Pharmacological Research 153 (2020) 104664

Contents lists available at ScienceDirect

Pharmacological Research

journal homepage: www.elsevier.com/locate/yphrs

Invited Review

The role of hepcidin and iron homeostasis in atherosclerosis

Florian Wunderer[a,1], Lisa Traeger[b,1], Haakon H. Sigurslid[c], Patrick Meybohm[a,d], Donald B. Bloch[b,e,2], Rajeev Malhotra[c,*,2]

[a] Department of Anesthesiology, Intensive Care Medicine and Pain Therapy, University Hospital Frankfurt, Goethe University, Frankfurt, Germany
[b] Anesthesia Center for Critical Care Research of the Department of Anesthesia, Critical Care and Pain Medicine, Massachusetts General Hospital and Harvard Medical School, Boston, United States
[c] Cardiovascular Research Center and the Cardiology Division of the Department of Medicine, Massachusetts General Hospital and Harvard Medical School, Boston, United States
[d] Department of Anaesthesiology, University Hospital Wuerzburg, Wuerzburg, Germany
[e] Division of Rheumatology, Allergy and Immunology of the Department of Medicine, Massachusetts General Hospital and Harvard Medical School, Boston, United States

ARTICLE INFO

Keywords:
Hepcidin
Iron
Atherosclerosis
Inflammation
Bone morphogenetic proteins
Macrophages

ABSTRACT

Atherosclerotic cardiovascular disease is a major burden on global health and a leading cause of death worldwide. The pathophysiology of this chronic disease is complex, involving inflammation, lipoprotein oxidation and accumulation, plaque formation, and calcification. In 1981, Dr. Jerome Sullivan formulated the 'Iron Hypothesis', suggesting that higher levels of stored iron promote cardiovascular diseases, whereas iron deficiency may have an atheroprotective effect. This hypothesis has stimulated research focused on clarifying the role of iron in the development of atherosclerosis. However, preclinical and clinical studies have produced contradictory results and the observation that patients with hemochromatosis do not appear to have an increased risk of atherosclerosis seemed incongruous with Sullivan's initial hypothesis. The 'paradox' of systemic iron overload not being accompanied by an increased risk for atherosclerosis led to a refinement of the iron hypothesis focusing on intracellular macrophage iron. More recent *in vitro* and animal studies have elucidated the complex signaling pathways regulating iron, with a particular focus on hepcidin, the master regulator of body iron homeostasis. Bone morphogenetic protein (BMP) signaling is the major pathway that is required for induction of hepcidin expression in response to increasing levels of iron. Strong links between iron homeostasis, BMP signaling, inflammation and atherosclerosis have been established in both mechanistic and human studies. This review summarizes the current understanding of the role of iron homeostasis and hepcidin in the development of atherosclerosis and discusses the BMP-hepcidin-ferroportin axis as a novel therapeutic target for the treatment of cardiovascular disease.

1. Introduction

Iron is an essential regulator of many cellular and biological processes including oxygen transport and energy metabolism. Intracellular and circulating iron levels are maintained by the hormone hepcidin, a master regulator of iron homeostasis. The transmembrane protein ferroportin is the only known "iron exporter", which serves to transport ferrous iron from within cells to the plasma [1]. Hepcidin regulates the amount of ferroportin at the surface of enterocytes, hepatocytes, macrophages and enterocytes, and thereby controls the concentration of iron in the circulation [2].

Because free iron participates in the Fenton reaction, which leads to the generation of reactive oxygen species (ROS), iron at high levels may contribute to DNA, protein or lipid damage and thereby cause cell death [3]. ROS-mediated changes to lipoproteins have a central role in the development of atherosclerosis. Oxidized low-density lipoproteins (oxLDL) trigger endothelial activation and induce inflammatory responses, which promote macrophage recruitment. Macrophages internalize oxLDL and become foam cells within vascular plaques. Vascular plaques can increase in size and thicken the vessel wall causing lumen narrowing. Plaques may also rupture and trigger thrombosis, which leads to acute ischemia and infarction [4–6]. Because of the involvement of iron in the formation of ROS, iron may contribute to the development and progression of atherosclerosis. Approximately 40 years

* Corresponding author at: Massachusetts General Hospital, 55 Fruit St., Boston, MA, 02114, United States.
E-mail address: rmalhotra@mgh.harvard.edu (R. Malhotra).
[1] These authors contributed equally (co-first author).
[2] These authors contributed equally (co-last author).

https://doi.org/10.1016/j.phrs.2020.104664
Received 27 September 2019; Received in revised form 20 December 2019; Accepted 24 January 2020
Available online 25 January 2020
1043-6618/

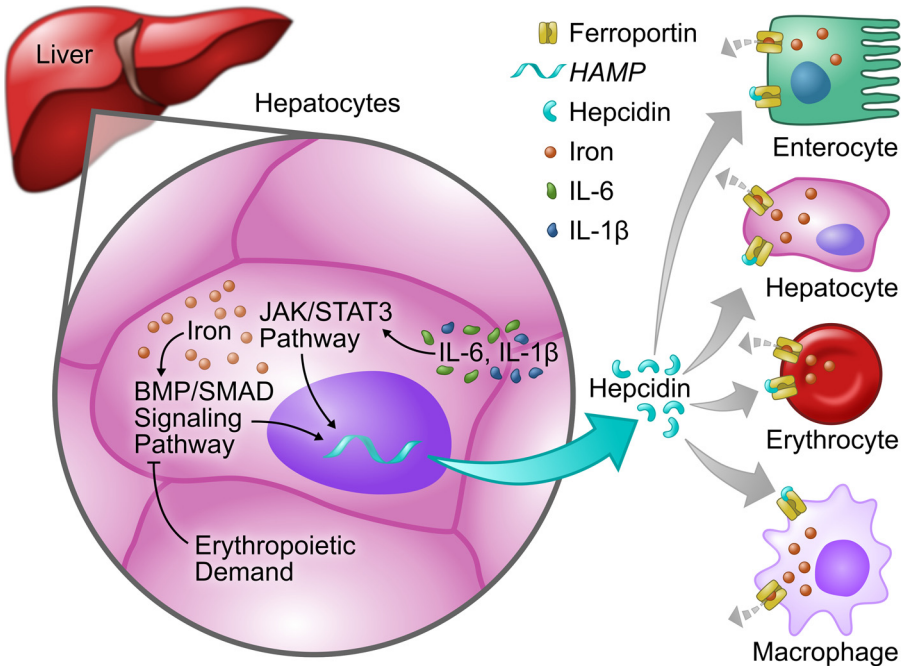

Fig. 1. Overview of the regulation of iron homeostasis. The hepatic hormone hepcidin is the central regulator of systemic iron homeostasis. The expression of the gene encoding hepcidin (hepcidin antimicrobial protein, "HAMP") is regulated at the transcriptional level by iron via the BMP/Smad signaling pathway, by inflammatory cytokines such as IL-6 and by IL-1β via the JAK/STAT3 pathway, and by erythropoietic demand. While iron and inflammation increase the expression of hepcidin, erythropoietic demand and iron deficiency cause suppression of hepcidin. Hepcidin regulates the level of the sole known iron exporter, ferroportin, at the cell surface of enterocytes, hepatocytes, erythrocytes and macrophages, and thereby controls the amount of iron in the circulation. IL-6, Interleukin-6; BMP, Bone morphogenetic protein; Smad, mothers against decapentaplegic; JAK/STAT3, Janus kinase/Signal Transducer and Activator of Transcription 3.

ago, the 'Iron Hypothesis' was formulated, suggesting that higher levels of stored iron promote cardiovascular diseases, whereas iron deficiency may have an atheroprotective effect.

1.1. Atherosclerosis

Atherosclerosis is a major burden on global health and cardiovascular disease (CVD) is the leading cause of death worldwide [7]. Modifiable risk factors for the development of atherosclerosis include smoking, hypertension, diabetes, and hyperlipidemia [8]. Over the last two decades, there has been increased appreciation of the role of oxidative stress and inflammation in the development of atherosclerosis. The opportunity to inhibit inflammation represents a potentially novel approach to prevent and treat atherosclerotic heart disease [9].

The pathophysiology of atherosclerosis is characterized by a chronic inflammatory response to endothelial injury with associated lipid deposition and infiltration by monocyte-derived macrophages, followed by calcification of arterial vessels [5,10]. In the early stages of atherogenesis, intimal accumulation of apolipoprotein B (apoB)-containing lipoproteins, particularly low-density lipoprotein cholesterol (LDL-C), activates the overlying endothelium [11]. Activated endothelial cells express leukocyte adhesion molecules that bind circulating monocytes, which then diapedese into the intimal wall where they differentiate into macrophages [12,13]. Atherosclerotic plaque progression is characterized by the intracellular accumulation of oxidized lipoproteins (oxLDL) and cholesterol esters within macrophages, leading to foam cell formation, with eventual apoptosis and growth of a necrotic core within the plaque [9,14]. Atheromatous plaques consist of lipids, fibrosis, and inflammatory cells [5]. Intra-plaque necrosis perpetuates the recruitment of more monocytes, which differentiate into macrophages and release cytokines and growth factors, further fueling the inflammatory process. The density of foam cells within atherosclerotic plaques and the size of the necrotic core are thought to be key determinants of the vulnerability of plaques to rupture with subsequent vessel occlusion [4,15].

In addition to treating hypertension and diabetes, intensive control of plasma lipid concentration is considered part of the first-line medical treatment for primary and secondary prevention of CVD [16–18]. Numerous randomized clinical trials support the benefits of decreasing the level of harmful lipids (LDL-C) using 3-hydroxy-3-methyglutaryl-CoA (HMG-CoA) reductase inhibitors (statins), to prevent CVD [19,20]. Nevertheless, in clinical practice, some patients do not reach the desired LDL-C target level using statins alone. In addition, some patients still have cardiovascular (CV) events, despite adequate control of LDL-C, diabetes, and hypertension. Efforts have been made to find and test alternative lipid-lowering agents for the primary and secondary prevention of CV-events. Novel lipid-lowering agents, such as cholesterol absorption inhibitors and proprotein convertase subtilisin/kexin type 9 (PCSK-9) inhibitors, have been developed and used in clinical settings [21].

Along with the increasing appreciation of the role of inflammation in atherosclerosis, the inflammatory response has become a promising target for novel therapies. However, because inflammation is often required for an adequate defense against infections, there is a narrow therapeutic window for the use of anti-inflammatory medications for the treatment of atherosclerotic disease [22,23]. The use of aggressive immunosuppressive therapy, as an approach to inhibiting inflammation in atherosclerosis, has only gradually been pursued as a therapeutic option [24]. Novel approaches are needed to specifically target aspects of inflammatory processes that may be unique to CVD.

Iron modulates Toll-like receptor (TLR) 4-activated inflammatory responses and increases oxidative stress through the generation of reactive oxygen and nitrogen species [25,26]. ROS activate the transcription factor NF-κB (nuclear factor kappa-B), which regulates expression of genes important for inflammation [27]. Because iron is a regulator of the body's inflammatory response, one promising approach to the prevention of atherosclerosis is to modify iron homeostasis [28].

1.2. Regulation of iron homeostasis

In a healthy individual, plasma iron levels are maintained at a constant level to provide iron for essential biological and cellular processes, while preventing excess iron accumulation, which can lead to the production of ROS. The central regulator of systemic iron homeostasis is the hepatic peptide hormone hepcidin (Fig. 1) [29]. Initially identified as a defensin-like antimicrobial peptide [2,30,31], hepcidin received its name because of high expression levels in the liver (hep-) and microbicidal activity (-cidin)31]. By targeting and down-regulating ferroportin (FPN), hepcidin reduces the export of iron from within duodenal enterocytes, macrophages, and hepatocytes [32,33].

Table 1
Studies on the association or effect of iron measures on atherosclerotic cardiovascular disease.

Study	Year	Sample Size	Design	Iron measure	Association with clinical disease
Salonen et al. [56]	1992	1,931 (men)	Prospective cohort study Endpoint: myocardial infarction	Serum ferritin	Men with high serum ferritin levels had a 2.2-fold (95 % CI, 1.2–4.0) higher risk of acute myocardial infarction
Kiechl et al. [57]	1997	826	Prospective cohort study Endpoint: progression of carotid atherosclerosis	Serum ferritin	Serum ferritin is a strong risk factor for carotid atherosclerosis progression (odds ratio, 1.50 per SD increase in ferritin; P = 0.0002)
Salonen et al. [60]	1998	2,862	Prospective cohort study Endpoint: risk of myocardial infarction	Iron depletion by blood donation	Frequent blood donation was associated with an 88 % reduced risk of acute myocardial infarction (95 % CI for hazard ratio, 0.02-0.86)
Duffy et al. [63]	2001	54	Acute intervention clinical study with CAD patients and healthy controls. Endpoint: vasomotor function in forearm resistance vessels	Iron depletion by iron chelation	Iron chelation with one dose of deferoxamine improved endothelium-dependent vasodilation in patients with coronary artery disease ($p < 0.01$ by 2-way repeated-measures ANOVA)
Meyers et al. [61]	2002	2,104	Retrospective cohort study Endpoint: cardiovascular events	Iron depletion by blood donation	Frequent blood donation was associated with reduced risk of cardiovascular events (odds ratio, 0.60; CI 0.43-0.83)
Zacharski et al. [65]	2007	1,277	Multicenter, randomized, controlled, single-blinded clinical trial Endpoint: all-cause mortality	Iron depletion by phlebotomy	Phlebotomy in patients with symptomatic peripheral arterial disease did not decrease all-cause mortality (hazard ratio, 0.85; 95 % CI, 0.67–1.08)
Menke et al. [58]	2009	2,662	Cross-sectional analysis Endpoint: association of ferritin and transferrin saturation with PAD	Serum ferritin and transferrin saturation	Borderline association in men of serum ferritin with peripheral arterial disease (PAD) (men: OR, 1.18; 95 % CI, 1.00–1.41; women: OR, 1.04; 95% CI, 0.87–1.25) but not transferrin saturation (men: OR, 1.45; 95% CI, 0.83–2.51; women: OR, 1.55; 95% CI, 0.98–2.45)
Sung et al. [59]	2012	12,033	Cross-sectional analysis Endpoint: association of ferritin and coronary artery calcium score (marker of atherosclerosis)	Serum ferritin	Increased ferritin levels were associated with the presence of coronary artery calcium (OR, 1.66; 95 % CI, 1.3–1.98)
Houschyar et al. [62]	2012	64	Randomized, controlled, single-blind clinical trial Endpoint: clinical markers of metabolic syndrome	Iron depletion by phlebotomy in metabolic syndrome patients	In patients with metabolic syndrome, phlebotomy reduced systolic blood pressure by 16.6 mmHg compared to the control group (95 % CI, -20.7 to -12.5 mmHg)
Kim et al. [67]	2012	5695	Prospective cohort study Endpoint: all cause-, cancer-, and cardiovascular mortality	Transferrin saturation, serum ferritin	Increasing quintiles of transferrin saturation were associated with reduced all-cause, cancer, and CV mortality, but there was no association with serum ferritin
Bagheri et al. [55]	2013	337	Cross sectional analysis Endpoint: association of iron levels with severity of coronary artery disease (CAD)	Serum iron	Increased serum iron levels in patients with severe atherosclerosis ($p = 0.01$) compared to those with mild and moderate amounts of coronary disease
Gill et al. [64]	2017	294,223	Mendelian randomization analysis Endpoint: Coronary artery disease risk	Serum iron, transferrin saturation, and ferritin	Protective effect of higher iron status on risk of coronary artery disease: OR 0.94 per SD increase in iron; 95 % CI, 0.88–1.00; OR, 0.95 per SD increase in transferrin saturation; 95% CI, 0.91-0.99; OR, 0.85 per SD increase in log-transformed ferritin; 95% CI, 0.73-0.98

Disturbances in the tight regulation of hepcidin expression are associated with various diseases including iron overload disorders, such as hereditary hemochromatosis [2,34], as well as anemia of inflammation [35]. The expression of the gene encoding hepcidin (*HAMP*) is regulated at the level of transcription [29,36]. Iron deficiency, hypoxia, and/or erythropoiesis inhibit the transcription of *HAMP*, whereas increased iron levels and inflammation stimulate hepcidin transcription [37]. Inflammatory mediators, such as IL-6, increase hepcidin expression through the Janus kinase/Signal Transducer and Activator of Transcription 3 (JAK/STAT3) pathway, resulting in sequestration of iron in macrophages, reduced duodenal iron absorption, and subsequent hypoferremia, which can lead to the development of anemia [38,39]. Increasing the level of hepcidin in states of inflammation (and thereby decreasing serum iron) may have evolved as a defense mechanism against invading pathogens, because iron is an obligate cofactor for the proliferation and growth of some microorganisms [33].

The bone morphogenetic protein (BMP) signal transduction system is the major pathway that is required for induction of hepcidin expression in response to increasing levels of iron, with BMP2 and BMP6 being the predominant ligands [33,40–43]. Upon binding of BMP2 or BMP6 to the BMP receptor complex, type II receptors (ActRIIA, ActRIIB, and BMPR2) phosphorylate type I receptors ALK2 (ACVR1) and ALK3 (BMPR1B). Activated type I BMP receptors phosphorylate SMAD1/5/8 proteins, which translocate to the nucleus together with SMAD4, bind to BMP response elements in the hepcidin promoter and induce the expression of hepcidin [44]. In mice, liver-specific disruption of BMP type I receptors ALK2 or ALK3 markedly decreases hepcidin expression, resulting in iron overload [45]. This effect is more severe in hepatocyte-specific *Alk3* knockout mice, indicating a predominant role for ALK3 in basal hepcidin expression. ALK3 is also required for induction of hepcidin expression by IL-6, showing an essential crosstalk between inflammatory-mediated (JAK/STAT) and iron-mediated (BMP/SMAD) hepcidin regulatory pathways [46]. Pharmacological inhibition of BMP type I receptors, with small molecules such as dorsomorphin and its derivative LDN-193189, inhibits hepcidin expression and decreases anemia of chronic disease in rodents treated with inflammatory agents [47,48]. Therefore, inhibitors of the BMP pathway may represent new therapeutic options for the treatment of (chronic) inflammatory processes with associated anemia of chronic disease.

1.3. The iron hypothesis

In 1981, Dr. Jerome Sullivan formulated the 'Iron Hypothesis', suggesting that higher levels of stored iron promote cardiovascular diseases, whereas iron deficiency may have an atheroprotective effect [49,50]. The hypothesis was primarily based on the observation that women have a lower risk of cardiovascular disease compared to men. The increased incidence of CVD that occurs in women after menopause, and the associated increase in iron stores, further suggested a link between iron and CVD [51]. Increased iron, in the form of free non-transferrin bound iron (NTBI), hemoglobin or heme, may catalyze the formation of ROS and promote lipid oxidation, thus enhancing intimal LDL-C retention and macrophage progression to foam cells [52–54].

The iron hypothesis was supported by clinical studies that reported an association between atherosclerosis and either increased serum iron [55] or increased levels of serum ferritin [56–59] (the latter is a marker of tissue iron stores). Studies demonstrated an association between iron depletion (either by blood donation [60–62]or iron chelation [63]) and a reduced risk of cardiovascular disease. However, several other studies found no evidence for, or demonstrated results that were even contrary to, the iron hypothesis [64–67]: Mendelian randomization analysis showed that higher levels of serum iron, as indicated by an increased percentage of transferrin saturation or increased serum ferritin, were associated with a reduced risk of coronary artery disease [64]; In a cohort study, transferrin saturation was inversely associated with cardiovascular mortality in men and post-menopausal women;67] and in a randomized controlled study, reduction of iron stores by phlebotomy in patients with peripheral arterial disease did not decrease all-cause mortality (Table 1) [65].

Murine models examining the role of iron in the development of atherosclerosis also produced conflicting results. In mice with iron overload disease, administration of iron chelators protected against the development of atherosclerosis [68]. Reduced macrophage iron, achieved through either an iron-deficient diet [69] or treatment with an iron chelator [70], was associated with decreased atherosclerosis suggesting a protective effect of decreased intracellular iron levels. Dietary iron loading worsened atherosclerosis by inducing inflammation in macrophages [71]. However, another study found that a high-iron diet decreased atherogenesis [72]. Kautz and colleagues reported that iron loading of macrophages had no effect on atherosclerosis. The authors suggested that excess iron was effectively stored in combination with ferritin within macrophages, thereby inhibiting ROS formation [73].

Patients with hereditary hemochromatosis (HH), a genetic disorder associated with hepcidin deficiency and progressive iron overload, do not appear to have an increased risk of atherosclerosis and ischemic heart disease [74–76]. The low hepcidin levels in hemochromatosis patients and the resulting increase in the amount of cell surface ferroportin leads to iron depletion of monocytes. Iron-depleted monocytes isolated from hemochromatosis patients were found to have a decreased ability to upregulate the cytokines monocyte chemoattractant protein (MCP)-1 and interleukin (IL)-6 [77]. The 'paradox' that chronic systemic iron overload in hemochromatosis was not accompanied by an increased risk for atherosclerosis, and even perhaps conferred protection from atherosclerotic heart disease, was taken as evidence against the original iron hypothesis. The hypothesis has subsequently been refined to focus on the level of intracellular macrophage iron and its impact on the development of atherosclerosis [78].

1.4. Macrophages in atherosclerosis

Macrophages have a vital role in systemic immunity and tissue remodeling as well as iron homeostasis. Macrophages have pro- and anti-inflammatory effects and these cells both protect from infection and resolve inflammation. Multiple macrophage phenotypes have been detected in atherosclerotic lesions, including macrophages with M1, M2, or M(Hb) phenotype (Table 2) [79].

Classically activated ('M1') macrophages promote inflammation and are characterized by high expression of tumor necrosis factor alpha (TNF-α), IL-6, IL-1, and inducible nitric oxide synthase (iNOS) [80]. M1 macrophages are the predominant phenotype found in advanced, unstable, atherosclerotic plaques, which have a large lipid-rich necrotic core [81]. Endogenous TLR ligands, such as free fatty acids and oxidized lipids, as well as cytokines such as interferon γ (IFN-γ), contribute to activation of macrophages to a M1-phenotype [79]. M1 polarized macrophages express high levels of the iron storage protein ferritin and low levels of FPN [82,83]. M1 macrophages express scavenger receptors on their surface, which internalize oxLDL, and M1 macrophages can transform into foam cells by lipid accumulation, which is a critical step in atherogenesis [84]. Factors such as platelet-derived growth factor (PDGF) or TGF-ß produced by M1 macrophages prompt proliferation of vascular smooth muscle cells (VSMC) and their migration from the arterial media into the intima [85]. Matrix metalloproteases (MMP-1, MMP-3, MMP-9), secreted by M1 macrophages, may destabilize the atherosclerotic plaque by hydrolysis of collagen within the fibrous cap [86]. In general, classically activated M1 macrophages have a pro-atherogenic phenotype.

Alternatively activated (M2) macrophages are induced by different cytokines, including IL-4 and IL-13, and produce anti-inflammatory cytokines, such as IL-10 [87]. M2 macrophages contribute to the resolution of vascular inflammation and to the stabilization of atheromatous plaques by clearing apoptotic cells and debris, by increasing the production of type I collagen within the plaque, and possibly by

Table 2
Characteristics of macrophages with a M1, M2, and M(Hb) phenotype.

	Classically activated M1 macrophages	Alternatively activated M2 macrophages	Hemorrhage-associated M(Hb) macrophages
Activation by	INFγ, fatty acids, oxidized lipids [79]	IL-4, IL-10, IL-13 [87]	Hemoglobin [97]
Characteristics	*Pro-inflammatory* [80,85,86] Promote inflammation Promote collagen hydrolysis ↑ TNFα, IL-1, IL-6, IL-12, iNOS ↑TGF-β, PDGF production ↑secretion of MMP1, MMP3 and MMP9	*Anti-inflammatory* [88,89,93,94] Promote tissue repair and vascular stability Promote collagen production ↑IL-10, arginase-1, CD206 ↑ornithine, VEGF-A production	*Anti- as well as pro-inflammatory properties* [97,105,106] Promote tissue repair and vascular stability Promote angiogenesis, vessel permeability, & inflammatory cell recruitment ↑ IL-10, CD206 ↑ HIF-α and VEGF-A
Lipid handling	*Increased intracellular lipid levels* [84] ↑ oxLDL scavenger receptors CD36 ↑foam cell formation	*Decreased intracellular lipid levels* [93,96] ↑ ABC transporters ↓ CD36 ↑ efferocytosis	*Decreased intracellular lipid levels* [97] ↑ ABC transporters ↓ CD36
Iron phenotype	*Iron sequestration phenotype* [82,83] ↑ Ferritin ↓ FPN, HO-1, CD163 ↑ intracellular iron	*Iron releasing phenotype* [83,90] ↓ Ferritin ↑ FPN, HO-1, CD163 ↓ intracellular iron	*Iron releasing phenotype* [103,104] ↓ Ferritin ↑ FPN, HO-1, CD163 ↓ intracellular iron
Atherosclerosis	**Pro-atherogenic**	**Atheroprotective**	**Pro-atherogenic and Atheroprotective**

Multiple macrophage phenotypes have been detected in atherosclerotic lesions, including macrophages with a M1, M2, and M(Hb) phenotype. The different types of macrophages have different effects on plaque progression. IFNγ, Interferon gamma; IL, Interleukin; TNFα, Tumor necrosis factor alpha; iNOS, inducible nitric oxide synthase; PDGF, Platelet-derived growth factor; MMP, Matrix metallopeptidase; VEGF-A, vascular endothelial growth factor; HIF, Hypoxia inducible factor; ABC transporter; ATP-binding cassette transporter; oxLDL, Oxidized low-density lipoprotein; FPN, Ferroportin; HO-1, Heme oxygenase 1.

inducing the proliferation of VSMC within the fibrous cap (before activation of the MMP cascade) [80,88,89]. Compared to M1 macrophages, M2 macrophages have high constitutive expression of arginase I, mannose receptor (CD206), hemoglobin-haptoglobin complex scavenging receptor (CD163) and IL-10. Relatively high levels of FPN and low levels of ferritin in M2 macrophages favor cellular iron efflux resulting in lower intracellular iron levels [83,90]. The activation of heme oxygenase 1 (HO-1), which catalyzes the degradation of heme into ferrous iron and carbon monoxide, further favors iron release [91,92].

In contrast to M1 macrophages, M2 macrophages have lower levels of intracellular lipids and do not transform into foam cells. In a process referred to as efferocytosis, M2 macrophages engulf apoptotic foam cells within atherosclerotic plaques, thereby promoting tissue repair and inhibiting further lesion progression [93,94]. However, in advanced atherosclerotic plaques, increased accumulation of cellular debris and lipids derived from apoptotic foam cells may exceed the efferocytosis capacity of M2 macrophages and promote necrotic core formation, contributing to further inflammation, necrosis, and thrombosis [95,96].

Hemorrhage-associated macrophages (M(Hb), also referred to as M(hem)), are induced by hemoglobin and are found in areas of neoangiogenesis and hemorrhage [97]. In humans, but not mice, intraplaque hemorrhage (IPH), as a consequence of neovascularization due to local hypoxia from intimal thickening, is observed in the late stages of atherosclerosis. IPH is associated with increased vessel permeability, red blood cell lysis, and free hemoglobin (Hb) release [98–100]. The CD163 heme-scavenger receptors on the surface of M(Hb) bind and internalize free hemoglobin-haptoglobin complexes. M(Hb) macrophages produce anti-inflammatory factors such as IL-10 [101,102]. In addition, M(Hb) macrophages are resistant to foam cell formation due to LXRα-induced up-regulation of cholesterol-exporting ATP-binding cassette (ABC) transporters ABCA-1 and ABCG-1 [97]. M(Hb) macrophages were therefore originally classified as atheroprotective and anti-inflammatory. However, LXRα also upregulates FPN mRNA expression and promotes iron export [103,104]. The resulting iron decrease in M(Hb) macrophages increases angiogenesis, vessel permeability, and inflammatory cell recruitment, leading to plaque progression [105]. M(Hb) macrophages are therefore classified as both atheroprotective and pro-atherogenic, as they have anti- as well as pro-inflammatory properties [106].

Differentiated macrophages have a “plastic” cell type in that they maintain the ability to change from one phenotype to another. For example, M1 macrophages can develop features of M2 macrophages, and may then revert to the M1 phenotype, depending on environmental signals [107,108]. Macrophage plasticity is reported to occur during atherosclerotic plaque progression, in both humans and mice [109]. Iron is one important factor that affects macrophage plasticity [110–112].

1.5. Effect of intracellular iron on macrophage polarization and function

Intracellular iron levels in macrophages are regulated by the hepcidin-ferroportin axis [37]. Iron handling and immune functions of macrophages are linked, and intracellular iron levels directly regulate macrophage polarization [110–112]. Although some studies have yielded conflicting results on the directionality of the effect of iron on the inflammatory response [113,114], most studies indicate that increased intracellular iron induces a pro-inflammatory M1 phenotype [115–119], potentially by increasing ROS production [120]. Intracellular iron *per se* has direct effects on macrophage polarization, as increased iron loading of M2 macrophages induces a rapid switch from an M2 to an M1 phenotype [117]. Furthermore, intracellular iron levels contribute to alterations in cytokine production. *Fpn*-deficient macrophages, which are characterized by increased intracellular iron levels due to lack of iron export, have an increased expression of inflammatory cytokines. Moreover, intracellular macrophage iron may catalyze the oxidation of LDL, thereby promoting the development of foam cells [121]. In contrast, treatment of macrophages with the iron chelator deferoxamine (DFO) has an anti-inflammatory effect and decreases the release of TNFα, IL-6, and the potent monocyte-attracting chemokine MCP-1 [122].

The pro-inflammatory effects of increased intracellular iron concentration were observed in a mouse model of wound healing. In mice with a macrophage-specific inactivation of FPN, wound closure was delayed, while the inflammatory response was prolonged. In these mice, high intracellular iron content inhibited the switch from pro-inflammatory M1 macrophages to anti-inflammatory M2 phenotype, and prolonged the wound healing phase [123]. In humans, an “unrestrained” population of pro-inflammatory M1 macrophages was identified adjacent to chronic venous leg ulcers. Localized iron overload induced by release of iron from degenerating, extracellular erythrocytes

may contribute to the formation of M1 macrophages and impair wound healing [115].

Mice that lack the *Hfe* gene are a well-studied murine model of hemochromatosis. Although the plasma iron concentrations in these mice are high, the intracellular iron levels in macrophages from *Hfe*$^{-/-}$ mice are low due to systemic hepcidin deficiency. Associated with the low intracellular iron levels in *Hfe*$^{-/-}$ macrophages, the cells have an impaired response to activation by TLR4 and impaired expression of inflammatory cytokines, including TNFα and IL-6. The reduced inflammatory response is likely the result of low intracellular iron levels within macrophages, as pro-inflammatory responses of macrophages can be increased by administration of exogenous iron [26,90,124]. The results of these studies suggest that macrophage iron content influences cytokine production in macrophages. The effect is, at least in part, attributed to the RNA-binding protein tristetraprolin (TTP). TTP is induced by iron deficiency [125,126] and destabilizes mRNA of pro-inflammatory cytokines including TNFα and IL-6 [127].

Because high intracellular iron levels induce the pro-inflammatory, pro-atherogenic M1 macrophage phenotype, factors that decrease intracellular iron may promote the switch from the M1 phenotype to the M2 phenotype and thereby protect against the development of atherosclerosis.

1.6. The role of hepcidin in atherosclerosis

Hepcidin gene expression is induced by increased levels of iron and by inflammation (Fig. 2). High levels of iron increase hepcidin by stimulating the bone morphogenetic protein (BMP) signal transduction pathway. Inhibition of the BMP signaling pathway has been demonstrated to effectively hinder atherogenesis in different mouse models of atherosclerosis [128–131].

LDN-193189 is a small molecule inhibitor of the BMP type I receptor. Treatment of Apolipoprotein E deficient (*Apoe*$^{-/-}$) mice (a model for the development of atherosclerosis) with LDN-193189 significantly reduced vascular macrophage accumulation and atherosclerotic lesion formation [130]. Inhibition of BMP signaling reduced hepcidin levels and increased the expression of FPN, which resulted in macrophage iron depletion and reduced intracellular ROS production. These changes were associated with an increase in transcription of genes encoding ABC transporters, which raised cholesterol efflux from macrophages and decreased the formation of foam cells and atherosclerosis [130]. Finn and colleagues further supported the interplay between cholesterol and iron metabolism in macrophages. In human monocytes derived from atherosclerotic plaques, hepcidin-induced down-regulation of FPN increased intracellular iron and increased lipid accumulation in macrophages [97]. By promoting the production of ROS, high intracellular iron levels inhibited LXRα-mediated transcription of genes encoding ABC transporters and hence hindered cholesterol efflux.

In LDL receptor-deficient (*Ldlr*$^{-/-}$) mice, a second model of atherosclerosis, inhibition of BMP signaling, either by treatment with LDN-193189 or with a soluble receptor-antibody fusion protein that sequesters BMP ligands (ALK3-Fc), reduced vascular calcification, vascular inflammation and atheroma formation [129]. Besides the direct effect of BMP inhibition on the vasculature, Derwall and colleagues found a potent effect of LDN-193189 on lipoprotein biosynthesis. LDN-193189 treatment reduced serum levels of total cholesterol and LDL, suggesting an additional and previously unknown role for BMP signaling in lipoprotein homeostasis [129].

These studies performed in *Apoe*$^{-/-}$ and *Ldlr*$^{-/-}$ mice described the essential role of BMP signaling in the development of atherosclerosis with intimal calcification. In addition, we and others have identified the BMP pathway as an important mediator of calcification of the vascular media both in humans and in mouse models with matrix Gla protein (MGP) deficiency or overexpression [128,132,133]. Pharmacological inhibition of BMP signaling with LDN-193189 or ALK3-Fc reduced medial vessel calcification in *Mgp*$^{-/-}$ mice and was associated with a decreased VSMC osteogenic phenotype [128]. Furthermore, BMP inhibition by overexpression of MGP, an antagonist of BMP signaling, reduced BMP activity, atherosclerotic lesion size and vascular calcification, without having an effect on cholesterol levels [131]. Intimal and medial calcification results from different underlying pathogenic mechanisms: Intimal calcification is associated with inflammation, lipid deposition and macrophage accumulation; medial calcification results from metabolite-induced (e.g., phosphate-induced) upregulation of osteogenic gene programs in the vasculature [134]. Despite the differing mechanisms of calcification, both processes are linked to BMP signaling.

BMP signaling also has a critical role in maintaining homeostasis in many tissues, including bone and cartilage formation [135]. Therefore, chronic inhibition of BMP signal transduction might have a broad range of adverse effects. To specifically determine the role of hepcidin-ferroportin signaling in the development of atherosclerosis, we investigated the effects of hepcidin deficiency on the progression of atherosclerosis in hepcidin- and LDL receptor-deficient (*Hamp*$^{-/-}$/*Ldlr*$^{-/-}$) mice [119]. The absence of hepcidin in these mice, and the resulting increase in ferroportin on the surface of macrophages, was associated with decreased atherosclerosis. A regression analysis showed that the benefits of hepcidin deficiency were not caused by differences in serum iron levels, body weight or serum LDL levels. The results

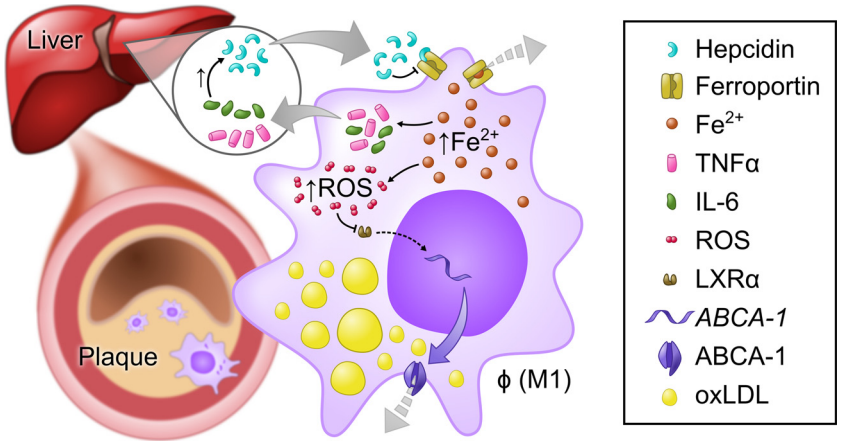

Fig. 2. Schematic representation of the role of hepcidin in foam cell formation and the pathogenesis of atherosclerosis. Hepcidin gene expression in the liver is induced by inflammatory cytokines IL-6 and IL1b. Hepcidin binds to ferroportin, the only known exporter of iron, and induces the degradation of ferroportin. Iron export from macrophages is therefore reduced, and high intracellular iron levels induce a pro-inflammatory, pro-atherogenic M1 macrophage phenotype. As a result of increased intracellular iron, the production of inflammatory cytokines, including IL-6 and TNFα, is increased. High levels of reactive oxygen species (ROS) are generated, which inhibit LXRα-induced expression of the gene encoding the ABCA-1 cholesterol transporter. As a consequence, cholesterol efflux is decreased, and lipids accumulate in the cell, leading to foam cell formation within the vascular plaque. Vascular plaques increase in size, causing lumen narrowing. The dotted grey arrow indicates reduced export. Fe^{2+}, ferrous iron; TNFα, Tumor necrosis factor α; IL-6, Interleukin-6; ROS, Reactive oxygen species; LXRα, Liver X receptor α; ABCA-1, ATP-binding cassette transporter 1; oxLDL, Oxidized low-density lipoprotein.

strongly suggest that the protective effect of hepcidin deficiency was caused by macrophage iron depletion. Decreased intracellular macrophage iron was associated with decreased levels of M1 phenotypic markers in aortic macrophages as well as reduced oxidized LDL uptake [119].

Although these studies show that mice treated with inhibitors of BMP signaling (thereby decreasing hepcidin levels) or mice with genetic deletion of hepcidin have decreased atherosclerosis, the precise role of hepcidin in the pathogenesis of atherosclerosis remains controversial. In a study published by Vinchi et al., *ApoE*-deficient/FPN$^{WT/C326S}$ knock-in mice, which carry a mutation in ferroportin that disrupts hepcidin binding, had significantly increased atherosclerosis compared to age-matched *ApoE*-deficient mice, despite reduced intracellular iron levels in macrophages [68]. Resolution of these seemingly contradictory results requires further investigation. One possible explanation for these disparate results [68,119] may be the intrinsic difference between the *ApoE*- and *Ldlr*-deficient mouse models of atherosclerosis [136]. ApoE, unlike LDLR, exerts additional atheroprotective effects (beyond its capacity to lower lipid levels), which include direct anti-inflammatory properties on macrophages that favor M2 polarization [137]. A second possible explanation for the differences between the two models of atherosclerosis is the complex interplay between serum and intracellular iron levels and the progression of atherosclerosis. The protective benefits of low macrophage iron, along with reduced hepcidin levels, may be partially offset by the detrimental effects of high serum iron. The degree of intracellular macrophage iron content might affect the anti-inflammatory properties of macrophages and explain the controversial results of the two models: while macrophages of homozygous FPN^{C326S} knock-in mice are iron depleted [138], heterozygous FPN$^{WT/C326S}$ knock-in mice may still have residual intracellular iron in macrophages as indicated by the presence of detectable iron within atherosclerotic plaques in *ApoE*-deficient/FPN$^{WT/C326S}$ knock-in mice [68]. Residual macrophage iron content may counteract the protective effect on atherosclerosis. It is also possible that the effect of hepcidin may be dependent on the stage of atherosclerotic disease [139]. In early- to mid-stage disease, low hepcidin levels might have beneficial effects by constraining the effects of pro-inflammatory macrophages [97]. In late-stage lesions however, depletion of macrophage iron might lead to HIF (hypoxia-inducible factor)-α and VEGF (vascular endothelial growth factor)-A mediated increased angiogenesis, vessel permeability, and inflammatory cell recruitment resulting in plaque progression [105,106].

1.7. Hepcidin and atherosclerosis in clinical studies

Inflammation induces hepcidin expression, and a variety of chronic inflammatory diseases are associated with increased levels of hepcidin, including rheumatoid arthritis [140], heart failure [141], chronic kidney disease [142], and non-alcoholic fatty liver disease (Table 3) [143,144]. In a study of patients with rheumatoid arthritis, increased serum hepcidin levels were associated with higher coronary artery calcium scores [145]. In patients with non-alcoholic fatty liver disease, higher serum ferritin and hepcidin levels were associated with increased vascular damage and the presence of carotid plaques [146]. In individuals with metabolic syndrome (diabetes mellitus, hypertension, and hyperlipidemia), who have a high risk of atherosclerosis, serum hepcidin levels and intracellular macrophage iron levels correlated with release of the pro-inflammatory cytokine MCP-1 and vascular damage [77,146]. Studies in patients with chronic kidney disease undergoing hemodialysis showed a relationship between elevated hepcidin levels and arterial stiffness [147] as well as between hepcidin levels and cardiovascular events [148]. In a recent population-based cohort study, increased hepcidin and an increased ratio of hepcidin to ferritin (an index of the amount of hepcidin relative to iron stores) were significantly associated with the presence of plaques in the carotid artery of post-menopausal women [149]. Together, these findings strongly suggest an important role for hepcidin in the pathogenesis of atherosclerosis and CVD events.

To date, there have been no clinical studies addressing the specific role of hepcidin in atherosclerosis. However, modulating the inflammatory response has become a promising target to reduce mortality and morbidity in CVD (Table 4) [22,150,151]. Increased expression of hepcidin is induced by inflammatory cytokines, including IL-6 and IL-1β [152]. Canakinumab is an inhibitor of IL-1β, a cytokine that is highly expressed in atherosclerotic lesions [153] and induces the expression of IL-6 and downstream CRP and hepcidin [154]. The CANTOS trail was designed to test whether canakinumab prevents recurrent cardiovascular events in men and women with prior myocardial infarction who have elevated levels of inflammation. Treatment with canakinumab resulted in reduction of IL-6 and CRP levels, with no effects on lipid levels. The incidence rate for myocardial infarction, stroke, and cardiovascular death was reduced in the canakinumab-treated group compared to the placebo group [22]. Reducing the inflammatory mediators IL-1β and IL-6 seems to be one of the key factors that were beneficial in reducing CVD events. The CANTOS trial is the first clinical trial to report that inhibition of inflammatory mediators improved CVD outcome.

The significance of the inflammatory cytokine IL-6 in CVD became evident with the finding that the Asp358Ala hypofunctional variant of the IL-6 receptor in humans is associated with reduced risk of coronary artery disease [155–157]. Expression of this variant in hepatocytes, monocytes, and macrophages reduces IL-6 signaling and leads to an attenuated expression of IL-6-induced proteins, including CRP. In a double-blind, randomized, placebo-controlled phase II trial, patients with non-ST-elevation MI received the IL-6 receptor antagonist tocilizumab to analyze the effect of IL-6 inhibition on the acute inflammatory response (primary endpoint), and to reduce troponin T release.

Treatment with tocilizumab attenuated inflammation and reduced percutaneous coronary intervention (PCI)-related troponin T release compared to the placebo group, which may indicate that IL-6 receptor antagonism is cardioprotective in ischemia-reperfusion injury. However, tocilizumab did not improve coronary flow reserve (a measure of endothelial function) [158,159]. The clinical trial was not powered to determine the effect of IL-6 inhibition on clinical outcome [158]. A definitive clinical trial to test the effects of specific IL-6 antagonism on CVD outcomes has not yet been performed. However, previous studies [160,161] found an association between high IL-6 levels and increased rates of coronary heart disease and CV-related mortality, as well as all-cause mortality. Increased hepcidin levels and iron deficiency were associated with higher IL-6 levels161], suggesting a link between hepcidin, inflammation and CV outcomes. Further investigation is needed to determine if the beneficial clinical effects of

Table 3
Epidemiological studies showing a correlation between circulating hepcidin levels and sub-clinical or clinical atherosclerosis.

Study	Year	Underlying disease	Sample size	Atherosclerosis Marker or Clinical Event
Abdel-Khalek et al. [145]	2011	Rheumatoid Arthritis	80	Coronary calcium score (CCS)
Valenti et al. [146]	2011	Nonalcoholic fatty liver disease (NAFLD)	143	Carotid plaque
Kuragano et al. [147]	2011	Chronic Kidney Disease patients on maintenance hemodialysis	168	Arterial stiffness
Van der Weerd et al. [148]	2013	Chronic Kidney Disease patients on chronic hemodialysis	405	Fatal and non-fatal CV events
Galesloot et al. [149]	2014	General population	766 (346 women)	Presence of plaque

Table 4
Clinical trials on the effect of targeting inflammation in cardiovascular disease.

Study	Year	Sample Size	Type of study	Drug	Result
Klevelnad et al. [158] and Holte et al. [159]	2016, 2017	117	Randomized, double-blind placebo-controlled phase II trial	Tocilizumab	Tocilizumab attenuated inflammation and Troponin-T release (primarily PCI-related, $p < 0.001$), but did not improve coronary flow reserve (a measure of endothelial function) in NSTEMI patients
Ridker et al. [22] CANTOS-trial	2017	10,061	Randomized, double-blind placebo-controlled phase III trial	Canakinumab	Treatment with canakinumab (at either a 150 mg or 300 mg dose) led to a statistically significant 15 % or 14 % reduction, respectively, in the composite of nonfatal MI, nonfatal stroke, or CV death
Ridker et al. [162] CIRT-trial	2019	4786	Randomized, double-blind, placebo-controlled trial	Methotrexate	Treatment with low-dose methotrexate did not reduce the occurrence of cardiovascular events including nonfatal myocardial infarction, nonfatal stroke or cardiovascular death (HR, 1.01; 95 % CI, 0.82–1.25).

decreased IL-6 or IL-1β signaling, as observed in humans carrying the Asp358Ala variant or in patients treated with tocilizumab or canakinumab, are in part mediated by decreased hepcidin expression, increased ferroportin levels, and low intracellular macrophage iron.

2. Clinical implications/conclusions

The “Iron Hypothesis” has led to research focused on clarifying the role of iron in the development of atherosclerotic heart disease. Early preclinical and clinical studies produced contradictory results and the observation that patients with hemochromatosis do not appear to have an increased risk of atherosclerosis seemed especially difficult to explain. This ‘paradox’ of systemic iron overload not being accompanied by an increased risk for atherosclerosis led to a refinement of the iron hypothesis focusing particularly on intracellular macrophage iron. More recent *in vitro* and animal studies have identified intracellular iron as an effector of macrophage polarization, cytokine production as well as lipid metabolism. High intracellular iron promotes a pro-inflammatory M1 macrophage phenotype with an increased expression of cytokines and reduced efflux of cholesterol. The effect on cholesterol transport is a key determinant of foam cell formation. Intracellular iron levels in macrophages are regulated by the hormone hepcidin, which induces the degradation of ferroportin and decreases iron export. BMP signaling is essential for the induction of hepcidin expression, and inhibition of BMP signaling in *in vivo* models has an atheroprotective effect. The strong links between iron homeostasis, BMP signaling, inflammation and atherosclerosis have been established in both mechanistic and human studies. However, while recent clinical trial results have underscored the benefits of targeting inflammation in cardiovascular disease, the precise clinical impact of modifying iron homeostasis warrants further investigation. The BMP-hepcidin-ferroportin axis may represent a novel therapeutic target for the treatment of cardiovascular disease.

Funding sources

Dr. Traeger was supported by the German Research Foundation (DFG TR 1642/1-1). Dr. Bloch was supported by the Leducq Foundation (Multidisciplinary Program to Elucidate the Role of Bone Morphogenetic Protein Signaling in the Pathogenesis of Pulmonary and Systemic Vascular Diseases) and by RO1DK082971 from the National Institute of Diabetes and Digestive and Kidney Diseases and by Luisa Hunnewell and Larry Newman. Dr. Malhotra was supported by the National Heart, Lung, and Blood Institute (K08HL111210 and R01HL142809), the American Heart Association (18TPA34230025), the Wild Family Foundation, and the Hassenfeld Scholar Award.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.phrs.2020.104664.

References

[1] E. Nemeth, M.S. Tuttle, J. Powelson, et al., Hepcidin regulates cellular iron efflux by binding to ferroportin and inducing its internalization, Science 306 (5704) (2004) 2090–2093.
[2] C. Pigeon, G. Ilyin, B. Courselaud, et al., A new mouse liver-specific gene, encoding a protein homologous to human antimicrobial peptide hepcidin, is overexpressed during iron overload, J. Biol. Chem. 276 (11) (2001) 7811–7819.
[3] S. Dev, J.L. Babitt, Overview of iron metabolism in health and disease, Hemodial. Int. 21 (1) (2017) S6–S20.
[4] E. Falk, Pathogenesis of atherosclerosis, J. Am. Coll. Cardiol. 47 (8 Suppl) (2006) C7–12.
[5] P. Libby, P.M. Ridker, G.K. Hansson, Progress and challenges in translating the biology of atherosclerosis, Nature 473 (7347) (2011) 317–325.
[6] R. Ross, J.A. Glomset, The pathogenesis of atherosclerosis (first of two parts), N. Engl. J. Med. 295 (7) (1976) 369–377.
[7] R. Lozano, M. Naghavi, K. Foreman, et al., Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010, Lancet 380 (9859) (2012) 2095–2128.
[8] E. Braunwald, Shattuck lecture—cardiovascular medicine at the turn of the millennium: triumphs, concerns, and opportunities, N. Engl. J. Med. 337 (19) (1997) 1360–1369.
[9] R. Ross, Atherosclerosis—an inflammatory disease, N. Engl. J. Med. 340 (2) (1999) 115–126.
[10] D.M. Small, Cellular mechanisms for lipid deposition in atherosclerosis (first of two parts), N. Engl. J. Med. 297 (16) (1977) 873–877.
[11] K.J. Williams, I. Tabas, The response-to-retention hypothesis of early atherogenesis, Arterioscler. Thromb. Vasc. Biol. 15 (5) (1995) 551–561.
[12] C.K. Glass, J.L. Witztum, Atherosclerosis. The road ahead, Cell 104 (4) (2001) 503–516.
[13] J. Mestas, K. Ley, Monocyte-endothelial cell interactions in the development of atherosclerosis, Trends Cardiovasc. Med. 18 (6) (2008) 228–232.
[14] G.K. Hansson, Inflammation, atherosclerosis, and coronary artery disease, N. Engl. J. Med. 352 (16) (2005) 1685–1695.
[15] K. Sakakura, M. Nakano, F. Otsuka, E. Ladich, F.D. Kolodgie, R. Virmani, Pathophysiology of atherosclerosis plaque progression, Heart Lung Circ. 22 (6) (2013) 399–411.
[16] H. Lawall, P. Huppert, C. Espinola-Klein, C.S. Zemmrich, G. Ruemenapf, German guideline on the diagnosis and treatment of peripheral artery disease—a comprehensive update 2016, VASA Z. Gefasskrankh. 46 (2) (2017) 79–86.
[17] D.K. Arnett, R.S. Blumenthal, M.A. Albert, et al., ACC/AHA guideline on the primary prevention of cardiovascular disease, Circulation 2019 (2019) Cir0000000000000678.
[18] A.L. Catapano, I. Graham, G. De Backer, et al., 2016 ESC/EAS Guidelines for the Management of Dyslipidaemias: The Task Force for the Management of Dyslipidaemias of the European Society of Cardiology (ESC) and European Atherosclerosis Society (EAS) Developed with the special contribution of the European Assocciation for Cardiovascular Prevention & Rehabilitation (EACPR), Atherosclerosis. 253 (2016) 281–344.
[19] C. Baigent, A. Keech, P.M. Kearney, et al., Efficacy and safety of cholesterol-lowering treatment: prospective meta-analysis of data from 90,056 participants in 14 randomised trials of statins, Lancet 366 (9493) (2005) 1267–1278.
[20] J. Fulcher, R. O'Connell, M. Voysey, et al., Efficacy and safety of LDL-lowering therapy among men and women: meta-analysis of individual data from 174,000 participants in 27 randomised trials, Lancet 385 (9976) (2015) 1397–1405.
[21] K. Fujisue, K. Tsujita, Current status of lipid management in acute coronary syndrome, J. Cardiol. 70 (2) (2017) 101–106.
[22] P.M. Ridker, B.M. Everett, T. Thuren, et al., Antiinflammatory therapy with canakinumab for atherosclerotic disease, N. Engl. J. Med. 377 (12) (2017) 1119–1131.
[23] P.M. Ridker, T.F. Luscher, Anti-inflammatory therapies for cardiovascular disease, Eur. Heart J. 35 (27) (2014) 1782–1791.
[24] D.P. Jones, J. Patel, Therapeutic approaches targeting inflammation in cardiovascular disorders, Biology (Basel) 7 (4) (2018).
[25] G. Weiss, G. Werner-Felmayer, E.R. Werner, K. Grunewald, H. Wachter, M.W. Hentze, Iron regulates nitric oxide synthase activity by controlling nuclear transcription, J. Exp. Med. 180 (3) (1994) 969–976.
[26] L. Wang, L. Harrington, E. Trebicka, et al., Selective modulation of TLR4-activated

inflammatory responses by altered iron homeostasis in mice, J. Clin. Invest. 119 (11) (2009) 3322–3328.
[27] C. Bubici, S. Papa, K. Dean, G. Franzoso, Mutual cross-talk between reactive oxygen species and nuclear factor-kappa B: molecular basis and biological significance, Oncogene 25 (51) (2006) 6731–6748.
[28] B.J. Cherayil, Iron and immunity: immunological consequences of iron deficiency and overload, Arch. Immunol. Ther. Exp. 58 (6) (2010) 407–415.
[29] T. Ganz, E. Nemeth, Hepcidin and iron homeostasis, Biochim. Biophys. Acta 9 (43) (2012) 26.
[30] A. Krause, S. Neitz, H.J. Magert, et al., LEAP-1, a novel highly disulfide-bonded human peptide, exhibits antimicrobial activity, FEBS Lett. 480 (2–3) (2000) 147–150.
[31] C.H. Park, E.V. Valore, A.J. Waring, G.T. Hepcidin, A urinary antimicrobial peptide synthesized in the liver, J. Biol. Chem. 276 (11) (2001) 7806–7810.
[32] G.T. Hepcidin, A key regulator of iron metabolism and mediator of anemia of inflammation, Blood 102 (3) (2003) 783–788.
[33] T. Ganz, E. Nemeth, Iron homeostasis in host defence and inflammation, Nat. Rev. Immunol. 15 (8) (2015) 500–510.
[34] G. Nicolas, M. Bennoun, I. Devaux, et al., Lack of hepcidin gene expression and severe tissue iron overload in upstream stimulatory factor 2 (USF2) knockout mice, Proc. Natl. Acad. Sci. U. S. A. 98 (15) (2001) 8780–8785.
[35] R.E. Fleming, W.S. Sly, Hepcidin: a putative iron-regulatory hormone relevant to hereditary hemochromatosis and the anemia of chronic disease, Proc. Natl. Acad. Sci. U. S. A. 98 (15) (2001) 8160–8162.
[36] C.Y. Wang, J.L. Babitt, Hepcidin regulation in the anemia of inflammation, Curr. Opin. Hematol. 23 (3) (2016) 189–197.
[37] M.U. Muckenthaler, S. Rivella, M.W. Hentze, B. Galy, A red carpet for iron metabolism, Cell 168 (3) (2017) 344–361.
[38] B. Chung, F. Verdier, P. Matak, J.C. Deschemin, P. Mayeux, S. Vaulont, Oncostatin M is a potent inducer of hepcidin, the iron regulatory hormone, FASEB J. 24 (6) (2010) 2093–2103.
[39] L.T. Goodnough, E. Nemeth, T. Ganz, Detection, evaluation, and management of iron-restricted erythropoiesis, Blood 116 (23) (2010) 4754–4761.
[40] D. Meynard, L. Kautz, V. Darnaud, F. Canonne-Hergaux, H. Coppin, M.P. Roth, Lack of the bone morphogenetic protein BMP6 induces massive iron overload, Nat. Genet. 41 (4) (2009) 478–481.
[41] P.S. Koch, V. Olsavszky, F. Ulbrich, et al., Angiocrine Bmp2 signaling in murine liver controls normal iron homeostasis, Blood 129 (4) (2017) 415–419.
[42] N.L. Parrow, R.E. Fleming, Bone morphogenetic proteins as regulators of iron metabolism, Annu. Rev. Nutr. 34 (2014) 77–94.
[43] N.W. Morrell, D.B. Bloch, P. ten Dijke, et al., Targeting BMP signalling in cardiovascular disease and anaemia, Nat. Rev. Cardiol. 13 (2) (2016) 106–120.
[44] A.U. Steinbicker, M.U. Muckenthaler, Out of balance—systemic iron homeostasis in iron-related disorders, Nutrients 5 (8) (2013) 3034–3061.
[45] A.U. Steinbicker, T.B. Bartnikas, L.K. Lohmeyer, et al., Perturbation of hepcidin expression by BMP type I receptor deletion induces iron overload in mice, Blood 118 (15) (2011) 4224–4230.
[46] C. Mayeur, L.K. Lohmeyer, P. Leyton, et al., The type I BMP receptor Alk3 is required for the induction of hepatic hepcidin gene expression by interleukin-6, Blood 123 (14) (2014) 2261–2268.
[47] P.B. Yu, C.C. Hong, C. Sachidanandan, et al., Dorsomorphin inhibits BMP signals required for embryogenesis and iron metabolism, Nat. Chem. Biol. 4 (1) (2008) 33–41.
[48] A.U. Steinbicker, C. Sachidanandan, A.J. Vonner, et al., Inhibition of bone morphogenetic protein signaling attenuates anemia associated with inflammation, Blood 117 (18) (2011) 4915–4923.
[49] J.L. Sullivan, Iron and the sex difference in heart disease risk, Lancet 1 (8233) (1981) 1293–1294.
[50] J.L. Sullivan, The iron paradigm of ischemic heart disease, Am. Heart J. 117 (5) (1989) 1177–1188.
[51] W.B. Kannel, M.C. Hjortland, P.M. McNamara, T. Gordon, Menopause and risk of cardiovascular disease: the Framingham study, Ann. Intern. Med. 85 (4) (1976) 447–452.
[52] P.J. Kraml, R.L. Klein, Y. Huang, A. Nareika, M.F. Lopes-Virella, Iron loading increases cholesterol accumulation and macrophage scavenger receptor I expression in THP-1 mononuclear phagocytes, Metabolism 54 (4) (2005) 453–459.
[53] D. Lapenna, S.D. Pierdomenico, G. Ciofani, et al., Association of body iron stores with low molecular weight iron and oxidant damage of human atherosclerotic plaques, Free Radic. Biol. Med. 42 (4) (2007) 492–498.
[54] T.P. Tuomainen, S. Loft, K. Nyyssonen, K. Punnonen, J.T. Salonen, H.E. Poulsen, Body iron is a contributor to oxidative damage of DNA, Free Radic. Res. 41 (3) (2007) 324–328.
[55] B. Bagheri, M. Shokrzadeh, V. Mokhberi, et al., Association between serum iron and the severity of coronary artery disease, Int. Cardiovasc. Res. J. 7 (3) (2013) 95–98.
[56] J.T. Salonen, K. Nyyssonen, H. Korpela, J. Tuomilehto, R. Seppanen, R. Salonen, High stored iron levels are associated with excess risk of myocardial infarction in eastern Finnish men, Circulation 86 (3) (1992) 803–811.
[57] S. Kiechl, J. Willeit, G. Egger, W. Poewe, F. Oberhollenzer, Body iron stores and the risk of carotid atherosclerosis: prospective results from the Bruneck study, Circulation 96 (10) (1997) 3300–3307.
[58] A. Menke, J.M. Fernandez-Real, P. Muntner, E. Guallar, The association of biomarkers of iron status with peripheral arterial disease in US adults, BMC Cardiovasc. Disord. 9 (2009) 34.
[59] K.C. Sung, S.M. Kang, E.J. Cho, J.B. Park, S.H. Wild, C.D. Byrne, Ferritin is independently associated with the presence of coronary artery calcium in 12,033 men, Arterioscler. Thromb. Vasc. Biol. 32 (10) (2012) 2525–2530.
[60] J.T. Salonen, T.P. Tuomainen, R. Salonen, T.A. Lakka, K. Nyyssonen, Donation of blood is associated with reduced risk of myocardial infarction. The Kuopio Ischaemic Heart Disease Risk Factor Study, Am. J. Epidemiol. 148 (5) (1998) 445–451.
[61] D.G. Meyers, K.C. Jensen, J.E. Menitove, A historical cohort study of the effect of lowering body iron through blood donation on incident cardiac events, Transfusion 42 (9) (2002) 1135–1139.
[62] K.S. Houschyar, R. Ludtke, G.J. Dobos, et al., Effects of phlebotomy-induced reduction of body iron stores on metabolic syndrome: results from a randomized clinical trial, BMC Med. 10 (2012) 54.
[63] S.J. Duffy, E.S. Biegelsen, M. Holbrook, et al., Iron chelation improves endothelial function in patients with coronary artery disease, Circulation 103 (23) (2001) 2799–2804.
[64] D. Gill, M.F. Del Greco, A.P. Walker, S.K.S. Srai, M.A. Laffan, C. Minelli, The effect of Iron status on risk of coronary artery disease: a mendelian randomization study-brief report, Arterioscler. Thromb. Vasc. Biol. 37 (9) (2017) 1788–1792.
[65] L.R. Zacharski, B.K. Chow, P.S. Howes, et al., Reduction of iron stores and cardiovascular outcomes in patients with peripheral arterial disease: a randomized controlled trial, Jama 297 (6) (2007) 603–610.
[66] C. Munoz-Bravo, M. Gutierrez-Bedmar, J. Gomez-Aracena, A. Garcia-Rodriguez, J.F. Navajas, Iron: protector or risk factor for cardiovascular disease? Still controversial, Nutrients 5 (7) (2013) 2384–2404.
[67] K.S. Kim, H.G. Son, N.S. Hong, D.H. Lee, Associations of serum ferritin and transferrin % saturation with all-cause, cancer, and cardiovascular disease mortality: Third National Health and Nutrition Examination Survey follow-up study, J. Prev. Med. Publ. Health 45 (3) (2012) 196–203.
[68] F. Vinchi, G. Porto, A. Simmelbauer, et al., Atherosclerosis is aggravated by iron overload and ameliorated by dietary and pharmacological iron restriction, Eur. Heart J. (2019).
[69] T.S. Lee, M.S. Shiao, C.C. Pan, L.Y. Chau, Iron-deficient diet reduces atherosclerotic lesions in apoE-deficient mice, Circulation 99 (9) (1999) 1222–1229.
[70] W.J. Zhang, H. Wei, B. Frei, The iron chelator, desferrioxamine, reduces inflammation and atherosclerotic lesion development in experimental mice, Exp. Biol. Med. (Maywood) 235 (5) (2010) 633–641.
[71] X. Hu, X. Cai, R. Ma, W. Fu, C. Zhang, X. Du, Iron-load exacerbates the severity of atherosclerosis via inducing inflammation and enhancing the glycolysis in macrophages, J. Cell. Physiol. 29 (10) (2019) 28518.
[72] E.A. Kirk, J.W. Heinecke, R.C. LeBoeuf, Iron overload diminishes atherosclerosis in apoE-deficient mice, J. Clin. Invest. 107 (12) (2001) 1545–1553.
[73] L. Kautz, V. Gabayan, X. Wang, et al., Testing the iron hypothesis in a mouse model of atherosclerosis, Cell Rep. 5 (5) (2013) 1436–1442.
[74] C. Ellervik, A. Tybjaerg-Hansen, P. Grande, M. Appleyard, B.G. Nordestgaard, Hereditary hemochromatosis and risk of ischemic heart disease: a prospective study and a case-control study, Circulation 112 (2) (2005) 185–193.
[75] J.L. Sullivan, Do hemochromatosis mutations protect against iron-mediated atherogenesis? Circ. Cardiovasc. Genet. 2 (6) (2009) 652–657.
[76] M. Miller, G.M. Hutchins, Hemochromatosis, multiorgan hemosiderosis, and coronary artery disease, Jama 272 (3) (1994) 231–233.
[77] L. Valenti, P. Dongiovanni, B.M. Motta, et al., Serum hepcidin and macrophage iron correlate with MCP-1 release and vascular damage in patients with metabolic syndrome alterations, Arterioscler. Thromb. Vasc. Biol. 31 (3) (2011) 683–690.
[78] J.L. Sullivan, L.R. Zacharski, Hereditary haemochromatosis and the hypothesis that iron depletion protects against ischemic heart disease, Eur. J. Clin. Invest. 31 (5) (2001) 375–377.
[79] N. Leitinger, I.G. Schulman, Phenotypic polarization of macrophages in atherosclerosis, Arterioscler. Thromb. Vasc. Biol. 33 (6) (2013) 1120–1126.
[80] K.J. Moore, F.J. Sheedy, E.A. Fisher, Macrophages in atherosclerosis: a dynamic balance, Nat. Rev. Immunol. 13 (10) (2013) 709–721.
[81] J.L. Stoger, M.J. Gijbels, S. van der Velden, et al., Distribution of macrophage polarization markers in human atherosclerosis, Atherosclerosis 225 (2) (2012) 461–468.
[82] R. Agoro, C. Mura, Inflammation-induced up-regulation of hepcidin and down-regulation of ferroportin transcription are dependent on macrophage polarization, Blood Cells Mol. Dis. 61 (2016) 16–25.
[83] G. Corna, L. Campana, E. Pignatti, et al., Polarization dictates iron handling by inflammatory and alternatively activated macrophages, Haematologica 95 (11) (2010) 1814–1822.
[84] P. Kraml, The role of iron in the pathogenesis of atherosclerosis, Physiol. Res. 66 (Supplementum 1) (2017) S55–S67.
[85] G.K. Owens, M.S. Kumar, B.R. Wamhoff, Molecular regulation of vascular smooth muscle cell differentiation in development and disease, Physiol. Rev. 84 (3) (2004) 767–801.
[86] K. Ley, Y.I. Miller, C.C. Hedrick, Monocyte and macrophage dynamics during atherogenesis, Arterioscler. Thromb. Vasc. Biol. 31 (7) (2011) 1506–1516.
[87] S. Gordon, Alternative activation of macrophages, Nat. Rev. Immunol. 3 (1) (2003) 23–35.
[88] H.J. Medbury, V. James, J. Ngo, et al., Differing association of macrophage subsets with atherosclerotic plaque stability, Int. Angiol. 32 (1) (2013) 74–84.
[89] R.N. Hanna, I. Shaked, H.G. Hubbeling, et al., NR4A1 (Nur77) deletion polarizes macrophages toward an inflammatory phenotype and increases atherosclerosis, Circ. Res. 110 (3) (2012) 416–427.
[90] S. Recalcati, M. Locati, A. Marini, et al., Differential regulation of iron homeostasis during human macrophage polarized activation, Eur. J. Immunol. 40 (3) (2010) 824–835.
[91] C.D. Ferris, S.R. Jaffrey, A. Sawa, et al., Haem oxygenase-1 prevents cell death by

regulating cellular iron, Nat. Cell Biol. 1 (3) (1999) 152–157.
[92] G. Cairo, S. Recalcati, A. Mantovani, M. Locati, Iron trafficking and metabolism in macrophages: contribution to the polarized phenotype, Trends Immunol. 32 (6) (2011) 241–247.
[93] N. Jetten, S. Verbruggen, M.J. Gijbels, M.J. Post, M.P. De Winther, M.M. Donners, Anti-inflammatory M2, but not pro-inflammatory M1 macrophages promote angiogenesis in vivo, Angiogenesis 17 (1) (2014) 109–118.
[94] I. Tabas, Macrophage death and defective inflammation resolution in atherosclerosis, Nat. Rev. Immunol. 10 (1) (2010) 36–46.
[95] G. Zizzo, B.A. Hilliard, M. Monestier, P.L. Cohen, Efficient clearance of early apoptotic cells by human macrophages requires M2c polarization and MerTK induction, J. Immunol. 189 (7) (2012) 3508–3520.
[96] I. Tabas, Consequences and therapeutic implications of macrophage apoptosis in atherosclerosis: the importance of lesion stage and phagocytic efficiency, Arterioscler. Thromb. Vasc. Biol. 25 (11) (2005) 2255–2264.
[97] A.V. Finn, M. Nakano, R. Polavarapu, et al., Hemoglobin directs macrophage differentiation and prevents foam cell formation in human atherosclerotic plaques, J. Am. Coll. Cardiol. 59 (2) (2012) 166–177.
[98] M.J. Nielsen, H.J. Moller, S.K. Moestrup, Hemoglobin and heme scavenger receptors, Antioxid. Redox Signal. 12 (2) (2010) 261–273.
[99] L. Guo, E. Harari, R. Virmani, A.V. Finn, Linking hemorrhage, angiogenesis, macrophages, and iron metabolism in atherosclerotic vascular diseases, Arterioscler. Thromb. Vasc. Biol. 37 (4) (2017) e33–e39.
[100] L. Parma, F. Baganha, P.H.A. Quax, M.R. de Vries, Plaque angiogenesis and intraplaque hemorrhage in atherosclerosis, Eur. J. Pharmacol. 816 (2017) 107–115.
[101] R.C. Landis, P. Philippidis, J. Domin, J.J. Boyle, D.O. Haskard, Haptoglobin genotype-dependent anti-inflammatory signaling in CD163(+) macrophages, Int. J. Inflam. 2013 (2013) 980327.
[102] P. Philippidis, J.C. Mason, B.J. Evans, et al., Hemoglobin scavenger receptor CD163 mediates interleukin-10 release and heme oxygenase-1 synthesis: antiinflammatory monocyte-macrophage responses in vitro, in resolving skin blisters in vivo, and after cardiopulmonary bypass surgery, Circ. Res. 94 (1) (2004) 119–126.
[103] G. Bories, S. Colin, J. Vanhoutte, et al., Liver X receptor activation stimulates iron export in human alternative macrophages, Circ. Res. 113 (11) (2013) 1196–1205.
[104] N. Harada, M. Kanayama, A. Maruyama, et al., Nrf2 regulates ferroportin 1-mediated iron efflux and counteracts lipopolysaccharide-induced ferroportin 1 mRNA suppression in macrophages, Arch. Biochem. Biophys. 508 (1) (2011) 101–109.
[105] L. Guo, H. Akahori, E. Harari, et al., CD163+ macrophages promote angiogenesis and vascular permeability accompanied by inflammation in atherosclerosis, J. Clin. Invest. 128 (3) (2018) 1106–1124.
[106] B. Pourcet, B. Staels, Alternative macrophages in atherosclerosis: not always protective!, J. Clin. Invest. 128 (3) (2018) 910–912.
[107] F. Porcheray, S. Viaud, A.C. Rimaniol, et al., Macrophage activation switching: an asset for the resolution of inflammation, Clin. Exp. Immunol. 142 (3) (2005) 481–489.
[108] S. Lee, S. Huen, H. Nishio, et al., Distinct macrophage phenotypes contribute to kidney injury and repair, J. Am. Soc. Nephrol. 22 (2) (2011) 317–326.
[109] J.E. Feig, J.X. Rong, R. Shamir, et al., HDL promotes rapid atherosclerosis regression in mice and alters inflammatory properties of plaque monocyte-derived cells, Proc. Natl. Acad. Sci. U. S. A. 108 (17) (2011) 7166–7171.
[110] A. Habib, A.V. Finn, The role of iron metabolism as a mediator of macrophage inflammation and lipid handling in atherosclerosis, Front. Pharmacol. 5 (2014) 195.
[111] M. Jung, C. Mertens, B. Brune, Macrophage iron homeostasis and polarization in the context of cancer, Immunobiology 220 (2) (2015) 295–304.
[112] S. Recalcati, M. Locati, E. Gammella, P. Invernizzi, G. Cairo, Iron levels in polarized macrophages: regulation of immunity and autoimmunity, Autoimmun. Rev. 11 (12) (2012) 883–889.
[113] A. Pagani, A. Nai, G. Corna, et al., Low hepcidin accounts for the proinflammatory status associated with iron deficiency, Blood 118 (3) (2011) 736–746.
[114] R. Agoro, M. Taleb, V.F.J. Quesniaux, C. Mura, Cell iron status influences macrophage polarization, PLoS One 13 (5) (2018) e0196921.
[115] A. Sindrilaru, T. Peters, S. Wieschalka, et al., An unrestrained proinflammatory M1 macrophage population induced by iron impairs wound healing in humans and mice, J. Clin. Invest. 121 (3) (2011) 985–997.
[116] K. Hoeft, D.B. Bloch, J.A. Graw, R. Malhotra, F. Ichinose, A. Bagchi, Iron loading exaggerates the inflammatory response to the toll-like receptor 4 ligand lipopolysaccharide by altering mitochondrial homeostasis, Anesthesiology 127 (1) (2017) 121–135.
[117] A. Kroner, A.D. Greenhalgh, J.G. Zarruk, R. Passos Dos Santos, M. Gaestel, S. David, TNF and increased intracellular iron alter macrophage polarization to a detrimental M1 phenotype in the injured spinal cord, Neuron 83 (5) (2014) 1098–1116.
[118] S. Zanganeh, G. Hutter, R. Spitler, et al., Iron oxide nanoparticles inhibit tumour growth by inducing pro-inflammatory macrophage polarization in tumour tissues, Nat. Nanotechnol. 11 (11) (2016) 986–994.
[119] R. Malhotra, F. Wunderer, H.J. Barnes, et al., Hepcidin deficiency protects against atherosclerosis, Arterioscler. Thromb. Vasc. Biol. 39 (2) (2019) 178–187.
[120] Y. Zhou, K.T. Que, Z. Zhang, et al., Iron overloaded polarizes macrophage to proinflammation phenotype through ROS/acetyl-p53 pathway, Cancer Med. 7 (8) (2018) 4012–4022.
[121] Y. Wen, D.S. Leake, Low density lipoprotein undergoes oxidation within lysosomes in cells, Circ. Res. 100 (9) (2007) 1337–1343.
[122] Z. Zhang, F. Zhang, P. An, et al., Ferroportin1 deficiency in mouse macrophages impairs iron homeostasis and inflammatory responses, Blood 118 (7) (2011) 1912–1922.
[123] S. Recalcati, E. Gammella, P. Buratti, et al., Macrophage ferroportin is essential for stromal cell proliferation in wound healing, Haematologica 104 (1) (2019) 47–58.
[124] L. Wang, E.E. Johnson, H.N. Shi, W.A. Walker, M. Wessling-Resnick, B.J. Cherayil, Attenuated inflammatory responses in hemochromatosis reveal a role for iron in the regulation of macrophage cytokine translation, J. Immunol. 181 (4) (2008) 2723–2731.
[125] M. Bayeva, A. Khechaduri, S. Puig, et al., mTOR regulates cellular iron homeostasis through tristetraprolin, Cell Metab. 16 (5) (2012) 645–657.
[126] S. Recalcati, E. Gammella, G. Cairo, Ironing out macrophage immunometabolism, Pharmaceuticals (Basel) 12 (2) (2019).
[127] S.A. Brooks, P.J. Blackshear, Tristetraprolin (TTP): interactions with mRNA and proteins, and current thoughts on mechanisms of action, Biochim. Biophys. Acta 1829 (6–7) (2013) 666–679.
[128] R. Malhotra, M.F. Burke, T. Martyn, et al., Inhibition of bone morphogenetic protein signal transduction prevents the medial vascular calcification associated with matrix Gla protein deficiency, PLoS One 10 (1) (2015) e0117098.
[129] M. Derwall, R. Malhotra, C.S. Lai, et al., Inhibition of bone morphogenetic protein signaling reduces vascular calcification and atherosclerosis, Arterioscler. Thromb. Vasc. Biol. 32 (3) (2012) 613–622.
[130] O. Saeed, F. Otsuka, R. Polavarapu, et al., Pharmacological suppression of hepcidin increases macrophage cholesterol efflux and reduces foam cell formation and atherosclerosis, Arterioscler. Thromb. Vasc. Biol. 32 (2) (2012) 299–307.
[131] Y. Yao, B.J. Bennett, X. Wang, et al., Inhibition of bone morphogenetic proteins protects against atherosclerosis and vascular calcification, Circ. Res. 107 (4) (2010) 485–494.
[132] S.U. Nigwekar, D.B. Bloch, R.M. Nazarian, et al., Vitamin K-dependent carboxylation of matrix gla protein influences the risk of calciphylaxis, J. Am. Soc. Nephrol. 28 (6) (2017) 1717–1722.
[133] S.U. Nigwekar, P. Jiramongkolchai, F. Wunderer, et al., Increased bone morphogenetic protein signaling in the cutaneous vasculature of patients with calciphylaxis, Am. J. Nephrol. 46 (5) (2017) 429–438.
[134] N. Sharma, Y. Lu, G. Zhou, et al., Myeloid Kruppel-like factor 4 deficiency augments atherogenesis in ApoE-/- mice–brief report, Arterioscler. Thromb. Vasc. Biol. 32 (12) (2012) 2836–2838.
[135] R.N. Wang, J. Green, Z. Wang, et al., Bone Morphogenetic Protein (BMP) signaling in development and human diseases, Genes Dis. 1 (1) (2014) 87–105.
[136] G.S. Getz, C.A. Reardon, Do the Apoe-/- and Ldlr-/- Mice Yield the Same Insight on Atherogenesis? Arterioscler. Thromb. Vasc. Biol. 36 (9) (2016) 1734–1741.
[137] D. Baitsch, H.H. Bock, T. Engel, et al., Apolipoprotein E induces antiinflammatory phenotype in macrophages, Arterioscler. Thromb. Vasc. Biol. 31 (5) (2011) 1160–1168.
[138] S. Altamura, R. Kessler, H.J. Grone, et al., Resistance of ferroportin to hepcidin binding causes exocrine pancreatic failure and fatal iron overload, Cell Metab. 20 (2) (2014) 359–367.
[139] A. Cornelissen, L. Guo, A. Sakamoto, R. Virmani, A.V. Finn, New insights into the role of iron in inflammation and atherosclerosis, EBioMedicine 47 (2019) 598–606.
[140] M.D. Demirag, S. Haznedaroglu, B. Sancak, et al., Circulating hepcidin in the crossroads of anemia and inflammation associated with rheumatoid arthritis, Intern. Med. 48 (6) (2009) 421–426.
[141] G.D. Lewis, R. Malhotra, A.F. Hernandez, et al., Effect of oral Iron repletion on exercise capacity in patients with heart failure with reduced ejection fraction and Iron deficiency: the IRONOUT HF randomized clinical trial, Jama 317 (19) (2017) 1958–1966.
[142] A. Santos-Silva, S. Ribeiro, F. Reis, L. Belo, Hepcidin in chronic kidney disease anemia, Vitam. Horm. 110 (2019) 243–264.
[143] A. Zimmermann, T. Zimmermann, J. Schattenberg, et al., Alterations in lipid, carbohydrate and iron metabolism in patients with non-alcoholic steatohepatitis (NASH) and metabolic syndrome, Eur. J. Intern. Med. 22 (3) (2011) 305–310.
[144] E. Senates, Y. Yilmaz, Y. Colak, et al., Serum levels of hepcidin in patients with biopsy-proven nonalcoholic fatty liver disease, Metab. Syndr. Relat. Disord. 9 (4) (2011) 287–290.
[145] M.A. Abdel-Khalek, A.M. El-Barbary, S.A. Essa, A.S. Ghobashi, Serum hepcidin: a direct link between anemia of inflammation and coronary artery atherosclerosis in patients with rheumatoid arthritis, J. Rheumatol. 38 (10) (2011) 2153–2159.
[146] L. Valenti, D.W. Swinkels, L. Burdick, et al., Serum ferritin levels are associated with vascular damage in patients with nonalcoholic fatty liver disease, Nutrition, metabolism, and cardiovascular diseases: NMCD 21 (8) (2011) 568–575.
[147] T. Kuragano, K. Itoh, Y. Shimonaka, et al., Hepcidin as well as TNF-alpha are significant predictors of arterial stiffness in patients on maintenance hemodialysis, Nephrol. Dial. Transplant. 26 (8) (2011) 2663–2667.
[148] N.C. van der Weerd, M.P. Grooteman, M.L. Bots, et al., Hepcidin-25 is related to cardiovascular events in chronic haemodialysis patients, Nephrol. Dial. Transplant. 28 (12) (2013) 3062–3071.
[149] T.E. Galesloot, S. Holewijn, L.A. Kiemeney, J. de Graaf, S.H. Vermeulen, D.W. Swinkels, Serum hepcidin is associated with presence of plaque in postmenopausal women of a general population, Arterioscler. Thromb. Vasc. Biol. 34 (2) (2014) 446–456.
[150] A.W. Aday, P.M. Ridker, Antiinflammatory therapy in clinical care: the CANTOS trial and beyond, Front. Cardiovasc. Med. 5 (62) (2018).
[151] P.M. Ridker, E. Danielson, F.A. Fonseca, et al., Reduction in C-reactive protein and LDL cholesterol and cardiovascular event rates after initiation of rosuvastatin: a prospective study of the JUPITER trial, Lancet 373 (9670) (2009) 1175–1182.
[152] G. Sebastiani, N. Wilkinson, K. Pantopoulos, Pharmacological targeting of the hepcidin/ferroportin axis, Front. Pharmacol. 7 (160) (2016).

[153] J. Galea, J. Armstrong, P. Gadsdon, H. Holden, S.E. Francis, C.M. Holt, Interleukin-1 beta in coronary arteries of patients with ischemic heart disease, Arterioscler. Thromb. Vasc. Biol. 16 (8) (1996) 1000–1006.
[154] A.B. Reiss, N.M. Siegart, J. De Leon, Interleukin-6 in atherosclerosis: atherogenic or atheroprotective? Clin. Lipidol. 12 (1) (2017) 14–23.
[155] N. Sarwar, A.S. Butterworth, D.F. Freitag, et al., Interleukin-6 receptor pathways in coronary heart disease: a collaborative meta-analysis of 82 studies, Lancet 379 (9822) (2012) 1205–1213.
[156] D.I. Swerdlow, M.V. Holmes, K.B. Kuchenbaecker, et al., The interleukin-6 receptor as a target for prevention of coronary heart disease: a mendelian randomisation analysis, Lancet 379 (9822) (2012) 1214–1224.
[157] J.S. Yudkin, M. Kumari, S.E. Humphries, V. Mohamed-Ali, Inflammation, obesity, stress and coronary heart disease: is interleukin-6 the link? Atherosclerosis 148 (2) (2000) 209–214.
[158] O. Kleveland, G. Kunszt, M. Bratlie, et al., Effect of a single dose of the interleukin-6 receptor antagonist tocilizumab on inflammation and troponin T release in patients with non-ST-elevation myocardial infarction: a double-blind, randomized, placebo-controlled phase 2 trial, Eur. Heart J. 37 (30) (2016) 2406–2413.
[159] E. Holte, O. Kleveland, T. Ueland, et al., Effect of interleukin-6 inhibition on coronary microvascular and endothelial function in myocardial infarction, Heart 103 (19) (2017) 1521–1527.
[160] J. Danesh, S. Kaptoge, A.G. Mann, et al., Long-term interleukin-6 levels and subsequent risk of coronary heart disease: two new prospective studies and a systematic review, PLoS Med. 5 (4) (2008) 0050078.
[161] G. Markousis-Mavrogenis, J. Tromp, W. Ouwerkerk, et al., The clinical significance of interleukin-6 in heart failure: results from the BIOSTAT-CHF study, Eur. J. Heart Fail. 21 (8) (2019) 965–973.
[162] P.M. Ridker, B.M. Everett, A. Pradhan, et al., Low-dose methotrexate for the prevention of atherosclerotic events, N. Engl. J. Med. 380 (8) (2019) 752–762.