

Safety aspects of iron in food

Klaus Schümann

Walther-Straub-Institut für Pharmakologie und Toxikologie
der Ludwig-Maximilians-Universität München

postal address:

Prof. Dr. med. Klaus Schümann

Walther-Straub-Institut für Pharmakologie und Toxikologie

Nußbaumstr. 26

80336 München

Tel.: +49 89 5160-7223

Fax.: +49 89 5160-7207

e-mail: K.Schuemann@lrz.uni-muenchen.de

key words: iron, USL, toxicity, cardiovascular, cancer, risk

abbreviations: AMI = acute myocardial infarction; BBM = brushborder membrane; DGE = Deutsche Gesellschaft für Ernährung; DFO = desferrioxamine; DMT-1 = divalent metal transporter at the duodenal BBM; GP = general practitioner, HFE = HLA = defect gene in hereditary hemochromatosis; IREG = Iron regulated transporter at the basolateral membrane of duodenal enterocytes; IRE = iron responsive element; IRP = iron regulatory protein; LDL = low density lipoprotein; LOAEL = lowest observed adverse effect level; NOAEL = no observed adverse effect level; NTA = nitrilotriacetic acid, RDI = recommended daily intake; ROS = reactive oxygen species; TfR = transferrin receptor; USL = upper safe level.

Introduction

Evolution's efforts to avoid the hazards of micronutrient deficiency and excess are shared by corresponding regulatory efforts. The "Recommended Daily Allowance" (RDI) is supposed to meet the nutritional requirement of 97.5% of the healthy population. For certain micronutrients beneficial health effects are observed at intake levels that exceed the requirement extensively. For example, selenium and vitamin E seem to counteract free radical damage. Thus, to optimise health it may be desirable to consume such micronutrients in excess of the RDI. This development urges regulatory agencies to establish an "Upper Safe Level" (USL) to mark the border line between favourable health affects and toxicity. The first step in this process is hazard identification, i.e. to spot adverse effects that may lead to persistent impairment of important physiological functions. The next step is to derive a dose-response relationship for such adverse effects and to estimate a "No Adverse Effect Level" (NOAEL) or on a "Lowest Adverse Effect Level" (LOEAL) from available data. An USL can be derived on this basis by use of appropriate safety factors and uncertainty factors. These factors make provisions for sensitive subpopulations and for doubts regarding the reliability of available data [1].

Dose-response assessments for iron present several problems. Firstly, bioavailability of non-haem iron varies by a factor of 10, depending on the composition of the diet [2]. In addition, haem-iron absorption is substantially higher than that of non-haem iron. It contributes 20 – 35% to total iron intake [3] corresponding to 1 – 5 mg Fe/d which is a considerable range of variation. Due to these uncertainties, it is difficult to assess absorbed iron quantities at known intake levels. This problem is circumvented by relating the endpoints of iron toxicity to the iron status of the organism.

2.5 – 4.0%. This data shows that the RDIs and corresponding recommendations of the DFG are solidly based and that iron deficiency in industrialised countries has become less of a general health problem. This is in part due to a diet favourable for iron bioavailability and partly to the mechanisms of iron homeostasis.

Regulation of intestinal iron absorption

Depending on its weight the iron-adequate human body contains 2.2–3.8 g of iron (distribution see Tab.1) [7]. Intestinal iron absorption is geared to the demand via adaptation of a specific receptor population in the duodenal brushborder membrane [8]. The iron supply from the blood to the immature enterocytes in the duodenal crypts modulates cellular IRP activity (details see below) which, in turn, seems to regulate the expression of a divalent metal transporter (= DMT-1) in the brushborder membrane (BBM). *This process gears non-heme iron uptake from the intestinal lumen to the body's iron requirements [9]. A second mechanism seems to modulate the function of DMT-1 in the BBM and the dislocation of this carrier from the membrane in response to recent iron absorption, which corresponds to a mucosal block [10]. Duodenal non-heme iron absorption is further modulated by the IREG transporter at the enterocytes basolateral membrane [11]. Body iron stores also influence the expression of a BBM reductase that reduces ferric food iron to the ferrous form to make it available to DMT-1. Heme iron, in contrast, is not taken up via DMT-1 but passes the BBM as an Fe-porphyrine complex [12] either by virtue of its lipophilicity or by use of a putative heme carrier. Heme iron, thus, circumvents down-regulation of the bottle neck for non-heme iron absorption in iron overload. The porphyrine ring is cleaved by heme oxygenase in the enterocytes [13]. The released iron enters the enterocytes' non-heme iron pool. Although there is some adaptation of heme binding to the BBM [14] and of heme oxygenase activity, down regulation of heme-iron absorption at adequate supply levels is by far not as effective as that of non-heme iron absorption [15].*

High iron contents in the diet will decrease Zn absorption which, however, needs pharmaceutical iron doses (22). The iron impact on Cu absorption is even less pronounced.

Iron homeostasis

Excess iron is sequestered into ferritin, from where it can be mobilised when the demand increases. A small family of messenger RNAs is equipped with specific base loops (= iron responsive element (IRE)) that bind to "iron regulatory protein" (IRP) in the cytosol. Binding of IRP to the IRE in the 5' untranslated region (UTR) of the mRNA represses ferritin translation; binding to multiple IREs in the 3'UTR, in contrast, stabilises the mRNA of the transferrin receptor. The IRP-IRE-affinity is increased in iron-deficient cells (Fig.1). This mechanism modulates the expression of ferritin and transferrin receptors and, thus, regulates the availability of low molecular iron in the cells [23]. Unfortunately, the same mechanism will also mobilise iron from ferritin and import it via transferrin receptors in situations of oxidative stress. Nitric oxide and superoxide radicals increase the IRP-IRE affinity [24]. Thus, cells under oxidative stress will behave like in iron deficiency: it will release iron from the stores and increase iron uptake from the extracellular space (Fig. 1). This misguided process may amplify oxidative stress. Besides, homeostasis can be overwhelmed by excess iron intake. In all these situation, iron may cause substantial harm to the body, depending on dose and duration of excess iron exposure.

Damage after acute iron exposure

Ingestion of an acute overdose of pharmaceutical iron preparations is known to cause local corrosion in the stomach and upper small intestine. High absorbed iron quantities cause shock symptoms due to postarteriolar dilatation, capillary leakage and heart failure. High iron concentrations damage hepatic mitochondria, leading to liver cell

[29]. Such cases are inappropriate to derive a LOAEL or NOAEL, as they deal with excessively high intakes and did not exclude hereditary hemochromatosis.

Homozygotes for hereditary hemochromatosis are a highly sensitive sub-population for iron overload. This disease is due to a defect in the HLA (=HFE) gene which increases iron absorption 2 – 3 fold. It is observed in 0.3 – 0.5% of the Caucasian population. The male/female ratio among homozygotes is 1:1, for serious manifestations it is about 5:1, though, if untreated. This is because women have constantly higher iron losses via menstrual bleeding. In hereditary hemochromatosis, transferrin saturation is frequently close to 100%. Free, bleomycin-detectable iron is often present and parenchymal cells can be damaged e.g. by Fenton chemistry.

Bantu siderosis is caused by excess oral iron intake with home-brewed beer, fermented in iron pots in sub-Saharan Africa. Regular consumption of such beer may serve as a model for chronic dietary iron overload, leading to hepatic cirrhosis and diabetes. The bioavailability of such iron is high. It parallels that of FeCl_3 salts and is approx. 1/3 of that of FeSO_4 . The average intake in the patients was 50 - 100 mg Fe/d [30]. A cumulative excess intake of approx. 28 g Fe was derived as a threshold for the development of fibrosis. The average consumption seems to have been less than 2 l of beer/d which is too little for clear-cut alcoholism. Still, substantial alcohol intake in some patients might have supported the development of cirrhosis. Iron absorption in cirrhotic subjects was not higher than in healthy controls [30], although a genetic failure distinct from HLA-linked hemochromatosis was later discussed to have increased intestinal iron absorption [31]. These findings lead to a LOAEL of 50 - 100 mg Fe to cause cirrhosis in the long run. Applying a safety factor of 2 seems sufficiently conservative, because we deal with a putatively sensitive sub-population, for which a genetic disposition and alcohol intake are discussed. Therefore, a USL of 25 - 50 mg Fe/d should be derived from this data. Bantu siderosis has become historical, because iron pots used in the 1950s and 60s have meanwhile been replaced by aluminium pots.

stopped by radical scavengers, e.g. α -tocopherol, lycopene or β -carotene, which are consumed in the process and must be regenerated or replaced (Fig. 2) [35].

Because of these mechanisms, the question: "what quantity of iron in the body is safe" is closely related to the question: "which conditions disturb the equilibrium between pro- and antioxidative mechanisms". In principle, such disturbances can, firstly, be caused by an increased basal generation rate of reactive oxygen species (ROS). This is observed under pathological conditions, such as inflammation and ischemia/reperfusion injury. Secondly, local iron availability will aggravate oxidative stress of any origin via Fenton chemistry. As excess iron cannot be excreted from the body, it is either incorporated into heme to serve specific functions or it is sequestered and stored in ferritin. Hemoxygenase 1 is induced in ischemia and liberates iron from heme [36]. Iron can also be liberated from ferritin under oxidative stress [37]. In addition, oxidative stress increases free iron concentration via IRP activation (Fig. 1) which promote the conversion of less-reactive to more-reactive radicals. Thirdly, the cell may run out of radical scavengers due to increased consumption under oxidative stress. In all 3 cases the equilibrium is shifted substantially to the pro-oxidative side.

Free iron seems to play a crucial role in the amplification of oxidative stress. The process is a self-amplifying vicious cycle. There is no place in the organism, neither in ferritin nor in the heme-bound form, where excess iron can be safely stored to prevent Fenton reaction to occur. A possible exception is haemoglobin-bound iron in erythrocytes, because these cells have highly developed intrinsic antioxidative defence mechanisms. Thus, excess iron stores can be compared to a powder keg: it is not wise to carry along a higher supply than needed for necessary requirements.

Iron promoted oxidative stress on the organ level: If these mechanistic considerations are valid, substitution or chelation of free iron should influence free radical production in ischemia-reperfusion injury. On the organ level, it should modulate lipid peroxidation and impair organ function. Indeed, all these effects were observed in corresponding animal

ischemia/reperfusion events, implying that high iron stores are disadvantageous and may aggravate the sequels of such events.

Epidemiological studies on the impact of iron status on AMI risk: Salonen et al., [48,49] found a 2.2-fold increased risk for AMI in Eastern Finish males at serum ferritin levels $>200 \mu\text{g/L}$. An additional intake of 1 mg Fe/d was calculated to increase cardiovascular risk by 5%. These assumptions have been criticised in so far as the impact of inflammatory diseases on serum ferritin should have been controlled more rigidly and the relationship between circulating ferritin and AMI risk should have been pursued for the whole range of values. Besides, it was argued, that heterozygotes for hemochromatosis with their slightly increased average iron stores should have an increased cardiovascular risk as well, if a ferritin value $>200 \mu\text{g/L}$ were to increase that risk. This had, indeed, not been observed before 1993; however, all of these requirements were fulfilled in the more recent studies on the subject as reported below.

No association was found between cardiovascular risk, on the one hand, and serum iron concentrations, transferrin saturation and total iron binding capacity (TIBC), on the other hand, in two subsequent reviews [50;51]. A transferrin saturation $>60\%$, however, accompanied an increased risk for AMI which fell short of statistical significance [51]. Serum ferritin did not seem to be associated with cardiovascular risk in a study from Iceland [52], although the risk was increased in people with low TIBC representing high iron stores. Diets high in highly bioavailable heme iron increased cardiovascular risk, but went along with high fat and cholesterol intake as well [53]. Morrison et al. [54] found a significantly increased risk for AMI at serum iron concentrations $> 175 \mu\text{g/dL}$ which supports the iron-hypothesis.

Most of these follow-up studies published between 1994 and 1997 did not support Salonen's findings. However, they raised concern about the choice of adequate biomarkers to define body iron stores. Serum iron concentrations and transferrin saturation represent no advantage over ferritin concentration; on the contrary, they are

AMI-risk. This is supported by a decreased AMI risk in blood donors, who have low body iron stores [60,61]. The latter study controlled the health of donors and non-donors alike to be comparable. Another trial showed significantly higher serum ferritin values in coronary artery disease patients with an early onset (age <46 years) as compared to others with a late onset (age >74 years) [62]. The most recent and best controlled epidemiological studies, thus, support the iron hypothesis of increased cardiovascular risk. However, to finally prove the causal association, an intervention trial would be needed; such would be highly unethical in the light of available data.

AMI-risk in heterozygotes for hereditary hemochromatosis: Due to slightly increased iron absorption, 25 – 30% of heterozygotes have elevated iron stores. The iron excess is available early in life [63] which might increase cardiovascular risk. Indeed, there seems to be a family aggregation of hereditary cardiovascular risk in 5-7% of the male population that cannot be explained by conventional risk factors such as diabetes and hypertension [56]. In a prospective nested case control study in 11,631 people, 531 cardiovascular casualties were compared to 535 controls that died from other causes. Among the cardiovascular deaths, 7.2% were heterozygous for hemochromatosis as compared to 4.1% in controls. In controls, hypertension and smoking increased cardiovascular risk by 2.2 as compared to 40.0 in heterozygotes [64]. This risk increment should be due to extra iron, because this is what characterises the heterozygotes. The self-amplifying effect of iron on ROS production described on the molecular level is likely to be the responsible mechanism.

In a prospective cohort study in 1150 Finnish males 6.7% were found to be heterozygous for hemochromatosis [65]. Ten point four percent of heterozygotes had myocardial infarction as compared to 5.6% in the control population. Again, smoking increased the risk significantly. An increment in serum ferritin of 100 µg/L increased AMI risk by 52%. These results take away one of most the severe objections to the iron-hypothesis of cardiovascular risk, namely that heterozygotes are not affected.

peritoneum and circulates in the blood. After glomerular filtration iron finds an optimal environment for Fenton reaction in the lumen of the proximal renal tubules. At this location, lipid peroxidation showed to be clearly associated with the induction of renal cancer [72]; both could be significantly reduced by vitamin E administration [73]. Chelated Fe^{3+} effectively catalysed DNA single and double strand breaks producing 8-OHGua as a marker for oxidative DNA damage [74] and inter-strand cross-links by site specific mechanisms [34]. Induction of lung cancer after asbestos inhalation relates to the iron content of different asbestos fibres [75] and iron and nitric oxide seem to be necessary for the mutagenic action of asbestos fibres [76]. Moreover, a high iron supply supports tumor cell proliferation [77]. Ample iron supply is likely to promote tumor growth whereas iron restriction can reduce cancer induction [78]. As a defence against iron-induced oxidative stress human tumor cells produce glutathion S-transferase pi. These mechanisms explain promoting effects of iron on dimethylnitrosamine- and dimethylbenz(a)anthracene-induced tumors in animal experiments [79]. Oxidative stress, moreover, may accelerate the conversion of pre-carcinogens to carcinogens [80].

Epidemiological observations: Stevens [81] first reported an association between iron stores and cancer; this was criticised because of the small differences in transferrin saturation (+2.4%) and total iron binding capacity (-1.5%) between cancer and non-cancer groups. Also, the risk of cancer induction was not stratified according to different periods of follow-up duration. It was suspected that these iron status indicators have been increased as a consequence of cancer that was present at the start of the study [82]. This objection was overcome by longer follow-up periods, after which the correlation between high iron stores and cancer was still present [83]. In addition, a dose-response relationship between transferrin saturation and relative risk for cancer was derived. The risk increased progressively at transferrin saturation levels above 40%. The cancer frequency in oesophagus, bladder, and in the colorectal area increased in parallel to iron stores. Dietary phytates, themselves known to decrease iron availability, seem to protect

intake of highly available iron with a food item was observed in Bantu siderosis, where chronic intakes between 50 – 100 mg Fe/d caused liver cirrhosis, diabetes and heart failure. Applying a safety factor of 2 would lead to an USL of 25 – 50 mg Fe/d *for conventional iron toxicity as an endpoint*

However, reports on an association between iron stores and cardiovascular and cancer risk urge to extend the data base for risk assessment. The molecular mechanism of how iron accumulates in specific tissues under oxidative stress and amplifies it are well investigated. Under this perspective the question “how much iron is safe” needs to be rephrased. We should ask to what extent we can rely on antioxidative mechanisms to prevent cardiovascular diseases and cancer as sequels of iron-amplified oxidative stress. In healthy humans, the answer is stochastic; in the vast majority of cases, antioxidative defence is sufficient. However, high iron stores seem to shift the odds to the worse as is suggested by the more recent, intensely controlled epidemiological studies [58,59, 64,65]. Looking at animal experiments and clinical data, the balance between oxidative stress and antioxidative defence seems clearly disturbed in ischemia/reperfusion damage. There is profound evidence that iron amplified oxidative stress participates in the pathophysiology of AMI, stroke and cancer, which are the most common causes of death in the industrialised countries. Even though the issue is not finally settled, available data do suggest not to set the USL beyond the RDI, *i.e. not beyond the requirement of growth and metabolic demand. These, however, must be met to avoid deficiency symptoms. The same rational applies for pharmaceutical iron substitution under medical supervision, e.g. to replete iron losses after bleeding.*