New insights into the role of iron in inflammation and atherosclerosis

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ABSTRACT

Iron is fundamental for life-essential processes. However, it can also cause oxidative damage, which is thought to trigger numerous pathologies, including cardiovascular diseases. The role of iron in the pathogenesis of atherosclerosis is still not completely understood. Macrophages are both key players in the handling of iron throughout the body and in the onset, progression and destabilization of atherosclerotic plaques. Iron itself might impact atherosclerosis through its effects on macrophages. However, while targeting iron metabolism within macrophages may have some beneficial effects on preventing atherosclerotic plaque progression there may also be negative consequences. Thus, the prevailing view of iron being capable of accelerating the progression of coronary disease through lipid peroxidation may not fully take into account the multi-faceted role of iron in pathogenesis of atherosclerosis. In this review, we will summarize the current understanding of iron metabolism in the context of the complex interplay between iron, inflammation, and atherosclerosis.

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1. Introduction

Iron is essential for physiologic processes and plays an important role in cellular metabolism through iron-containing and -sequestering enzymes and proteins involved in oxidative stress, inflammation, and atherosclerosis.
proteins and enzymes that maintain mitochondrial function, DNA synthesis and repair, as well as cell growth and death [1]. As the main component of haemoglobin, it is crucial for erythropoiesis and oxygen transport. However, iron can also be toxic due to its ability to generate reactive oxygen species (ROS) along with the oxidation of biomolecules via the generation of toxic hydroxyl radicals generated by the Fenton reaction.

Iron homeostasis is tightly controlled by the interplay of various iron-processing tissues and cells, including macrophages, erythrocytes, hepatocytes and duodenal epithelial cells, and regulated by the hepcidin-ferroportin axis. Most of the iron required for life is recycled from senescent red cells by the reticuloendothelial system with additional demand fine-tuned by adjusting the amount of absorbed iron by enterocytes. Distortions in the intake/output of iron can result in disease. Iron deficiency (ID) is the most common cause of anaemia and represents a global health problem. In some disease states such as haemochromatosis, excess iron enters the body and may harm parenchymal organs including the liver, pancreas and heart.

The role of iron in the pathogenesis of atherosclerosis and coronary artery disease (CAD) has been investigated for more than 35 years when Sullivan proposed iron as a cardiovascular risk factor, suggesting that it might explain the lower incidence of cardiovascular disease in premenopausal women [2], given that measures of iron stores increase as women age, especially after menopause. Free iron released might accelerate the oxidation of low density lipoproteins (LDL) which can then be taken up by the LDL receptor on macrophages leading to their development into foam cells. Foam cell infiltration and necrotic core expansion are key events in atherogenesis [3]. Over the years, numerous in vitro [4–6] and in vivo [7–10] studies have been conducted to investigate the relationship between iron and cardiovascular diseases. With major advances in our understanding of iron metabolism as well as the role of inflammation in atherosclerosis, it appears that the initial interplay between iron and CAD proposed by Sullivan might be overly simplistic. Our knowledge of the role of iron in cardiovascular disease is evolving and it is likely that the effect of iron on atherosclerosis is context-dependent as will be explained [11]. Overall, in this review we will summarize the current understanding of iron in the development and progression of atherosclerosis.

2. Iron metabolism: the basics

2.1. Iron consumption and uptake

90% of daily iron needs are obtained from the breakdown of senescent erythrocytes, recycled by macrophages. The sum of iron losses and iron required for growth and during anaemia or pregnancy is regulated by adjusting absorbed dietary iron, which has two main chemical forms. The majority enters the body as non-haem ferric iron (Fe3+) that is present in plants and requires reduction by ferrireductase in the gut mucosa before absorption. Since haem ferrous iron (Fe2+) from animal source foods can be directly absorbed, the bioavailability of haem iron is higher. From an average daily consumption of 12 to 15 mg iron, only 1–2 mg is ultimately absorbed [12].

In 1992, a Finnish study gave cause for concerns about dietary iron consumption, as iron intake was associated with myocardial infarction (MI) [13]. Other studies showed rather conflicting data, and it later became obvious that the risk of MI was in fact associated with the consumption of processed meat, containing several harmful components that have been associated with an increased mortality risk [14].

2.2. Hepcidin-ferroportin axis: immediate control of available and circulating iron

Dietary iron is absorbed in the duodenum and the upper jejunum (Fig. 1) [15]. Cellular iron efflux into the systemic circulation at the basolateral site of the enterocytes is mediated by iron transporter ferroportin (FPN) [16]. FPN is abundantly expressed in cells that are involved in regulating plasma iron levels, notably duodenal enterocytes, macrophages, and hepatocytes [16]. The peptide hormone hepcidin is a key regulator of FPN and mediates its internalization and degradation [17]. Hepcidin expression in the liver is triggered by high systemic iron levels. By decreasing FPN expression on macrophages, hepcidin inhibits iron export from macrophages, and serum iron decreases. The hepcidin–FPN axis maintains iron homeostasis in response to changing requirements. For the purposes of this review we will focus on the hepcidin–FPN axis in macrophages as major regulator of plasma iron levels [18].

2.3. Iron transportation and storage

Transferrin is the major iron binding protein in the serum and delivers ferric iron (Fe3+) to the cells. After intracellular reduction to ferrous (Fe2+) iron, non-utilized iron is taken up into ferritin, storing up to 4500 iron atoms [1]. The vast majority of iron, however, is dedicated to haemoglobin synthesis, and the haemoglobin storage accounts for two thirds of 3–5 g iron stored in the human body [18].

2.4. Catalytic effect of iron and links to lipoprotein oxidation

Iron is a redox active metal and powerful catalyst. The transfer of electrons between the ferrous and ferric states contributes to the formation of ROS through the Fenton reaction [19].

\[ \text{Fe}^2+ + \text{O}_2^- \rightarrow \text{Fe}^3+ + \text{O}_2 \]

\[ \text{Fe}^2+ + \text{H}_2\text{O}_2 \rightarrow \text{OH}^- + \text{OH}^- + \text{Fe}^{3+} \]

Iron storage in ferritin and haemoglobin reduces its toxicity. However, during haemolysis, free iron from accumulated free haemoglobin may cause tissue damage by the formation of ROS with subsequent oxidative modification of biomolecules. The oxidation of lipoproteins is one of the critical events in atherogenesis. Oxidized LDL (oxLDL) promotes endothelial dysfunction with activation of the inflammatory transcription factor Nuclear Factor kappa-light-chain-enhancer of activated B cells (NF-κB) and induces a significant dose-dependent increase in the production of ROS [20]. Ox-LDL is taken up by high-affinity LDL receptors on macrophages, eventually driving their development into foam cells. Foam cells upregulate proteolytic enzymes such as matrix metalloproteases (MMPs) and degrade extracellular matrix structure, leading to plaque rupture and MI [21]. Necrosis of foam cells and the failure to clear these dead cells leads to further growth of the plaque and finally to the formation of a necrotic core. Ferroptosis is an iron-dependent form of necrosis and characterized by accumulation of lipid peroxides [22]. Although to our best knowledge, ferroptosis has not been investigated in atherosclerosis, it might be an important mechanism for plaque destabilization [23]. Glutathione peroxidase-4 (Gpx4), one of the major anti-oxidant enzymes in the human body, is a key regulator of ferroptosis [24] capable of removing oxidative lipid modifications. Overexpression of Gpx4 was shown to inhibit plaque development in apoE−/− mice [25]. Ferrostatin-1 has been identified as a potent inhibitor of ferroptosis in vitro, but lacks adequate chemical stability for in vivo applications [26]. Novel ferroptosis inhibitors containing amide and sulphonamide moieties have been reported to exhibit an improved stability, but it remains uncertain if these agents are suitable for in vivo application [27].

Although the pro-oxidative effect of iron on lipoproteins has been shown in vitro [3], the data on associations of systemic iron parameters and the progression of atherosclerosis in vivo is rather conflicting. Whereas some population surveys have demonstrated associations...
between ferritin levels and carotid atherosclerosis [7,28], other studies did not [29], and a meta-analysis of twelve prospective studies involving 7800 subjects did not find a clear association between parameters of iron status (ferritin, transferrin saturation, total iron-binding capacity, and serum iron) and CAD [30]. However, ferritin is regulated both by iron and by inflammation, which is also evident in atherosclerosis, and might represent a non-causal indicator rather than a surrogate of body iron stores.

Likewise, whereas lowering iron levels by phlebotomy was proposed to be athero-protective by increased flow-mediated vasodilation in the brachial artery and decreased oxidative stress in blood donors [31], another study did not detect altered vascular function when compared with sham phlebotomy [32].

3. Iron status and genetic disorders associated with abnormal iron metabolism and their effects on atherosclerosis

3.1. Hereditary hemochromatosis

In hereditary hemochromatosis, polymorphisms in the hfe gene, Cys282Tyr (rs1800562) or His63Asp (rs1799945) lead to a reduced expression or impaired function of hepcidin [33]. The subsequent overexpression of FPN results in excessive gut absorption and pathologic deposition of iron in tissues, especially in heart, liver, and pancreas. Despite the reported increased oxidative stress, patients are not at higher risk for cardiovascular events [34,35]. Studies also do not exclude a degree of protection against atherosclerosis in hemochromatosis [36]. In fact, an autopsy study even found the extent of CAD to be less (OR 0.18) in 41 cases of hemochromatosis versus age, race and sex-matched controls [37], and intima-media-thickness was significantly lower even in heterozygous patients [38]. These data challenge the hypothesis of iron as a pro-oxidant being capable of accelerating atherosclerosis.

To shed light on these paradox observations, it needs to be emphasized that patients carrying the homozygous form of hfe are also deficient of hepcidin [39]. Serum hepcidin, hepcidin:transferrin, and hepcidin:ferritin ratios were positively correlated with non-invasive measurements of atherosclerosis (NIMA) in postmenopausal women [40]. Similar results were found in a genetic study, in which 12 iron-related genes were assessed [41]. In a large clinical study of 759 patients with ACS and 526 patients with stable CAD, plasma hepcidin was found positively associated with all-cause and CVD mortality in ACS patients [42]. However, other studies have found no significant associations [43,44]. Thus, the influence of iron status on CAD remains incompletely understood.

3.2. SNPs associated with iron status based on Mendelian Randomization estimates

Over the last decade, Mendelian Randomization (MR) studies aimed to obtain unbiased estimates of the effects of iron on CAD without confounding environmental or lifestyle factors, mimicking the randomization in a clinical trial and hence allowing for assessment of causality of iron status [45].

Genome-wide association studies (GWAS) have facilitated the design of MR studies ensuring adequate power by the availability of very large genetic datasets [46]. A meta-analysis of GWAS including 48,978 individuals from 11 European-population studies with replication in eight additional cohorts found 11 loci being associated with at least one of the iron parameters at a genome-wide level [47,48]. A study investigating SNP associations between iron status and NIMA in 549 subjects did not provide evidence for a causal role of iron parameters on atherosclerosis, except of rs651007, which is associated with a decrease in ferritin concentration, and revealed a decreased risk of atherosclerosis [47]. In contrast, rs411988, which has a similar effect on ferritin, showed far weaker or even no association with NIMA. A reverse approach of investigating correlations between NIMA-associated SNPs with iron parameters failed to provide evidence for a role of ferritin, iron, total iron binding capacity, and transferrin saturation (TS) in atherosclerosis. However, significant associations were found between two of six investigated NIMA-related SNPs and hepcidin/ferritin ratio [47], suggesting that traditional iron parameters might not be adequate to investigate the impact of iron in atherosclerosis, and a more refined analysis is needed. More recently, a two-sample MR approach was used to

Fig. 1. Systemic iron regulation. Iron is absorbed in the duodenum and upper jejunum. Elemental iron is taken up from the intestinal lumen via divalent metal transporter 1 (DMT1). High systemic iron levels lead to increased hepcidin expression in the liver. Hepcidin binds ferroportin, leading to its internalization and degradation with subsequent drop of systemic serum iron, but an increase in intracellular free iron levels. Accordingly, hepcidin levels are decreased in iron deficiency, which increases iron absorption and cellular iron release.
estimate the effect of iron status on CAD risk investigating three loci (rs1800562 and rs1799945 in the hfe gene and rs855791 in tmprss6) that are associated with high serum iron, TS, ferritin, and transferrin based on the GWAS dataset [48]. This study combined results of a GWAS meta-analysis of 60,801 CAD cases and 123,504 controls [49] with those of a meta-analysis of 63,746 CAD cases and 130,681 controls obtained from Metabochip [50] to achieve combined MR estimates for each marker. The pooled MR estimates across the three genetic variants for serum iron, ferritin, transferrin and TS, suggested that higher iron status lowers the risk of CAD. Further studies from the same group using MR analysis investigated the genetically determined high iron status and found a strong association with venous thromboembolism, and lower risk of carotid plaque, but no significant effect on carotid artery intima-media thickness [51].

3.3. Macrophages as key effectors of inflammation in atherosclerosis

Macrophages play a central role in atherosclerosis progression, and different subtypes of macrophages have been detected within atherosclerotic plaques [52]. Lipid ingestion is the primary stimulus for M1 macrophage differentiation in plaques which induces inflammatory cytokine production and foam cell formation [53]. Furthermore, M1 macrophages through paracrine effects are able to induce SMC proliferation and migration from the media into the intima and are therefore considered pro-atherogenic. In addition, M1 macrophages are stimulated by Th2 type cytokines (i.e. IL-10, IL-4, and IL-13) and produce anti-inflammatory cytokines. M2 macrophages are thought to counterbalance inflammatory responses, promoting resolution of inflammation and tissue repair. The M1/M2 paradigm provides a simplified framework for our understanding of the macrophages' function in the setting of injury, but it is based on mouse data or in vitro differentiation studies and cannot be directly translated to the situation in humans. The mechanisms by which macrophages orchestrate inflammation and its resolution to promote tissue repair remain incompletely understood.

Iron turnover is different in M1 and M2 cells. Due to a low expression of FPN and haem oxygenase 1 (HO-1), M1 macrophages are rich in ferritin and prone to iron accumulation, whereas M2 are able to metabolize and export iron, resulting in lower intracellular iron concentrations [55]. Macrophage iron levels can alter their polarization [56]. Low intracellular iron levels were shown to inhibit the expression of pro-inflammatory cytokines and prone to iron accumulation, whereas M2 are able to metabolize iron and migrate from the media into the intima and are therefore considered athero-protective. In addition, M1, M2 and M1 macrophages are stimulated by Th2 type cytokines (i.e. IL-10, IL-4, and IL-13) and produce anti-inflammatory cytokines. M2 macrophages are thought to counterbalance inflammatory responses, promoting resolution of inflammation and tissue repair. The M1/M2 paradigm provides a simplified framework for our understanding of the macrophages' function in the setting of injury, but it is based on mouse data or in vitro differentiation studies and cannot be directly translated to the situation in humans. The mechanisms by which macrophages orchestrate inflammation and its resolution to promote tissue repair remain incompletely understood.

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Fig. 2. Intraplaque haemorrhage in fibroatheroma with a core in a late stage of necrosis (Panels A, B, C, D, and E) and thin-cap fibroatheroma (panels F, G, H, I, and J). Panel A shows a low-power view of a fibroatheroma with a late-stage necrotic core (NC) (Movat pentachrome, ×20). Panel B shows intense staining of CD68-positive macrophages within the necrotic core (×200). Panel C shows extensive staining for glycophorin A in erythrocyte membranes localized with numerous cholesterol clefts within the necrotic core (×200). Panel D shows iron deposits (blue pigment) within foam cells (Mallory’s stain, ×200). Panel E shows microvessels bordering the necrotic core with perivascular deposition of von Willebrand factor (vWF) (×400). Panel F shows a low-power view of a fibroatheroma with a thin fibrous cap (arrow) overlying a relatively large necrotic core (Movat pentachrome, ×20). The fibrous cap is devoid of smooth-muscle cells (not shown) and is heavily infiltrated by CD68-positive macrophages (Panel G, ×200). Panel H shows intense staining for glycophorin A in erythrocyte membranes within the necrotic core, together with cholesterol clefts (×100). Panel I shows an adjacent coronary segment with iron deposits (blue pigment) in a macrophage-rich region deep within the plaque (Mallory’s stain, ×200). Panel J shows diffuse, perivascular deposits of von Willebrand factor in microvessels, indicating that leaky vessels border the necrotic core (×400). Reproduced with permission from Kolodgie FD et al. N Engl J Med 2003; 349:2316–2325.

Fig. 3. Intraplaque haemorrhage. Fragile and permeable intraplaque vasa vasorum drive haemorrhage inside the plaque. The pro-oxidant environment of the plaque promotes haemolysis as well as the formation of cholesterol crystals. Free iron from haemoglobin might further catalyse these reactions. Haptoglobin binds free haemoglobin, and haemoglobin:haptoglobin complexes are cleared via CD163+ macrophages. These M(Hb) macrophages have been associated with plaque progression, microvascularity, and upregulation of hypoxia inducible factor-1α (HIF-1α).
membranes require the removal of haemoglobin [67]. Further research is warranted to determine the role of M(Hb) macrophages in the context of vascular calcification.

4. Opportunities and future directions: modulation of iron metabolism as potential therapy target in atherosclerosis?

4.1. Effects of blockade/downregulation of hepcidin

Hepcidin induces degradation of FPN, leading to an increase of intracellular iron and a decrease of serum iron levels. Genetic disorders resulting in hepcidin deficiency, such as hemochromatosis, are characterized by low levels of intracellular iron in macrophages [17]. While hemochromatosis is well-known to be associated with organ toxicity to liver, heart, and pancreas, paradoxically, several lines of evidence suggest hemochromatosis is associated with dampening of inflammation within macrophages. Mutations in the hfe gene lead to aberrant regulation of hepcidin expression, resulting in low hepcidin levels in spite of iron overload. Under normal conditions, hepcidin is strongly induced during infections [68], and hfe<sup>−/−</sup> mice showed attenuated inflammatory responses to Salmonella infection [58]. Macrophages, aiming to withhold iron from invading pathogens to limit their growth [69], produce endogenous hepcidin independently of iron levels upon stimulation with LPS in a TLR-dependent manner, whereas liver hepcidin expression is independent of TLR4, but mediated by macrophage cytokine production (IL6 and IL1) and iron [68,70]. TLR4 signalling pathways have been implicated in the initiation and progression of atherosclerosis [71], potentially driving hepcidin production in macrophages.

Recently, the effect of serum and tissue iron overload on atherosclerosis has been investigated in apoE<sup>−/−</sup> FPN<sup>WT/C282Y</sup> knock-in mice with disrupted interaction of hepcidin and FPN causing a constitutive efflux of iron into the blood stream [72]. Exceeding the capacity of transferrin to bind iron, the generation of non-transferrin bound free iron was associated with reduced atherosclerosis, driven by elevated pro-inflammatory mediators, enhanced endothelial activation and dysfunction, and iron deposits in the vascular media. Conversely, in another study deletion of the hepcidin gene (hamp) was associated with reduced atherosclerosis in hamp<sup>−/−</sup>/Ldlr<sup>−/−</sup> mice along with reduced pro-inflammatory markers in macrophages [73]. In addition, fasting serum LDL was decreased, which might in part be attributable to a genetic overlap in iron and lipid loci [47]. Thus, these experiments show nicely how altering hepcidin-FPN axis through loss or gain of function profoundly affects the development of atherosclerosis in experimental models. However, the exact mechanisms by which such changes remain uncertain but experimental evidence points towards their effects within macrophages, although data from knock in/out models are not necessarily comparable to human genetic data.

Links between hepcidin pathway and macrophage cholesterol metabolism have been shown. The promoter elements of hepcidin are activated by SMAD 1/5/8 transcription which are activated through bone morphogenetic protein signalling. Blockade of BMP type 1 receptors with small molecular inhibitors dorsomorphin or LDN leads to

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**Fig. 4.** Effect of hepcidin on macrophages phenotype and function in the setting of atherosclerosis. Hepcidin may play different roles in atherogenesis depending upon the stage of atherosclerosis. a) In early and mid-stage plaques, M1 Macrophages are predominant. Hepcidin induces degradation of FPN, leading to an increase of intracellular iron in macrophages. Intracellular iron accumulation results in an increased ox-LDL cholesterol incorporation via scavenger receptors such as CD36 and LOX-1 (LDL receptor-1), and increased inflammatory (LPS-stimulated) signalling through TLR-4, decreasing cholesterol efflux, and intracellular reactive oxygen species (ROS) generation. Overall, these cells exhibit a phenotype consistent with pro-inflammatory foamy macrophage phenotype. Under these conditions, macrophages contribute to atherosclerosis progression. In an environment with low or without hepcidin, intracellular iron is actively exported out of the macrophages via FPN. Lowering intracellular iron within the macrophage suppresses LDL uptake and increases its oxidation within macrophages. M(Hb) Macrophages are abundant. Iron is an essential cofactor for PHD that mediates degradation of HIF-1α, promoting VEGF target gene expression and leading to further intraplaque angiogenesis, endothelial permeability, and inflammatory signalling. Thus, in early- to mid-stage plaques, inhibition of hepcidin may have beneficial effects by restraining the effects of pro-inflammatory macrophages, while in late stage lesions (i.e. those with IPh), lowering of macrophage iron may promote plaque progression through VEGF-mediated increases in angiogenesis, permeability, and inflammatory cells recruitment.

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**Legend:**
- **Hepcidin Expression↑**
- **Hepcidin Expression↓**
- M1 Macrophages
- M(Hb) Macrophages
- HIF-1α
- PHD
- Iron
- Cholesterol
- ABCA1, ABCG1
- LPS
- M1 Macrophage
- M(Hb) Macrophage
- HIF-1α
- PHD
- Iron-PHD-HIF-1α
- VEGF
- Cholesterol Crystal
- Erythrocyte
- lysed Erythrocytes
- Free Cholesterol
- Haemoglobin
decreased hepcidin expression and lowered macrophage iron [74]. The administration of LDN to apoE\(^{-/-}\) mice increased the expression of the cholesterol exporters in intraplaque macrophages, which in turn decreased foam cell formation and delayed atherosclerotic plaque progression [75]. Simultaneously, the production of pro-inflammatory cytokines and ROS in macrophages decreased. Exogenous hepcidin administration reversed all preceding LDN-induced effects. These data suggest the hepcidin–FPN axis might potentially be able to reduce atherosclerosis through its effects on macrophages (Fig. 4).

Because hepcidin deficiency is associated with increased serum iron but decreased macrophage iron, the question remains whether increasing serum iron might be the mechanism behind the anti–atherogenic effects seen both in hamp\(^{-/-}\) mice and after LDN administration rather than decreased macrophage iron. However, treatment of hamp\(^{+/+}\)/Ldlr\(^{-/-}\) mice with iron dextran, producing a 2-fold increase in serum iron, did not decrease atherosclerosis. These findings suggest the key to reducing atherosclerosis by alterations in iron metabolism is through lowering macrophage iron (Fig. 4), modulating their immune functions and cytokine expression. However, some important caveats exist to this strategy. Given the pivotal role of hepcidin in maintaining iron homeostasis, its chronic inhibition might result in iron overload–like state. Moreover, it remains possible that hepcidin may play different roles in atherogenesis depending upon the stage of atherosclerosis. In early–to mid-stage plaques, inhibition of hepcidin may have beneficial effects by restraining the effects of pro-inflammatory macrophages, while in late stage lesions (i.e. those with IPH), lowering of macrophage iron may promote plaque progression through VEGF-mediated increases in angiogenesis, permeability, and inflammatory cells recruitment (Fig. 4).

5. Conclusion

Iron metabolism is carefully balanced by complex networks to meet the daily iron demands and to prevent iron overload. Macrophages play a central role in iron homeostasis, and their regulatory mechanisms are increasingly recognized. Modification of the intracellular iron metabolism of macrophages might alter their inflammatory and lipid handling responses, which impacts atherogenesis in experimental models, and thus might be a potential therapeutic target in cardiovascular diseases. However, hepcidin, which is the main regulator of iron homeostasis, may play a two-faced role over the course of atherosclerosis progression. The inhibition of hepcidin might be beneficial in early plaques by affecting macrophage pro-inflammatory activity whereas in areas of IPH its effect may actually be detrimental because of its pro-angiogenic effect. Further research is warranted to elucidate further the complex relationship between iron metabolism and atherosclerosis.

6. Outstanding questions

Despite the large number of studies published to date, there are still open questions to fully elucidate the relationship between iron metabolism and atherosclerosis.

1. How handling of iron within the body affects atherosclerosis remains uncertain.
2. Experimental data suggests that diseases of iron overload might paradoxically reduce atherosclerosis progression through its effects of macrophages, but it remains uncertain how such findings apply to individuals with normal iron metabolism.
3. Further research is warranted to understand the role of hepcidin in pathways beyond iron metabolism that contribute to atherosclerosis.
4. It is unclear if ferroptosis, an iron-dependent form of necrosis characterized by accumulated lipid peroxides, might play a role in cell death of macrophages, one of the key features of atherosclerotic plaque progression, and if ferroptosis inhibitors alleviate atherosclerosis.
5. Although it has been demonstrated that M(Hb) are associated with plaque progression, it is unclear if this connection is causal, given that IPH per se leads to plaque progression. M(Hb) macrophages clear haem and might be mere bystanders responding to iron release rather than actively causing pathology. A more comprehensive analysis of cause and effect is warranted to fully understand the macrophages’ role in plaque progression and calcification.

Search strategy and selection criteria

Data for this review were identified by searches of MEDLINE, PubMed, and references from relevant articles using the search terms ‘iron’, ‘atherosclerosis’, and ‘cardiovascular’. Only articles published in English between 1980 and 2019 were included.

Originality of figures

The authors confirm originality of Figs. 1, 3, and 4. These figures have not been published previously. Fig. 2 was reproduced with written permission from Kolodgie FD et al. N Engl J Med 2003; 349:2316–2325.

Author contributions

A.C., L.G., and A.V.F. drafted the article. A.S. and R.V. critically revised the manuscript. A.C., L.G., A.S., R.V., and A.V.F. gave final approval of the version to be published.

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