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Treatment of Anemia in Heart Failure: Potential Risks and Benefits of Intravenous Iron Therapy in Cardiovascular Disease

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Abstract

Iron-deficiency anemia is common in patients with heart failure (HF), but the optimum diagnostic tests to detect iron deficiency and the treatment options to replete iron have not been fully characterized. Recent studies in patients with HF indicate that intravenous iron can rapidly replenish iron stores in patients having iron-deficiency anemia, with resultant increased hemoglobin levels and improved functional capacity. Preliminary data from a sub-group analysis also suggests that supplemental intravenous iron therapy can improve functional capacity even in those subjects without anemia. The mechanisms responsible for this observation are not fully characterized, but may be related to beneficial effects of iron supplementation on mitochondrial respiration in skeletal muscle. The long-term safety of using intravenous iron supplementation in HF populations is not known. Iron is a known pro-oxidant factor that can inhibit nitric oxide signaling and irreversibly injury cells. Increased iron stores are associated with vascular endothelial dysfunction and increased risk of coronary heart disease events. Additional clinical trials are needed to more fully characterize the therapeutic potential and safety of intravenous iron in HF patients.

Keywords

heart failure; anemia; iron metabolism; reactive oxygen species; exercise; clinical trials

Physiologic Role of Iron

Iron is an essential trace element that plays an important role in numerous homeostatic biological processes, but is also recognized to be a potentially toxic agent capable of generating reactive oxygen species that may impair cellular function. Major physiological functions of iron include oxygen transport, as a component of hemoglobin and myoglobin, and maintenance of energy production through oxidative phosphorylation as an integral component of cytochromes, NADPH and succinate dehydrogenases.^{1–3} Iron plays an important role in immune regulation as a component of peroxide- and nitrous oxide generating enzymes required by some immune cells for normal host defenses.^{1–3} Iron is also increasingly recognized to be an important transcription factor for a variety of other signal pathways related to neurotransmission, cell growth, and inflammation.^{1–3}

Total body iron stores are regulated exclusively through control of iron absorption, as there are no known natural metabolic pathways for iron excretion.^{4,5} Heparin is a liver-derived 25 amino acid peptide hormone that appears to play a critical role in the regulation of iron

absorption.^{6,7} The physiological signals that regulate hepcidin secretion are not fully characterized but appear to be related to iron-dependent redox signaling related to pathways involving the HFE, TRF2, and HJV genes, red cell mass, and cytokine signaling.^{6,8–10} In a negative feedback loop, hepcidin is secreted in response to increased body iron stores and interacts with ferroportin and other iron transport proteins in the enterocyte to inhibit gut iron absorption.^{6,7} The cellular mechanisms for hepatic sensing of iron stores have not yet been fully characterized. HFE, the protein associated with the most common form of inherited hemochromatosis, forms complexes with the transferrin type 1 and type 2 receptors with varying affinities that are modified by the presence of iron binding to transferrin. In iron replete states, when HFE binding to transferrin receptor type 2 is high, the HFE-transferrin complex interacts on the hepatocyte cell surface with hemojuvelin and bone morphogenetic protein receptors to activate a Smad-dependent signaling pathway that activates nuclear transcription for hepcidin. Hepcidin synthesis is also regulated by other signaling pathways that do not appear to be directly regulated by iron stores.¹¹ Activation of hepcidin synthesis by interleukin-6 appears to be an important mechanism contributing to altered iron metabolism and anemia in acute and chronic inflammatory diseases. The alterations in iron metabolism in response to inflammatory signals is thought to be protective against microbial growth in certain acute infections.¹²

Once iron is absorbed via the enterocyte, almost all iron is bound to specific iron transport proteins (transferrin) and iron storage proteins (ferritin) in a highly-regulated system that controls iron availability to the bone marrow for erythropoiesis. In non-heme tissues, two iron regulatory proteins (IRP-1 and IRP-2) play an important role in maintaining intracellular iron homeostasis. In response to changes in iron availability and redox signals, IRP-1 and IRP-2 bind iron-response elements that regulate transcription of the transferrin receptor, ferritin and other proteins.^{5,13} There also exists a small amount of low-molecular weight, non-protein bound iron complexed to citrate and other small molecules in the extracellular and intracellular compartments outside of the reticuloendothelial system.^{14–16} Low molecular weight non-transferrin bound iron is present in the serum of normal human subjects in concentrations ranging from 0.08–0.19 μM .¹⁷ Increased levels of non-transferrin-bound iron have been reported in patients with iron overload secondary to end-stage renal disease, hemolytic anemias, and hemochromatosis.^{18,19} Low molecular weight iron appears to be transported across cell membranes by poorly characterized transporter proteins distinct from the transferrin receptor, as patients with chronic iron overload manifest increased intracellular iron despite downregulation of transferrin receptors.²⁰ The low molecular weight iron pool is readily chelatable, is available for participation in redox reactions via Fenton chemistry and interactions with nitric oxide, and regulates synthesis and activity of iron-containing proteins.^{14,15,16}

Iron stores in the body may be assessed by a variety of clinically available tests. Normal total body iron stores range from 30–40 mg/kg, with 1.8 gm contained within red blood cells (depending on blood volume and hemoglobin concentration), and an additional 0.5–1.5 gm in storage in liver parenchymal cells and the reticuloendothelial system.^{2,3,21} Less than 5% of total body iron is contained in other iron-containing proteins in muscle (myoglobin) and mitochondria. Normal daily loss of iron from sloughing of gastrointestinal mucosal cells is approximately 1–2 mg/day (<0.5% of total stores) with losses generally matched by gastrointestinal absorption of an equal amount.^{2,3,21,22} Bone marrow iron stores are considered the gold standard for the evaluation of iron deficiency anemia, but this procedure is invasive and therefore not routinely used in clinical evaluation of anemia.²³ Blood markers of iron stores include total iron binding capacity (circulating transferrin), serum iron, percent transferrin saturation, plasma ferritin, sideroblast percent, red blood cell protoporphyrin level, and erythrocyte morphology (Table 1).

In clinically overt iron deficiency anemia, the erythrocytes become hypochromic and microcytic, total iron binding capacity goes up while the rest of the parameters exhibit a downward trend. Clinical manifestations associated with iron deficiency include the symptoms of anemia (weakness and fatigue), glossitis, stomatitis, Plummer Vinson syndrome, pica, and restless legs syndrome.³ Impaired thermoregulation and immune function and decreased endurance exercise capacity have also been attributed to iron deficiency.³

Clinically overt iron overload occurs in hemochromatosis, some thalassemias and other transfusion dependent hematological disorders, some sideroblastic anemias, chronic liver disease, porphyria cutanea tarda, and hemosiderosis.² In these conditions serum ferritin is increased, in some cases to 10–20 fold above normal values. Excess stored iron in these conditions is estimated to range from as low as 1 gm to as much as 40 gm.

While laboratory assessment of iron stores provide fairly reliable diagnostic accuracy in most cases with overt signs of iron overload or deficiency, very little data exist on the optimal levels of iron required for normal physiological function. Available evidence suggests that there may be subclinical states of iron depletion and/or excess that may be impacting iron-dependent biological processes without overt evidence of iron-deficiency or overload (Figure 1). This review article will consider potential implications of altered iron homeostasis relevant to evaluation and treatment of anemia in patients with heart failure (HF).

Potential Effects of Subclinical Iron Deficiency in Heart Failure

Anemia is common in patients with HF, and is associated with greater impairment of functional capacity, and increased risk of hospitalization and mortality.^{24–34} The etiology of anemia in HF is often difficult to determine and in many cases may be multifactorial. Possible mechanisms include insufficient erythropoietin secretion (due to inhibition of the renin angiotensin system and/or co-morbid chronic kidney disease) or resistance to the effects of endogenous erythropoietin (due to chronic inflammation), hemodilution, and iron deficiency.^{34–45}

Iron deficiency is thought to be a common contributing factor to anemia in the HF population. In the OPTIME-HF clinical trial, 21% of the study population had iron deficiency anemia diagnosed by standard blood biomarkers.²⁵ Nanas and colleagues determined that 73 % of patients with advanced HF and anemia had reduced iron stores based on the iron content of bone marrow biopsies.⁴⁶ The etiology of iron deficiency in HF patients is likely multifactorial; some of the postulated causes are poor nutrition, malabsorption due to edematous bowel wall, increased gastrointestinal blood loss due to chronic anti-platelet and/or oral anticoagulation use, and altered iron homeostasis due to pro-inflammatory cytokine activation.^{30,47–49}

The presence of chronic inflammation in many HF patients may mask the diagnosis of iron deficiency anemia.^{50–52} An interesting feature of anemia of chronic disease is the retention of iron within macrophages, thus limiting the availability of iron for erythropoiesis.⁵³ Pro-inflammatory signaling molecules including tumor necrosis factor-alpha, interferon gamma, and lipopolysaccharide increase macrophage iron content by increasing expression of divalent metal transporter-1 (an iron uptake protein) and decreasing expression of ferroportin (an iron exporter protein).⁵⁴ Hepcidin can also contribute to anemia of chronic disease by blocking iron release from macrophages by downregulation of ferroportin.^{6,55} These changes in iron transport protein expression increase reticuloendothelial iron stores, but limit release of iron to the bone marrow for hematopoiesis. The clinical correlate of these molecular changes is a change in biomarkers consistent with the diagnosis of anemia

of chronic disease (low hemoglobin in the presence of elevation of serum ferritin). Weiss and Goodnough suggest that in patients with evidence of inflammation and anemia, a transferrin saturation <16% and serum ferritin value of <30 ng/ml, the diagnosis of iron deficiency anemia is likely. If serum ferritin is >100 ng/ml in the setting of reduced transferrin saturation, anemia of chronic disease (functional iron deficiency) is the more likely diagnosis.⁵³

Based on these proposed definitions, iron deficiency may be more common than previously reported in anemic patients with heart failure. Several investigators have reported on the effects of iron supplementation in patients with HF with and without anemia. These studies are reviewed in chronological order based on date of publication below and are summarized in Table 2.

Studies on the Use of Intravenous Iron Supplementation in Patients with Heart Failure

In a prospective, uncontrolled study, Bolger and colleagues studied the effects of intravenous iron sucrose in 17 patients with anemia and chronic systolic HF.⁵⁶ Patients were receiving treatment with guideline-recommended heart failure medications for ≥ 6 weeks and had anemia as defined by hemoglobin of ≤ 12 g/dl. Patients with serum ferritin >400 ng/ml were excluded. Patients received bolus intravenous injections of 200 mg undiluted iron sucrose on days 1, 3 and 5 in an outpatient setting. Serum ferritin was measured on day 12. If ferritin was <400 ng/ml on day 12, further doses of intravenous iron sucrose were administered on days 15 and 17. Most of the study subjects received all five doses of intravenous iron (total 1 g). Symptoms and exercise capacity were assessed by New York Heart Association (NYHA) functional classification, the Minnesota Living with Heart Failure (MLHF) questionnaire and the 6-minute walk test respectively. In response to intravenous iron treatment, hemoglobin, ferritin, serum iron and transferrin saturation all significantly increased from baseline values. At entry 9 patients were in NYHA class II symptoms and the remainder in class III. At follow-up all patients were in NYHA functional class II ($p < 0.02$). The MLHF score also significantly improved ($p = 0.02$). Mean 6-min walk distance increased from 242 ± 78 m to 286 ± 72 m ($p = 0.01$). Changes in these later two outcome variables were strongly associated with increased hemoglobin levels ($r = 0.76$, $p = 0.002$ and $r = 0.56$, $p = 0.03$ respectively). Intravenous iron sucrose was well-tolerated in the study population.

In a randomized, double-blind study, Toblli and colleagues investigated the effects of intravenous iron sucrose (200 mg weekly for five weeks) vs. matching normal saline placebo in 40 patients with anemia and chronic systolic HF (hemoglobin <12.5 gm/dl in men or <11.5 gm/dl in women) with evidence of iron deficiency (serum ferritin <100 ng/ml and/or transferrin saturation $\leq 20\%$) and co-morbid renal insufficiency (estimated creatinine clearance ≤ 90 ml/min).⁵⁷ Primary endpoints were to determine the effects of study drug on iron stores, hemoglobin values, N-terminal-pro-brain natriuretic peptide (NT-ProBNP levels), systemic inflammation as assessed by C-reactive protein, and renal function at 6 months. Secondary endpoints were to determine the effects of study treatment on NYHA functional class, the MLHF questionnaire score, the 6-minute walk test, and left ventricular ejection fraction (LVEF) assessed by transthoracic echocardiography. Intravenous iron significantly increased hemoglobin, ferritin and transferrin saturation when compared with placebo (all $p < 0.01$). Intravenous iron significantly reduced NT-proBNP ($p < 0.01$) and CRP ($p < 0.01$) when compared with placebo. Symptomatic improvement was evidenced by significant improvements in NYHA class and MLHF questionnaire score ($p < 0.01$) and increased 6-minute walk distance (estimated mean treatment effect 54 m, $p < 0.01$). A significant increase in LVEF was also observed ($p < 0.01$). Although not powered to detect difference in clinical event rates, there no hospitalizations in the intravenous iron group and

five hospitalizations in the placebo group over six months of follow-up. Intravenous iron sucrose was well-tolerated with no reported side effects.

The effects of Intravenous iron sucrose on exercise tolerance in HF patients were studied in two European centers in an open-label trial.⁵⁸ Thirty-five subjects with chronic systolic heart failure were enrolled and randomized in a 2:1 manner to intravenous iron sucrose vs. control (placebo or standard care depending on the enrolling center). Key eligibility criteria included NYHA functional class II or III symptoms at study entry; exercise limitation characterized by a peak oxygen uptake (VO_2)/kg ≤ 18 ml/kg/min; hemoglobin concentration of <12.5 g/dl for anemic subjects and 12.5–14.5 g/dl for non-anemic subjects; serum ferritin of <100 ng/ml (or serum ferritin 100–300 ng/ml with transferrin saturation $<20\%$) and a LVEF of $\leq 45\%$. Patients in the intravenous iron group received drug weekly for 3 weeks (therapeutic phase) followed by doses at 4, 8, 12 and 16 weeks (maintenance phase). Iron therapy was withheld if ferritin was ≥ 500 ng/ml or Hb ≥ 16.0 g/dl or transferrin saturation was $\geq 45\%$ at any stage. The primary endpoint was the change in absolute peak VO_2 (ml/min) from baseline to week 18. Secondary end points included changes in peak VO_2 adjusted for body weight (ml/kg/min) from baseline to week 18, exercise duration, hemoglobin, biomarkers of iron stores, NYHA functional class, changes on 7-point patient Global Assessment scale, the MLHF questionnaire score and a fatigue score. The mean dose of iron sucrose administered during the study was 928 ± 219 mg. For all patients receiving iron sucrose, absolute peak VO_2 did not significantly increase when compared with placebo (mean treatment effect 96 ml/min, $p=0.08$) but peak VO_2 normalized to body weight did significantly increase when compared with placebo (mean treatment effect 2.2 ml/kg/min, $p=0.01$). In the anemic subgroup iron sucrose significantly increased absolute peak VO_2 (mean treatment effect 205 ml/min, $p=0.02$) and a peak VO_2 /kg adjusted for body weight when compared with placebo (mean treatment effects 3.9 ml/kg/min, $p=0.009$). In the non-anemic subgroup, iron sucrose was not associated with any change in peak VO_2 . Changes in peak VO_2 were associated with changes in transferrin saturation in anemic subjects ($n=18$, $r=0.62$, $p=0.006$) but not changes in hemoglobin concentration ($r=0.37$, $p=0.08$).

Usmanov and colleagues studied the effects of intravenous iron in 32 anemic patients (hemoglobin <11 gm/dl) with NYHA Class III–IV systolic heart failure and co-morbid chronic renal insufficiency (serum creatinine 1.5–3.9 mg/dl) in an uncontrolled longitudinal study.⁵⁹ The outcome variables were laboratory markers of anemia and iron stores, functional status and LV structure and function as assessed by transthoracic echocardiography. Intravenous iron sucrose was administered at a dose of 100 mg over 30 minutes three times weekly for 3 weeks followed by once weekly dosing for 23 weeks. The total dose was 3200 mg of elemental iron over the 26-week study. The hemoglobin values increased significantly from 10.7 to 13.7 gm/dl in the NYHA Class III subjects and from 9.4 to 12.7 gm/dl in the NYHA Class IV subjects. Intravenous iron sucrose improved symptoms to NYHA class II in nine of the subjects with baseline NYHA Class III symptoms (47.4%, $p<0.01$), but did not improve functional class in any of the 13 patients with NYHA IV symptoms at baseline. Echocardiographic parameters including posterior wall thickness, LV mass index, septal thickness, LV end diastolic volume and end systolic volume improved significantly when compared with pre-treatment baseline in the NYHA Class III subjects but not in the NYHA Class IV subjects.

In a multicenter, prospective, double-blind study, Anker and colleagues investigated the effects of intravenous ferric carboxymaltose vs. matching placebo in 459 heart failure subjects with biomarkers of iron deficiency, with or without anemia (Ferrinject Assessment in Patients with Iron Deficiency and Chronic Heart Failure (FAIR-HF)).⁶⁰ Study drug was randomly assigned in a 2:1 ratio: 304 subjects received ferric carboxymaltose and 155 subjects received placebo. Key inclusion criteria included chronic NYHA functional class II

or III symptoms, LV systolic function (EF $\leq 40\%$ in patients with NYHA class II symptoms or $\leq 45\%$ for NYHA class III symptoms), iron deficiency (defined by a ferritin < 100 ng/ml or serum ferritin 100–200 ng/ml with transferrin saturation $< 20\%$), and hemoglobin value 9.5–13.5 gm/dl. Intravenous bolus injection of ferric carboxymaltose 200 mg or placebo was administered weekly until iron stores were repleted (correction phase, total number of weekly infusions based on estimated iron deficit derived from baseline hemoglobin values), followed by a dosing interval of 4 weeks starting week 8 or week 12 (maintenance phase). Study drug was administered in a double-blind manner to maintain a transferrin saturation of 25–45% and a serum ferritin of 400–800 ng/ml. Primary endpoints for the study were a self-reported 7-point Patient Global Assessment Scale and Investigator-assessed NYHA functional class at week 24. Secondary end points included the distance on the 6 minute walk test and the overall score on the Kansas City Cardiomyopathy Questionnaire (KCCQ) at week 24. Intravenous iron carboxymaltose significantly increased serum ferritin and hemoglobin levels when compared with placebo at week 24 (mean treatment difference for serum ferritin was 246 ± 20 ng/ml and mean treatment difference for hemoglobin, 0.6 gm/dl (both $p < 0.001$). For the primary end point at week 24, 50% of patients in the intravenous iron carboxymaltose group reported improvement on the Patient Global Assessment scale compared to 28% in the placebo group (odds ratio; 2.51; 95% CI, 1.75 TO 3.61; $P < 0.001$). Likewise 47% of subjects receiving intravenous iron carboxymaltose showed improved to NYHA class I or II compared to 30 % in the placebo group (odd ratio 2.40, 95% CI 1.55 TO 3.71; $P < 0.001$). For secondary endpoints at week 24, the 6-minute walk distance and KCCQ score significantly improved in subjects randomized to iron carboxymaltose when compare with placebo (mean study treatment effect for 6-minute walk test 35 ± 8 m, and mean study treatment for KCCQ effect +7 points, both $p < 0.001$). In a pre-specified subgroup analysis, the magnitude of the treatment effect did not differ in subjects with vs. without baseline anemia (defined as hemoglobin ≤ 12.0 g/dl), even though the hemoglobin level did not change in response to intravenous iron in the non-anemic subgroup ($n = 221$) when compared with placebo (13.3 ± 0.1 vs. 13.2 ± 0.1 g/dl, $p = 0.21$). The overall rate of adverse events was similar for both groups, but the rate of first hospitalization for cardiovascular events was lower in the subjects randomized to receive intravenous iron carboxymaltose when compared with placebo (hazard ratio, 0.53; 95 % CI 0.25 TO 1.09; P value=0.08).

Taken together, these independently conducted clinical trials provide a consistent signal of improved exercise capacity and quality of life in response to intravenous iron therapy in patients with HF and iron-deficiency. In most of the studies, the improved symptoms associated with iron therapy are accompanied by a significant increase in hemoglobin values. In these studies, the presumed mechanism underlying the clinical benefit is increased peripheral oxygen delivery associated with increased hemoglobin levels. The same mechanism has been proposed for the observed increased in exercise capacity in response to erythropoiesis-stimulating protein therapy in anemic patients with HF.⁶¹ However, the reported findings of the non-anemic subgroup of the FAIR-HF study suggest that other mechanisms may also be operative, since this subgroup demonstrated significant improvement in functional capacity with no significant change in hemoglobin values in response to iron therapy.

Potential Mechanisms of Improved Functional Capacity after Iron Supplementation

The favorable response to iron therapy in the non-anemic group of the FAIR-HF study may be attributable to the effects of iron supplementation on oxygen utilization in skeletal muscle. In HF, reduced cardiac output reserve, impaired regulation of skeletal muscle blood flow, and abnormalities in skeletal muscle mass and metabolism all appear to be important factors in the pathophysiology of exercise intolerance.⁶² Many of the major proteins responsible for oxygen transport and transfer to skeletal muscle (hemoglobin, myoglobin,

guanylyl cyclase), and for oxygen utilization in skeletal muscle (cytochromes and iron-sulfur enzymes involved in electron transport in mitochondria) require iron as an essential component for normal enzyme activity.⁶³

The effects of iron deficiency on skeletal muscle metabolism and exercise performance have been investigated in animals and human subjects.^{3,64} In rats fed a low iron diet, iron deficiency develops over several weeks and is associated with proportional reductions of hemoglobin and iron-containing cytochromes in skeletal muscle. In animal models of iron deficiency, exchange transfusion allows investigators to evaluate the independent effects of iron deficiency while maintaining experimental control of the hemoglobin level. In such experiments, endurance exercise performance increases in direct proportion to the increase in hemoglobin concentration in iron-replete rats, but not in iron-deficient rats.^{65–69} A comparable pattern of response to iron-deficiency was observed in an isolated limb perfusion study that allowed precise control of skeletal muscle oxygen delivery.⁷⁰ These findings suggest that changes in iron-dependent abnormalities in skeletal muscle substrate utilization reduce endurance performance independently of hemoglobin levels. Data from human subjects on the relation between iron stores and aerobic capacity are more difficult to interpret, as reduced hemoglobin levels confound interpretation of the direct effects of iron deficiency in skeletal muscle. Nonetheless, several small double-blind clinical trials suggest that iron deficiency is associated with impairment of exercise endurance at submaximal levels in non-anemic subjects with biomarkers of iron deficiency.^{71–75} Taken together with the experimental findings in animal models, these findings in human subjects support the hypothesis that improved functional capacity after intravenous iron supplementation in non-anemic HF patients may be partly attributable to increased oxidative capacity associated with repletion of iron-containing oxidative enzymes.

Potential Cardiovascular Effects of Subclinical Iron Overload

The findings in the non-anemic subgroup of the FAIR-HF study (**AQ ref here**) suggest that iron supplementation may be a novel form of therapy to improve endurance performance in patients with heart failure with biochemical evidence of iron deficiency regardless of their hemoglobin status. This hypothesis requires testing in prospective randomized trials and raises issues regarding the optimal targets for biomarkers of iron stores during repletion and maintenance therapy and the long-term safety of iron supplementation in the HF population. Several lines of evidence suggest that moderately increased iron stores without overt evidence of organ dysfunction associated with severe iron overload may confer an increased risk of cardiovascular events.

In biological systems, iron may exist in several different oxidative states, a property that allows iron to participate in redox reactions. These states include ferrous (+2), ferric (+3) and ferryl (+4) ions. Free ferrous ion is considered most reactive and can catalyze the formation of free hydroxyl radicals that can damage cells irreversibly and cause reperfusion injury.⁷⁶ Almost all iron is stored in non-reactive states by specific binding proteins, but increased pools of labile reactive iron has been linked to increased total body iron stores.^{77–84} Indeed this reversible interconversion of iron between the highly reactive ferrous form and stable ferric form (in labile and bound pools respectively), while essential to its life supporting functions, has also been deemed the culprit for many of its deleterious effects in humans, most notable among them potential causal relationships to carcinogenesis and atherogenesis.^{85–89}

NADPH oxidase can generate superoxide radicals in the vascular wall and thus initiate activation of a cascade of redox-sensitive transcription factors that contribute to the initial stages of atherosclerosis.^{89–93} The catalytic subunit of NADPH oxidase, cytochrome b₅₅₈

contains 2 molecules of heme.⁹⁴ Blockade of heme synthesis or removal of iron by heme-oxygenase-1 lowers NADPH oxidase activity.^{95,96} Human aortic endothelial cells incubated with $\geq 1 \mu\text{g/ml}$ of lipopolysaccharide showed a significant increase in NADPH oxidase activity, an effect that was eliminated by pretreatment with the iron chelator desferrioxamine.⁹⁷ Desferrioxamine also abolished the increase in NADPH oxidase activity induced by treatment of human aortic endothelial cells with tumor necrosis factor- α . Marx and colleagues studied the effects of non-transferrin bound iron on monocyte-endothelial interaction.⁹⁸ Non-transferrin-bound iron enhanced monocyte adhesion to endothelium and increased expression of vascular cell adhesion molecule-1, intracellular adhesion molecule-1 and endothelial selectins. These effects were completely inhibited by iron chelators and oxygen free radical scavengers.

A number of animal and human studies have focused on the presence of iron in atherosclerotic plaques. Watt and colleagues demonstrated increased iron content in newly formed atherosclerotic lesions in New Zealand white rabbits fed a 1% cholesterol diet.^{99,100} Repeated bleeding or treatment with iron chelators lead to a decrease in the average level of intralesion iron and also reduced lesion area.^{82,101} Lee and colleagues studied the effects of iron on atherosclerosis in Apo-E deficient mice. They observed that aortic iron content and area of atherosclerotic lesions were significantly smaller in the low-iron group when compared with that in the control group.⁷⁸ Redox-active iron was found to be present in human atherosclerotic lesions in a 1992 study.¹⁰²

The Iron-Heart Hypothesis

Sullivan first proposed in 1981 that reduction of iron stores protects against development of coronary heart disease.¹⁰³ His original hypothesis was based on epidemiological observations that demonstrated temporally coincident age-dependent increases in serum ferritin and increased coronary risk in men and women with heterozygous familial hypercholesterolemia (with onset of clinical heart disease 20 years later in women vs. men from the same family) and from the observation that hysterectomy without oophorectomy was associated with increased coronary heart disease risk in women.^{104–106} Negative findings of controlled clinical trials of hormone replacement therapy in post-menopausal women indirectly support this alternative iron hypothesis as an explanation for increased cardiovascular risk in post-menopausal women.^{107,108} Moreover this hypothesis has gained credence over years with direct support coming from studies demonstrating redox active iron in atherosclerotic plaques and studies demonstrating beneficial effects of iron depletion by chelation therapy or phlebotomy on plaque biology.^{79–83}

Epidemiological Evidence in Support of the Iron-Heart Hypothesis

A prospective cohort study carried out on Finnish population testing the association between serum ferritin and risk of coronary heart disease events provided support to the iron-heart hypothesis.¹⁰⁹ Men with serum ferritin levels $>200 \text{ ng/ml}$ had a more than 2-fold increased risk of acute myocardial infarction (MI) compared with those having lower levels. Numerous other retrospective and prospective studies have reported on the relationship between various biochemical markers of iron stores and cardiovascular risk. A meta-analysis of all the published data demonstrated no overall association between various measures of iron stores and cardiovascular risk in the pooled data set.¹¹⁰ Interpretation of findings of this meta-analysis is extremely limited as incomplete measures of body iron stores (including serum iron, transferrin saturation and dietary iron intake) were reported in most of the studies. The potential confounding effects of systemic inflammation or chronic diseases (cancer and chronic kidney disease) on markers of iron stores were also not considered in any of these studies.

Blood Donation and Cardiovascular Risk

Blood donation is known to deplete body iron stores. A single 500 ml standard blood donation contains approximately 250 mg elemental iron and reduces serum ferritin by nearly half.^{111,112} Accordingly, study of the association between blood donation and cardiovascular risk is potentially the most relevant test of the proposed iron hypothesis. Four published studies on the association between blood donation and cardiovascular risk are summarized in Table 3.^{113–116} Three of these 4 published studies reported a substantial reduction in cardiovascular risk in association with blood donation, whereas 1 study showed a neutral effect for all MI but a substantial reduction in the risk of fatal MI. Comparison between these 4 studies are difficult given differences in baseline characteristics of the study subjects, differences in the study design, and different methods for ascertaining cardiac events. None of these studies enrolled a substantial number of high-frequency blood donors with very low iron stores or documented donation-associated changes in biomarkers of iron stores in all of the study subjects. These results are also possibly confounded by the healthy donor effect due to the health screening criteria used by blood banks before donation.

The FeAST Trial was a prospective randomized controlled study in which phlebotomy was carried out to achieve iron reduction in patients with peripheral arterial disease in order to determine the effects of reduction in body iron stores on cardiovascular outcomes.¹¹⁷ The primary end-point was all-cause mortality and the secondary end-points were death plus nonfatal MI and stroke. The amount of blood to be removed by phlebotomy was calculated for each subject to achieve serum ferritin in the range of 20–60 ng/ml. Subjects completed 72% of planned phlebotomies with a mean serum ferritin of 79.7 ng/ml. Although the study results failed to demonstrate a significant effect of iron reduction on cardiovascular events in the entire study population (hazard ratio 0.85; 95% confidence interval (CI), 0.67–1.08; $p=0.17$), there was a significant reduction in all-cause mortality and in combined death plus non-fatal MI and stroke in a pre-specified subgroup analysis based on age. In the youngest age group (43–61 years), the reduction in all-cause mortality was 54% ($p < 0.019$) with a 57% reduction in death plus non-fatal MI and stroke ($p < 0.001$). The findings of this trial are clearly relevant to the originally proposed iron hypothesis, but interpretation is limited by the selection of a secondary prevention study population with established clinical vascular disease and the limited compliance with phlebotomy and consequent inability to achieve reduction of iron stores to the target levels proposed in the study design.

Zheng and colleagues studied the effects of serial blood donation in high-frequency and low-frequency blood donors age 50–75 years on flow-mediated endothelium-dependent dilation of the brachial artery, markers of iron stores and vascular inflammation.¹¹⁸ Forty high-frequency blood donors (defined by a verified history of ≥ 8 blood donations in the last 2 years) and 42 low-frequency blood donors (defined by a verified history of 1–2 donations in the past 2 years) were recruited. Serum markers of iron stores were significantly decreased in high-frequency donors when compared with low frequency donors (median serum ferritin 17 vs 52 ng/ml, $p < 0.001$, and mean transferrin saturation 22 vs 28%, $p=0.03$) but there was no difference in hemoglobin between groups. Serum levels of 3-nitrotyrosine, a marker of oxidative stress were significantly decreased in high frequency donors when compared with low frequency donors (35 vs 43 nmol/L, $P=0.02$). Flow-mediated dilatation in the brachial artery, a physiological marker of vascular endothelial function known to be independently linked to cardiovascular outcomes, was significantly increased in high-frequency blood donors when compared with that of low frequency blood donors ($5.5 \pm 2.6\%$ vs $3.8 \pm 1.6\%$, $p=0.0003$). In multivariate models, high-frequency blood donation remained significantly associated with increased flow-mediated dilatation in the brachial artery when adjusting for traditional and non-traditional cardiovascular risk factors.

Acute Vascular Effects of Altered Iron Availability

In a double-blind, placebo-controlled, cross-over trial, Zheng and colleagues studied the effects of dexrazoxane, an intracellular iron chelator, on vascular endothelial function in a model of transient homocysteine-induced endothelial dysfunction in normal subjects.¹¹⁹ Dexrazoxane infusion did not affect the rise in homocysteine after methionine ingestion but did abrogate homocysteine-induced reduction in flow-mediated dilatation in the brachial artery when compared with placebo. In a study involving both normal subjects and patients with coronary artery disease, Duffy and colleagues (AQ provide ref) studied forearm blood flow in response to endothelium-dependent vasodilation with methacholine and endothelium-independent vasodilation with nitroprusside before and after acute iron chelation with the extracellular iron chelator desferrioxamine. Methacholine-induced endothelium-dependent vasodilation was impaired in patients with coronary artery disease. Iron chelation with desferrioxamine was associated with improvement in endothelium-dependent response back towards normal values. The beneficial effect of desferrioxamine was linked to increased bioavailability of nitric oxide as the effects of desferrioxamine were mitigated by administration of the nitric oxide synthase inhibitor L-NMMA.

Zheng and colleagues studied the acute effects of intravenous iron sucrose 100 mg vs. matching placebo on vascular endothelial function in healthy volunteers.¹²⁰ Subjects in the treatment group were preloaded with oral methionine to increase homocysteine levels. Serum markers of iron stores, homocysteine and nitrotyrosine levels, and endothelium-dependent and -independent dilatation were measured before and after iron sucrose administration. Intravenous iron sucrose significantly increased percent transferrin saturation (iron sucrose 31 to 87% vs. placebo 21 to 32%) and non-transferrin bound iron (iron sucrose 0.54 to 2.54 μ M vs. placebo 0.64 to 0.66 μ M). Flow-mediated dilation in the brachial artery significantly decreased from baseline 1 hour after administration of iron sucrose when compared with placebo. There was no change in nitroglycerine-mediated vasodilatation and no difference in homocysteine and nitrotyrosine levels before and after administration of iron sucrose and placebo. Rooyackers and colleagues administered intravenous ferric saccharate to subjects with normal kidney function.¹²¹ Administration of iron was associated with a 4-fold increase in non-transferrin bound iron, increased generation of superoxide, and a transient decrease in vascular endothelial function.

Iron Supplementation and Risk of Cardiac Events in Chronic Kidney Disease

In 1998, Besarab et al reported that raising the hematocrit to normal values in hemodialysis patients with end-stage renal disease and chronic HF or ischemic heart disease significantly increased the risk of death or first non-fatal MI. In a randomized, prospective, open-label trial, 1233 patients with end stage renal disease on hemodialysis were enrolled.¹²² All patients had chronic heart failure or ischemic heart disease and a serum transferrin saturation of 20% or higher. Patients were randomized to 2 groups: in the normal hematocrit group (n=618), patients were administered increasing doses of erythropoietin to achieve hematocrit target of 42%; in the low hematocrit group (n=615), patients were administered erythropoietin to achieve hematocrit target of 30%. The study was stopped prematurely. There were a total of 183 deaths and 19 first nonfatal myocardial infarctions in the normal hematocrit group and 150 deaths and 14 nonfatal MIs in the low hematocrit group. (risk ratio, 1.3, 95 % CI, 0.9 –19). Intravenous iron dextran was administered in conjunction with erythropoietin to achieve hematocrit targets according to investigator discretion. In the high hematocrit group, survivors received less iron dextran over a 4-week period when compared with non survivors (152 $\pm$ 150 vs. 214 $\pm$ 190 mg, p<0.001). The risk of mortality was increased in subjects who received intravenous iron dextran when compared with those subjects who did not (relative risk 2.4, p<0.001). Since the randomization in this trial was to a treatment strategy, rather than to a specific single agent, it is not possible to determine whether higher

doses of erythropoietin, higher doses of intravenous iron, their combination or other factors may have contributed towards higher event rates observed in the normal hematocrit group in this study.

Conclusions

The optimal iron stores in HF patients remain unknown. Despite concerns about potential adverse effects of chronic iron overload, there are accumulating data indicating that short term administration of intravenous iron is associated with improved symptoms in HF patients. These important studies suggest that subclinical iron deficiency may occur commonly in heart failure patients and may contribute to symptoms related to reduced exercise capacity. Further work is needed to more accurately characterize iron stores in the HF population and determine the mechanisms of benefit and long-term risks associated with supplemental iron therapy. Until such data are available, the use of intravenous iron in heart failure patients should be limited to those with anemia and evidence of absolute or functional iron deficiency not responsive to oral iron supplementation.

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References

1. Failla ML. Trace elements and host defense: recent advances and continuing challenges. *J Nutr* 2003;133:1443S–7S. [PubMed: 12730439]
2. Gordeuk VR, Bacon BR, Brittenham GM. Iron overload: causes and consequences. *Annu Rev Nutr* 1987;7:485–508. [PubMed: 3300744]
3. Beard JL. Iron biology in immune function, muscle metabolism and neuronal functioning. *J Nutr* 2001;131:568S–579S. [PubMed: 11160590]
4. Beutler E, Hoffbrand AV, Cook JD. Iron deficiency and overload. *Hematology Am Soc Hematol Educ Program* 2003:40–61. [PubMed: 14633776]
5. Beutler E. Cell biology. “Pumping” iron: the proteins. *Science* 2004;306:2051–3. [PubMed: 15604397]
6. Nemeth E, Tuttle MS, Powelson J, et al. Heparin regulates cellular iron efflux by binding to ferroportin and inducing its internalization. *Science* 2004;306:2090–3. [PubMed: 15514116]
7. Ganz T. Heparin, a key regulator of iron metabolism and mediator of anemia of inflammation. *Blood* 2003;102:783–8. [PubMed: 12663437]
8. Swinkels DW, Janssen MC, Bergmans J, et al. Hereditary hemochromatosis: genetic complexity and new diagnostic approaches. *Clin Chem* 2006;52:950–68. [PubMed: 16627556]
9. Tomosugi N, Kawabata H, Wakatabe R, et al. Detection of serum hepcidin in renal failure and inflammation by using ProteinChip System. *Blood* 2006;108:1381–7. [PubMed: 16621968]
10. Nemeth E. Iron regulation and erythropoiesis. *Curr Opin Hematol* 2008;15:169–75. [PubMed: 18391780]
11. Collins JF, Wessling-Resnick M, Knutson MD. Heparin regulation of iron transport. *J Nutr* 2008;138:2284–8. [PubMed: 18936232]
12. McCord JM. Iron, free radicals, and oxidative injury. *J Nutr* 2004;134:3171S–3172S. [PubMed: 15514292]
13. Meyron-Holtz EG, Ghosh MC, Rouault TA. Mammalian tissue oxygen levels modulate iron-regulatory protein activities in vivo. *Science* 2004;306:2087–90. [PubMed: 15604406]
14. Hider RC. Nature of nontransferrin-bound iron. *Eur J Clin Invest* 2002;32 (Suppl 1):50–4. [PubMed: 11886432]
15. Richardson DR, Ponka P. The molecular mechanisms of the metabolism and transport of iron in normal and neoplastic cells. *Biochim Biophys Acta* 1997;1331:1–40. [PubMed: 9325434]

16. Ponka P. Cellular iron metabolism. *Kidney Int Suppl* 1999;69:S2–11. [PubMed: 10084280]
17. Ali A, Zhang Q, Dai J, et al. Calcein as a fluorescent iron chemosensor for the determination of low molecular weight iron in biological fluids. *Biometals* 2003;16:285–93. [PubMed: 12572687]
18. Breuer W, Hershko C, Cabantchik ZI. The importance of non-transferrin bound iron in disorders of iron metabolism. *Transfus Sci* 2000;23:185–92. [PubMed: 11099894]
19. Brittenham GM, Weiss G, Brissot P, et al. Clinical Consequences of New Insights in the Pathophysiology of Disorders of Iron and Heme Metabolism. *Hematology Am Soc Hematol Educ Program* 2000:39–50. [PubMed: 11701534]
20. Pietrangelo A. Physiology of iron transport and the hemochromatosis gene. *Am J Physiol Gastrointest Liver Physiol* 2002;282:G403–14. [PubMed: 11841990]
21. Andrews NC. Disorders of iron metabolism. *N Engl J Med* 1999;341:1986–95. [PubMed: 10607817]
22. Beard J. Iron status of free-living elderly individuals. *Am J Clin Nutr* 2001;73:503–4. [PubMed: 11237923]
23. Barron BA, Hoyer JD, Tefferi A. A bone marrow report of absent stainable iron is not diagnostic of iron deficiency. *Ann Hematol* 2001;80:166–9. [PubMed: 11320902]
24. Horwich TB, Fonarow GC, Hamilton MA, et al. Anemia is associated with worse symptoms, greater impairment in functional capacity and a significant increase in mortality in patients with advanced heart failure. *J Am Coll Cardiol* 2002;39:1780–6. [PubMed: 12039491]
25. Felker GM, Gattis WA, Leimberger JD, et al. Usefulness of anemia as a predictor of death and rehospitalization in patients with decompensated heart failure. *Am J Cardiol* 2003;92:625–8. [PubMed: 12943893]
26. Mozaffarian D, Nye R, Levy WC. Anemia predicts mortality in severe heart failure: the prospective randomized amlodipine survival evaluation (PRAISE). *J Am Coll Cardiol* 2003;41:1933–9. [PubMed: 12798560]
27. Kosiborod M, Smith GL, Radford MJ, et al. The prognostic importance of anemia in patients with heart failure. *Am J Med* 2003;114:112–9. [PubMed: 12586230]
28. Tanner H, Moschovitis G, Kuster GM, et al. The prevalence of anemia in chronic heart failure. *Int J Cardiol* 2002;86:115–21. [PubMed: 12400591]
29. Cromie N, Lee C, Struthers AD. Anaemia in chronic heart failure: what is its frequency in the UK and its underlying causes? *Heart* 2002;87:377–8. [PubMed: 11907017]
30. Ezekowitz JA, McAlister FA, Armstrong PW. Anemia is common in heart failure and is associated with poor outcomes: insights from a cohort of 12 065 patients with new-onset heart failure. *Circulation* 2003;107:223–5. [PubMed: 12538418]
31. Kalra PR, Bolger AP, Francis DP, et al. Effect of anemia on exercise tolerance in chronic heart failure in men. *Am J Cardiol* 2003;91:888–91. [PubMed: 12667582]
32. Szachniewicz J, Petruk-Kowalczyk J, Majda J, et al. Anaemia is an independent predictor of poor outcome in patients with chronic heart failure. *Int J Cardiol* 2003;90:303–8. [PubMed: 12957766]
33. Anand I, McMurray JJ, Whitmore J, et al. Anemia and its relationship to clinical outcome in heart failure. *Circulation* 2004;110:149–54. [PubMed: 15210591]
34. Silverberg DS, Wexler D, Iaina A. The importance of anemia and its correction in the management of severe congestive heart failure. *Eur J Heart Fail* 2002;4:681–6. [PubMed: 12453537]
35. Weiss G. Pathogenesis and treatment of anaemia of chronic disease. *Blood Rev* 2002;16:87–96. [PubMed: 12127952]
36. Anker SD, Volterrani M, Pflaum CD, et al. Acquired growth hormone resistance in patients with chronic heart failure: implications for therapy with growth hormone. *J Am Coll Cardiol* 2001;38:443–52. [PubMed: 11499736]
37. Torre-Amione G, Bozkurt B, Deswal A, et al. An overview of tumor necrosis factor alpha and the failing human heart. *Curr Opin Cardiol* 1999;14:206–10. [PubMed: 10358791]
38. Sharma R, Anker SD. Cytokines, apoptosis and cachexia: the potential for TNF antagonism. *Int J Cardiol* 2002;85:161–71. [PubMed: 12163221]
39. Kalra PR, Anagnostopoulos C, Bolger AP, et al. The regulation and measurement of plasma volume in heart failure. *J Am Coll Cardiol* 2002;39:1901–8. [PubMed: 12084586]

40. Anand IS, Chandrashekhar Y, Ferrari R, et al. Pathogenesis of oedema in chronic severe anaemia: studies of body water and sodium, renal function, haemodynamic variables, and plasma hormones. *Br Heart J* 1993;70:357–62. [PubMed: 8217445]
41. Iversen PO, Woldbaek PR, Tonnessen T, et al. Decreased hematopoiesis in bone marrow of mice with congestive heart failure. *Am J Physiol Regul Integr Comp Physiol* 2002;282:R166–72. [PubMed: 11742835]
42. Gossmann J, Burkhardt R, Harder S, et al. Angiotensin II infusion increases plasma erythropoietin levels via an angiotensin II type 1 receptor-dependent pathway. *Kidney Int* 2001;60:83–6. [PubMed: 11422739]
43. Pratt MC, Lewis-Barned NJ, Walker RJ, et al. Effect of angiotensin converting enzyme inhibitors on erythropoietin concentrations in healthy volunteers. *Br J Clin Pharmacol* 1992;34:363–5. [PubMed: 1457271]
44. Chatterjee B, Nydegger UE, Mohacsi P. Serum erythropoietin in heart failure patients treated with ACE-inhibitors or AT(1) antagonists. *Eur J Heart Fail* 2000;2:393–8. [PubMed: 11113716]
45. Ishani A, Weinhandl E, Zhao Z, et al. Angiotensin-converting enzyme inhibitor as a risk factor for the development of anemia, and the impact of incident anemia on mortality in patients with left ventricular dysfunction. *J Am Coll Cardiol* 2005;45:391–9. [PubMed: 15680718]
46. Nanas JN, Matsouka C, Karageorgopoulos D, et al. Etiology of anemia in patients with advanced heart failure. *J Am Coll Cardiol* 2006;48:2485–9. [PubMed: 17174186]
47. Witte KK, Desilva R, Chattopadhyay S, et al. Are hematinic deficiencies the cause of anemia in chronic heart failure? *Am Heart J* 2004;147:924–30. [PubMed: 15131553]
48. Westenbrink BD, Voors AA, van Veldhuisen DJ. Is anemia in chronic heart failure caused by iron deficiency? *J Am Coll Cardiol* 2007;49:2301–2. author reply 2302. [PubMed: 17560297]
49. Berry C, Norrie J, Hogg K, et al. The prevalence, nature, and importance of hematologic abnormalities in heart failure. *Am Heart J* 2006;151:1313–21. [PubMed: 16781243]
50. Katz SD, Rao R, Berman JW, et al. Pathophysiological correlates of increased serum tumor necrosis factor in patients with congestive heart failure. Relation to nitric oxide-dependent vasodilation in the forearm circulation. *Circulation* 1994;90:12–6. [PubMed: 8025986]
51. Levine B, Kalman J, Mayer L, et al. Elevated circulating levels of tumor necrosis factor in severe chronic heart failure. *N Engl J Med* 1990;323:236–41. [PubMed: 2195340]
52. Tang YD, Katz SD. Anemia in chronic heart failure: prevalence, etiology, clinical correlates, and treatment options. *Circulation* 2006;113:2454–61. [PubMed: 16717164]
53. Weiss G, Goodnough LT. Anemia of chronic disease. *N Engl J Med* 2005;352:1011–23. [PubMed: 15758012]
54. Ludwiczek S, Aigner E, Theurl I, et al. Cytokine-mediated regulation of iron transport in human monocytic cells. *Blood* 2003;101:4148–54. [PubMed: 12522003]
55. Nemeth E, Rivera S, Gabayan V, et al. IL-6 mediates hypoferremia of inflammation by inducing the synthesis of the iron regulatory hormone hepcidin. *J Clin Invest* 2004;113:1271–6. [PubMed: 15124018]
56. Bolger AP, Bartlett FR, Penston HS, et al. Intravenous iron alone for the treatment of anemia in patients with chronic heart failure. *J Am Coll Cardiol* 2006;48:1225–7. [PubMed: 16979010]
57. Toblli JE, Lombrana A, Duarte P, et al. Intravenous iron reduces NT-pro-brain natriuretic peptide in anemic patients with chronic heart failure and renal insufficiency. *J Am Coll Cardiol* 2007;50:1657–65. [PubMed: 17950147]
58. Okonko DO, Grzeslo A, Witkowski T, et al. Effect of intravenous iron sucrose on exercise tolerance in anemic and nonanemic patients with symptomatic chronic heart failure and iron deficiency FERRIC-HF: a randomized, controlled, observer-blinded trial. *J Am Coll Cardiol* 2008;51:103–12. [PubMed: 18191732]
59. Usmanov RI, Zueva EB, Silverberg DS, et al. Intravenous iron without erythropoietin for the treatment of iron deficiency anemia in patients with moderate to severe congestive heart failure and chronic kidney insufficiency. *J Nephrol* 2008;21:236–42. [PubMed: 18446719]
60. Anker SD, Comin Colet J, Filippatos G, et al. Ferric carboxymaltose in patients with heart failure and iron deficiency. *N Engl J Med* 2009;361:2436–48. [PubMed: 19920054]

61. Mancini DM, Katz SD, Lang CC, et al. Effect of erythropoietin on exercise capacity in patients with moderate to severe chronic heart failure. *Circulation* 2003;107:294–9. [PubMed: 12538431]
62. Katz SD, Zheng H. Peripheral limitations of maximal aerobic capacity in patients with chronic heart failure. *J Nucl Cardiol* 2002;9:215–25. [PubMed: 11986567]
63. Rouault TA, Klausner RD. Iron-sulfur clusters as biosensors of oxidants and iron. *Trends Biochem Sci* 1996;21:174–7. [PubMed: 8871401]
64. Haas JD, Brownlie Tt. Iron deficiency and reduced work capacity: a critical review of the research to determine a causal relationship. *J Nutr* 2001;131:676S–688S. discussion 688S–690S. [PubMed: 11160598]
65. Finch CA, Miller LR, Inamdar AR, et al. Iron deficiency in the rat. Physiological and biochemical studies of muscle dysfunction. *J Clin Invest* 1976;58:447–53. [PubMed: 956378]
66. Davies KJ, Maguire JJ, Brooks GA, et al. Muscle mitochondrial bioenergetics, oxygen supply, and work capacity during dietary iron deficiency and repletion. *Am J Physiol* 1982;242:E418–27. [PubMed: 7091311]
67. Davies KJ, Donovan CM, Refino CJ, et al. Distinguishing effects of anemia and muscle iron deficiency on exercise bioenergetics in the rat. *Am J Physiol* 1984;246:E535–43. [PubMed: 6742115]
68. Willis WT, Gohil K, Brooks GA, et al. Iron deficiency: improved exercise performance within 15 hours of iron treatment in rats. *J Nutr* 1990;120:909–16. [PubMed: 2199635]
69. Perkio MV, Jansson LT, Henderson S, et al. Work performance in the iron-deficient rat: improved endurance with exercise training. *Am J Physiol* 1985;249:E306–11. [PubMed: 2994490]
70. McLane JA, Fell RD, McKay RH, et al. Physiological and biochemical effects of iron deficiency on rat skeletal muscle. *Am J Physiol* 1981;241:C47–54. [PubMed: 6264804]
71. Brutsaert TD, Hernandez-Cordero S, Rivera J, et al. Iron supplementation improves progressive fatigue resistance during dynamic knee extensor exercise in iron-depleted, nonanemic women. *Am J Clin Nutr* 2003;77:441–8. [PubMed: 12540406]
72. Brownlie, Tt; Utermohlen, V.; Hinton, PS., et al. Marginal iron deficiency without anemia impairs aerobic adaptation among previously untrained women. *Am J Clin Nutr* 2002;75:734–42. [PubMed: 11916761]
73. Brownlie, Tt; Utermohlen, V.; Hinton, PS., et al. Tissue iron deficiency without anemia impairs adaptation in endurance capacity after aerobic training in previously untrained women. *Am J Clin Nutr* 2004;79:437–43. [PubMed: 14985219]
74. Hinton PS, Giordano C, Brownlie T, et al. Iron supplementation improves endurance after training in iron-depleted, nonanemic women. *J Appl Physiol* 2000;88:1103–11. [PubMed: 10710409]
75. McClung JP, Karl JP, Cable SJ, et al. Randomized, double-blind, placebo-controlled trial of iron supplementation in female soldiers during military training: effects on iron status, physical performance, and mood. *Am J Clin Nutr* 2009;90:124–31. [PubMed: 19474138]
76. Yuan XM, Li W. Iron involvement in multiple signaling pathways of atherosclerosis: a revisited hypothesis. *Curr Med Chem* 2008;15:2157–72. [PubMed: 18781942]
77. Sullivan JL. Is stored iron safe? *J Lab Clin Med* 2004;144:280–4. [PubMed: 15614249]
78. Lee TS, Shiao MS, Pan CC, et al. Iron-deficient diet reduces atherosclerotic lesions in apoE-deficient mice. *Circulation* 1999;99:1222–9. [PubMed: 10069791]
79. Facchini FS, Saylor KL. Effect of iron depletion on cardiovascular risk factors: studies in carbohydrate-intolerant patients. *Ann N Y Acad Sci* 2002;967:342–51. [PubMed: 12079862]
80. Lee HT, Chiu LL, Lee TS, et al. Dietary iron restriction increases plaque stability in apolipoprotein-e-deficient mice. *J Biomed Sci* 2003;10:510–7. [PubMed: 12928591]
81. Patt A, Horesh IR, Berger EM, et al. Iron depletion or chelation reduces ischemia/reperfusion-induced edema in gerbil brains. *J Pediatr Surg* 1990;25:224–7. discussion 227–8. [PubMed: 2303992]
82. Ponraj D, Makjanic J, Thong PS, et al. The onset of atherosclerotic lesion formation in hypercholesterolemic rabbits is delayed by iron depletion. *FEBS Lett* 1999;459:218–22. [PubMed: 10518022]

83. van Jaarsveld H, Pool GF. Beneficial effects of blood donation on high density lipoprotein concentration and the oxidative potential of low density lipoprotein. *Atherosclerosis* 2002;161:395–402. [PubMed: 11888523]
84. Kruszewski M. The role of labile iron pool in cardiovascular diseases. *Acta Biochim Pol* 2004;51:471–80. [PubMed: 15218543]
85. McCord JM. Iron, free radicals, and oxidative injury. *Semin Hematol* 1998;35:5–12. [PubMed: 9460805]
86. Gey KF. Prospects for the prevention of free radical disease, regarding cancer and cardiovascular disease. *Br Med Bull* 1993;49:679–99. [PubMed: 8221032]
87. Corti MC, Gaziano M, Hennekens CH. Iron status and risk of cardiovascular disease. *Ann Epidemiol* 1997;7:62–8. [PubMed: 9034408]
88. Wardman P, Candeias LP. Fenton chemistry: an introduction. *Radiat Res* 1996;145:523–31. [PubMed: 8619017]
89. Li L, Fink GD, Watts SW, et al. Endothelin-1 increases vascular superoxide via endothelin(A)-NADPH oxidase pathway in low-renin hypertension. *Circulation* 2003;107:1053–8. [PubMed: 12600921]
90. Rajagopalan S, Kurz S, Munzel T, et al. Angiotensin II-mediated hypertension in the rat increases vascular superoxide production via membrane NADH/NADPH oxidase activation. Contribution to alterations of vasomotor tone. *J Clin Invest* 1996;97:1916–23. [PubMed: 8621776]
91. Pagano PJ, Clark JK, Cifuentes-Pagano ME, et al. Localization of a constitutively active, phagocyte-like NADPH oxidase in rabbit aortic adventitia: enhancement by angiotensin II. *Proc Natl Acad Sci U S A* 1997;94:14483–8. [PubMed: 9405639]
92. Kriegelstein CF, Granger DN. Adhesion molecules and their role in vascular disease. *Am J Hypertens* 2001;14:44S–54S. [PubMed: 11411765]
93. Ley K, Huo Y. VCAM-1 is critical in atherosclerosis. *J Clin Invest* 2001;107:1209–10. [PubMed: 11375406]
94. Quinn MT, Gauss KA. Structure and regulation of the neutrophil respiratory burst oxidase: comparison with nonphagocyte oxidases. *J Leukoc Biol* 2004;76:760–81. [PubMed: 15240752]
95. Yu L, Zhen L, Dinanuer MC. Biosynthesis of the phagocyte NADPH oxidase cytochrome b558. Role of heme incorporation and heterodimer formation in maturation and stability of gp91phox and p22phox subunits. *J Biol Chem* 1997;272:27288–94. [PubMed: 9341176]
96. Taille C, El-Benna J, Lanone S, et al. Induction of heme oxygenase-1 inhibits NAD(P)H oxidase activity by down-regulating cytochrome b558 expression via the reduction of heme availability. *J Biol Chem* 2004;279:28681–8. [PubMed: 15123630]
97. Li L, Frei B. Prolonged exposure to LPS increases iron, heme, and p22phox levels and NADPH oxidase activity in human aortic endothelial cells: inhibition by desferrioxamine. *Arterioscler Thromb Vasc Biol* 2009;29:732–8. [PubMed: 19251588]
98. Marx JJ, Kartikasari AE, Georgiou NA. Can iron chelators influence the progression of atherosclerosis? *Hemoglobin* 2008;32:123–34. [PubMed: 18274990]
99. Minqin R, Watt F, Huat BT, et al. Correlation of iron and zinc levels with lesion depth in newly formed atherosclerotic lesions. *Free Radic Biol Med* 2003;34:746–52. [PubMed: 12633751]
100. Thong PS, Selley M, Watt F. Elemental changes in atherosclerotic lesions using nuclear microscopy. *Cell Mol Biol (Noisy-le-grand)* 1996;42:103–10. [PubMed: 8833671]
101. Minqin R, Rajendran R, Pan N, et al. The iron chelator desferrioxamine inhibits atherosclerotic lesion development and decreases lesion iron concentrations in the cholesterol-fed rabbit. *Free Radic Biol Med* 2005;38:1206–11. [PubMed: 15808418]
102. Smith C, Mitchinson MJ, Aruoma OI, et al. Stimulation of lipid peroxidation and hydroxyl-radical generation by the contents of human atherosclerotic lesions. *Biochem J* 1992;286 (Pt 3): 901–5. [PubMed: 1329721]
103. Sullivan JL. Iron and the sex difference in heart disease risk. *Lancet* 1981;1:1293–4. [PubMed: 6112609]
104. Sullivan JL. Are menstruating women protected from heart disease because of, or in spite of, estrogen? Relevance to the iron hypothesis. *Am Heart J* 2003;145:190–4. [PubMed: 12595829]

105. Stone NJ, Levy RI, Fredrickson DS, et al. Coronary artery disease in 116 kindred with familial type II hyperlipoproteinemia. *Circulation* 1974;49:476–88. [PubMed: 4813182]
106. Kannel WB, Hjortland MC, McNamara PM, et al. Menopause and risk of cardiovascular disease: the Framingham study. *Ann Intern Med* 1976;85:447–52. [PubMed: 970770]
107. Hulley S, Grady D, Bush T, et al. Randomized trial of estrogen plus progestin for secondary prevention of coronary heart disease in postmenopausal women. Heart and Estrogen/progestin Replacement Study (HERS) Research Group. *JAMA* 1998;280:605–13. [PubMed: 9718051]
108. Manson JE, Hsia J, Johnson KC, et al. Estrogen plus progestin and the risk of coronary heart disease. *N Engl J Med* 2003;349:523–34. [PubMed: 12904517]
109. Salonen JT, Nyyssönen K, Korpela H, et al. High stored iron levels are associated with excess risk of myocardial infarction in eastern Finnish men. *Circulation* 1992;86:803–11. [PubMed: 1516192]
110. Danesh J, Appleby P. Coronary heart disease and iron status: meta-analyses of prospective studies. *Circulation* 1999;99:852–4. [PubMed: 10027804]
111. Finch CA, Cook JD, Labbe RF, et al. Effect of blood donation on iron stores as evaluated by serum ferritin. *Blood* 1977;50:441–7. [PubMed: 884321]
112. Milman N, Kirchhoff M. The influence of blood donation on iron stores assessed by serum ferritin and hemoglobin in a population survey of 1359 Danish women. *Ann Hematol* 1991;63:27–32. [PubMed: 1878420]
113. Tuomainen TP, Salonen R, Nyyssönen K, et al. Cohort study of relation between donating blood and risk of myocardial infarction in 2682 men in eastern Finland. *BMJ* 1997;314:793–4. [PubMed: 9080998]
114. Meyers DG, Strickland D, Maloley PA, et al. Possible association of a reduction in cardiovascular events with blood donation. *Heart* 1997;78:188–93. [PubMed: 9326996]
115. Ascherio A, Rimm EB, Giovannucci E, et al. Blood donations and risk of coronary heart disease in men. *Circulation* 2001;103:52–7. [PubMed: 11136685]
116. Meyers DG, Jensen KC, Menitove JE. A historical cohort study of the effect of lowering body iron through blood donation on incident cardiac events. *Transfusion* 2002;42:1135–9. [PubMed: 12430669]
117. Zacharski LR, Chow BK, Howes PS, et al. Reduction of iron stores and cardiovascular outcomes in patients with peripheral arterial disease: a randomized controlled trial. *JAMA* 2007;297:603–10. [PubMed: 17299195]
118. Zheng H, Cable R, Spencer B, et al. Iron stores and vascular function in voluntary blood donors. *Arterioscler Thromb Vasc Biol* 2005;25:1577–83. [PubMed: 15961703]
119. Zheng H, Dimayuga C, Hudaihed A, et al. Effect of dexrazoxane on homocysteine-induced endothelial dysfunction in normal subjects. *Arterioscler Thromb Vasc Biol* 2002;22:E15–8. [PubMed: 12117747]
120. Zheng H, Huang X, Zhang Q, et al. Iron sucrose augments homocysteine-induced endothelial dysfunction in normal subjects. *Kidney Int* 2006;69:679–84. [PubMed: 16395269]
121. Rooyackers TM, Stroes ES, Kooistra MP, et al. Ferric saccharate induces oxygen radical stress and endothelial dysfunction in vivo. *Eur J Clin Invest* 2002;32 (Suppl 1):9–16. [PubMed: 11886426]
122. Besarab A, Bolton WK, Browne JK, et al. The effects of normal as compared with low hematocrit values in patients with cardiac disease who are receiving hemodialysis and epoetin. *N Engl J Med* 1998;339:584–90. [PubMed: 9718377]

Figure 1.

A schematic illustration of the effects of alterations in iron availability on distribution of body iron stores and clinical manifestations. Shaded areas in the 3 compartments of iron storage (erythropoiesis, other iron containing enzymes, and other tissue iron storage) indicate the relative states of repletion or depletion for each compartment. The distribution of total body iron is highly regulated to provide sufficient quantities for incorporation into hemoglobin during erythropoiesis and synthesis of other iron-containing enzymes. To minimize potentially toxic effects of reactive iron species, almost all iron is bound to hemoglobin, other iron-containing enzymes, or to iron storage proteins ferritin and transferrin. In iron deficient anemia due to nutritional deficiency and/or blood loss, iron loss from all compartments is detected by low serum ferritin values and clinical manifestations of microcytic anemia and associated syndromes. Repletion of iron stores with oral or intravenous iron administration will reverse these clinical manifestations. The iron hypothesis proposes that repletion of iron just above the minimum stores necessary for erythropoiesis may be associated with reduced risk of coronary heart disease events. In functional iron deficiency associated with inflammatory diseases, iron availability for incorporation into hemoglobin and other iron containing enzymes is reduced, but total body iron stores may actually be increased in the storage compartment. Serum ferritin may be elevated, and the anemia is typically not microcytic. Administration of supplemental iron in these disease states can increase erythropoiesis and raise hemoglobin levels, but the full range of biological effects associated with further increases of non-heme iron stores is uncertain. Studies from chronic kidney disease populations indicated that excess iron stores could be associated with increased risk of coronary heart disease events. Iron overload states associated with hemochromatosis or transfusion dependent anemias are associated with end-

organ damage. These disease states are characterized by very large amounts of increased storage iron and very high values of serum ferritin, well above those typically observed in response to intravenous iron administration. Data on risk of coronary heart disease have yielded conflicting findings

Biomarkers of iron stores in normal conditions and in disease states of increased or decreased body iron content

Table 1

	Normal	Iron Overload	Iron deficiency anemia
Marrow Iron stores	2-3 +	4+	0
Transferrin Iron-Binding Capacity (ug/dl)	330±30	<300	>390
Plasma Ferritin (ng/ml)	100±60	>300	<10
Plasma iron (ug/dl)	115±50	>175	<40
Transferrin saturation (%)	35±15	>60	<10
Sideroblasts (%)	40-60	40-60	<10
RBC protoporphyrin (ug/dl RBC)	30	30	200
Erythrocytes	Normal	Normal	Microcytic/Hypochromic

Adapted from Herbert V: Anemias. In Paige DM (ed.): Clinical Nutrition. St Louis, CV Mosby, 1988, p 593

Table 2
Summary of clinical trials of intravenous iron supplementation in heart failure subjects

Investigators	Study design	Study duration	Patient number, n	Salient Patient characteristics	Major Findings
Bolger et al ⁵⁶	Prospective, uncontrolled	12-day treatment phase; follow-up at 92± 6 days	17	Systolic heart failure patients NYHA II and III; Hb≤ 12g/dl	<p>1 <i>NYHA</i>: at follow-up all patients were in class II ($P<0.02$)</p> <p>2 <i>MLHF</i>: Improved from 33± 19 To 14 ±19 ($p=0.02$)</p> <p>3 <i>Mean 6-min walk distance</i>: improved from 242± 78 to 286± 72 ($p=0.01$)</p>
Toblli et al ⁵⁷	Randomized, placebo controlled study	6 months	40, Iron sucrose group=20 Placebo group= 20	Anemic heart failure patients with renal insufficiency; NYHA class II–IV; LVEF≤ 35%	<p>1 <i>LVEF, MLHF questionnaire, 6-min walk test</i> improved significantly in Fe-sucrose group ($p<0.01$)</p>
Okonko et al ⁵⁸	Randomized controlled study	18 weeks	35, Iron sucrose=24 control=11	CHF patients with NYHA class II or III; Exercise limitation (peak VO2/KG < 18ml/kg/min); Hb <12.5 g/dl for anemic or 12.5–14.5 g/dl for non-anemic	<p>1 <i>Exercise capacity</i>: in anemic group, absolute peak VO2 significantly improved ($p=0.02$); peak VO2 also improved ($p=0.009$).</p> <p>2 <i>NYHA</i>: In 44% of patient ($n=8$) NYHA class also improved. Improvement at week 18 in anemic patients was significant ($p= 0.048$)</p>
Usmanov et al ⁵⁹	Prospective controlled study	6 months	Iron=32; Control group=22	CHF Class III and IV; renal insufficiency; Hb <11g/dl	<p>1 <i>NYHA</i> = 9 patients in NYHA III showed improvement to II (47.4%, $P<0.01$). No change in class IV</p> <p>2 Most echocardiographic parameters improved significantly in both class III and IV</p>
Anker SD et al ⁶⁰	Randomized, placebo-controlled prospective study	26 weeks	N=459 Placebo group:155 Study drug: 304	CHF class II or III; LVEF ≤40% (Class II) or ≤45% (class III); functional iron deficiency (ferritin <100 ng/ml or ferritin 100–299 ng/mL with transferrin saturation <20%); Hb: 9.5–13.5 g/dL	<p>1 <i>Primary end-points</i> (week-24):</p> <p>a. <i>PGA</i>: 50% pts in IV iron group showed improvement ($p<0.001$)</p> <p>b. 47% improved to NYHA class I or II ($p<0.001$)</p> <p>2 <i>Secondary end-points</i> (week 4 & 12): PGA and NYHA improved significantly ($p<0.001$) 6-min walk test and quality of life assessment also showed significant improvement ($p<0.001$)</p>

CHF, chronic heart failure; Fe, iron; Hb, hemoglobin; LVEF, left ventricular ejection fraction; MLHF, Minnesota Living with Heart Failure (Questionnaire); NYHA, New York Heart Association; PGA, Patient Global Assessment

Table 3

Population studies to estimate the risk of cardiovascular events in blood donors.

Study	Design	Population	n	Cardiovascular Event Rates			OR/RR (95%CI)	p-values
Tuomainen et al. ¹¹³	Prospective cohort study	Middle-aged Finnish men	2682	AMI	Donors (n=153) 1.2 events/1000 pt-yr	Non-donors (n= 2529) 19 events/1000 pt year	RR=0.14 (CI=0.02-0.97)	0.048
Meyers et al. ¹¹⁴	Prospective cohort study	Men and women > 40 years of age from the Nebraska diet heart study (1985-1987)	4762 (889 lost to follow-up; 18 refused to participate)	AMI	Donors, n=655 1.8/1000 pt-yrs	Non-Donors n= 3200 3.6/1000 pt-yrs	OR=0.51 (CI= 0.26-0.93)	0.017
Ascherio et al. ¹¹⁵	Prospective	Men from the Health professional follow-up study with no history of prior cardiovascular events	38, 244	Total Fatal and non-fatal MI	Donors n=3680 (10-20 donations) 5.1 events/1000 pt-yrs	Non-donors n=10735 5.0 events/1000 pt-yrs	RR=1	NS
Meyers et al. ¹¹⁶	Retrospective	Men > 39 years, women > 50 years from a local blood center	Frequent donors, n=1508 Casual Donors n=1508	Fatal MI MI, PTCA and CABG	0.8 events/1000 pt-yr Frequent blood donors (>1 donation in 3 year period) 6.0 %	1.3 events/1000 pt-yr Casual donors (1 donation during 3 years) 9.6 %	RR=0.61 (CI=0.43-0.87) OR=0.56 (CI=0.42-0.74)	< 0.01 P<0.001

AMI, Acute Myocardial Infarction; CABG, Coronary Artery Bypass Graft; CI, Confidence Interval; OR, Odds Ratio; PTCA, Percutaneous Transluminal Coronary Angiography; RR, Relative Risk