EBioMedicine 47 (2019) 598–606

Contents lists available at ScienceDirect

EBioMedicine

journal homepage: www.ebiomedicine.com

EBioMedicine
Published by THE LANCET

Review

New insights into the role of iron in inflammation and atherosclerosis

Anne Cornelissen [a,b], Liang Guo [a], Atsushi Sakamoto [a], Renu Virmani [a], Aloke V. Finn [a,c,*]

[a] CVPath Institute, Gaithersburg, MD, USA
[b] University Hospital RWTH Aachen, Department of Cardiology, Aachen, Germany
[c] University of Maryland, School of Medicine, Baltimore, MD, USA

ARTICLE INFO

Article history:
Received 15 July 2019
Received in revised form 5 August 2019
Accepted 6 August 2019
Available online 12 August 2019

Keywords:
Iron metabolism
Hepcidin-ferroportin axis
Atherosclerosis
Intraplaque haemorrhage
Macrophages

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.

Contents

1. Introduction . . . 598
2. Iron metabolism: the basics . . . 599
 2.1. Iron consumption and uptake . . . 599
 2.2. Hepcidin-ferroportin axis: immediate control of available and circulating iron . . . 599
 2.3. Iron transportation and storage . . . 599
 2.4. Catalytic effect of iron and links to lipoprotein oxidation . . . 599
3. Iron status and genetic disorders associated with abnormal iron metabolism and their effects on atherosclerosis . . . 600
 3.1. Hereditary hemochromatosis . . . 600
 3.2. SNPs associated with iron status based on Mendelian Randomization estimates. . . . 600
 3.3. Macrophages as key effectors of inflammation in atherosclerosis . . . 601
 3.4. Intraplaque haemorrhage and plaque progression . . . 601
4. Opportunities and future directions: modulation of iron metabolism as potential therapy target in atherosclerosis? . . . 603
 4.1. Effects of blockade/downregulation of hepcidin . . . 603
5. Conclusion . . . 604
6. Outstanding questions . . . 604
Search strategy and selection criteria . . . 604
Originality of figures . . . 604
Author contributions . . . 604
Funding . . . 604
Disclosures . . . 604
References . . . 604

* Corresponding author at: CVPath Institute, Inc., 19 Firstfield Road, Gaithersburg, MD 20878, USA.
E-mail addresses: acornelissen@cvpath.org (A. Cornelissen), lguo@cvpath.org (L. Guo), asakamoto@cvpath.org (A. Sakamoto), rvirmani@cvpath.org (R. Virmani), afinn@cvpath.org (A.V. Finn).

1. Introduction

Iron is essential for physiologic processes and plays an important role in cellular metabolism through iron-containing and -sequestering

https://doi.org/10.1016/j.ebiom.2019.08.014

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 hemochromatosis, 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 (Fe^{3+}) that is present in plants and requires reduction by ferrireductase in the gut mucosa before absorption. Since haem ferrous iron (Fe^{2+}) 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 (Fe^{3+}) to the cells. After intracellular reduction to ferrous (Fe^{2+}) 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].

$$Fe^{3} + +O_2{}^{-} \rightarrow O_2 + Fe^{2+}$$

$$Fe^{2+} + H_2O_2 \rightarrow OH^{\cdot} + OH^{-} + Fe^{3+}$$

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

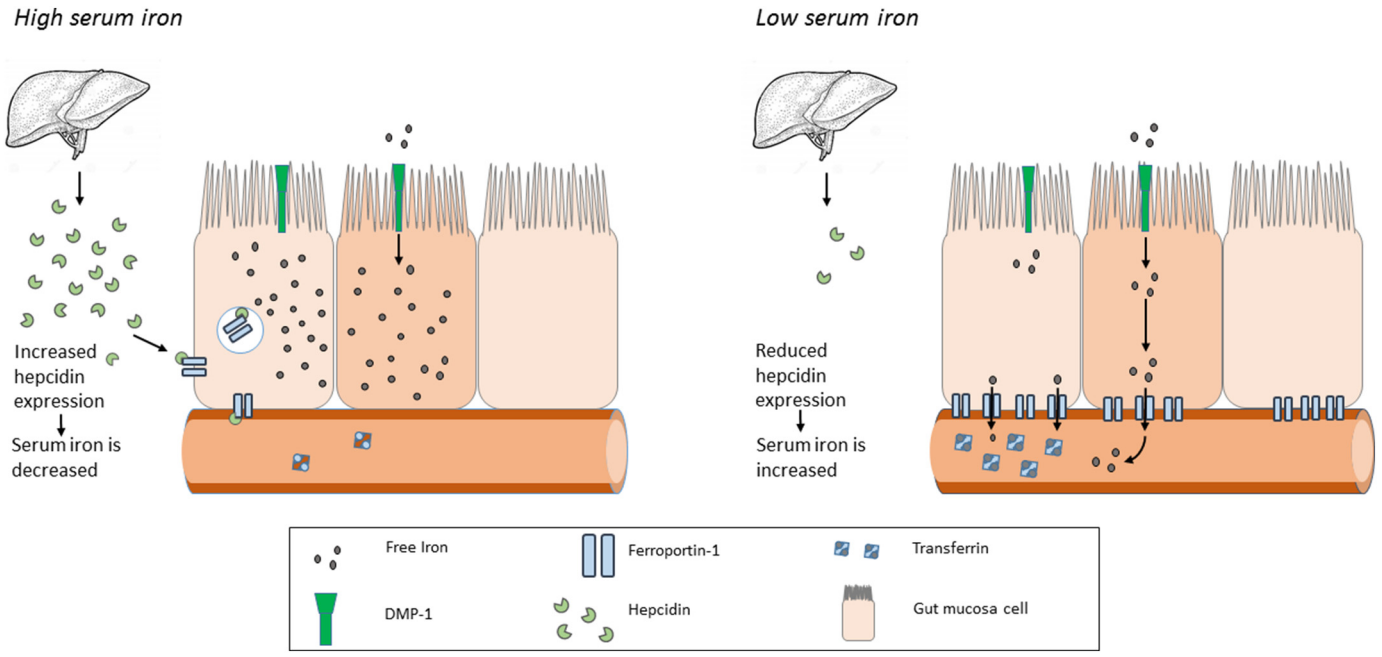

Fig. 1. Systemic iron regulation. Iron is absorbed in the duodenum and upper jejunum. Elemental iron is taken up from the intestinal lumen via divalent metal transporter 1 (DMT1). High systemic iron levels lead to increased hepcidin expression in the liver. Hepcidin binds ferroportin, leading to its 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.

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 [46]. This data has been used for subsequent studies investigating genomic correlations between iron parameters and atherosclerosis [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

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, MMP-1, MMP-3, and MMP-9 released from M1 cells may hydrolyse collagen fibres within the fibrous cap and thus contribute to destabilization of plaques [54]. On the other hand, M2 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 [57,58], whereas increased levels promote a pro-inflammatory response [59,60]. Recent studies have demonstrated additional complexity to the M1/M2 paradigm. In general, the M2 phenotype has been divided into subgroups depending upon their activation stimuli with M2a macrophages induced by IL-4 or IL-13 and M2b induced by IL-1β or LPS. However, other subtypes have also been described, such as M4 macrophages induced by CXCL4, Mox macrophages induced by oxidized LDL, and M(Hb) or Mhaem macrophages induced by haemoglobin in the setting of intraplaque haemorrhage (IPH). For the purposes of this review we will focus on the iron/haemoglobin-driven effects on macrophages, which are especially relevant in the setting of IPH.

3.4. Intraplaque haemorrhage and plaque progression

More than 75 years ago, Wartman proposed that IPH might be an important contributor to plaque progression. Our group as well as others have previously shown that IPH is a critical event in atherogenesis that fundamentally alters the plaque microenvironment by increasing the content of free cholesterol derived from red blood cell membranes, causing necrotic core expansion and plaque progression (Fig. 2) [61]. IPH is thought to derive from fragile immature permeable microvessels, originating from vasa vasorum, which extend into the intima more frequently from the adventitia or less frequently from lumen (Fig. 3) [62]. During IPH, the pro-oxidant environment of the plaque promotes haemolysis along with deposition of unesterified free cholesterol from erythrocyte membranes. Free cholesterol is deposited into the plaque, some of which is organized in crystals, which are highly toxic for cells and membranes. Free iron released from haemoglobin is thought to further contribute to oxidative reactions such as the oxidation of LDL.

The plasma protein haptoglobin binds free haemoglobin, and haemoglobin:haptoglobin (HH) complexes are formed. These complexes are cleared via CD163, an HH scavenger receptor that is exclusively expressed on macrophages. After internalization of the HH complexes, macrophages differentiate into a phenotype referred to as M(Hb) by our group or Mhaem by others, characterized by high levels of CD163 [63]. The haem subunit of Hb is degraded by HO-1, which produces the anti-oxidants carbon monoxide and biliverdin and also releases free iron, which is either utilized by the cells, stored as ferritin in a redox inactive form, or exported out of the cell via FPN to a less redox active ferric (Fe^{3+}) form. Because M(Hb) demonstrate reduced pro-inflammatory cytokine production, lower expression of ROS, and lack of lipid ingestion due to decreased expression of scavenger receptors, but increased expression of the cholesterol efflux transporters ABCA1 and ABCG1, they were initially considered athero-protective [63]. Some of these changes are driven by haem-mediated activation of activating transcription factor-1 (ATF-1), which co-induces HO-1, and liver X receptors, which drive transcription of ABCA1 [64].

However, iron metabolism has a complex array of effects on macrophages. One central aspect of haemoglobin ingestion on M(Hb) macrophages is the changes that occur in intracellular iron (Fig. 4). M(Hb) macrophages exhibit reduced intracellular iron accumulation, owing in part to increased FPN expression. In human carotid plaques, M(Hb) are associated with plaque progression and microvascularity, both features of advanced atherosclerosis. These findings suggest that these cells may be actively involved in these processes rather than merely bystanders.

A relationship between iron and the pro-angiogenic transcription factor hypoxia inducible factor-1α (HIF-1α) can be found through its interactions with prolyl hydroxylase (PHD) family of enzymes (Fig. 4). In the setting of normoxia or hyperoxia, HIF-1α becomes hydroxylated by PHDs on proline residues 402 and 564 within its oxygen-dependent domain. This allows it to be recognized by von Hippel–Lindau tumour suppressor protein (pVHL), which targets it for degradation. Hypoxia is recognized as an activator of HIF-1α. Iron is indirectly related to the activation of HIF-1α because it serves as an essential cofactor for the activity of PHDs. Our recent work shows that within M(Hb) cells activation of HIF-1α via inhibition of iron-dependent PHDs promotes vascular endothelial growth factor (VEGF)-mediated increase in intraplaque angiogenesis, vascular permeability, inflammatory cell recruitment and plaque progression [65]. Knockout of CD163 in a mouse model of atherosclerosis decreased plaque progression in aged (one-year old) mice's brachiocephalic arteries and decreased intraplaque angiogenesis and permeability. Treatment of cultured endothelial cells with supernatants from cultured M(Hb) cells results in greater tubing formation in an angiogenesis assay, greater permeability, and activation of NF-κB. In human plaques, M(Hb) localized to areas of vascularity and permeability (as measured by Evans Blue dye), and CD163 expression was associated with greater vascular inflammation. Thus, the M(Hb) response to intraplaque haemorrhage might actually initiate a vicious circle of angiogenesis, inflammatory cell recruitment, vascular permeability and further haemorrhage, eventually driving plaque progression [65].

Previous studies suggested that intraplaque haemorrhage may be associated with calcification of atherosclerotic lesions [66]. Recently, it has been shown that only the membrane fraction of lysed, but not intact human erythrocytes promoted mineralization of human arterial SMCs and murine aortic rings, and the osteogenic effects of lysed erythrocyte

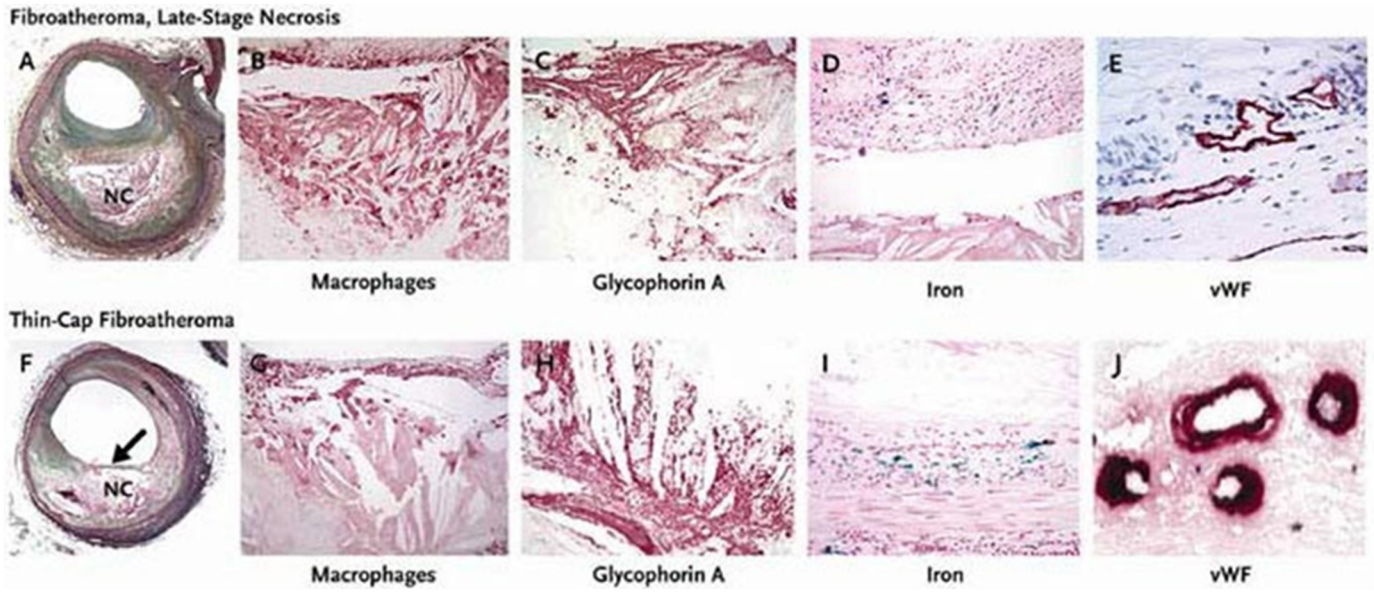

Fig. 2. Intraplaque haemorrhage in fibroatheroma with a core in a late stage of necrosis (Panels A, B, C, D, and E) and thin-cap fibroatheroma (panels F, G, H, I, and J). Panel A shows a low-power view of a fibroatheroma with a late-stage necrotic core (NC) (Movat pentachrome, ×20). Panel B shows intense 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.

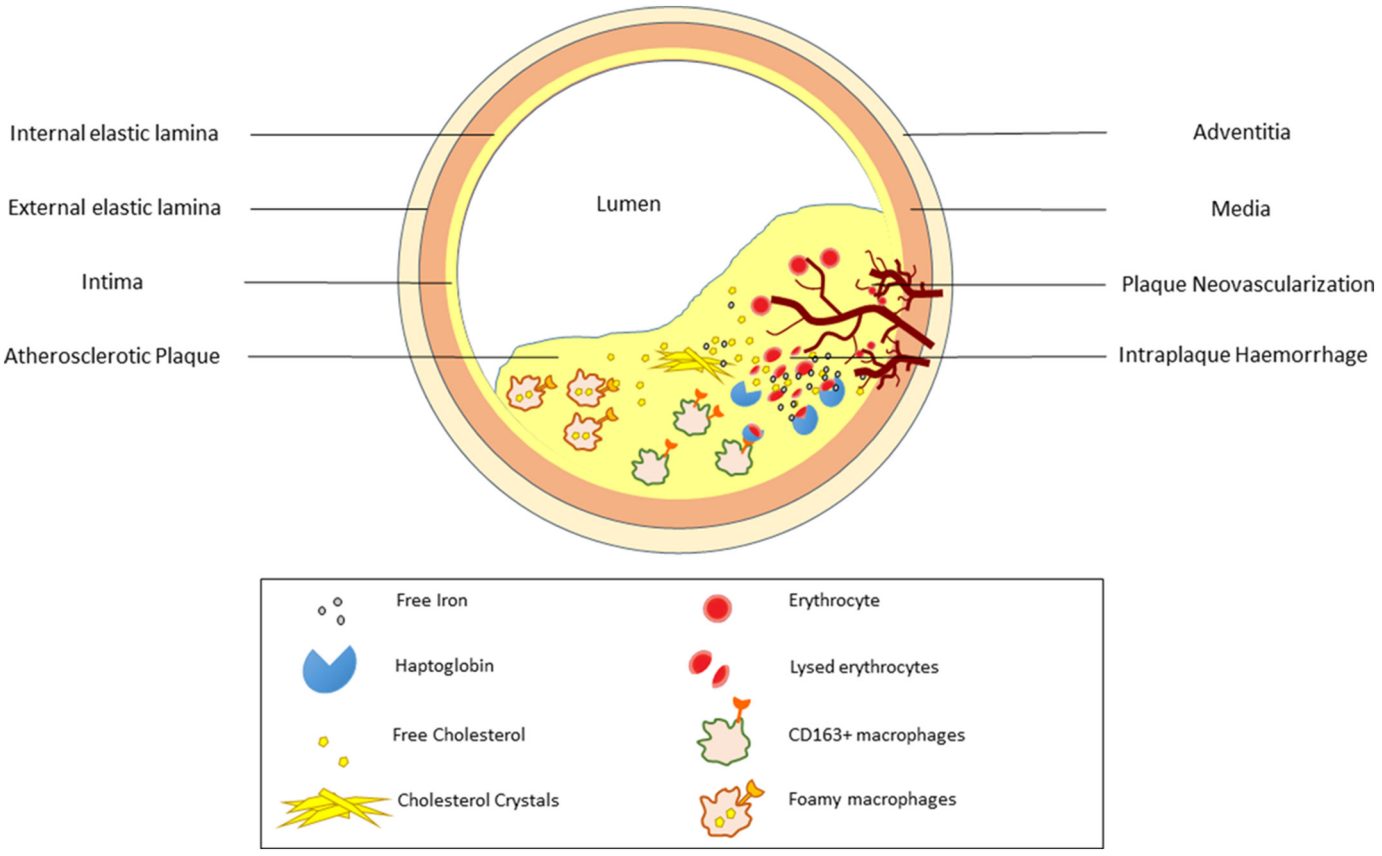

Fig. 3. Intraplaque haemorrhage. Fragile and permeable intraplaque vasa vasorum drive haemorrhage inside the plaque. The pro-oxidant environment of the plaque promotes haemolysis as well as the formation of cholesterol crystals. Free iron from haemoglobin might further catalyse these reactions. Haptoglobin 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).

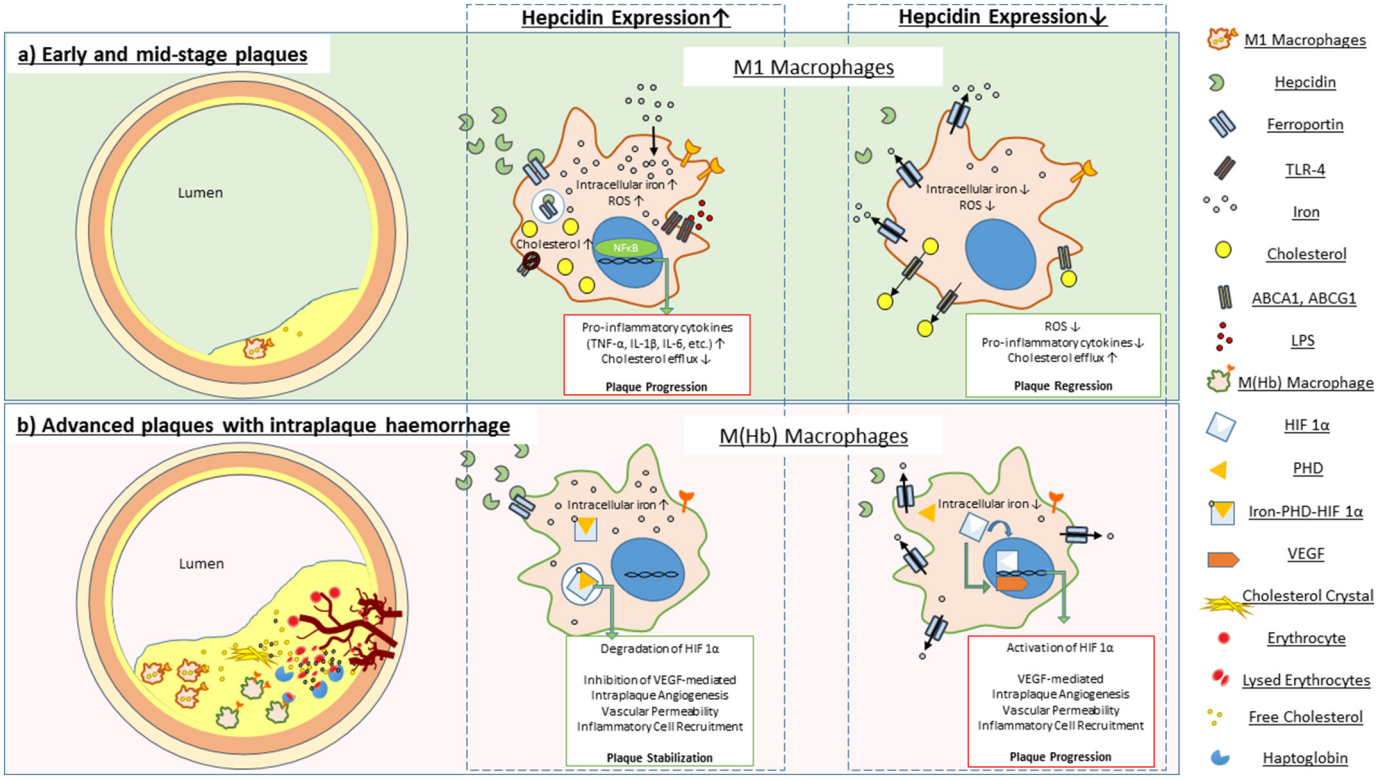

Fig. 4. Effect of hepcidin on macrophages phenotype and function in the setting of atherosclerosis. Hepcidin may play different roles in atherogenesis depending upon the stage of atherosclerosis. a) In early and mid-stage plaques, M1 Macrophages are predominant. Hepcidin induces degradation of FPN, leading 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 via 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 only 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 export via ABCA1 and ABCG1, lowers TLR-4-dependent inflammatory signalling and ROS production. These effects are thought to be anti-atherogenic. b) In advanced plaques with intraplaque haemorrhage, M(Hb) Macrophages are abundant. Iron is an essential cofactor for PHD that mediates degradation of HIF 1α. Low iron levels promote nuclear translocation 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.

membranes require the removal of haemoglobin [67]. Further research is warranted to determinate 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 $hfe^{-/-}$ 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 $apoE^{-/-}$ $FPN^{wt/C326S}$ 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 aggravated 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 $hamp^{-/-}/Ldlr^{-/-}$ 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

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-faceted 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 it 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.

Funding

This study was sponsored by CVPath Institute, a non-profit organization dedicated to cardiovascular research. All authors are employees of CVPath Institute.

Disclosures

A.C. receives research grants from University Hospital RWTH Aachen. R.V. and A.V.F. have received institutional research support from R01 HL141425 Leducq Foundation Grant; 480 Biomedical; 4C Medical; 4Tech; Abbott; Accumedical; Amgen; Biosensors; Boston Scientific; Cardiac Implants; Celonova; Claret Medical; Concept Medical; Cook; CSI; DuNing, Inc.; Edwards LifeSciences; Emboline; Endotronix; Envision Scientific; Lutonix/Bard; Gateway; Lifetech; Limflo; MedAlliance; Medtronic; Mercator; Merill; Microport Medical; Microvention; Mitraalign; Mitra assist; NAMSA; Nanova; Neovasc; NIPRO; Novogate; Occulotech; OrbusNeich Medical; Phenox; Profusa; Protembis; Qool; Recor; Senseonics; Shockwave; Sinomed; Spectranetics; Surmodics; Symic; Vesper; W.L. Gore; Xeltis. A.V.F. has received honoraria from Abbott Vascular; Biosensors; Boston Scientific; Celonova; Cook Medical; CSI; Lutonix Bard; Sinomed; Terumo Corporation; and is a consultant to Amgen; Abbott Vascular; Boston Scientific; Celonova; Cook Medical; Lutonix Bard; Sinomed. R.V. has received honoraria from Abbott Vascular; Biosensors; Boston Scientific; Celonova; Cook Medical; Cordis; CSI; Lutonix Bard; Medtronic; OrbusNeich Medical; CeloNova; SINO Medical Technology; ReCore; Terumo Corporation; W. L. Gore; Spectranetics; and is a consultant Abbott Vascular; Boston Scientific; Celonova; Cook Medical; Cordis; CSI; Edwards Lifescience; Lutonix Bard; Medtronic; OrbusNeich Medical; ReCore; Sinomededical Technology; Spectranetics; Surmodics; Terumo Corporation; W. L. Gore; Xeltis. The other authors declare no competing interests.

References

[1] Muckenthaler MU, Rivella S, Hentze MW, Galy B. A red carpet for iron metabolism. Cell 2017;168(3):344–61.
[2] Sullivan JL, et al. Lancet (London, England) 1981;1(8233):1293–4.
[3] Balla G, Jacob HS, Eaton JW, Belcher JD, Vercellotti GM. Hemin: a possible physiological mediator of low density lipoprotein oxidation and endothelial injury. Arterioscl Thromb 1991;11(6):1700–11.

[4] Smith C, Mitchinson MJ, Aruoma OI, Halliwell B. Stimulation of lipid peroxidation and hydroxyl-radical generation by the contents of human atherosclerotic lesions. Biochem J 1992;286(Pt 3):901–5.
[5] Juckett MB, Balla J, Balla G, Jessurun J, Jacob HS, Vercellotti GM. Ferritin protects endothelial cells from oxidized low density lipoprotein in vitro. Am J Pathol 1995;147(3):782–9.
[6] Silva G, Jeney V, Chora A, Larsen R, Balla J, Soares MP. Oxidized hemoglobin is an endogenous proinflammatory agonist that targets vascular endothelial cells. J Biol Chem 2009;284(43):29582–95.
[7] Kiechl S, Aichner F, Gerstenbrand F, et al. Body iron stores and presence of carotid atherosclerosis. Results from the Bruneck Study. Arterioscl Thromb 1994;14(10):1625–30.
[8] Syrovatka P, Kraml P, Hulikova K, et al. Iron stores are associated with asymptomatic atherosclerosis in healthy men of primary prevention. Eur J Clin Invest 2011;41(8):846–53.
[9] Tuomainen TP, Punnonen K, Nyyssonen K, Salonen JT. Association between body iron stores and the risk of acute myocardial infarction in men. Circulation 1998;97(15):1461–6.
[10] Tuomainen TP, Kontula K, Nyyssonen K, Lakka TA, Helio T, Salonen JT. Increased risk of acute myocardial infarction in carriers of the hemochromatosis gene Cys282Tyr mutation: a prospective cohort study in men in eastern Finland. Circulation 1999;100(12):1274–9.
[11] Munoz-Bravo C, Gutierrez-Bedmar M, Gomez-Aracena J, Garcia-Rodriguez A, Navajas JF. Iron: protector or risk factor for cardiovascular disease? Still controversial. Nutrients 2013;5(7):2384–404.
[12] von Haehling S, Ebner N, Evertz R, Ponikowski P, Anker SD. Iron deficiency in heart failure: An overview. JACC Heart Fail 2019;7(1):36–46.
[13] Salonen JT, Nyyssonen K, Korpela H, Tuomilehto J, Seppanen R, Salonen R. High stored iron levels are associated with excess risk of myocardial infarction in eastern Finnish men. Circulation 1992;86(3):803–11.
[14] Wolk A. Potential health hazards of eating red meat. J Intern Med 2017;281(2):106–22.
[15] Hurrell R, Egli I. Iron bioavailability and dietary reference values. Am J Clin Nutr 2010;91(5):3.
[16] Donovan A, Lima CA, Pinkus JL, et al. The iron exporter ferroportin/Slc40a1 is essential for iron homeostasis. Cell Metab 2005;1(3):191–200.
[17] Nemeth E, Tuttle MS, Powelson J, et al. Hepcidin regulates cellular iron efflux by binding to ferroportin and inducing its internalization. Science 2004;306(5704):2090–3.
[18] Ganz T, Nemeth E. Iron imports. IV. Hepcidin and regulation of body iron metabolism. Am J Physiol Gastrointest Liver Physiol 2006:290(2).
[19] Crichton RR, Wilmet S, Legssyer R, Ward RJ. Molecular and cellular mechanisms of iron homeostasis and toxicity in mammalian cells. J Inorg Biochem 2002;91(1):9–18.
[20] Cominacini L, Pasini AF, Garbin U, et al. Oxidized low density lipoprotein (ox-LDL) binding to ox-LDL receptor-1 in endothelial cells induces the activation of NF-kappaB through an increased production of intracellular reactive oxygen species. J Biol Chem 2000;275(17):12633–8.
[21] Sakakura K, Nakano M, Otsuka F, Ladich E, Kolodgie FD, Virmani R. Pathophysiology of atherosclerosis plaque progression. Heart Lung Circ 2013;22(6):399–411.
[22] Stockwell BR, Friedmann Angeli JP, Bayir H, et al. Ferroptosis: a regulated cell death nexus linking metabolism, redox biology, and disease. Cell 2017;171(2):273–85.
[23] Martinet W, Coornaert I, Puylaert P, De Meyer GRY. Macrophage death as a pharmacological target in atherosclerosis. Front Pharmacol 2019:10(306).
[24] Yang WS, SriRamaratnam R, Welsch ME, et al. Regulation of ferroptotic cancer cell death by GPX4. Cell 2014;156(1–2):317–31.
[25] Guo Z, Ran Q, Roberts 2nd LJ, et al. Suppression of atherogenesis by overexpression of glutathione peroxidase-4 in apolipoprotein E-deficient mice. Free Radic Biol Med 2008;44(3):343–52.
[26] Dixon SJ, Lemberg KM, Lamprecht MR, et al. Ferroptosis: an iron-dependent form of nonapoptotic cell death. Cell 2012;149(5):1060–72.
[27] Hofmans S, Vanden Berghe T, Devisscher L, et al. Novel ferroptosis inhibitors with improved potency and ADME properties. J Med Chem 2016;59(5):2041–53.
[28] Wolff B, Volzke H, Ludemann J, et al. Association between high serum ferritin levels and carotid atherosclerosis in the study of health in Pomerania (SHIP). Stroke 2004;35(2):453–7.
[29] Moore M, Folsom AR, Barnes RW, Eckfeldt JH. No association between serum ferritin and asymptomatic carotid atherosclerosis. The Atherosclerosis Risk in Communities (ARIC) Study. Am J Epidemiol 1995;141(8):719–23.
[30] Danesh J, Appleby P. Coronary heart disease and iron status: meta-analyses of prospective studies. Circulation 1999;99(7):852–4.
[31] Zheng H, Cable R, Spencer B, Votto N, Katz SD. Iron stores and vascular function in voluntary blood donors. Arterioscler Thromb Vasc Biol 2005;25(8):1577–83.
[32] Jelani QU, Harchandani B, Cable RG, et al. Effects of serial phlebotomy on vascular endothelial function: results of a prospective double-blind randomized study. Cardiovasc Ther 2018;36(6):1755–5922.
[33] Klingler KR, Zech D, Wielckens K. Haemochromatosis: automated detection of the two point mutations in the HFE gene: Cys282Tyr and His63Asp. Clin Chem Lab Med 2000;38(12):1225–30.
[34] van der A DL, Rovers MM, Grobbee DE, et al. Mutations in the HFE gene and cardiovascular disease risk: an individual patient data meta-analysis of 53 880 subjects. Circ Cardiovasc Genet 2008;1(1):43–50.
[35] Pilling LC, Tamosauskaite J, Jones G, et al. Common conditions associated with hereditary haemochromatosis genetic variants: cohort study in UK Biobank. BMJ 2019:16(364).
[36] Sullivan JL. Do hemochromatosis mutations protect against iron-mediated atherogenesis? Circ Cardiovasc Genet 2009;2(6):652–7.
[37] Miller M, Hutchins GM. Hemochromatosis, multiorgan hemosiderosis, and coronary artery disease. JAMA 1994;272(3):231–3.
[38] Engberink MF, Povel CM, Durga J, et al. Hemochromatosis (HFE) genotype and atherosclerosis: increased susceptibility to iron-induced vascular damage in C282Y carriers? Atherosclerosis 2010;211(2):520–5.
[39] Lee PL, Beutler E. Regulation of hepcidin and iron-overload disease. Annu Rev Pathol 2009;4:489–515.
[40] Galesloot TE, Holewijn S, Kiemeney LA, de Graaf J, Vermeulen SH, Swinkels DW. Serum hepcidin is associated with presence of plaque in postmenopausal women of a general population. Arterioscler Thromb Vasc Biol 2014;34(2):446–56.
[41] Galesloot TE, Janss LL, Burgess S, et al. Iron and hepcidin as risk factors in atherosclerosis: what do the genes say? BMC Genet 2015;16:79.
[42] Li X, Ding D, Zhang Y, et al. Associations of plasma hepcidin with mortality risk in patients with coronary artery disease. Oncotarget 2017;8(65):109497–508.
[43] Pechlaner R, Kiechl S, Mayr M, et al. Correlates of serum hepcidin levels and its association with cardiovascular disease in an elderly general population. Clin Chem Lab Med 2016;54(1):151–61.
[44] Risko P, Platenik J, Buchal R, Potockova J, Kraml PJ. Long-term donors versus non-donor men: iron metabolism and the atherosclerotic process. Atherosclerosis 2018;272:14–20.
[45] Emdin CA, Khera AV, Kathiresan S. Mendelian randomization. JAMA 2017;318(19):1925–6.
[46] Benyamin B, Esko T, Ried JS, et al. Novel loci affecting iron homeostasis and their effects in individuals at risk for hemochromatosis. Nat Commun 2014;5(4926).
[47] Galesloot TE, Janss LL, Burgess S, et al. Iron and hepcidin as risk factors in atherosclerosis: what do the genes say? BMC Genet 2015;16(79):015–0246.
[48] Gill D, Del Greco MF, Walker AP, Srai SKS, Laffan MA, Minelli C. The effect of Iron status on risk of coronary artery disease: a Mendelian randomization study-brief report. Arterioscler Thromb Vasc Biol 2017;37(9):1788–92.
[49] Nikpay M, Goel A, Won HH, et al. A comprehensive 1,000 Genomes-based genome-wide association meta-analysis of coronary artery disease. Nat Genet 2015;47(10):1121–30.
[50] Deloukas P, Kanoni S, Willenborg C, et al. Large-scale association analysis identifies new risk loci for coronary artery disease. Nat Genet 2013;45(1):25–33.
[51] Gill D, Brewer CF, Monori G, et al. Effects of genetically determined Iron status on risk of venous thromboembolism and carotid atherosclerotic disease: a Mendelian Randomization Study. J Am Heart Assoc 2019;8(15):16.
[52] Bouhlel MA, Derudas B, Rigamonti E, et al. PPARgamma activation primes human monocytes into alternative M2 macrophages with anti-inflammatory properties. Cell Metab 2007;6(2):137–43.
[53] Gordon S. Alternative activation of macrophages. Nat Rev Immunol 2003;3(1):23–35.
[54] Ley K, Miller YI, Hedrick CC. Monocyte and macrophage dynamics during atherogenesis. Arterioscler Thromb Vasc Biol 2011;31(7):1506–16.
[55] Boytard L, Spear R, Chinetti-Gbaguidi G, et al. Role of proinflammatory CD68(+) mannose receptor(−) macrophages in peroxiredoxin-1 expression and in abdominal aortic aneurysms in humans. Arterioscler Thromb Vasc Biol 2013;33(2):431–8.
[56] Handa P, Thomas S, Morgan-Stevenson V, et al. Iron alters macrophage polarization status and leads to steatohepatitis and fibrogenesis. J Leukoc Biol 2019;105(5):1015–26.
[57] Wang L, Johnson EE, Shi HN, Walker WA, Wessling-Resnick M, Cherayil BJ. Attenuated inflammatory responses in hemochromatosis reveal a role for iron in the regulation of macrophage cytokine translation. J Immunol 2008;181(4):2723–31.
[58] Wang L, Harrington L, Trebicka E, et al. Selective modulation of TLR4-activated inflammatory responses by altered iron homeostasis in mice. J Clin Invest 2009;119(11):3322–8.
[59] She H, Xiong S, Lin M, Zandi E, Giulivi C, Tsukamoto H, et al. Am J Physiol Gastrointest Liver Physiol 2002;283(3).
[60] Zhang Z, Zhang F, An P, et al. Ferroportin1 deficiency in mouse macrophages impairs iron homeostasis and inflammatory responses. Blood 2011;118(7):1912–22.
[61] Kolodgie FD, Gold HK, Burke AP, et al. Intraplaque hemorrhage and progression of coronary atheroma. N Engl J Med 2003;349(24):2316–25.
[62] Guo L, Harari E, Virmani R, Finn AV. Linking hemorrhage, angiogenesis, macrophages, and iron metabolism in atherosclerotic vascular diseases. Arterioscler Thromb Vasc Biol 2017;37(4):e33–9.
[63] Boyle JJ, Harrington HA, Piper E, et al. Coronary intraplaque hemorrhage evokes a novel atheroprotective macrophage phenotype. Am J Pathol 2009;174(3):1097–108.
[64] Boyle JJ, Johns M, Kampfer T, et al. Activating transcription factor 1 directs Mhem atheroprotective macrophages through coordinated iron handling and foam cell protection. Circ Res 2012;110(1):20–33.
[65] Guo L, Akahori H, Harari E, et al. CD163+ macrophages promote angiogenesis and vascular permeability accompanied by inflammation in atherosclerosis. J Clin Invest 2018;128(3):1106–24.
[66] Lin R, Chen S, Liu G, Xue Y, Zhao X. Association between carotid atherosclerotic plaque calcification and intraplaque hemorrhage: a magnetic resonance imaging study. Arterioscler Thromb Vasc Biol 2017;37(6):1228–33.
[67] Tziakas DN, Chalikias G, Pavlaki M, et al. Lysed erythrocyte membranes promote vascular calcification: possible role of erythrocyte-derived nitric oxide. Circulation 2019;5(10):037166.
[68] Theurl I, Theurl M, Seifert M, et al. Autocrine formation of hepcidin induces iron retention in human monocytes. Blood 2008;111(4):2392–9.
[69] Weinberg ED. Iron loading and disease surveillance. Emerg Infect Dis 1999;5(3):346–52.

[70] Peyssonnaux C, Zinkernagel AS, Datta V, Lauth X, Johnson RS, Nizet V. TLR4-dependent hepcidin expression by myeloid cells in response to bacterial pathogens. Blood 2006;107(9):3727–32.
[71] Pasterkamp G, Van Keulen JK, De Kleijn DP. Role of Toll-like receptor 4 in the initiation and progression of atherosclerotic disease. Eur J Clin Invest 2004;34(5):328–34.
[72] Vinchi F, Porto G, Simmelbauer A, et al. Atherosclerosis is aggravated by iron overload and ameliorated by dietary and pharmacological iron restriction. Eur Heart J 2019;20(5416182).
[73] Malhotra R, Wunderer F, Barnes HJ, et al. Hepcidin deficiency protects against atherosclerosis. Arterioscler Thromb Vasc Biol 2019;39(2):178–87.
[74] Yu PB, Hong CC, Sachidanandan C, et al. Dorsomorphin inhibits BMP signals required for embryogenesis and iron metabolism. Nat Chem Biol 2008;4(1):33–41.
[75] Saeed O, Otsuka F, Polavarapu R, et al. Pharmacological suppression of hepcidin increases macrophage cholesterol efflux and reduces foam cell formation and atherosclerosis. Arterioscler Thromb Vasc Biol 2012;32(2):299–307.