Iron recycling involves 10–20 times greater iron flux than intestinal absorption, that makes approximately 20–25 mg of iron circulating daily, an amount sufficient to ensure erythropoiesis needs. This role is played by macrophages in the spleen, bone marrow, and liver (Küpffer cells) [11]. Iron losses are due to intestinal desquamation and menstruation and should balance absorbed amounts (average 1–2 mg/day). However, hemorrhages, intense menstrual blood loss [12], pregnancy, and intense growth are frequent causes of iron deficiency anemia (IDA).

In iron overload conditions, such as hereditary hemochromatosis, transferrin becomes saturated with iron and the excess occurs as non-transferrin-bound iron (NTBI) that may be toxic [13].

The discovery of the intracellular iron regulatory proteins and that of the key regulator, hepcidin, has triggered a revolution in iron metabolism research. Hepcidin, now accepted as a true

hormone, was initially named liver-expressed antimicrobial peptide (LEAP-1) and shortly later renamed as hepcidin because it is expressed in the liver (hep-) and exhibits antimicrobial activity (-cidin) [14, 15].

Figure 1 shows a scheme of the role of hepcidin on systemic iron homeostasis under conditions of high or low iron level. Hepatic hepcidin synthesis is stimulated, secreted into the circulation, and released into tissues when iron levels are high. In different cells but mainly in hepatocytes, enterocytes, and macrophages, hepcidin inhibits iron export, thus decreases absorption, recycling, and circulation of iron. This hormone therefore is a negative regulator of iron status. The mechanism of action is binding to its receptor, the cellular iron exporter ferroportin (FPN), and subsequent internalization and degradation of the hepcidin-ferroportin complex. In contrast, under physiological or pathological situations of low iron levels, hepcidin synthesis is minimized resulting in an enhanced iron flux from liver and macrophages stores and an increased transport through the duodenal basolateral membrane.

The gene-encoding hepcidin, *HAMP*, is expressed primarily in hepatocytes, although there is also evidence of expression in duodenal enterocytes, liver Kupffer cells, splenic macrophages, and placental syncytiotrophoblasts [16]. Sequencing of *HAMP* reveals several mutations that are either not functional [17] or related to a rare form of hemochromatosis [18] indicating low variability and that this gene is highly conserved in humans while the common iron metabolism alterations, either iron deficiency or hemochromatosis, have been associated with polymorphisms in other genes [19–23].

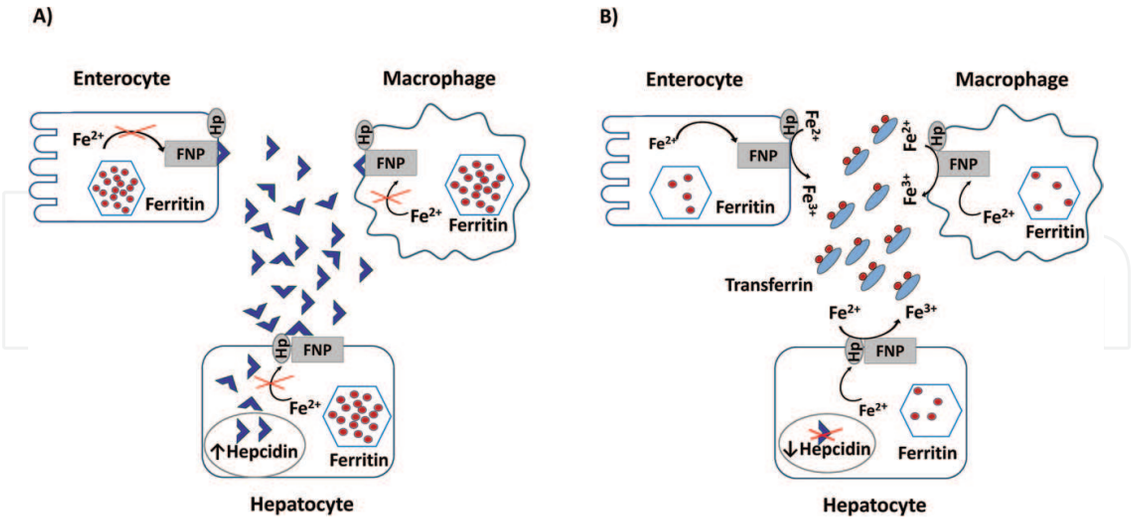

Figure 1. Role of hepcidin in systemic iron homeostasis. (A) High iron level conditions. Hepatic hepcidin expression and circulating hepcidin levels are increased; in hepatocytes, enterocytes, and macrophages, hepcidin is bound to the complex ferroportin-hephaestin and ferroportin is internalized and degraded; consequently, iron efflux is inhibited. (B) Low iron level conditions. Hepatic hepcidin synthesis is inhibited and serum hepcidin levels are negligible; consequently, iron crosses the membrane and is delivered into the circulation and transported to tissues by transferrin that is highly saturated (FNP: ferroportin; HP: hephaestin). Modified from Blanco-Rojo R. [24].

On the other hand, hepcidin regulation is a very active field. Many stimuli affect hepcidin transcription; the main factors are iron level, as explained above; inflammation, as hepcidin behaves as an acute phase protein; hypoxia, through the hypoxia inducible factor; and erythropoiesis signals. The details of hepcidin regulation and intracellular iron regulatory proteins involved in transcription are far beyond this revision and have been reviewed by others [16, 19, 24–27].

1.2. Role of macrophages in iron recycling

Hemoglobin in erythrocytes constitutes the major iron pool of the body. Senescent or damaged erythrocytes are phagocytosed by macrophages in the spleen, bone marrow, and liver. This activity is very efficient, as daily 20–25 mg of iron is delivered from macrophages into circulation and recycled, and the amount of iron that has to be absorbed for body functions is only 1–2 mg per day. Moreover, macrophages can work as a reservoir and participate in iron homeostasis [11].

Figure 2 shows the erythrophagocytic activity of macrophages. Once the macrophage detects an alteration or damage in the erythrocyte, the phagocytosis process is triggered. First, the erythrocyte is incorporated into the phagosome and heme is released. Then, heme is catabolized by hemoxigenase-1, and carbon monoxide, biliverdin, and Fe^{2+} are released. Fe^{2+} is transported across the phagosome membrane by DMT1 and natural resistance-associated

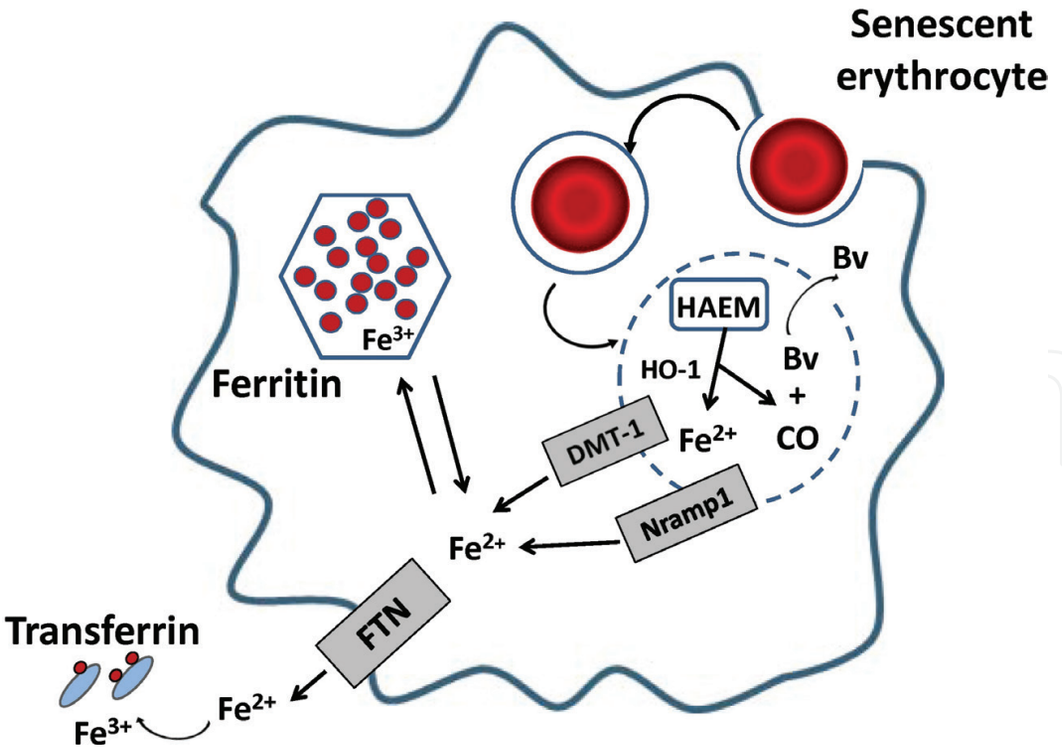

Figure 2. Erythrophagocytic activity of macrophages. CO: carbon monoxide; HO-1: hemoxygenase-1; Bv: biliverdin; DMT-1: dimetal transporter-1; Nramp1: natural resistance-associated macrophage protein; FTN: ferroportin. Modified from Blanco-Rojo R. [24].

macrophage protein (Nramp1). It seems that the presence of both transporters makes recycling more efficient. If iron is not needed for erythropoiesis, it is stored as ferritin in the form of Fe^{3+}. Finally, iron is released into the circulation via ferroportin and hephaestin (HP), and the iron is donated to transferrin to be reutilized [28].

2. Role of iron in oxidative status

The redox potential of iron, that is the switch between Fe^{2+} and Fe^{3+}, is essential for many biochemical reactions but is also a potential threat. Iron toxicity is based on the Fenton and Haber-Weiss reaction, which generates •OH (hydroxyl radicals) from H_2O_2 (hydrogen peroxide) and superoxide ($\bullet O_2^-$) in the presence of catalytic amounts of iron. The first step of the catalytic cycle involves reduction of ferric ion to ferrous:

$$Fe^{3+} + \bullet O_2^- \rightarrow Fe^{2+} + O_2 \quad (1)$$

The second step is:

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + \bullet OH \quad (2)$$

Net reaction:

$$\bullet O_2^- + H_2O_2 \rightarrow \bullet OH + OH^- + O_2 \text{ (where Fe acts as a catalytic metal)} \quad (3)$$

The catalytic action of iron also leads to the formation of organic reactive oxygen species (ROS), such as peroxyl radicals (ROO•), alkoxyl radicals (RO•), thiyl radicals (RS•), sulfonyl radicals (ROS•), thiyl peroxyl radicals (RSOO•), and disulfides (RSSR). Similarly, heme iron catalyzes the formation of ROS, via the formation of oxoferryl intermediates. In addition, ferrous iron can also contribute as a reactant to free radical generation [29].

It is worth mentioning that ROS are normally produced by the mitochondria aerobic metabolism through the incomplete reduction of molecular oxygen. ROS can also be generated by the membrane-bound NADPH oxidase complex that is an important tool for the antimicrobial defense and is mainly expressed not only in phagocytic macrophages but also in neutrophils and other cell types.

ROS are highly reactive species and promote the oxidation of proteins, nucleic acids, and membrane lipids. Any increase in the ROS levels beyond the antioxidant capacity of the organism causes oxidative stress [29]. In this regard, in primary and secondary iron overload conditions, such as in hereditary hemochromatosis and thalassemia, respectively, oxidative stress is observed as the iron-binding capacity of transferrin gets saturated and high levels of non-transferrin-bound iron reach the cell, are internalized, and induce tissue damage [30, 31].

Excess iron is involved in the pathophysiology of chronic inflammation, Alzheimer disease, diabetes, atherosclerosis, and, generally, cardiovascular diseases (CVD).

3. Iron excess and cardiovascular risk

3.1. The iron hypothesis

Early in the 1980s, Sullivan published in *The Lancet* the hypothesis that higher-stored iron in men and postmenopausal women compared to premenopausal women increase the risk of heart diseases and that iron deficiency is a protective factor [32]. This was supported by epidemiological studies; positive associations between serum ferritin (marker of iron stores), and the cholesterol transported by low-density lipoproteins (LDL-cholesterol) that were reported in men [33]. But other data do not support this hypothesis [34, 35] and the debate still continues. Our research group and others have reported that iron-deficient and anemic women present low lipid levels that increase during pharmacological treatment with iron salts, although final values are in normal range [36, 37]. Other studies coincide in the gender and age differences in iron metabolism and lipoprotein metabolism and the lower cardiovascular risk in women compared to men [38]. However, there may be several interacting factors. In this regard, estrogens are related to higher levels of cholesterol transported by high-density lipoproteins (HDL-cholesterol) and aldosterone that may partly explain lower atherosclerosis and hypertension risk [39]. However, age has been suggested to exert higher influence than hormones as estrogens explain about 25% of the phenotype differences related to cardiovascular risk, and thus menopausal women have higher cardiovascular risk than fertile women mainly due to age [40]. Studies in older men and women do not support the Sullivan's hypothesis and are rather opposite, with a subgroup of individuals who have low iron stores and higher cardiovascular risk [41]. Likewise, our findings in an elderly population consuming a variant of the Mediterranean diet show that prevalence of anemia is higher than that of high ferritin [42].

Therefore, it is likely that iron is only one of the players in the pathophysiological process of cardiovascular diseases.

3.2. Iron excess and atherosclerosis

Atherosclerosis is a chronic inflammatory disease affecting the arterial intima [43, 44]. Endothelial dysfunction induces recruitment of LDL particles and blood monocytes that differentiate into macrophages, phagocyte lipid material, and are transformed into foam cells [45]. The possibility that iron plays an interacting role emerges from its capacity to enhance the formation of ROS and LDL oxidation and its presence in macrophages, where a reservoir of intracellular iron may remain if body iron is high. In this regard, high iron levels hypothetically increase atherosclerosis risk.

However, there are many doubts on this hypothesis. Results from the ARIC study, carried out in the 1990s, did not find an association between ferritin values and LDL oxidation [43] or ferritin and asymptomatic carotid atherosclerosis [44]. Likewise, a recent systematic analysis by Hosseini [46] concludes that iron intake/status is not associated with carotid intima media thickness. In this issue, it could be speculated that the effect of iron is related to the "labile pool" or unbound iron more than to the total amount of iron in the body.

Results from the MONICA study in France [47] show that carotid atherosclerosis was positively associated with serum ferritin in individuals free from subclinical inflammation. In another study [48], atherosclerotic plaque specimens, which were removed from carotids of patients as a stroke reduction strategy, were analyzed. The study compared symptomatic and asymptomatic plaques considering that stroke symptoms occur when carotid bifurcation plaque ruptures and clots move into the cerebral circulation. It has been assumed that iron accumulates in atherosclerosis plaques following plaque rupture and hemorrhage since phagocytosed erythrocytes have been identified in plaque macrophages. It was found that in the symptomatic plaque (causing stenosis and cerebrovascular symptoms), iron is associated with the patient's LDL-cholesterol level. Furthermore, iron is abundant in such unstable plaques within thrombus, in the presence of macrophages, and away from calcium and zinc, elements that co-localize in areas of plaque mineralization. Finally, iron in asymptomatic plaque (causing stenosis but not neurological symptoms) was present as ferritin and was observed in association with CD68-positive macrophages.

Therefore, iron may be involved both in the initial step of atherosclerosis by activating LDL oxidation and in the final step linked to the vessel lesion within the plaque. Interestingly, increasing the iron levels in circulating macrophages do not increase atherosclerosis [49]. In a mouse model of atherosclerosis (ApoE-/-), mice were fed with a high-fat diet and their tissue iron was increased by parenteral iron administration and a genetic mutation in ferroportin [49]. Iron loading produced an iron level increase in macrophages, liver, and spleen and resulted in the activation of the macrophage antioxidant defenses and in the storage of iron in the form of ferritin. Clearly, this regulation reduced NTBI and toxicity.

With the hypothesis that blood donation reduces cardiovascular risk by lowering body iron status, a study was done in 819 healthy blood donors in the Netherlands [50]. Data included blood donation frequency, body iron status parameters, and a measure of the carotid intima-media thickness (CIMT). Body iron status was not related to CIMT, but CIMT was slightly and not significantly reduced in frequent donors. Therefore, blood donation might give some protection against atherosclerosis in individuals predisposed to accumulate iron in excess, but the mechanism may be independent of total body iron.

The possibility that heme instead of iron is the inductor of LDL oxidation has also been investigated [51, 52]. Heme oxygenase-1, the heme-catabolizing enzyme, is therefore crucial for heme detoxification (see HO-1 in **Figure 2**). HO-1 induction results in an increase in free iron and ferritin upregulation, which means iron storage and protection of the cell [51]. Interestingly, a child with HO-1 deficiency showed elevated plasma heme levels, extensive LDL oxidation, severe endothelial damage, and accelerated atherosclerosis, and thus the possibility of a HO-1 therapy that mitigates some of the symptoms is a matter of research [52, 53].

3.3. Lessons from hemochromatosis and other iron overload disturbances

Type 1 hereditary hemochromatosis (HH) is a genetic disease defined as homozygous for the C282Y mutation of the *HFE* gene. This gene is located in chromosome 6 and encodes the major histocompatibility complex class I-like protein HFE. The prevalence of HH is approximately 0.1% in the population of Caucasian origin. However, low morbidity has been found

in HH and most of the C282Y +/+ have no iron overload phenotype or are asymptomatic until adulthood. Cash et al. [30] compared vascular function, biochemical endothelial markers, and antioxidant status between HH patients (C282Y homozygous and high serum-ferritin levels) and controls. Noninvasive pulse wave analysis and pulse wave velocity were applied to carotid and radial arteries to estimate endothelial dysfunction. They reported that male HH patients had higher pulse wave velocity; however, this effect disappeared after adjusting for hypertension. In both sexes, HH was associated with diminished antioxidant levels but neither increase in lipid peroxidation nor alteration in the systemic inflammation marker (i.e., C-reactive protein) could be demonstrated [30]. Thus, controversy remains on the idea that cardiovascular risk is high in hemochromatosis patients [54].

Iron overload is usually a complication of thalassemia, particularly in patients who require red blood cell transfusions. Among the three types of thalassemia, thalassemia intermedia is characterized by ineffective erythropoiesis, anemia, medullary expansion, and extramedullary hematopoiesis. In contrast to HH, thalassemic patients show a proatherogenic biochemical phenotype which may contribute to enhance cardiovascular risks [31].

There are other iron overload pathological situations, where bone medulla is inefficient and iron overload results from repeated transfusions. In such syndromes, the amount of body iron can reach very high values and myocardial damage is the most frequent collateral effect of the treatment. In these cases, iron chelation therapy may result in higher quality of life and reduction of cardiac events [55].

4. Iron deficiency anemia and cardiovascular risk

Oxidative stress results from disequilibrium between oxidants and antioxidants. While iron excess may be involved in the generation of ROS, as commented above, anemia due to iron deficiency anemia (IDA) may affect the functioning of many enzymatic systems (cytochromes, catalases, hydroxylases, etc.) related to immunity, antioxidant status, and DNA integrity, among others [56].

In this regard, Aslan et al. [57] compared total plasma antioxidant capacity and lymphocyte DNA damage between two groups of IDA and control adults and concluded that both oxidative stress and DNA damage increased in IDA. In another study, four groups were compared: patients recently diagnosed with IDA who were not receiving any treatment at the beginning of the study; patients with IDA at the sixth week of an iron-replacement program (considered the time of hemoglobin normalization); patients with IDA at the end of the iron-replacement treatment (time of saturation of body iron stores); and age- and sex-matched healthy controls. Results show that untreated IDA patients present high lipid peroxidation, assessed by plasma malondialdehyde, and low activities of the antioxidant enzymes glutathione peroxidase, superoxide dismutase, and catalase and that the values did not differ between the sixth week and the end of the treatment, suggesting that recovery from IDA reduces oxidative stress [58]. Unfortunately, in these studies, the changes within a patient were not analyzed. In animal models of IDA, where all experimental conditions are controlled, high oxidative stress and DNA damage were not demonstrated [59, 60].

Another aspect that has been studied is the possible changes in lipid levels in IDA. Old animal studies reported dyslipidemia with altered triglycerides and total cholesterol in serum. However, in most animal experiments, there were important confounders. For instance, iron deficiency induces low appetite and a reduction in food intake that often was not adequately controlled. In this regard, there are inconsistent results, but clearly the direction of change was toward reduction in circulating lipids and profound modifications in lipoprotein metabolism [61].

In humans, our research group found low values of total cholesterol, HDL-cholesterol, glucose, and uric acid in IDA women at fertile age, which significantly increased during anemia recovery [36]. These results coincide with that of others who also reported low serum triglycerides [62] and LDL-cholesterol [63]. It is noteworthy that despite significant increases after treatment, the observed lipid values were very low in the severe anemic patients from these studies and still did not reach levels of non-anemic controls after recovery (reported mean values were approximately 150–170 mg/dL for total cholesterol and 60–70 mg/dL for triglycerides).

The above results can be explained by inhibition of lipid biosynthesis due to iron deficiency. Kamei et al. [64] performed a transcriptome analysis to determine the effects of iron deficiency on hepatic gene expression. Rats on an iron-deficient diet were compared with rats pair-fed a control diet with a normal iron level. In agreement with human studies, these authors observed that iron deficiency decreases cholesterol and triglycerides in serum and liver. In addition, they found that serum glucose and insulin increased. Expressions of genes encoding gluconeogenic enzymes were upregulated, lactate was increased, and the urea cycle was activated. These results are explained by the insufficiency of iron for its enzymatic functions and the situation of hypoxia due to anemia. Nevertheless, the results of high glucose and insulin do not agree with human observations.

Iron deficiency may affect cardiovascular health by indirect mechanisms. In this regard, iron participates in the hydroxylation of vitamin D to the active metabolites, 25 hydroxyvitamin D and 1,25-hydroxyvitamin D, and vitamin D acts as an antioxidant and may have protective cardiovascular effects, decreasing LDL-cholesterol, and blood pressure [37]. Moreover, iron supplementation alone increases vitamin B12 and folic acid levels [36]. This is attributed to a general increase in intestinal mucosa that favors nutrient absorption.

5. Dysmetabolic iron, type 2 diabetes, and cardiometabolic alterations

5.1. Iron excess and type 2 diabetes mellitus (T2DM)

T2DM is the most common and an ever-increasing form of diabetes [65]. It is characterized by disorders in insulin secretion or action either of which maybe the predominant feature. The association between iron overload and T2DM came from the observation that the frequency of diabetes is increased in classic hereditary hemochromatosis [65]. A link between red meat consumption, one of the highest iron bioavailability source, and T2DM has been reported [66]. Moreover, some reports show a relationship between high ferritin and the risk of gestational diabetes [67].

The positive association between iron excess and T2DM is feasible although the underlying mechanisms still remain to be fully determined. First, iron is a powerful pro-oxidant and catalyst molecule, which promotes the formation of hydroxyl radicals and could attack pancreatic β cells by increasing oxidative stress thus resulting in impaired insulin synthesis and secretion [68]. Second, iron excess can diminish insulin utilization in muscle tissue leading to a shift from glucose to free acid oxidation, which may result in enhanced insulin resistance [69]. Third, increasing free fatty acid, main substrate for hepatic gluconeogenesis, would provoke higher glucose production [69]. Thus, the possible mechanisms are insulin deficiency, insulin resistance, and hepatic dysfunction [70].

Several studies report an association between the heme iron intake and risk of T2DM. The prospective cohort within the Nurses' Health Study found that higher intake of heme iron was associated with higher intake of fat (total and saturated), red meat, and protein and with lower intake of carbohydrates. However, the association was not entirely explained by the red meat intake. Total dietary iron, non-heme iron, or supplemental iron were not related to diabetes risk [71].

Other studies reveal that vegetarians have higher insulin sensitivity than omnivores, and this was mainly attributed to their lower body iron [72]. In this regard, blood donation, by reducing iron stores, may increase insulin sensitivity [72, 73]. However, there is controversy in this issue [74–76].

We have studied some cardiovascular risk markers in a population of 595 T2DM from the DIabetes and CArdiovascular RIsk VAllecas (DICARIVA) study according to ferritin levels (**Table 1**).

Diabetic dyslipidemia is a cluster of altered plasma lipids and lipoproteins [77] though LDL-cholesterol levels are normal or reduced. It is characterized by high triglycerides and low HDL-cholesterol levels and by increased number of small and dense LDL particles [77]. Altogether, these features are known as the lipidic triad. In addition, other alterations are often observed:

- Increased concentration of very low density lipoproteins (VLDL) due to an increased production or a lower clearance of triglycerides and apolipoprotein (apo) B.
- Increased production of apo B-LDL as well as an increment in glycosylation and oxidation of LDL particles.

	n	Mean	Standard deviation	P25	Median	P75
Males	265	150.5	149.3	50.5	107	200.5
Females	330	67.6	83	21.8	41.5	78

The distribution of ferritin in male and femaleT2DM was significantly different ($p < 0.0001$).

Table 1. Male and female ferritin levels (ng/mL) in type 2 diabetes population belonging to the DIabetes and CArdiovascular RIsk VAllecas (DICARIVA) study P25 and P75, 25th and 75th percentiles.

- Higher clearance of apo A1 with decrease in the high-size HDLs and decrease of the cholesterol reverse transport.
- Lower clearance of chylomicrons and remnant particles (i.e., intermedium density lipoproteins or IDL).

The diabetic dyslipidemia is associated with insulin resistance, visceral obesity, and liver fat content. Furthermore, insulin resistance is related to an excessive flux of substrates (free fatty acids and glucose) to participate in the formation of VLDL in the liver and with a positive control of mechanisms that produces an excess of large VLDL. These lipoprotein metabolism anomalies are not disconnected facts but are closely related to each other [78]. It is known that lipid metabolism in T2DM is modulated by several factors, such as the degree of glucose control and insulin resistance. The hypertriglyceridemia is very prevalent in T2DM and is also frequent in prediabetes, preceding the presence of chronic hyperglycemia [79]. When there is insulin resistance, mesenteric or "central" adipocytes are full and are unable to retain more fatty acids, consequently fatty acids reach the liver in very high quantities.

Since LDL-size assessment requires special methodology, other approaches have been proposed. The triglycerides/HDL-cholesterol molar ratio has been widely used as a surrogate marker of LDL-size in clinical practice [80]. Earlier a value < 1.33 for this ratio was considered adequate and indicative of large LDL particles. In contrast, individuals with high triglyceride/LDL-cholesterol molar ratio present a high amount of small, dense, oxidizable, and, thus, highly atherogenic LDL particles [81].

It has been confirmed that triglyceridemia is the determinant of LDL size [82]. In fact, it has been proposed that highly enriched in triglyceride VLDL subtype (VLDL1) are the predecessors of dense and small LDL particles [83].

According to the data in **Table 2**, T2DM women presented higher triglyceridemia and higher HDL-cholesterol levels but lower triglyceride/HDL-cholesterol levels than men.

This study also shows that triglyceride levels increase in parallel to the level of ferritin in men and women. Triglycerides were 36 and 23% higher in men and women, respectively, belonging to the 4th ferritin quartile *versus* their 1st counterparts. LDL particles appear 38% smaller in men and 24% smaller in women at the highest quartile *versus* the lowest, according to the triglyceride/HDL-cholesterol molar ratio. Taking into account these data and the significant correlation between ferritin and this molar ratio ($p < 0.001$), it can be speculated that body iron contributes to this theoretically higher oxidability and atherogenicity of the LDL.

When the T2DM sample belonging to the 1st or 4th quartile for ferritin was stratified according to the presence of normo or hypertriglyceridemia, low or high levels of HDL-cholesterol, and small or large LDL particles, it was observed that the prevalence of altered triglycerides was higher (odd ratio 1.78; $p=0.011$) in T2DM patients belonging to the highest quartile for ferritin. Similarly, the odd ratios for high levels of HDL-cholesterol or the presence of small LDLs was 0.54 ($p = 0.010$) and 1.93 ($p = 0.004$), respectively in the T2DM patients of the 4th quartile *versus* the 1st quartile of ferritin.

To insist even more in this idea, the prevalence of T2DM presenting the lipid triad was compared to that of patients who did not present any of the three components of the triad. The

						95% CI			
		Ferritin quartiles	**N**	**Mean**	**SD**	**Lower limit**	**Upper limit**	**ANOVA *p***	**P25 *vs* P75**
Men	Triglycerides	<P25	66	128.6	63.1	113.0	144.1	0.038	0.006
	mg/dL	P25-<P75	133	157.8	114.3	138.2	177.4		
		≥P75	66	174.8	118.5	145.7	203.9		
	HDL-cholesterol	<P25	66	48.4	12.5	45.3	51.5	0.99	0.96
	mg/dL	P25-<P75	133	48.6	12.7	46.4	50.7		
		≥P75	66	48.2	12.5	45.4	51.5		
	TG/HDLc*	<P25	66	1.29	0.82	1.08	1.49	0.013	0.020
	mol/mol	P25-<P75	133	1.63	1.66	1.35	1.92		
		≥P75	66	1.78	1.47	1.42	2.14		
Women	Triglycerides	<P25	82	152.8	95.4	131.9	173.8	0.027	0.17
	mg/dL	P25-<P75	163	138.1	101.6	122.4	153.8		
		≥P75	84	187.9	213.1	141.7	234.2		
	HDL-cholesterol	<P25	82	59.2	14.9	55.9	62.4	0.025	0.007
	mg/dL	P25-<P75	163	56.5	14.1	54.3	58.7		
		≥P75	84	53.2	13.3	50.3	56.1		
	TG/HDLc*	<P25	82	1.33	1.20	1.07	1.59	0.038	0.11
	mol/mol	P25-<P75	163	1.21	1.27	1.01	1.41		
		≥P75	84	1.65	1.40	1.35	1.96		

P, percentile; ANOVA *p*, P value for <P25, P25-<75 and ≥P75.

*TG/HDLc, Triglycerides/HDL-cholesterol molar ratio.

Table 2. Triglyceride, HDL-cholesterol, and the Triglyceride/HDL-cholesterol molar ratio in men and women from the DIabetes and CArdiovascular RIsk VAllecas (DICARIVA) study stratified according to ferritin quartiles.

concurrence of high ferritin and all the three components of the triad was higher than the concurrence of low ferritin and the three components of the triad. On the contrary, the absence of any of the three components of the triad was less prevalent in T2DM patients with high ferritin values than in their low ferritin counterparts. The odd ratio for the lipid triad/ferritin association was 2.23 ($p = 0.010$), suggesting the hypothesis that altered CVD risk factors is more prevalent in T2DM patients presenting high iron body stores.

6. Conclusions, remarks, and future research

From all the above, there clearly exists a connection between iron regulation, lipoprotein metabolism, and insulin resistance.

Experimental evidence in animals and humans indicates that dietary fat may be important in iron metabolism. Despite increasing evidence that dietary fat can influence iron absorption and retention, there is a paucity of information about the mechanism implicated [84, 85]. These may be related directly to changes occurring within the intestinal lumen in the enterocytes at luminal or apical membranes. Many aspects of iron absorption and its regulation are still unknown, such as the mechanisms of ferric iron transport, the role of mucines, and so on [8, 9, 11].

Droke et al. [86] demonstrate that palmitate increased iron transport to a greater extent than stearate, and this is followed by far by oleate, which could be due to fatty acid metabolism within the cells and the elongation of palmitic to stearic acid. However, the results suggest that fatty acids affected iron uptake to a greater extent than iron transport. One of the most striking effects of dietary fat on mineral metabolism is the finding of the enhancement of iron uptake and utilization by saturated fat. The effects are prominent when dietary iron is limiting and thus indicate a novel role in promoting an adequate iron status in human [86].

A genome-wide association study (GWA study or GWAS) and epigenome-wide association study (EWAS) together with metabolomic studies would help much to understand the mechanisms involved in the conjoint iron-lipid metabolism that, in turn, affects CVD risks. This information will be useful in the dietary personalization to optimize human health and function.

Meanwhile, as saturated fatty acids (mainly palmitic) increase the iron store [86], total cholesterol, and LDL cholesterol, and induce negative effects on insulin resistance compared to unsaturated fat [87], the authors of the present review claim insisting in the need that T2DM patients show a high adherence to present dietary recommendations for diabetes (American Diabetes Association [88]), which textually include that fat quality (eating monounsaturated and polyunsaturated fats and avoiding *trans* fats and saturated fats) appears to be more important than quantity.

In conclusion, iron is a key metal involved in cardiovascular health. Mild iron deficiency may reduce cardiovascular risk; contrarily, severe anemia induces alterations in the antioxidant iron-dependent enzymes and can be a threat. Iron overload appears to be more important than deficiency in triggering insulin resistance. In this regard, dysmetabolic iron overload

syndrome has been related to liver fat accumulation and visceral adiposity [89]. Whether hepcidin resistance is linked to insulin resistance should be a matter of further research.

Our data in an ample sample of adults diagnosed with T2DM suggest that body iron stores, evaluated as serum ferritin, are clearly related with some key markers of the so-called lipidic triad of the T2DM (high triglyceride and low HDL-cholesterol levels together with the presence of small and dense LDL particles) which also is in the frame of the dysmetabolic iron overload syndrome.

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Abbreviations

CVD	cardiovascular diseases
DMT1	divalent metal transporter
FPN	ferroportin
IDA	iron deficiency anemia
HDL-cholesterol	cholesterol transported by high density lipoproteins
NTBI	non-transferrin-bound iron
ROS	reactive oxygen species
T2DM	type 2 diabetes mellitus
TfR1	transferrin receptor

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