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Do Hemochromatosis Mutations Protect Against Iron-Mediated Atherogenesis?

Jerome L. Sullivan, MD, PhD

It has been proposed that modest levels of stored iron, far less than conventional iron overload, promote cardiovascular disease and that sustained iron depletion is protective against it.¹⁻⁶ This so-called “iron hypothesis” was initially presented as an explanation for the sex difference in cardiovascular disease and the increase in disease after menopause. The idea, although continually debated for >25 years, has achieved some standing as a plausible and testable hypothesis.⁷⁻¹⁸

No definitive test of the hypothesis has yet been published. A first randomized clinical trial to partially address the hypothesis was recently reported.⁷ The first randomized clinical trial⁷ had 2 key limitations as a general test of the idea: (1) it was a trial of secondary prevention and (2) the iron reduction protocol fell far short of achieving full iron depletion. Zacharski et al⁷ reported that reducing iron stores significantly improves survival for patients with symptomatic but stable peripheral arterial disease, if iron reduction begins before the age of 60 years. The first randomized clinical trial provides compelling support for a new trial designed to fully test the original hypothesis.

Controversial results from multiple epidemiological studies investigating a variety of atherosclerotic events using all kinds of variable parameters of body iron load have presented a confusing picture regarding the iron hypothesis.¹⁹ Confusion became complete when it appeared that patients with homozygous hemochromatosis who were afflicted with serious life-long iron overload had no increase in atherosclerosis and might even be protected against atherosclerosis. In the debate on the hypothesis, the disease pattern in homozygous hemochromatosis has been perceived as perhaps the most persuasive evidence against the hypothesis.²⁰ This “hemochromatosis paradox” is seen as an anomaly that makes the hypothesis untenable for some observers. How can normal stored iron levels be bad for the vascular system, when massive amounts of stored iron in genetic iron overload do not seem to provoke increased atherosclerosis?

Hemochromatosis and Atherosclerosis: More to It Than Iron Load Alone

One of the early corollaries to the iron hypothesis was the proposal that heterozygous hemochromatosis might be a significant risk factor for premature myocardial infarction.²¹

This was proposed despite a general impression at the time that homozygous hemochromatosis was not prominently associated with increased atherosclerosis. In the absence of definitive data, this was not viewed as necessarily incompatible with the iron hypothesis.²¹⁻²³ An impact on cardiovascular event rates in hemochromatosis could not have been excluded based on available data. In addition, even without promotion of atherosclerosis by genetic iron overload, relevant issues that were (and continue to be) unresolved include roles of hemochromatosis mutation-associated iron overload in myocardial reperfusion injury^{2,23-25} and endothelial dysfunction.^{26,27} Future investigations are needed, as long-term exposure to even low levels of nontransferrin bound iron in genetic iron overload may contribute to life-long progression of atherosclerosis as it promotes monocyte-endothelium interaction and inflammatory pathways.

Mutational effects other than promotion of an increase in total body iron were not considered in the formulation of the 1990 hypothesis relating heterozygosity to early onset of myocardial infarction.²¹ The idea that total body iron load was the only factor that might influence cardiovascular disease expression in hemochromatosis was reiterated as recently as 2007 in a *JAMA* editorial on the status of the iron hypothesis by Hu⁸:

“The 1996 discovery of HFE gene mutations responsible for most cases of hereditary hemochromatosis . . . has led to the use of genetic markers of iron stores (ie, heterozygosity for the C282Y mutation in the HFE gene as a marker of lifelong moderate iron overload) in epidemiological studies. In contrast to biomarkers, genetic markers of iron overload can be measured exactly and are not influenced by such factors as inflammation, recent blood loss, diet, and use of medications (eg, aspirin).”²⁸

The corollary hypothesis that heterozygosity might be associated with myocardial infarction²¹ led to a number of investigations, especially after the identification of the disease-causing mutation in most cases of hemochromatosis in 1996.²⁸ Early findings seemed to support some increase in cardiovascular events among heterozygotes.^{22,29,30} However, these studies taken together with a number of subsequent investigations³¹⁻³⁵ do not support an increase in myocardial infarction, stroke, or atherosclerosis in patients who are

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heterozygous for hemochromatosis. In fact, the body of relevant work, including some older studies^{36,37} does not exclude a degree of protection against atherosclerosis in hemochromatosis. In an autopsy series that examined coronary artery disease in heavily hemosiderotic individuals, Miller and Hutchins³⁶ reported an odds ratio of coronary artery disease with iron overload of 0.18. This is suggestive of a marked protective effect in patients presumably homozygous for hemochromatosis who comprised 80% of the autopsy cases reviewed by Miller and Hutchins.³⁶ Was it possible that some poorly understood feature of homozygous hemochromatosis might confound the relationship between iron load and atherosclerosis?

Hepcidin and a Resolution of the Hemochromatosis Paradox

An iron-loading mutation is not simply “a marker of lifelong moderate iron overload” as indicated by Hu.⁸ Hemochromatosis mutations also markedly alter the distribution of body iron.³⁸ The appearance of iron-poor Kupffer cells adjacent to iron-loaded hepatocytes is a classic finding in hereditary hemochromatosis.³⁸ Another classic finding in homozygotes is a relative scarcity of coronary artery iron deposition, despite extensive iron deposits in myocardium.^{38,39}

In 1998, Moura et al⁴⁰ observed that monocytes from patients with hereditary hemochromatosis released twice as much iron in the low molecular weight form as normal human monocytes after erythrocyte phagocytosis. Thus, even before the discovery and understanding of the iron regulatory hormone, hepcidin,^{41–43} there was evidence for “a macrophage defect in hemochromatosis leading to a constriction . . . of the macrophage/reticuloendothelial iron pool.”²³ This macrophage defect⁴⁰ in hereditary hemochromatosis was proposed as a factor that might “protect homozygotes from foam cell formation and thus, to a degree, gives some specific protection against atherosclerosis,”²³ with a partial protective effect in heterozygotes.

The discovery of hepcidin^{41–43} and the details of its influence on iron metabolism^{44–48} clarified patterns of macrophage iron retention and led to a conceptual *volte-face* on the possibility of diminished atherosclerosis in homozygotes.^{4,6}

Hepcidin is the major regulator of the amount of iron retained within macrophages. Production of hepcidin is itself regulated by iron intake and a number of interrelated factors. Elevated levels, favoring macrophage iron retention, are encountered with increased iron intake, infection, and inflammation. Iron loading in secondary iron overload in wild-type individuals is associated with an increase in hepcidin expression. Reduced hepcidin levels and iron-poor macrophages are associated with iron deficiency, hypoxia, anemia, and hereditary hemochromatosis. Hepcidin binds to the iron exporter protein, ferroportin (FPN), leading to the internalization and subsequent intracellular degradation of FPN. Loss of the iron exporter function of FPN from macrophages leads to intracellular retention of iron and to reduced extracellular serum iron levels. In intestinal epithelial cells, hepcidin-induced loss of FPN results in reduced iron transfer into the systemic circulation.

Remarkably, the most extreme reductions in hepcidin level are encountered at the opposite extremes of total body iron load (ie, in iron deficiency anemia and in homozygous hemochromatosis).⁴⁹ Loss of hepcidin expression can be caused by mutations in hepcidin, hemojuvelin, *TFR2*, and *HFE*.⁵⁰ Mutations at these sites can lead to hereditary iron overload. In this discussion, the term “hemochromatosis” indicates hereditary iron overload associated with 1 of the mutations causing loss of hepcidin expression. The homozygous *HFE* C282Y mutation is by far the most common cause of hereditary iron overload and is associated with lower liver expression of hepcidin mRNA.⁵⁰

The very low hepcidin levels seen in homozygous hemochromatosis are associated with systemic iron loading because reduced hepcidin levels permit unregulated FPN-mediated transfer of iron from intestinal epithelial cells into the systemic circulation. In general, the more extreme the degree of hepcidin deficiency, the more severe the level of parenchymal iron load and also the more extreme the macrophage iron retention deficit.

These patterns offer a potential resolution of the paradox of the proposed protection by iron depletion in wild-type subjects against cardiovascular disease in spite of the lack of increased atherosclerosis in genetic iron overload.^{4,6} Hepcidin may act as an iron-dependent risk factor for atherosclerosis by favoring iron loading of plaque macrophages with promotion of foam cell formation. According to this proposal, hepcidin amplifies the plaque iron loading effects of an increased iron load as the iron load itself upregulates hepcidin concentration. At the other end of the iron status spectrum, iron deficiency downregulates hepcidin and accelerates removal of iron from plaque macrophages. In hemochromatosis, the associated hepcidin deficiency is hypothesized to reduce progressive iron accumulation within arterial walls and the resulting foam cell formation. Patients with hemochromatosis may thus enjoy a specific protection against plaque progression in proportion to the severity of the hepcidin deficiency. Hepcidin deficiency does not protect these patients from direct iron-mediated injury to heart muscle from parenchymal iron accumulation in myocardial tissue. The corollary hypothesis that identifies hepcidin as a risk factor for atherogenesis⁴ may explain the conundrum of diminished atherosclerosis in the face of massive iron loading and provide additional justification for the contention that the macrophage has a key role in atherogenesis.

Older studies, especially the work of Miller and Hutchins³⁶ and Pirart and Barbier,³⁷ raised the possibility of a protective effect of hereditary hemochromatosis against atherosclerosis. An unknown “*facteur constitutionnel*”³⁷ linked to hemochromatosis that enhances resistance to vascular lesions was suggested. A mechanistic hypothesis to explain the findings^{36,37} was not proposed as the studies were done before identification of either the principal iron overloading genotypes or the iron regulatory hormone hepcidin.

More recent evidence in favor of the hypothesis that hemochromatosis-associated hepcidin deficiency is protective against atherosclerosis has been reported.⁵¹ Valenti et al⁵¹ studied vascular disease, iron status, hepcidin levels, and *HFE* mutations in a group of 506 consecutive patients with

nonalcoholic fatty liver disease. None of the patients were homozygous for hereditary hemochromatosis. Serum ferritin was associated with common carotid intima-media thickness ($P=0.048$) and with prevalence of atherosclerotic carotid plaques ($P=0.0004$), except in patients whose heterozygous *HFE* mutations diminish hepcidin levels. Hyperferritinemia was associated with increased vascular damage only in patients with wild-type *HFE* genotypes ($P<0.0001$). Hepcidin was elevated in those without such an *HFE* mutation and was an independent predictor of the presence of carotid atherosclerosis.

Iron, Hepcidin, Inflammation, and Vascular Disease

Inflammation promotes atherogenesis.⁵² The mechanism may involve, in part, iron- and hepcidin-mediated mechanisms.^{4,6} Hepcidin is upregulated by interleukin-6, a cytokine induced by many inflammatory processes. Interleukin-6 has also been reported to be a cardiovascular disease risk factor.⁵³ An important end result of any process that induces interleukin-6 is increased deposition of iron within reticuloendothelial cells, presumably including atherosclerotic plaque macrophages, because of hepcidin upregulation. Continued inflammation-mediated hepcidin synthesis acts to maintain iron in storage sites even in the face of a low hematocrit as in the anemia of inflammation (ie, the “anemia of chronic disorders”).

It seems possible that hepatic hepcidin may be normally upregulated in inflammation even in hemochromatosis homozygotes who usually have markedly low hepcidin levels.⁵⁴ The effects of inflammatory processes in patients with hemochromatosis on possible redistribution of iron from parenchymal cells to the reticuloendothelial compartment, including arterial plaque macrophages, are currently unknown. Interactions between mutational effects and inflammation-induced effects on hepcidin level may produce complex epidemiological patterns in studies of cardiovascular disease expression in patients with hemochromatosis.

Blunted-Inflammatory Responses in Macrophages in Hemochromatosis or Induced Iron Depletion

A recent study of macrophages in the *Hfe* knockout (*Hfe*^{-/-}) mouse⁵⁵ is pertinent to the discussion of iron, inflammation, and atherosclerosis. Wang et al⁵⁵ report attenuated inflammatory responses in a mouse model of human hemochromatosis and reduced translation of cytokine mRNAs in *Hfe*^{-/-} macrophages in response to *Salmonella* and lipopolysaccharide exposure. Intramacrophage iron levels were reduced in the *Hfe*^{-/-} mice in association with upregulation of macrophage iron exporter FPN. *Salmonella*- and lipopolysaccharide-induced inflammatory responses were attenuated in the *Hfe* knockout animals. Less severe enterocolitis was observed in vivo and reduced macrophage tumor necrosis factor and interleukin-6 secretion was seen in vitro.

Of special significance in this discussion, the reduced translation of cytokine mRNAs of the mutant macrophages could be reproduced in wild-type cells by decreasing the intracellular iron concentration with chelation. Atheroscle-

rotic plaque macrophages in patients with hemochromatosis mutations associated with diminished hepcidin may also display similar attenuated inflammatory responses such as those from *Hfe*^{-/-} mice,⁵⁵ and thereby a diminished tendency to form atherosclerotic foam cells.

Iron, Hemochromatosis, and Other Cell Types in Vascular Disease

Iron plays a role in vascular disease in cell types other than the macrophage (eg, endothelial cells^{3,9,14,18,56–58} and vascular smooth muscle cells^{59–61}). Patients with hemochromatosis have endothelial dysfunction, which can be improved by iron reduction therapy.⁶² This suggests that iron overload itself rather than mutational effects of iron overload genes predominates in influencing endothelial function. Proliferation of vascular smooth muscle cells^{59–61} seems to require iron. How hemochromatosis mutations might modify iron-mediated atherogenic processes in these cell types will require additional studies.

Serum Cholesterol Level, Hemochromatosis, Macrophage Iron Loss, and Cardiovascular Disease

Adams et al⁶³ reported that patients with hemochromatosis homozygous for C282Y have lower serum cholesterol and low-density lipoprotein levels. Systemically, lower cholesterol and low-density lipoprotein may represent an additional mechanism by which patients with hemochromatosis are relatively protected from atherosclerosis. This could be related to the iron retention deficit in mutant macrophages. A role for macrophage iron metabolism in regulation of cellular lipid level has been reported.⁶⁴ As noted earlier, the most extreme reductions in hepcidin level are encountered at the opposite extremes of total body iron load (ie, in both iron deficiency anemia and in homozygous hemochromatosis). Consistent with a hepcidin level similar to that in hemochromatosis, iron deficiency has also been found to be associated with lower systemic levels of serum cholesterol and low-density lipoprotein.^{12,65,66} Future studies are needed to determine whether lower macrophage iron level in iron deficiency or inherited iron overload can negatively regulate systemic cholesterol level.

Mutational Protection Against Atherogenesis: Epidemiological Implications

The epidemiological literature on the role of iron in cardiovascular disease in the general population is contradictory and inconsistent, as has often been noted.⁸ There have been misconceptions regarding the hypothesis, which led to inadequate study designs.^{19,67} Another key limitation of previous epidemiological studies that has not been addressed is the possibility of a protective effect of hemochromatosis mutations against iron-mediated atherogenesis. If hemochromatosis mutations confer specific protection against atherogenesis, previous epidemiological studies of iron and atherosclerosis may be critically flawed. The highest serum ferritin levels in population groups whose hemochromatosis gene status has not been ascertained will select a disproportionate share of subjects in the population who are heterozygous or homozy-

gous for hemochromatosis. These high-serum ferritin individuals may have less disease because of mutational protection against atherosclerosis and may thus confound underlying associations of iron load and atherosclerosis in normal subjects.

Penetrance and Testing the Hepcidin Hypothesis

This problem of clinical penetrance of the hemochromatosis mutations needs to be taken into account in the design of a study to test the hepcidin hypothesis. Clearly, there is a variable impact of genotype on hepcidin expression. Genotype of subjects in a study to test the hypothesis would need to be determined; however, testing the hypothesis would not rely directly on showing an association of genotype with disease. The hypothesis implies that protection against atherogenesis is inversely proportional to hepcidin expression. In an epidemiological study, the hypothesis suggests that, among those with any 1 of a number of iron overloading genotypes, protection against atherogenesis would be seen as a function of the degree of life-long hepcidin downregulation.

It would not be appropriate to simply look at a group of all subjects with hepcidin expression below some prespecified level. It would be necessary to exclude the iron-deficient subjects from a group defined by such a criterion, as iron deficiency is also associated with quite low hepcidin levels. A future interventional study of the effect of long-term iron deficiency-induced reduction in hepcidin expression on atherogenesis would also be of interest.

Conclusions and Future Directions

The hypothesis that iron depletion protects against atherosclerosis may apply even in the case of hemochromatosis homozygotes because of the mutational effect of selective iron depletion of the macrophage, a key cell type in atherogenesis. In homozygotes, a sea of tissue iron deposition surrounds small islands of iron-depleted cells of the reticuloendothelial system. Low hepcidin expression is a mutational effect of hemochromatosis and a feature of systemic iron deficiency that may protect against iron-mediated atherogenesis in both conditions. What is known at present about disease patterns in genetic iron overload is compatible with the hypothesis that iron depletion protects against cardiovascular disease. Hereditary hemochromatosis may represent a special case of selective cellular iron depletion that inhibits atherogenesis.

More detailed investigations are needed on hepcidin as a risk factor for atherosclerosis including additional studies of atherosclerotic disease in patients with hemochromatosis mutations. More work is also needed on the effects of the inflammatory response on iron metabolism, especially the impact of inflammatory processes on hepcidin and macrophage iron in patients with hemochromatosis mutations.

It would be of interest to replicate the low hepcidin levels of those with hemochromatosis mutations in normal subjects and to assess the effects of such low hepcidin levels on atherogenesis. One well-established and safe method that would have the effect of reducing hepcidin production in normal subjects is induced iron depletion. Long-term modest reduction in storage iron is achievable in patients with

established vascular disease and is associated with decreased cancer mortality⁶⁸ and, among younger participants, decreased cardiovascular mortality.⁷

In humans with intact hepcidin responses, atherosclerotic plaque has been reported to have a substantially higher iron concentration than that in healthy arterial wall.¹⁵ Increased lesional iron is also found in cholesterol-fed animals. In a series of studies with rabbits fed a 1% cholesterol diet, Watt and coworkers^{69–73} used nuclear microscopy to show a 7-fold increase in iron concentration within newly formed atherosclerotic lesions compared with healthy artery tissue. Iron accumulation was observed at the onset of lesion formation.

A role for iron in foam cell formation and lesion progression has been suggested by numerous observations and experiments.^{4–6,74–82} More importantly, recent work shows that iron can be mobilized out of atherosclerotic plaque by manipulation of body iron status and that this process may be associated with reduction in lesion size. Animal experiments suggest that systemic lowering of stored iron levels can reduce intralesional iron content and also the size of atherosclerotic plaques.^{69,83} It is well known that iron-deficient erythropoiesis can mobilize and relocate essentially all stored iron in the body to maturing erythroid precursors. In iron deficiency, the process of mobilization is facilitated by extreme downregulation of hepcidin. Key questions in future human studies include the following: What duration and degree of iron reduction therapy is required for restoring iron levels in atherosclerotic vessel segments to the much lower level seen in healthy vascular tissue? How much reduction in the level of hepcidin is needed to facilitate the relocation of stored iron from intralesional macrophages to erythroid precursors? Is it possible in normal subjects to inhibit the formation of atherosclerotic foam cells by making their macrophages as iron poor as in those with hemochromatosis mutations?

Disclosures

None.

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KEY WORDS: atherosclerosis ■ hemochromatosis ■ hepcidin ■ iron ■ macrophages