

# The hazards of iron loading†

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Excessive or misplaced tissue iron now is recognized to pose a substantial health risk for an extensive array of endocrinological, gastrointestinal, infectious, neoplastic, neurodegenerative, obstetric, ophthalmic, orthopedic, pulmonary and vascular diseases. Ingested, injected, inhaled and decompartmentalized iron contributes not only to disease, but also to aging and mortality. Iron is dangerous by catalyzing free radical formation and by serving as an essential nutrient for microbial and neoplastic cell invaders. Our body cells exhibit wide variation in sensitivity to iron toxicity. Efficacy of our iron withholding defense system is modulated by numerous environmental, behavioral and genetic factors. A notable variety of methods for prevention and therapy of iron toxicity are now becoming available.

## Introduction

During the past half century, research scientists and clinical investigators have reported that excessive/misplaced iron in specific tissue, cellular and subcellular sites can promote a vast array of acute and chronic illnesses. Iron now is recognized to be a serious risk factor for endocrinological, gastrointestinal, infectious, neoplastic, neurodegenerative, obstetric, ophthalmic, orthopedic, pulmonary and vascular diseases. Moreover, the metal accelerates development of sarcopenia and, as well, can be teratogenic (Tables 1 and 2). Increasingly, it is becoming

apparent that “life was designed to exist at the very interface of iron deficiency and iron sufficiency.”<sup>1</sup>

Iron toxicity ensues from two distinct attributes of the metal. The first is its ability, in redox active form, to generate oxygen-based free radicals. Although short-lived, the latter can destroy proximate cells by initiating lipid peroxidation, enzyme denaturation, polysaccharide depolymerization and DNA strand rupture.<sup>2</sup> The second attribute of iron that potentiates disease is its role as an essential growth factor for nearly all pathogenic bacteria, fungi and protozoa<sup>3</sup> and for all neoplastic cells.<sup>4</sup> In response, hosts have evolved a highly structured iron withholding defense system that continuously attempts to purge ‘free’ iron from all body tissues. Furthermore, during episodes of microbial and neoplastic cell invasion, the system promptly lowers the amount of liganded iron in body fluids (Table 3).

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† Electronic supplementary information (ESI) available: Tables detailing: (1) Conditions for which excessive/misplaced iron can be a risk factor; (2) Association of iron with morbidity; (3) Iron withholding defense system; (4) Conditions that can result in iron loading and (5) Enhanced mortality associated with elevated transferrin iron saturation. See DOI: 10.1039/c0mt00023j

## Cellular variation in iron sensitivity

Research in the past score of years has considerably expanded awareness of the pervasiveness of iron toxicity. Not surprisingly, minimal toxic concentrations of iron vary with cell type. Disparity in iron susceptibility might arise from differing capacities to synthesize antioxidants or ferritin. Anterior pituitary cells provide an example of very high sensitivity. In culture, these cells are killed by 1  $\mu\text{M}$  iron whereas cultured hepatocytes survive in the presence of 10–100  $\mu\text{M}$  iron.<sup>60</sup> Thus young thalassemic-major children who begin to load iron early in life are deprived of growth hormone; as adults, they are short in stature. Hemochromatotic persons who begin to load iron in late teens or early adulthood can have abnormally low levels of gonadotrophic hormones that result in amenorrhea and low fertility in women; testicular atrophy in men.

In untreated hemochromatotic patients, the pancreas can accumulate 50–100 times its normal amount of iron. The exocrine cells of the gland acquire the heavier portion of the metal yet remain functional. In contrast, the beta cells acquire less iron but are highly sensitive to the metal with resulting impairment of insulin synthesis.<sup>61</sup>



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*by restricting their supply host of iron. He pioneered in the discovery of the host iron withholding defense system. Recently, he has been concerned with iron as a risk factor in osteoporosis and in complications of pregnancy.*

**Table 1** Conditions for which excessive/misplaced iron can be a risk factor

<b>Aging</b> Sarcopenia	<b>Obstetric</b> gestational diabetes GRACILE syndrome neonatal hemochromatosis pre-eclampsia teratogenicity	<b>Pediatric</b> Down syndrome epilepsy sudden infant death
<b>Dermal</b> Porphyria cutanea tarda Rosacea		<b>Pulmonary</b> cystic fibrosis ozone lung injury pneumoconiosis PAP
<b>Endocrine</b> Diabetes Endometriosis Growth deficiency Hypogonadism Hypothyroidism	<b>Oncologic</b> breast colorectal esophageal hepatic Kaposi sarcoma leukemia lung	<b>Renal</b> aminoglycoside & vancomycin toxicity
<b>Hepatic &amp; Intestinal</b> Cirrhosis Inflammatory bowel Steatosis/NAFLD Viral hepatitis	<b>Ophthalmic</b> cataract macular degeneration	<b>Vascular</b> arrhythmia atheroclerosis cardiomyopathy hypertension ischemic stroke venous leg ulcer
<b>Infectious</b> Microbial & viral infections of all body systems	<b>Orthopedic</b> gout hemophilic synovitis osteoarthritis osteoporosis	
<b>Neurologic</b> ALS Alzheimer's depression Friedreich's ataxia Huntington's multiple sclerosis Parkinson's peripheral neuropathy PKAN prion disease	<b>Otologic</b> aminoglycoside toxicity	

Modified from Table 1.<sup>5</sup>

**Table 2** Association of iron with morbidity**Iron, by itself, can initiate the condition**

arrhythmia,<sup>6</sup> cardiomyopathy,<sup>7</sup> growth deficiency,<sup>8</sup> hemophilic synovitis,<sup>9</sup> hypogonadism,<sup>10</sup> lung cancer,<sup>11</sup> osteoporosis,<sup>12</sup> pneumoconiosis,<sup>13</sup> teratogenicity<sup>14</sup>

**Iron can be a cofactor in promoting the condition**

Alzheimer's,<sup>15</sup> atherosclerosis,<sup>16</sup> bacterial infections,<sup>3</sup> diabetes,<sup>17</sup> endometriosis,<sup>18</sup> esophageal adenocarcinoma,<sup>19</sup> fungal and protozoan infections,<sup>20</sup> gout,<sup>21</sup> hepatoma,<sup>22</sup> multiple sclerosis,<sup>23</sup> osteoarthritis,<sup>24</sup> oto- and renal toxicity,<sup>25</sup> ozone lung injury,<sup>26</sup> peripheral neuropathy,<sup>27</sup> viral infection<sup>28</sup>

**Iron deposits are observed in condition-associated tissues sites**

basal ganglia in PKAN,<sup>29</sup> bronchoalveolar fluid in PAP,<sup>30</sup> hepatocytes in cirrhosis/NAFLD and viral hepatitis,<sup>31</sup> lens in cataract,<sup>32</sup> microglia in Huntington's,<sup>33</sup> mitochondria in Friedreich's ataxia,<sup>34</sup> pulmonary secretions in cystic fibrosis,<sup>35</sup> retina in macular degeneration,<sup>36</sup> skeletal muscle in aging,<sup>37</sup> skin cells in rosacea,<sup>38</sup> soft tissue in Kaposi's sarcoma,<sup>39</sup> substantia nigra in Parkinson's,<sup>40</sup> thyroid in hypothyroidism<sup>41</sup>

**Body iron loading is associated with above normal incidence of condition**

amyotrophic lateral sclerosis,<sup>42</sup> breast cancer,<sup>43</sup> colorectal cancer,<sup>44</sup> depression,<sup>45</sup> Down syndrome,<sup>46</sup> epilepsy,<sup>47</sup> gestational diabetes,<sup>48</sup> GRACILE syndrome,<sup>49</sup> hypertension,<sup>50</sup> inflammatory bowel disease,<sup>51</sup> ischemic stroke,<sup>52</sup> leukemia,<sup>53</sup> pre-eclampsia,<sup>54</sup> porphyria cutanea tarda,<sup>55</sup> prion disease,<sup>56</sup> sudden infant death syndrome,<sup>57</sup> venous leg ulcer<sup>58</sup>

**Maternal antibodies can impair fetal iron metabolism**

Fetal or neonatal death in neonatal hemochromatosis<sup>59</sup>

Modified from Table 2.<sup>5</sup>

Bone remodeling offers an example of tissue metabolism controlled by cells of quite dissimilar iron sensitivity. Osteoclastic cells that constantly destroy bone are of macrophage lineage and thus are highly resistant to iron. On the other hand, osteoblastic cells that constantly rebuild bone are of fibroblast lineage and are exceptionally sensitive to iron. Consequently, osteoporosis is a common manifestation in persons who have any one of the various iron loading disorders.<sup>12</sup>

A biologic process whose phases differ markedly in iron susceptibility is that of mammalian gestation. During a specific time in the differentiation (embryogenesis) phase, but not at all during the fetal growth phase, iron is teratogenic.<sup>14</sup> For instance, in pregnant mice, intraperitoneal injection of ferric gluconate on day seven caused extensive encephalic damage to 1% of the fetuses, on day eight to 33% and on day nine to 17%. Exposure to iron on either day six or ten caused no damage.<sup>14</sup>

**Table 3** Iron withholding defense system**Constitutive components***Siderophilins*

Transferrin in plasma, lymph, cerebrospinal fluid

Lactoferrin in secretion of lachrymal and mammary glands and of pulmonary, gastrointestinal and genital tracts.

*Ferritin within host cells***Processes induced at times of microbial and neoplastic cell invasions***Prompt reduction in plasma iron and in dietary iron absorption*

Increased hepatic synthesis of hepcidin (ferroportin inactivator) to suppress release of iron by macrophages and duodenal enterocytes.

Increased synthesis of macrophage ferritin and decreased synthesis of ferroportin.

*Removal of iron from sites of invasion*

Release of neutrophils from bone marrow into circulation and then into invasion sites. Release of apolactoferrin from neutrophil granules followed by binding of iron at invaded sites. Macrophage scavenging of hololactoferrin. Hepatic release of haptoglobin and hemopexin (to bind extracellular hemoglobin and hemin).

*Macrophage suppression of microbial iron metabolism*

Synthesis of lipocalins (to bind siderophores), of nitric oxide (to disrupt invader iron metabolism), and of Nramp1 (to withhold growth-essential iron from invaders).

*Induction in B Lymphocytes of immunoglobulins (I<sub>G</sub>)*Synthesis of I<sub>G</sub> that bind microbial cell surface receptors for either ferrated siderophores, ferrated siderophilins, hemoglobin or heme.Modified from Table 2.<sup>3</sup>**Contravention of iron withholding defense**

Conditions that can compromise the iron withholding defense system are listed in Table 4. Generally, a combination of factors modulates the abnormal condition. For example, in hereditary hemochromatosis (HH), about one fourth of persons who are homozygous for an HFe mutated gene, C282Y, develop organ damage.<sup>62</sup> Among environmental factors that enhance penetrance are ingestion of excessive quantities of red meat, ascorbic acid and ethanol. Genes that modify extent of iron loading in HH include those that encode hepcidin, hemojuvelin, transferrin receptor 2 and ceruloplasmin.<sup>63</sup> Moreover, the amount of iron-induced organ damage also would be influenced by gene products involved in antioxidant defense, fibrogenesis and tissue repair.

Lipid metabolism also can have an impact on iron toxicity. In rabbits that had elevated cholesterol and iron, significantly more atherosclerosis was observed than in those animals that had an increase in only one of the two factors.<sup>64</sup> In a cohort of 3410 U.S. adults, followed for 18 y, at baseline 27.3% had LDL > 160 mg/dl and 1.64% had transferrin iron saturation > 55%.<sup>65</sup> In persons with either elevated LDL or iron, the relative risk (RR) of dying with atherosclerosis was

1.4 or 1.57, respectively. If both factors were high, the RR was 5.21.

In a set of 6558 U.S. adults, aged >40 y, followed for 20 y, the RR for Alzheimer's disease was 1.35 if only iron was elevated and 1.6 if only total cholesterol was high.<sup>66</sup> However, if both factors were raised, the RR was 3.0. Fortunately, in HH, persons homozygous for the C282Y mutation tend to have low levels of LDL and of cholesterol.<sup>67</sup>

**Ingested iron**

Excessive iron, by itself, has been observed to cause abnormal conditions. For instance, feeding iron to iron replete human toddlers caused substantial suppression of normal growth.<sup>68</sup> In young healthy women given 98 mg iron/d for six weeks, malonylaldehyde, a marker of oxidative stress, accumulated in their plasma.<sup>69</sup> Moreover, moderately increased dietary iron can be a risk factor for such conditions as hepatitis C and non-alcoholic fatty liver disease (NAFLD).<sup>70</sup>

Increasing awareness of the association of ingestion of iron from food or supplements with development of obesity has been summarized recently.<sup>71</sup> For example, in a group of

**Table 4** Conditions that can result in iron loading**Genetic disorders**

Aceruloplasminemia

African siderosis

Hemochromatosis

Types 1, 2, 3, 4

Hemoglobinopathies

Sickleemia

Thalassemia

Myelodysplasia

**Behavioral factors**

Ingestion of excessive amounts: heme (red meat); ethanol; iron supplements; ascorbic acids; iron-adulterated food

Inhalation of items that contain or are contaminated with iron asbestos; coal; sand; tobacco smoke; industrial sources of iron; urban &amp; subway air particulates

Injection of excessive amounts of iron saccharates; whole blood or erythrocytes

**Pathological conditions**

Release of body iron into plasma in hemolytic conditions; hepatitis; myelo-ablative conditioning prior to cell/tissue transplant

Splenectomy

25 obese, menstruating females as compared with non-obese controls, serum ferritin values were elevated by 56% and plasma malonylaldehyde by 240%.<sup>72</sup> In a different set of 20 menstruating women, serum ferritin values were doubled as compared with 20 non-obese controls.<sup>72</sup> Serum ferritin values of 248 post-menopausal women and men, 58 + 3.9 y, were directly correlated with levels of visceral and subcutaneous fat.<sup>73</sup>

In a well designed study of iron and oxidative stress, 105 elderly males in Crete were compared with 139 men of similar age in the Netherlands.<sup>74</sup> The former had significantly lower serum levels of iron, ferritin and transferrin (Tf) iron saturation. In the Crete group, the mean level of serum ferritin (sFt) was 67.9 (56–81.3) ng/ml; in the Netherlands group, 137.1 (117.1–160.4) ng/ml ( $p < 0.0001$ ). Not surprisingly, the proportion of men with a history of stroke or cancer was four times lower among men from Crete as compared with those from the Netherlands. Unexpectedly, differences in rate of diabetes and of levels of total and HDL cholesterol were not significant. The authors noted that the Mediterranean diet is known for its abundance of fruit and vegetables and the use of olive oil.

Citrus fruits, however, fail to protect against iron loading<sup>75</sup> apparently because of their high content of ascorbic acid which facilitates duodenal absorption of non-heme iron. In contrast, in non-citrus fruits, as well as in tea, coffee and cocoa, numerous polyphenolic compounds bind non-heme iron and strongly limit its absorption.<sup>76</sup> Moreover, polyphenols have recently been observed to impede basolateral heme iron release from Caco-2 cells. Accordingly, such suppression might also occur *in vivo* in humans. Additionally, intake of grains that contain phytate, a strong iron chelator, causes suppression of non-heme iron loading.<sup>76</sup>

In the U.S., iron compounds are added to various foods. The U.S. Food and Drug Administration requires addition of 20 mg iron to each pound of flour and 13–26 mg iron/pound to corn meal, farina and rice. Moreover, further quantities of iron, in some cases quite excessive, are added by some food processors to most ready-to-eat cereals.<sup>77</sup> The rationale for addition of iron to foods in the U.S. is questionable. A survey of iron status in the U.S. reported that 99.8% of men, 98.1% of post-menopausal women and 97.4% of pre-menopausal women were iron replete.<sup>78</sup> Subsequently, 7.3% of U.S. adults were observed to have Tf iron saturation values  $>45\%$ .<sup>79</sup> Normal values are 25–35%. It has generally been assumed that ingestion of foods artificially suffused with iron is safe because of hepcidin inactivation of ferroportin. Nevertheless, there exists much variation in the amount of iron loading among the general population. In “normal” persons, the serum level of ferritin (the defense protein synthesized primarily to sequester iron) varies from 20 ng/ml to as much as 200 ng/ml in women and 300 ng/ml in men.

## Injected iron

Parenteral administration of iron is even more hazardous than ingested iron because the metal bypasses the natural barrier to excessive enteral uptake provided by hepcidin. A 500 ml unit of whole blood contains 200–250 mg iron, mainly

in the erythrocytes. Patients who receive either chronic or acute transfusions are at risk. For instance, 511 patients with myelodysplasia or other hematopoietic disorders who received chronic transfusion therapy were compared with a corresponding number of non-transfused patients.<sup>80</sup> Receipt of transfusions was associated with onset of cardiomyopathy (RR = 1.62), conduction/rhythm disorders (RR = 4.18), hepatic diseases (RR = 3.31) and diabetes (RR = 5.06).

Among transfusion-dependent thalassemia-major TM patients, cardiac complications account for the greatest amount of mortality.<sup>81</sup> Endocrinopathies are the most frequent cause of morbidity. Sixty percent of TM patients develop one or more of diabetes, hypogonadism, hypothyroidism or hypoparathyroidism.<sup>81</sup> Osteoporosis and enhanced infection, especially with *Klebsiella*, also are observed in TM patients.<sup>12</sup>

Particularly perilous to recipients of whole blood or packed erythrocytes are blood products stored more than fourteen days.<sup>81</sup> Marked increase in non-transferrin bound iron and a decrease in antioxidant capacity occurs in the products. Prolonged storage of the blood is associated with an increased risk of nosocomial infection. Unfortunately, the average length of storage of transfused erythrocytes for critically ill U.S. patients has been reported to be 21 days; one fourth of the units are held more than 30 days.<sup>82</sup>

The antithesis to iron loading *via* blood transfusion is iron withdrawal by bleeding. Accordingly, most women are able to delay acquisition of dangerous levels of body iron (as compared with men) for about thirty years. In normal menstruation, approximately one unit (500 ml) is shed per year. Post-menopausal women and all men can possibly achieve this quantity of bleeding by ingesting an aspirin tablet daily. Intake of at least 1.3 g aspirin tablet/d caused intestinal bleeding of one unit/y.<sup>83</sup>

In a study of regular blood donation, 30 men, 30–60 y, achieved a 67% reduction in serum ferritin by four phlebotomies/y.<sup>84</sup> Several investigators have recorded an association of regular blood donation with lowering of cardiovascular and other chronic disorders.<sup>85</sup> In a comparison of 40 high frequency voluntary blood donors ( $>$ eight donations in past two y) with 42 low frequency donors (1–2 donations in past 2 y), the former had a decreased iron burden (mean sFt 17 ng/ml vs. 52 ng/ml;  $p < 0.001$ ), decreased oxidative stress and enhanced flow mediated dilation.<sup>86</sup> In both groups, hematocrits were similar.

In a cohort of 636 adults who donated blood periodically to maintain a low level of body iron (monitored by keeping sFt at 25–60 ng/ml) for an average of 4.5 y, 38 cancers of various types developed. In a control group of 641 adults who donated no blood, 60 cancers occurred (RR = 0.65;  $p = 0.017$ ).<sup>87</sup> In this study, significant reduction in all cause mortality and in vascular pathology occurred in the youngest age quartile of blood donors.<sup>88</sup> In a study of HH patients, impaired endothelial function was normalized by phlebotomies.<sup>89</sup>

## Inhaled iron

In addition to enteral and parenteral routes of iron uptake, the metal can enter the body *via* inhalation. Industrial workers who inhale ferrous materials (*e.g.* welders, foundry

workers, iron miners) have been observed to have significantly increased risk for pulmonary tract neoplasms as compared with workers in non-ferriferous employment.<sup>11</sup> Moreover, inhaled iron can be disseminated *via* macrophages to other body tissues to result in systemic iron loading.<sup>90,91</sup>

Non-industrial sources of inhaled iron include urban air particulates, especially in subways,<sup>92</sup> and active and passive tobacco smoke. Leaves of tobacco contain approximately 100 µg iron/g; one-tenth percent is present in mainstream smoke. As compared with non-smokers, alveolar macrophages of light smokers have a fourfold increase in iron content; heavy smokers a nine-fold increase.<sup>11</sup> Host iron accumulation is aided by particulate matter in tobacco smoke.<sup>93</sup>

Tobacco smoke is a complex mixture of >4000 organic and inorganic compounds, many of which contribute to health risks.<sup>94</sup> If the tobacco plant is cultivated in soil contaminated by cadmium and/or nickel, these carcinogens may be found in the smoke.<sup>95</sup> In contrast, iron (an essential nutrient for plant growth) consistently must be present in or added to soil, and is a constant component of the smoke. In addition to sharing carcinogenic activity with cadmium and nickel, iron also is a notable risk factor of smokers for impaired hearing, bacterial and viral infections, osteoporosis and vascular disease.<sup>96</sup>

Systemic diseases that elevate pulmonary tract iron result in lowered resistance to lung infections. For instance, the progression of human immunodeficiency virus (HIV) toward its more advanced stages is accompanied by increasing iron deposits. The metal occurs in excess in macrophages, microglia, endothelial cells and monocytes.<sup>97</sup> The greatly increased incidence in HIV patients of such iron dependent infections as tuberculosis, cryptococcosis and pneumocystosis is well recognized.<sup>98</sup>

Patients with cystic fibrosis (CF) have elevated iron in pulmonary epithelial cells. The metal stimulates replication and biofilm synthesis of *Pseudomonas* and other bacterial pathogens in the lungs.<sup>35,99</sup>

A group of 14 non-smoking persons, 53 + 4 y, occupationally exposed to asbestos for >10 y were compared with ten non-smoking unexposed controls, 40 + 3 y, by bronchoscopy with broncho-alveolar lavage.<sup>100</sup> In the lavage samples of the exposed group, iron concentration was doubled. As an indication of engagement in the exposed persons of iron withholding defense, Tf receptors were increased two to threefold, lactoferrin and ferritin were raised threefold.

Inhalation of such iron-rich asbestos fibers as crocidolite and amosite can result in bronchogenic carcinoma, interstitial fibrosis, and malignant mesothelioma. In cultures of macrophages, pulmonary epithelial cells, or mesothelial and endothelial cells, asbestos cytotoxicity can be attenuated by iron chelators.<sup>101</sup> Chrysotile fibers, with very low iron, are not cytotoxic. In contrast, synthetic, iron loaded chrysotile induced DNA strand breakage and lipoperoxidation.<sup>102</sup>

Furthermore, non-asbestos silicate fibers can be highly efficient in mobilizing host iron deposits. Erionite, an aluminium silicate, is more strongly carcinogenic than either crocidolite or amosite. Its cage-like structure with a large surface area enables it to mobilize a large quantity of host iron.<sup>103</sup> Unlike native, iron-free erionite, the iron coated fibers cause DNA

damage similar to that of crocidolite but at a much lower ratio of iron to mg of fiber.

## Decompartmentalized iron

Iron can be released from its normal intracellular milieu by hemolysis or other insults to cell integrity. For example, hyperferremia results from destruction of erythrocytes in malaria and bartonellosis, and from damaged hepatocytes in viral hepatitis. In each of the three diseases, patients are at enhanced risk for iron-dependent bacterial pathogens such as *Salmonella*.<sup>3</sup> Moreover, the elevated serum iron level in hepatitis carriers predisposes to development of hepatocellular carcinoma.<sup>104</sup> Recipients of cell or tissue transplants who have been myeloblastically conditioned with cytotoxic chemotherapy temporarily suppress erythropoiesis and become hyperferremic with increased susceptibility to infection.<sup>105</sup>

## Senescence and mortality

As cells age, their iron content increases. During senescence of cultures of human primary fibroblasts, intracellular iron rose tenfold.<sup>106</sup> In aging rats, gastrocnemius muscle had an increase of 23% of non-heme iron content with an accompanied heightened oxidative damage of 85%.<sup>107</sup> During the lifetime of rats, non-heme iron loading of mitochondria rose substantially especially between 29 and 37 months of age.<sup>108</sup> Caloric restriction was associated with lower rates of both non-heme iron accumulation and development of sarcopenia.<sup>109</sup>

Persons whose iron loading is untreated tend to have abbreviated lifespans. In a cohort of 10 714 U.S. adults, 2.3% had Tf iron saturation values >55%.<sup>110</sup> During the subsequent 22 years, their rate of death was significantly more rapid than those with values <55% (Table 5). Except for a higher amount of diabetes and hepatic cirrhosis in the elevated iron population, causes of death in the two groups were similar.

In a similar investigation, 9,229 persons, 35–70 y, were followed for 12 y.<sup>79</sup> Those with both elevated iron and high dietary iron died more rapidly than did persons with either one of the raised factors. Evidence for iron-enhanced mortality is provided also by an analysis of NHANES III data for sFt.<sup>111</sup> The mean sFt for U.S. men crested at age 60 y at 150 ng/ml. The accelerated loss of iron loaded men resulted at age 90 y of a mean sFt of 80 ng/ml.

**Table 5** Enhanced mortality associated with elevated transferrin iron saturation (Tfsat)

Years after Tfsat observation	Mortality (%)	
	Tfsat < 55%	Tfsat > 55%
0	0	0
4	3.5	6
8	8	16
12	13	24
16	20	32
20	25.5	34
22	26.5	36

Data from Fig. 1.<sup>101</sup>

## Perspectives

An increasingly large number of clinical reports on the manifold dangers of iron loading emphasize the importance of including Tf iron saturation percentage and serum ferritin tests in routine biochemical profiles of both male and female adults of all ages. Presently, routine screening of blood pressure and lipid levels affords specific evidence of the possible necessity of diet modification and long term use of specific drugs. Similarly, evidence of iron loading would guide the patient to modify nutritional behavior if necessary and, as well, to consider blood donations.

Fortunately, clinical interest is growing in regard to the association between elevated sFt level and chronic disease. For instance, in men, 20–49 y, sFt >150 ng/ml is markedly associated with lack of cardiovascular fitness.<sup>112</sup> In middle aged adults, each increase of 10 ng/ml of sFt has been observed to be correlated with a 3% heightened risk of carotid atherosclerotic plaque.<sup>113</sup> Moreover, in a group of 2443 persons, 45–79 y (1200 women), the association of elevated sFt with carotid atherosclerosis was potentiated by high LDL cholesterol.<sup>114</sup> However, a positive association between cardiovascular illness and high sFt has not been observed in every study.<sup>115</sup>

The percentage of iron loaded young or middle-aged adults consistently is higher in men than in women. For example, in a survey of recreational marathon runners, excessive body iron was found in 15% of 127 men, but in only 4.7% of 43 women.<sup>116</sup> In efforts to maximize performance, iron supplements are commonly consumed by athletes. Upon retirement from competition, any excess deposits of iron should be removed promptly by phlebotomies or oral iron chelator drugs.

Consumption of iron-containing vitamin or nutritional supplements is observed not only in athletes. In a group of 1277 U.S. iron replete adults with advanced but stable peripheral arterial disease, 17.6% used iron supplements.<sup>85</sup> After counseling, usage fell to 6.7%. Furthermore, there is special concern for the injudicious administration of supplemental iron to pregnant women in the absence of medical evidence of iron deficiency. Iron loading during the embryonic phase (3–9 wks) can be teratogenic.<sup>14</sup> During the fetal growth phase (10–39 wks) elevated iron is a well documented risk factor for gestational diabetes<sup>54</sup> and pre-eclampsia.<sup>48</sup>

If an item is beneficial in small dose (as is iron), should it not be even more valuable as the dose is increased? To pharmacologists, the answer is beware. An unlimited increase of a useful drug generally will be detrimental. Similarly, physiologists will cite the toxicity of elevated levels of oxygen; and nutritional scientists, the hazards of high quantities of vitamins A, C, and E.

But acknowledgment of iron toxicity is difficult for some nutritional scientists, clinicians and laypersons. After all, iron is symbolic of strength, vigor, power. Surely, we are told, routine consumption of non-prescription iron products by all who are tired or pregnant, as well as continual ingestion by all consumers of iron-“fortified” foods, can result in unlimited good and can do no harm. Recently, for example, all women in Western countries were strongly urged to alter their diet so as to raise their body iron values to those of men.<sup>117</sup> Not acknowledged by these authors is the increasing accumulation

of medical evidence that the naturally lower iron values of women are optimal not only for child bearing but also for outliving men.

*Pharmacotherapy.* The natural iron chelators, transferrin (Tf) and lactoferrin (Lf), are now available for therapy at specific iron loading body sites. Transferrin has been extracted from pooled human serum, de-ironed and extensively purified.<sup>118</sup> Recombinant human Tf, with iron chelating ability comparable to the natural product, is secreted by a strain of the yeast *Pichia pastoris*.<sup>119</sup> Recombinant human Lf is produced by strains of a variety of yeasts, molds, plant cells and animal cells.<sup>120</sup> Each of the two proteins will become increasingly useful in treatment of severe sepsis, either as an adjunct of or as a replacement for antibiotics. As in natural defense, Tf will be most useful in vascular infections, Lf in non-vascular sites.

During the past century, numerous low molecular mass compounds that possess iron binding substituents have been developed into useful drugs. Examples include anti-inflammatory salicylates, anti-infective tetracyclines and anti-neoplastic anthracyclines. However, emphasis has not been placed on their possible ability to alter iron homeostasis of either hosts or invaders.<sup>121</sup> In recent decades, large numbers of novel compounds with iron chelating sites have been screened for possible therapeutic use in transfusional iron loaded patients.<sup>122</sup> With additional research, some of these might be found effective in infection, cancer or chronic disorders associated with iron-induced oxidative damage.

To be medically acceptable, an iron chelator must neither deplete tissues of essential iron nor redistribute the metal to such sensitive organs as heart and brain. The chelator must be highly specific for iron and be far less avid for zinc, copper and manganese. The compound should not serve as a siderophore. In the chelated form, it should be excreted in either bile or urine. Useful compounds should be easily administered (orally, if possible) and be available at reasonable cost.

Presently, three iron chelators are effective in patients with transfusional iron overload: deferoxamine (DFO), deferiprone (DP) and deferasirox (DFRA). Increasingly, DFO and DP are used together. The latter is smaller and more lipophilic and easily enters host cells. It then transfers, in plasma, the metal to DFO, the stronger chelator.<sup>123</sup> DFO and DP have brief plasma elimination half-lives; 5–10 min and 47–134 min, respectively. Not orally absorbed, DFO is infused subcutaneously three nights/wk. DP is taken orally, three times/d. In contrast, the long half life of DFRA,  $19 \pm 6.5$  h, permits efficacy with a single daily dose.

When serum ferritin value is lowered below 500 ng/ml, DFO and DFRA may begin to withdraw metabolically essential iron.<sup>124</sup> In contrast, DP appears to be safer than DFO and DFRA for use in non-iron loaded patients. For example, in a murine model, DP protects against retinal degeneration.<sup>125</sup> The drug also has shown efficacy in reduction of renal damage in human patients who have diabetic nephropathy.<sup>126</sup> At one-fourth of the dosage employed in iron loaded patients, DP has been effective in alleviation of Friedreich's ataxia apparently by removing excessive mitochondrial iron.<sup>127</sup> In iron loaded patients, DFRA is effective in therapy of systemic fungal infections, either alone or with liposomal amphotericin B.<sup>128</sup>

Clinical experience with long term use of DFO, DP and DFRA in transfusional iron loaded human patients has spanned four, two and one half, and one decade, respectively. With each drug, side effects have been reported in a minority of patients. In some cases, dose reduction has helped; in others, patients are shifted to an alternate drug. Because DFO is a siderophore, reawakening of latent infections can occur. Ophthalmic, otic and neurologic toxicity of DFO also has been documented.<sup>129</sup> Arthralgia can develop in either DFO or DP patients.

In patients on DP, possible agranulocytosis requires weekly monitoring of peripheral leukocytes. Plasma zinc level, especially in diabetic patients, should be assayed annually.<sup>129</sup> In rats and rabbits, DP is teratogenic.<sup>130</sup> Thus it would be prudent to recommend avoidance of DP by pregnant patients especially during the embryonic growth phase.

In patients receiving DFRA, careful monitoring of kidney function is essential. Elevation of creatinine may be alleviated by reduction in dose. However, acute renal failure and Fanconi syndrome have been reported.<sup>131</sup>

**Immunoprophylaxis.** Suppression of infections can be obtained also by development of vaccines that stimulate the host to specifically inhibit bacterial iron acquisition. For example, uropathogenic strains of *Escherichia coli*, to survive in the urinary tract, must upregulate their outer membrane receptors for siderophoric iron. Production of the receptor antigen is obtained by growing the pathogen in low iron culture medium.<sup>132</sup> An iron acquisition protein of *Staphylococcus aureus* induces an excellent immune response in cattle and appears to be a good vaccine candidate to prevent bovine mastitis.<sup>133</sup> A receptor for human Tf that is utilized by *Neisseria meningitidis* has been identified as a vaccine candidate. Persons recovering from invasion by this pathogen have high antibody titer to the receptor whereas non-infected adults have almost none. In animal models, the vaccine has demonstrated efficacy.<sup>134</sup>

## Conclusions

(1) For excellent health and longevity, body iron levels should be maintained at a moderately low value consistent with the metabolic functions of the metal.

(2) Routine monitoring of iron values (along with those for blood pressure and lipids) is essential for health maintenance.

(3) Our iron withholding defense system can not only be damaged by ingestion of excessive iron, but also by injection, inhalation and decompartmentalization of the metal.

(4) Published studies are validating methods for prevention and therapy of iron toxicity.

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This review is dedicated to Leo R. Zacharski, MD, in recognition of his pioneering demonstrations that targeted reduction in human iron burden substantially can prevent development of neoplasms and vascular damage.

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