Iron, Atherosclerosis, and Ischemic Heart Disease

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Objective: To review the epidemiological and experimental data concerning iron and the development of atherosclerosis and ischemic heart disease.

Data Sources: The English-language literature was searched from 1981 through 1998 manually and using MEDLINE and Current Contents. Important references in the articles that were found were also included in this review.

Results: There is growing epidemiological evidence for a relationship between iron levels and cardiovascular disease. Some experimental data support the role of iron in the process of lipid peroxidation, the first step in the formation of atherosclerotic lesions. Macrophages and endothelial cells are involved in this process, but the exact mechanism and the sites of the interactions between these cells, iron, and low-density lipoprotein are still unknown.

Conclusions: Strong epidemiological evidence is available that iron is an important factor in the process of atherosclerosis. Epidemiological studies, eg, prospective follow-up studies in blood donors, may clarify the cardiovascular benefits of iron depletion. Knowledge of the molecular mechanism of iron-related cardiovascular disease is still limited. We speculate that catalytically active iron species modify low-density lipoprotein levels to interact with the macrophage oxidized low-density lipoprotein receptor. Both nontransferrin-bound plasma iron and hemoglobin are candidates for such interactions.

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THE IRON HYPOTHESIS OF THE RISK OF CARDIOVASCULAR DISEASE

In 1981, Sullivan1 proposed that iron depletion protects against ischemic heart disease. He argued that the difference in the incidence of heart disease between men and women could be explained by differences in levels of stored iron. He argued in support of his theory that myocardial failure occurs in patients with iron storage disorders and that there is accumulation of stored iron with age in men and after menopause in women. Because the results of the Framingham Study2-4 showed that the risk of heart disease in women increased equally by natural menopause or by surgical menopause (independent of oophorectomy), uterine factors, especially regular menstrual blood losses, rather than hormonal factors, might be responsible for the protection of premenopausal women against ischemic heart disease.5 A study by Lauffer6 showed a significant correlation between iron stores and cardiovascular mortality. In men, iron stores, assessed by serum ferritin concentration,7 rise after adolescence. In women, iron stores remain low and only begin to rise after the age of 45 years.8 In young men, there is a parallel increase in risk of coronary heart disease (CHD) and iron load.1,5 The maximal sex difference in serum ferritin level is reached at approximately 45 years of age and is about 300%. The maximal sex difference in heart disease is also reached at approximately 45 years and is also about 300%.9

In familial hyperlipoproteinemia, the sex difference of CHD is preserved despite high levels of lipoprotein in both male and premenopausal female heterozygote patients, suggesting a protecting factor against the cardiovascular effects of elevated cholesterol levels in premenopausal women.5,10-12 Besides explaining the sex difference of CHD, the iron hypothesis could also explain the low prevalence of CHD in areas with a high prevalence of iron deficiency.1 However, this deficiency may be caused by insufficient diet, which is probably less atherogenic. The iron hypothesis could also explain the protective effect of medication that causes gastrointestinal blood loss, eg, aspirin, or inhibits iron absorption, eg, cholestyramine, and the risk
of an increasing effect of oral contraceptives, which are known to decrease menstrual blood loss.\textsuperscript{1,3,13-15} If the iron hypothesis is true, easy and effective ways to reduce the risk of cardiovascular events would be through blood donation\textsuperscript{5,16} and abolition of iron fortification of food and multivitamin preparations.

**EPIDEMIOLOGICAL STUDIES**

**Iron Stores and Coronary Heart Disease**

**Study Findings in Favor of the Iron Hypothesis.** In 1992, Salonen et al\textsuperscript{17} published a prospective study of 1931 randomly selected eastern Finnish men that renewed the discussion about the iron hypothesis of CHD.\textsuperscript{18-21} They found that a high level of stored iron, assessed by elevated serum ferritin concentrations, was a risk factor for CHD. The difference in ferritin levels was not explained by alcohol consumption or inflammatory processes, well-known causes of high ferritin levels. Men with serum ferritin concentrations higher than 200 µg/L had a 2.2-fold higher chance of myocardial infarction than men with lower values. The association was stronger in men with high serum low-density lipoprotein (LDL)–cholesterol levels, suggesting a synergistic role of high iron stores and high LDL-cholesterol levels, which was also found by Laufer.\textsuperscript{8} An elevated serum ferritin concentration was associated with an excess risk of myocardial infarction only when serum LDL-cholesterol levels were high. This finding was confirmed by other investigators\textsuperscript{9,10} and supported by animal studies, which showed that iron overload stimulated the formation of atherosclerotic lesions in hypercholesterolemic rabbits.\textsuperscript{23} In another study, the ratio of serum transferrin receptor to serum ferritin was used as an estimation of body iron stores in the same cohort of Finnish men.\textsuperscript{9} Men with high body iron stores (low transferrin-ferritin ratio) at baseline had a 2- to 3-fold increased risk of myocardial infarction compared with men with low body iron stores.

Morrison et al\textsuperscript{25} studied a large cohort of both men and women in Canada. They found an association between serum iron levels and risk of fatal acute myocardial infarction in male and female subjects. Serum ferritin concentration was not studied. The rate ratio in the highest category of serum iron levels (≥31.3 µmol/L [175 µg/dL]) was 2.18 for men and 5.53 for women. Like Salonen et al,\textsuperscript{17} they found a higher risk for men with high serum iron levels and high serum cholesterol levels (relative risk, 4.60) compared with men with high serum iron levels and low cholesterol levels (relative risk, 1.46). This study and others using serum iron levels as a parameter can be biased by the inclusion of subjects with inflammatory and chronic diseases, who can have lower serum iron levels and higher mortality rates. Also, results may be influenced by the inclusion of subjects who have high serum iron levels because of hereditary or secondary hemochromatosis,\textsuperscript{26} a subpopulation of patients who are supposedly at risk for CHD.\textsuperscript{27} Therefore, although there is evidence that the total amount of iron in the body is related to the development of atherosclerotic disease, it is not clear whether the relationship is gradual or whether the effect is attributable only to certain conditions with higher iron stores (such as hemochromatosis) or confounding factors with higher mortality rates (such as chronic inflammatory diseases), which can influence study results.

In a study of asymptomatic carotid atherosclerosis assessed by duplex sonography, there was a strong correlation between atherosclerosis and iron stores in men and women, which was more prominent when associated with hypercholesterolemia.\textsuperscript{22} A prospective study by the same group revealed serum ferritin levels as one of the strongest risk predictors of progression of atherosclerosis. Changes in iron stores during the 5-year follow-up period modified the risk of atherosclerosis: the lowering of iron stores was beneficial, and further iron accumulation increased cardiovascular risk. Ferritin and LDL-cholesterol levels showed a synergistic association with incidence of cardiovascular disease and death.\textsuperscript{28} However, these results could not be confirmed in some studies using ultrasonographically measured carotid intima-media thickness as a parameter of early asymptomatic atherosclerosis.\textsuperscript{29,30}

Strong support for the relationship between iron levels and increased risk for cardiovascular mortality comes from a recent study investigating the Cys282Tyr mutation of the \textit{HFE} gene, which is present in patients with hereditary hemochromatosis\textsuperscript{31} and porphyria cutanea tarda.\textsuperscript{32} The association between this mutation and cardiovascular death in postmenopausal women was studied, using a nested case control design, in a cohort study of 12,239 women with a follow-up period of 16 to 18 years.\textsuperscript{33} A significantly increased risk of total cardiovascular and cerebrovascular death was found, with risk ratios of 1.6 and 2.4, respectively. This is the first large scale follow-up study that identified a significant correlation between a single genetic polymorphism and cardiovascular mortality. While the Cys282Tyr mutation of the \textit{HFE} gene is probably responsible for the metabolic defects in hereditary hemochromatosis,\textsuperscript{34} this finding supports a role for iron in the development of atherosclerotic disease. In contradiction with these results, Nassar et al\textsuperscript{35} found no difference in presence of the Cys282Tyr mutation between 2 groups of 150 subjects with early-onset CHD (<50 years of age) or late-onset CHD (>65 years of age). In this study, men but not women with early-onset CHD had higher iron stores, assessed by serum ferritin concentrations, than subjects with late-onset CHD, but this finding was not related to the Cys282Tyr mutation of the \textit{HFE} gene. This mutation was present in 8% of the younger subjects and in 11% of the older subjects, slightly more than reported by Feder et al.\textsuperscript{31}

Two studies on the effect of blood donation on cardiovascular events support the iron hypothesis.\textsuperscript{36,37} In a cohort from Nebraska, blood donation was associated with reduced risk of cardiovascular disease only in nonsmoking men with an odds ratio of 0.67.\textsuperscript{36} The benefit of donation was greater in men with higher levels of serum LDL cholesterol. No significant effect of blood donation was seen in women. Because the iron hypothesis suggests that the protective effect of blood donation would be more prominent in men, who have higher iron load than women, these results are in agreement with the hypothesis. In a prospective cohort study of 2682 Finnish men followed up for 5.5 years, Tuomainen et al\textsuperscript{37} found that blood donation reduced the risk of myocardial infarction by 86%.
They reported notably fewer myocardial infarctions in donors (0.7%) than in nondonors (9.8%). However, the results of these 2 studies could be biased by the selection of healthier persons for blood donation.

**Studies Weakening the Iron Hypothesis.** A number of studies failed to show a correlation between iron stores and CHD. A nested case study in middle-aged dyslipemic men,38 a retrospective study,39 and a prospective study40 showed no association between ferritin levels and CHD. A Canadian study failed to show a correlation between serum ferritin levels and angiographically determined coronary artery disease.41 Two reports using iron transferrin saturation (TS) for assessment of iron stores did not show a protective effect of iron deficiency. One of these studies found a relative risk of acute myocardial infarction of 1.3 in subjects with a TS greater than or equal to 62%, which was not statistically significant.42 Giles et al43 found a relative risk of 0.8 for men and of 2.6 for women in cases with a high TS (≥60%), which was also not statistically significant. Unfortunately, there is a poor correlation between TS in the normal range and body iron stores,43,44 making interpretation of these studies difficult. In an Icelandic study45 of men and women followed up for an average of 8.3 years, no association was found between serum ferritin levels and the risk of myocardial infarction. However, a high total iron-binding capacity (TIBC) was a negative risk factor. Each increase in TIBC of 1 µmol/L was associated with a 5.1% decrease in the risk of myocardial infarction. This finding suggests a possible protection by iron deficiency,46 but iron depletion was uncommon in the population studied.47

In the First National Health and Nutrition Examination Survey Epidemiologic Follow-up Study, TIBC and TS were not related to myocardial infarction. However, serum iron levels and TS were inversely associated with CHD (ie, myocardial infarction and other forms of ischemic heart disease) in both men and women.48 Data from the same study showed a U-shaped association of TS with risk of stroke and stroke mortality in white women aged 45 to 74 years. No association was found in white men or blacks.49 Sempos et al50 also found an inverse association between TS and risk of CHD; however, they did not measure serum ferritin concentrations, thus weakening their conclusion.51,52 Regnstrom et al53 studied serum ferritin levels, serum iron levels, and TIBC in 94 young male survivors of myocardial infarction 4 to 6 months after the event. They found no differences in ferritin levels in patients compared with healthy controls. The patients had lower serum iron levels and a higher TIBC. The number and severity of coronary artery lesions shown by angiography were not associated with iron status. However, the use of aspirin after the event (causing blood loss) in combination with phlebotomy for diagnostic purposes could have lowered iron storage levels,54 and the number of subjects studied was small.

**Dietary Iron Intake and CHD**

Genetic, pathological, and environmental factors may contribute to iron-related cardiovascular risk. Salonen et al55 found a significant association of iron intake and CHD in the Finnish cohort. For each milligram of iron consumed, there was an increase of 5% in the risk of CHD. The relationship between iron intake and coronary disease was partly confirmed by Ascherio et al,56 who found (after adjustment for saturated fat and dietary cholesterol) an increased risk of myocardial infarction among men with a higher intake of heme iron (red meat), which was positively correlated with total iron stores. However, they could not find an association between total iron intake and CHD.

In a study of 329 Greek men and women with an electrocardiographically confirmed first coronary infarct, a first abnormal coronary arteriogram, or both, dietary iron intake was positively associated with risk for coronary disease among men and also especially among women aged 60 years or older.56 In a study of the relationship between iron parameters and carotid atherosclerosis, determined by ultrasonographic measurements of the intima-media thickness, no correlation between iron intake and asymptomatic carotid atherosclerosis was found.57 Also, no correlation between iron intake and CHD was found in the First National Health and Nutrition Examination Survey Epidemiologic Follow-up Study.58 Morrison et al55 did not find a relationship between total iron intake or iron supplementation and risk of fatal acute myocardial infarction. Dietary iron intake in the 2 latter studies55,58 was assessed by using a 24-hour recall questionnaire, which is not an optimal estimate of long-term dietary iron intake. In these studies,55,58 no differentiation between heme and nonheme iron intake was made. Snowdon et al59 found a 60% increase in the risk of fatal coronary disease among men who consumed meat 6 times a week compared with men who consumed meat less than once a week. Meat is an important source of heme iron. The feedback mechanism of iron absorption functions better for nonheme iron than for heme iron. At any serum ferritin level, the percentage of heme iron absorption is greater than that of nonheme iron absorption. Inhibition of heme absorption at higher serum ferritin levels is less than inhibition of nonheme iron absorption.58 Differences in feedback of heme and nonheme iron absorption could account for the relationship between meat intake and ferritin levels and CHD, and for the absence of a relationship between nonheme iron intake and CHD.57 Thus, the absence of an association between nonheme or total iron intake and CHD does not exclude the possibility of a relationship between iron stores or heme intake and CHD. A lack of correlation between nonheme iron intake and CHD suggests that dietary nonheme iron does not contribute to an increased cardiovascular risk, except perhaps among patients with hemochromatosis, including heterozygotes.10

**Exercise, Iron Levels, and CHD**

Exercise is associated with reduced mortality from cardiovascular disease.59-61 The reduction of mortality cannot be explained solely by improvements in risk factors such as lipid profile or blood pressure.59 Differences in these risk factors only account for 10% of the reduction of cardiovascular risk in physically trained groups.61

Lauffer59,62,63 proposed that the beneficial effects of ex-
Exercise may be attributable to lower body iron stores associated with physical exertion. Physical training reduces iron stores by creating a negative iron balance, as shown in athletes. There are several mechanisms by which physical exertion could decrease body iron stores: gastrointestinal blood loss caused by intestinal ischemia or stress-induced gastritis; intravascular hemolysis with urinary excretion of hemoglobin owing to mechanical or osmotic destruction of erythrocytes or to oxidative stress; exercise acedia with early release of iron from transferrin; and iron loss through sweating. Also, building muscle mass by training leads to increased need of iron for myoglobin.

In a study among eastern Finnish men, duration and frequency of physical activity were inversely related to serum ferritin levels. Regular physical activity 2 to 3 hours a week reduced serum ferritin levels. Along with monocytes and LDL and thus promote the progression of oxidized LDL has cytotoxic capacity that induces changes in vessel wall. Thereby, oxidant damage in cardiovascular disease. The mechanism by which iron may stimulate atherogenesis is not only induced by iron but that it could also be promoted by other, still unknown, factors as well. The production of apoferit in the macrophages and endothelial cells could be a protective mechanism against the damaging effects of free iron. Heme derived from lysed red blood cells is taken up rapidly by endothelial cells and releases its iron, thus promoting oxidant damage. Although transferrin does not release its iron at a normal pH, at a low pH, which may be the case in arterial walls, iron can be released from transferrin and induce oxidation of LDL. The finding of a 10-fold higher expression of both H-ferritin and L-ferritin messenger RNA in human and animal atherosclerotic aortas compared with normal aortas strongly indicates that these cells are exposed to high amounts of low-molecular-weight iron complexes.

In a time course experiment, the induction of ferritin expression occurred in parallel with the progression of the lesions. Furthermore, Prussian blue stain showed the presence of iron deposits in advanced lesions but not in the early lesions of rabbit and human aortas. This finding suggests that the ferritin gene expression is not only induced by iron but that it could also be promoted by other, still unknown, factors as well. The production of apoferitin within the macrophages and endothelial cells could be a protective mechanism against the damaging effects of free iron. Heme derived from lysed red blood cells is taken up rapidly by endothelial cells and releases its iron, thus promoting oxidant damage. Although transferrin does not release its iron at a normal pH, at a low pH, which may be the case in arterial walls, iron can be released from transferrin and induce oxidation of LDL. The finding of a 10-fold higher expression of both H-ferritin and L-ferritin messenger RNA in human and animal atherosclerotic aortas compared with normal aortas strongly indicates that these cells are exposed to high amounts of low-molecular-weight iron complexes.

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LDL oxidation, thus promoting atherosclerosis. Yuan et al. detected iron in the lysosomal apparatus of foam cells. They also showed that iron- or hemoglobin-loaded macrophages can oxidize LDL and then endocytose the oxidized LDL and become foam cells. The process of oxidation was shown to involve secretion of iron and was inhibited by desferrioxamine. These authors hypothesize that macrophages after erythrophagocytosis contain secondary lysosomes with catalytically active iron complexes. In the arterial wall close to LDL particles, these macrophages might exocytose iron along with the production of superoxide, for instance, leading to production of catalytically active ferrous iron, which initiates oxidation of LDL and thus promotes the transformation of these macrophages into foam cells.

Whether nontransferrin-bound iron is important in atherogenesis in vivo is not well known. It is found in conditions of iron overload or transferrinemia but may be present in small amounts in normal subjects and thus be responsible for lipid peroxidation in vivo. We have recently shown that monocytes and macrophages from patients with hereditary hemochromatosis release twice as much low-molecular-weight iron as cells from normal controls, explaining the higher plasma concentration of potentially catalytically active iron in these patients. Iron overload was shown to augment the formation of atherosclerotic lesions in hypercholesterolemic rabbits, probably by stimulating LDL oxidation in the arterial intima or by influencing lipoprotein synthesis in the liver, which could lead to increased susceptibility to oxidation in the intima. A small study by Salonen et al. performed in healthy subjects, showed a reduction of lipid oxidation susceptibility after 3 phlebotomies, removing 1500 mL of whole blood (containing approximately 700 mg of iron). This study, and the experiments by Matthews et al. demonstrating that the iron chelator deferiprone (L1) prevented the oxidation of LDL and diminished the cytotoxic capacity of LDL in vitro and reduced rabbit thoracic aorta cholesterol content in vivo, also suggests a role for iron, maybe in the nontransferrin-bound state, in the process of lipid peroxidation and atherogenesis in vivo.

Ischemia/Reperfusion Damage in Coronary Artery Disease

Atherosclerosis can lead to coronary artery disease, causing myocardial ischemia and infarction. Reperfusion damage, caused by restoration of aerobic metabolism after a period of ischemia, is dependent on the presence of free radicals. During reoxygenation, oxygen free radicals are produced. These radicals probably damage cells by oxidizing various cellular components and could be important in inducing myocardial “stunning” and reperfusion-induced arrhythmias. By catalyzing the Haber-Weiss reaction, iron plays a role in the generation of oxygen free radicals. Evidence suggests that iron promotes the damage that occurs during ischemia and reperfusion, even in the absence of iron overload. An iron-supplemented diet increased the degree of oxidative injury in ischemic rat hearts. Some, but not all, studies of the effect of the chelator desferrioxamine in ischemic myocardial events in animals show a beneficial effect of pretreatment with the drug on reperfusion damage. There is some evidence that iron is mobilized during organ ischemia, thus being available for catalyzing free-radical generation. In addition to the effect of antioxidants in ischemia, these data suggest a role for iron in the occurrence of reperfusion damage that complicates atherosclerosis and ischemic heart disease.

CONCLUSIONS

There is increasing epidemiological evidence concerning the role of iron in atherosclerosis and ischemic heart disease. Studies in this field are performed using different and sometimes inappropriate estimations of iron stores, which makes them difficult to compare. Further epidemiological studies are needed, in particular prospective follow-up studies comparing cardiovascular events in healthy blood donors with those in healthy controls. Iron could play a role in the process of atherosclerosis by catalyzing the formation of free radicals and thus enhancing peroxidation of lipoproteins, both the lipid and protein moiety, and formation of oxidized LDL. Iron-catalyzed generation of free radicals also contributes to reperfusion damage. The exact mechanism of iron-induced LDL peroxidation is still unresolved. Studies should focus on the exact site where the detrimental interaction between iron and LDL occurs, information that is necessary to design preventive strategies, such as long-standing antioxidant or iron chelation therapy. Further studies should also clarify what the source of the catalytically active iron is and which role nontransferrin-bound iron plays in peroxidation in vivo. Of particular importance may be the development of nontoxic oral iron chelators for the prevention of iron-induced cardiovascular damage.

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