Nutritional iron deficiency

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Iron deficiency is one of the leading risk factors for disability and death worldwide, affecting an estimated 2 billion people. Nutritional iron deficiency arises when physiological requirements cannot be met by iron absorption from diet. Dietary iron bioavailability is low in populations consuming monotonous plant-based diets. The high prevalence of iron deficiency in the developing world has substantial health and economic costs, including poor pregnancy outcome, impaired school performance, and decreased productivity. Recent studies have reported how the body regulates iron absorption and metabolism in response to changing iron status by upregulation or downregulation of key intestinal and hepatic proteins. Targeted iron supplementation, iron fortification of foods, or both, can control iron deficiency in populations. Although technical challenges limit the amount of bioavailable iron compounds that can be used in food fortification, studies show that iron fortification can be an effective strategy against nutritional iron deficiency. Specific laboratory measures of iron status should be used to assess the need for fortification and to monitor these interventions. Selective plant breeding and genetic engineering are promising new approaches to improve dietary iron nutritional quality.

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Epidemiology

Estimates of occurrence of iron deficiency in industrialised countries are usually derived from nationally representative samples with specific indicators of iron status.[1] By contrast, estimates from developing countries are often based only on haemoglobin measurements from restricted regions or target populations, and should be interpreted with caution. Prevalence estimates of iron deficiency anaemia (ie, iron deficiency and low haemoglobin) based on haemoglobin alone are overestimations because they fail to account for other causes of anaemia, such as nutritional deficiencies (eg, vitamin A deficiency), infectious disorders (particularly malaria, HIV disease, and tuberculosis), haemoglobinopathies, and ethnic differences in normal haemoglobin distributions.[2,3] For example, in Côte d'Ivoire, iron deficiency was detected with specific indicators of iron status in about 50% of anaemic women and children.[4] Even in industrialised countries, haemoglobin alone, which is used to detect iron deficiency anaemia, has poor sensitivity and specificity.[5] Anaemia is regarded as a public health problem when the frequency of low haemoglobin values is more than 5% in the population.[6]

WHO estimates that 39% of children younger than 5 years, 48% of children between 5 and 14 years, 42% of all women, and 52% of pregnant women in developing countries are anaemic,[6] with half having iron deficiency anaemia.[7] According to WHO, the frequency of iron deficiency in developing countries is about 2·5 times that of anaemia.[6] Iron deficiency is also common in women and young children in industrialised countries. In the UK, 21% of female teenagers between 11 and 18 years, and 18% of women between 16 and 64 years are iron deficient.[8] In the USA, 9–11% of non-pregnant women aged between 16 and 49 years are iron deficient, and 2–5% have iron deficiency anaemia, with more than twofold higher frequency in poorer, less educated, and minority populations.[9] In pregnant women of low-income areas in the USA, the frequency of iron deficiency anaemia in the first, second, and third trimesters is 2%, 8%, and 27%, respectively.[9] In France, iron deficiency and iron deficiency anaemia affect 29% and 4% of children younger than 2 years;[10] in the USA, 2% of children between 1 and 2 years have iron deficiency anaemia.[1]

Search strategy and selection criteria

We searched PubMed, Current Contents Connect, and ISI Web of Science for articles in English, French, German, and Spanish. We searched for "iron", "iron deficiency", "anaemia", "nutrition", "haemoglobin", "bioavailability", "supplementation", "fortification", "plant breeding", and "genetic engineering". We mainly selected publications from the past 5 years, but did not exclude highly regarded earlier publications.

Physiology

Human beings are unable to excrete iron actively, so its concentration in the body must be regulated at the site of iron absorption in the proximal small intestine (figure). Diets contain both haem and non-haem (inorganic) iron; each form has specific transporters. A putative intestinal haem iron transporter (HCP1) has been identified, which is upregulated by hypoxia and iron deficiency, and might also transport folate.[11,12] Transport of non-haem iron from the intestinal lumen into the enterocytes is mediated by the divalent metal ion transporter 1 (DMT1).[13] DMT1 transports only ferrous iron, but most dietary iron that enters the duodenum is in the ferric form. Therefore, ferric iron must be first reduced to ferrous iron, possibly by the brush border ferric reductase, duodenal cytochrome b (DCYTB),[14] or by other reducing agents, such as ascorbic acid. Once inside the enterocyte, iron that is not directly transferred to the circulation is stored as ferritin and ultimately is lost when the cell is sloughed at the villus tip. Efflux of iron across the basolateral membrane into the blood is mediated by the transport protein ferroportin 1, and the iron oxidase, hephaestin. Ferroportin 1 also mediates export of iron from other

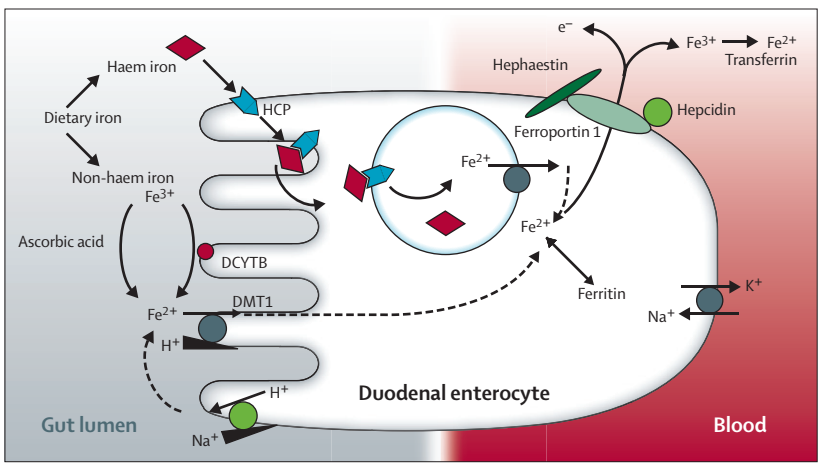

Figure: **Regulation of intestinal iron uptake**
Haem iron is taken up by the haem iron transporter (HCP), undergoes endocytosis, and Fe^{2+} (ferrous iron) is liberated within the endosome or lysosome. Non-haem iron includes Fe^{2+} and Fe^{3+} (ferric iron) salts. Fe^{3+} is reduced to Fe^{2+} by ascorbic acid in the lumen or by membrane ferrireductases that include duodenal cytochrome B (DCYTB). At the apical membrane, the acid microclimate provides an H^+ electrochemical gradient that drives Fe^{2+} transport into the enterocyte via the divalent metal-ion transporter (DMT1). At the basolateral membrane, iron transport to transferrin in the circulation is mediated by ferroportin 1, in association with hephaestin. Hepcidin, produced by the liver, binds to ferroportin 1, causing its internalisation and degradation and decreasing iron transfer into the blood.

cells, including macrophages.[15] Iron deficiency and hypoxia stimulate duodenal expression of DMT1, DCYTB, and ferroportin, and thereby increase iron absorption.[14,16]

Hepcidin is a regulatory hormone secreted by the liver that inhibits both the absorption and release of iron from macrophages and other cell types.[17] Hepcidin seems to bind to ferroportin 1 at the basolateral membrane of the enterocyte, causing its internalisation and degradation.[18] The internalisation and degradation processes decrease iron transfer into the blood, and additional iron is lost in sloughed enterocytes. In iron deficiency, hepcidin release from the liver is decreased, thereby increasing iron absorption to the maximum.[19,20] In the erythroid iron cycle, senescent red cells are broken down mainly by macrophages in the spleen, and the extracted iron is returned to the circulation where it binds to transferrin. Transferrin binds to specific transferrin receptors (TfRs) on erythroid precursors in the bone marrow, and the cycle is completed when new erythrocytes enter the circulation in the following 7–10 days. Iron deficiency increases iron transfer through the cycle to the maximum by stimulating increased ferroportin expression on macrophages,[21] hepatic synthesis of transferrin, and expression of TfR1 in the bone marrow and other tissues.[22]

Within cells, iron status upregulates or downregulates various proteins that are implicated in iron homoeostasis (notably ferritins and TfR1) at the post-transcriptional level by binding of iron regulatory proteins to specific non-coding sequences in their mRNAs, known as iron-responsive elements.[23–25] Scarce data from DNA microarrays suggest that various genes are modulated by iron status, including those encoding retinoblastoma (RB), p21, cyclin D3, cyclin E1, v-myc myelocytomatosis viral oncogene homolog (MYC), cyclin-dependent kinase 2 (CDK2), cyclin A, FAS ligand (FASL), and inducible nitric oxide synthase (iNOS); many of these genes are not directly related to iron metabolism.[26,27] Additionally, haemochromatosis (HFE), TfR2, haemochromatosis type 2 (HFE2), and SMAD family member 4 (SMAD4) in hepatocytes have been identified as regulators of hepcidin expression, and thus of intestinal iron transport and homoeostasis.[28]

During gestation, the fetus stores about 250 mg of iron. These stores are drawn on during breastfeeding, because breastmilk supplies only about 0·15 mg of absorbed iron per day, whereas requirements for absorbed iron are about 0·55 mg per day.[29] Low birthweight infants do not store an adequate amount of iron during fetal life and are at high risk of developing iron deficiency while being breastfed. During growth in childhood, about 0·5 mg of iron per day is absorbed in excess of body losses; adequate amounts of iron during growth typically results in a 70-kg man accumulating about 4 g of body iron.[30] About 2·5 g of body iron is within haemoglobin and about 1 g is stored as ferritin or haemosiderin, mainly in the liver. Men absorb and excrete about 0·8 mg of iron per day, and women, during childbearing years, should absorb almost twice as much (1·4 mg per day) to cover menstrual losses.[30] The usual diet of a population strongly affects iron bioavailability[31] (see below); thus, recommended intakes for iron depend on diet characteristics (table 1).

	Children (1–3 years)	Children (4–6 years)	Women (19–50 years)	Women during pregnancy (second trimester)	Women during breastfeeding (0–3 months lactation)	Men (19–50 years)
15%	3·9	4·2	19·6	>50·0	10·0	9·1
10%	5·8	6·3	29·4	>50·0	15·0	13·7
5%	11·6	12·6	58·8	>50·0	30·0	27·4

Numbers are mg per day. Recommended daily intake for iron depends on the bioavailability of the diet: diet rich in vitamin C and animal protein=15%; diet rich in cereals, low in animal protein, but rich in vitamin C=10%; diet poor in vitamin C and animal protein=5%.[31]

Table 1: **Selected recommended daily intakes for iron,[31] by estimated dietary iron bioavailability**

Causation

Nutritional iron deficiency arises when physiological requirements cannot be met by iron absorption from diet. Dietary iron bioavailability is low in populations consuming monotonous plant-based diets with little meat.[32] In meat, 30–70% of iron is haem iron, of which 15–35% is absorbed.[33] However, in plant-based diets in developing countries most dietary iron is non-haem iron, and its absorption is often less than 10%.[32,33] The absorption of non-haem iron is increased by meat and ascorbic acid, but inhibited by phytates, polyphenols, and calcium.[33] Because iron is present in many foods, and its intake is directly related to energy intake,[30] the risk of deficiency is highest when iron requirements are greater than energy needs. This

situation happens in infants and young children, adolescents, and in menstruating and pregnant women. During infancy, rapid growth exhausts iron stores accumulated during gestation and often results in deficiency, if iron-fortified formula or weaning foods are not supplied. Excessive early consumption of cows' milk can also contribute to early-childhood iron deficiency.[34] In a study of infants aged 6 months, frequency of iron deficiency anaemia was lowest in infants fed iron-fortified formula (about 1%) but occurred in 15% of breastfed infants, and 20% of infants fed cows' milk or non-fortified formula.[35] In the USA, the introduction of iron-fortified weaning foods in the 1970s was associated with a reduction in the frequency of iron deficiency anaemia in infants and preschool children.[36] In many developing countries, plant-based weaning foods are rarely fortified with iron, and the frequency of anaemia exceeds 50% in children younger than 4 years.[6] In schoolage children, iron status typically improves as growth slows and diets become more varied.

The frequency of iron deficiency begins to rise again, mainly in female individuals, during adolescence, when menstrual iron losses are superimposed with needs for rapid growth. Because a 1 mL loss of blood translates into a 0·5 mg loss of iron, heavy menstrual blood loss (>80 mL per month in about 10% of women) sharply increases the risk for iron deficiency.[37] Other risk factors for iron deficiency in young women are high parity, use of an intrauterine device, and vegetarian diets.[38] During pregnancy, iron requirements increase three-fold because of expansion of maternal red-cell mass and growth of the fetal–placental unit.[36] The net iron requirement during pregnancy is about 1 g (equal to that contained in about 4 units of blood), most of which is needed in the last 2 trimesters.[39] During lactation, because only about 0·25 mg of iron per day is excreted into breastmilk and most women are amenorrhoeic, iron requirement is low—only half of that of non-pregnant, non-lactating women.[30]

Increased blood loss from gastrointestinal parasites aggravates dietary deficiencies in many developing countries. Infections with *Trichuris trichiura* (whipworm) and *Necator americanus* (hookworm) cause intestinal blood loss and are important causes of iron deficiency anaemia.[40–43] Revised estimates indicate that hookworms afflict more than 700 million people in tropical and subtropical regions.[44] In endemic areas, hookworm infection is estimated to account for 35% of iron deficiency anaemia and 73% of its severe form,[45] and deworming decreases the occurrence of anaemia.[44,46,47] In a trial in Nepal, women who were given albendazole in the second trimester of pregnancy had a lower rate of severe anaemia during the third trimester, gave birth to infants of greater weight, and mortality of infants at 6 months decreased.[48] Iron deficiency anaemia can also be caused by impaired iron absorption. Gastric acid is needed to maintain ferric iron forms in solution, and achlorhydria might be a substantial cause of iron deficiency, mainly in elderly people, in whom atrophic gastritis is common.[49] Other common causes of lowered iron absorption and iron deficiency are mucosal atrophy in coeliac disease[50,51] and, possibly, *Helicobacter pylori* infection,[52] although a study of iron absorption showed no effect of *H pylori*.[53]

Adverse effects

The high frequency of iron deficiency anaemia in the developing world has substantial health and economic costs. In an analysis of ten developing countries, the median value of physical productivity losses per year due to iron deficiency was about US$0·32 per head, or 0·57% of the gross domestic product.[54] In the WHO African subregion, it is estimated that if iron fortification reached 50% of the population, it would avert 570 000 disability adjusted life years (DALYs) every year.[55] During the first two trimesters of pregnancy, iron deficiency anaemia increases the risk for preterm labour, low birthweight, infant mortality, and predicts iron deficiency in infants after 4 months of age.[56,57] Estimates are that anaemia accounts for 3·7% and 12·8% of maternal deaths during pregnancy and childbirth in Africa and Asia, respectively.[58] Data for the adverse effects of iron deficiency on cognitive and motor development in children are equivocal because environmental factors limit their interpretation.[59–61] Several studies reported adverse effects of iron deficiency anaemia on infant development that might be only partly reversible.[59,60] Other studies suggest that no convincing evidence exists that iron deficiency anaemia affects mental or motor development in children younger than 2 years, but that iron deficiency adversely affects cognition in school children.[61] Anaemic school-children have decreased motor activity, social inattention, and decreased school performance.[60] Whether adverse effects of iron deficiency on neuromotor development are due to anaemia or absence of iron in the developing brain is unclear.[62] Iron deficiency anaemia increases susceptibility to infections, mainly of the upper respiratory tract, which happen more often and have a longer duration in anaemic than in healthy children.[63] A recent study showed no positive effect of iron supplementation on physical growth during childhood.[64] The response to iodine prophylaxis is reduced in goitrous children with deficiencies of both iodine and iron,[65,66] probably because of impairment of the haem-dependent enzyme, thyroid peroxidase.[67] Iron supplementation can increase low serum retinol concentrations in iron-deficient children.[68,69] Iron deficiency might increase the risk for chronic lead poisoning in children exposed to environmental lead.[70] In adults, physical activity is reduced,[71] and manual labourers in developing countries are more productive if they are given iron and treated for hookworm and other infections.[72] Iron deficiency, even in the absence of anaemia, might cause fatigue and reduce work performance.[73,74]

Laboratory diagnosis

Table 2 shows useful indicators for diagnosis of iron deficiency anaemia in population studies. The major diagnostic challenge is to differentiate between iron deficiency anaemia in otherwise healthy individuals and anaemia of chronic disease. Inflammatory disorders increase circulating hepcidin concentrations,[90] and hepcidin blocks iron release from enterocytes and the reticuloendothelial system,[17] resulting in iron-deficient erythropoiesis. If chronic, inflammation can produce anaemia of chronic disease. The distinction between anaemia of chronic disease and iron deficiency anaemia is difficult because increased serum ferritin concentration in anaemia does not exclude iron deficiency anaemia in the presence of inflammation. A widely used marker of inflammation is the C-reactive protein (CRP), but the extent of increase of CRP concentration that invalidates the use of serum ferritin to diagnose iron deficiency is unclear; CRP values higher than 10–30 mg/L have been used. Moreover, during the acute-phase response, the increase of CRP concentration is typically of shorter duration than the increase of serum ferritin. Alternative markers such as α1-acid glycoprotein (AGP) might be useful because AGP tends to increase later during infection than CRP, and remains high for several weeks.[90] A distinct advantage of the soluble transferrin receptor (sTfR) is that it might differentiate iron deficiency anaemia from anaemia of chronic disease.[79,91] Thus, in surveys in developing countries with a high frequency of infection, in addition to serum ferritin and haemoglobin measurements,[80] laboratory assessment should include sTfR, zinc protoporphyrin (ZPP), and CRP, AGP, or both,[4] although the sensitivity and specificity of sTFR and ZPP are low in these settings.[78] In an anaemic individual with high CRP, AGP, or both, high sTfR and ZPP concentrations are likely to mean concurrent iron deficiency, despite high serum ferritin.

	Selected cutoff values to define iron deficiency	Comments
Haemoglobin (g/L)	6 months–5 years <110 6 years–11 years <115 Non-pregnant women <120 Pregnant women <110	When used alone, it has low specificity and sensitivity
Mean corpuscular volume (MCV) (cu μm)	Children older than 11 years and adults <82	A reliable, but late indicator of iron deficiency Low values can also be due to thalassaemia
Reticulocyte haemoglobin content (CHr) (pg)	In infants and young children <27·5 In adults ≤28·0	A sensitive indicator that falls within days of onset of iron-deficient erythropoiesis[75,76] False normal values can occur when MCV is increased and in thalassaemia[76] Wider use is limited because it can only be measured on a few models of analyser
Erythrocyte zinc protoporphyrin (ZPP) (μmol/mol haem)	5 years or younger >70 Children older than 5 years >80 Children older than 5 years on washed red cells >40	It can be measured directly on a drop of blood with a portable haematofluorometer[77] A useful screening test in field surveys, particularly in children,[78] in whom uncomplicated iron deficiency is the primary cause of anaemia Red cells should be washed before measurement[78] because circulating factors, including serum bilirubin, can spuriously increase values Lead poisoning can increase values, particularly in urban and industrial settings[70]
Transferrin saturation	<16%	It is inexpensive, but its use is limited by diurnal variation in serum iron and by many clinical disorders that affect transferrin concentrations[27,79]
Serum ferritin (SF) (μg/L)	5 years or younger <12 Children older than 5 years <15 In all age groups in the presence of infection <30	It is probably the most useful laboratory measure of iron status;[80] a low value of SF is diagnostic of iron deficiency anaemia in a patient with anaemia In healthy individuals, SF is directly proportional to iron stores: 1 μg/L SF corresponds to 8–10 mg body iron or 120 μg storage iron per kg bodyweight[81] As an acute-phase protein, SF increases independent of iron status by acute or chronic inflammation; it is also unreliable in patients with malignancy, hyperthyroidism, liver disease, or heavy alcohol intake[27]
Serum transferrin receptor (sTfR)	Cutoff varies with assay, and with patient age and ethnic origin	Main determinants are the erythroid mass in the bone marrow and iron status; thus, sTfR is increased by enhanced erythropoiesis and iron deficiency[79,82] sTfR is not substantially affected by the acute-phase response,[79] but it might be affected by malaria,[83,84] age, and ethnicity[78] Its application limited by high cost of commercial assays and lack of an international standard
sTfR-to-SF ratio		This ratio is a quantitative estimate of total body iron; the logarithm of this ratio is directly proportional to the amount of stored iron in iron-replete patients and the tissue iron deficit in iron deficiency[85] In elderly people, this ratio might be more sensitive than other laboratory tests for iron deficiency[86] This ratio cannot be used in individuals with inflammation because SF might be high independent of iron stores This ratio is assay specific[85] Although it is only validated for adults,[85] this ratio has been used in children[4,32,87,88]

Table 2: **Indicators of iron deficiency anaemia**

Strategies

Three main strategies for correcting iron deficiency in populations exist, alone or in combination: education combined with dietary modification or diversification, or both, to improve iron intake and bioavailability; iron supplementation (provision of iron, usually in higher doses, without food); and iron fortification of foods. A new approach is biofortification via plant breeding or genetic engineering. Although dietary modification and diversification is the most sustainable approach, change of dietary practices and preferences is difficult, and foods that provide highly bioavailable iron (such as meat) are expensive.

Supplementation

Iron supplementation can be targeted to high-risk groups (eg, pregnant women), and can be cost effective,[55] but the logistics of distribution and absence of compliance are major limitations. For oral supplementation, ferrous iron salts (ferrous sulphate and ferrous gluconate) are preferred because of their low cost and high bioavailability. Standard therapy for iron deficiency anaemia in adults is a 300-mg tablet of ferrous sulphate (60 mg of iron) three or four times per day. Although absorption is enhanced when given on an empty stomach, nausea and epigastric pain might develop. If these side-effects arise, lower doses between meals should be attempted, or iron should be provided with meals, although food reduces absorption of medicinal iron by about two-thirds.[79] Alternatively, oral iron supplements can be supplied every few days; this regimen might increase fractional iron absorption.[92] In studies supported by WHO in southeast Asia, iron and folic acid supplementation every week to women of childbearing age improved iron nutrition and reduced iron deficiency anaemia.[92] In industrialised countries, universal iron supplementation of pregnant women is widely advocated even though so far little evidence exists that it improves maternal or fetal outcomes. However, in two controlled trials of prenatal iron supplementation in iron-replete, non-anaemic low-income pregnant women in the USA, iron supplementation increased birthweight, reduced incidence of preterm delivery, or both, but did not affect prevalence of anaemia during the third trimester.[93,94] Iron supplementation during pregnancy is advisable in developing countries, where women often enter pregnancy with low iron stores.[1]

Untargeted iron supplementation in children in tropical countries, mainly in areas of high transmission of malaria, is associated with increased risk of serious infections.[95,96] In a region of endemic malaria in east Africa, untargeted supplementation with iron (12·5 mg per day) and folic acid in preschool children increased risk of severe illness and death.[97] Although iron supplements were thought to be the cause, provision of folic acid might have reduced the effectiveness of anti-folate antimalarial drugs,[98] and thereby contributed to morbidity.[99] A similar study in Nepal, which is a non-malarial area, showed no effects of iron and folic acid on infection-related morbidity.[100] A recent WHO report stated that iron and folic acid supplementation should be targeted to children who are anaemic and at risk of iron deficiency, and concurrent protection from malaria and other infectious diseases should be provided.[101]

Fortification

Iron fortification is probably the most practical, sustainable, and cost-effective long-term solution to control iron deficiency at the national level.[55,102,103] Overall cost-effectiveness for iron fortification is estimated to be $66–70 per DALY averted.[103] Fortification of foods with iron is more difficult than it is with other nutrients, such as iodine in salt and vitamin A in cooking oil. The most bioavailable iron compounds are soluble in water or diluted acid, but often react with other food components to cause off-flavours, and colour changes, fat oxidation, or both.[33] Thus, less soluble forms of iron, although less well absorbed, are often chosen for fortification to avoid unwanted sensory changes. Fortification with low iron doses is more similar to the physiological environment than is supplementation and might be the safest intervention.[101,102] Iron fortification of milk or cereals does not increase infection-related morbidity in children younger than 18 months.[95] In an analysis of four studies of infants receiving iron-fortified foods, the regimen did not cause visible adverse effects and significantly protected against the development of respiratory tract infections (incidence rate ratio 0·92, 95% CI 0·86–0·98; p=0·02).[96]

Industrialised countries

Although little direct evidence exists, the reduction in occurrence of iron deficiency in young children in industrialised countries has been attributed to iron fortification of infant formulas and weaning foods. Iron-fortified foods distributed through the Special Supplemental Nutrition Program for Women, Infants, and Children (WIC) have probably contributed to the fall of iron deficiency in underprivileged preschool children in the USA.[104] At present, the low frequency of iron deficiency anaemia in adolescent and young women in the USA might be at least partly due to consumption of iron-fortified wheat flour, although other factors, including open-market fortification of food products, and use of vitamin and mineral supplements, have also had a role. More-specific evidence is provided by retrospective studies from Sweden that reported decrease of iron intake[105] and increase of iron deficiency in young women[106] since iron fortification of wheat flour was discontinued in 1994. By contrast, findings from Denmark, where iron fortification of wheat flour was discontinued in 1987, suggest no change in the frequency of iron deficiency in adults older than 40 years,[107,108] but the data might have been confounded by the effects of increasing bodyweight, alcohol consumption, or both, contributing to increased values or serum ferritin.

Panel 1: Failure to determine the effectiveness of iron fortification programmes in developing countries[3,33]

Failure of effectiveness

- Use of iron compounds with low bioavailability or failure to enhance absorption from inhibitory diets
- Inadequate iron fortification
- Consumption of fortified food too low to deliver adequate iron
- High frequency of parasitic infections that cause blood loss (eg, hookworm)
- High frequency of infection, inflammation, or both, that impairs iron metabolism and erythropoiesis (eg, malaria)

Failure to detect effectiveness

- Failure to define iron status with specific indicators clearly
- Failure to recognise other causes of anaemia
- Poor programme control and enforcement

Developing countries

Universal iron fortification is generally recommended for countries where the risk of developing iron deficiency is high for all groups other than adult men and postmenopausal women.[102] Up to now, no clear indication of efficacy of iron fortification in developing countries existed, because of several factors (panel 1). However, recent studies have shown convincingly that iron fortification can be effective.[66,88,109–112] The iron compound and type of fortification should be chosen on the basis of the fortification vehicle, iron requirements of the target population, and iron bioavailability of the local diet (panel 2). Efficacy should be monitored with measurements of serum ferritin and, when possible, serum transferrin receptor, in addition to haemoglobin.[66,80,88,109–113] Iron fortification efforts have been accelerated by the Global Alliance for Improved Nutrition (GAIN), an alliance of United Nation agencies, national governments, development agencies, and the private sector, funded mainly by the Bill & Melinda Gates Foundation. GAIN has awarded $38 million in grants to food fortification programmes in 14 countries, including iron fortification of soy sauce in China, fish sauce in Vietnam, and wheat and maize flour in South Africa.

The foods most often used for mass fortification are the staple cereal flours. Iron is only poorly absorbed from high-extraction flours because of the presence of phytate and other inhibitory factors.[114,115] Dried ferrous sulphate can be used in wheat flour that is consumed shortly after it is milled, but in most developing countries flour is stored for longer periods. Thus, elemental iron powders, which are less reactive, are widely used, despite their lower bioavailability.[109,115,116] Findings from an efficacy trial in Thailand suggest that two forms of elemental iron, electrolytic iron and hydrogen-reduced iron, might be useful for fortification, but their bioavailability is only 50–79% that of ferrous sulphate.[109] Two other forms of reduced iron, carbon-monoxide-reduced and atomised iron, are poorly absorbed and unlikely to be useful for food fortification. A trial in Sri Lanka failed to show a reduction in anaemia occurrence after 2 years of fortification of low-extraction wheat flour with either electrolytic or reduced iron, but fortification was probably too low.[117] Wheat flour fortification with ferrous sulphate in Chile at 30 mg/kg has probably contributed to a strong decrease in iron deficiency.[118] Fortification of maize flour in South Africa with ferrous fumarate has shown effectiveness in lowering anaemia, and improving iron status and motor development of infants in poor settings.[119] Clear guidelines on wheat flour fortification have recently been published.[120]

Sodium iron ethylenediaminetetraacetic acid (NaFeEDTA) has shown effectiveness as a fortificant in sugar in Guatemala,[121] curry powder in South Africa,[122] soy sauce in China,[123] fish sauce in Vietnam,[110] and maize flour in Kenya.[124] NaFeEDTA is absorbed 2–3 times more than ferrous sulphate from diets high in phytic acid,[125] but is approved as a food additive only at 0·2 mg iron a day as NaFeEDTA per kg bodyweight, which limits its usefulness as a fortificant for infants and children.[126] NaFeEDTA does not promote fat oxidation in stored cereals and is the only soluble iron compound that does not precipitate peptides in fish and soy sauces. Use of micronised ground ferric pyrophosphate, a white-coloured iron compound with good bioavailability, has allowed successful fortification of colour-sensitive food vehicles, such as low-grade salt in Africa[66,113] and rice in India.[88] A micronised, dispersible ferric pyrophosphate[127] and ferrous bisglycinate, an aminoacid chelate,[111] are iron fortificants particularly useful for liquid products.

Infants and young children in developing countries are at high risk of iron deficiency and might not be reached by universal fortification programmes. Chile has shown convincing evidence of the benefit of targeted fortification

Panel 2: Iron compound that can be used for iron fortification of food in order of preference[102]

Most foods (eg, cereal flours)

- Ferrous sulphate
- Ferrous fumarate
- Encapsulated ferrous sulphate or fumarate
- Electrolytic iron (at twice the amount *vs* ferrous sulphate)
- Ferric pyrophosphate (at twice the amount *vs* ferrous sulphate)
- NaFeEDTA

For high phytate cereal flours and high peptide sauces (eg, fish and soy sauce)

- NaFeEDTA

For liquid milk products

- Ferrous biglycinate
- Micronised dispersible ferric pyrophosphate
- Ferric ammonium citrate

of powdered milk with ferrous sulphate and ascorbic acid, with frequency of anaemia decreasing from 27% to 9%.[128] By contrast, distribution of a milk-based iron-fortified weaning food in Mexico for 1 year did not improve iron status, possibly because of the poor bioavailability of the reduced iron used as a fortificant.[129] Complementary food supplements that are added to the infant's food immediately before consumption have been developed. Three types of supplements have been tested: powders (sprinkles), crushable tablets, and fat-based spreads.[130–132] Iron status was improved in Ghanaian infants with home fortification with powder containing encapsulated ferrous fumarate.[132]

Biofortification

The variation in the iron content of cultivars of wheat, bean, cassava, maize, rice, and yam[133–137] suggests that selective breeding might increase iron content of staple foods. However, although differences in iron content exist in wheat (25–56 mg/kg) and rice (7–23 mg/kg), most of the iron is removed during the milling process. Thus, to increase iron concentration in milled wheat up to 40 mg/kg, which is the fortification level commonly used in wheat flour, might be difficult.[120] This problem was evident when the effectiveness of a rice cultivar high in iron was tested in a feeding trial in Filipino women consuming either the high-iron rice (3·21 mg/kg) or a local variety (0·57 mg/kg) for 9 months.[135] Possibly because the high-iron rice added only an extra 1·5 mg of iron a day to the diet, no clear benefit of iron status was seen. Iron absorption from other cereals and legumes (many of which have high native iron content) is low because of their high contents of phytate and polyphenols.[138] Donangelo and colleagues[139] compared iron bioavailability from two varieties of red beans: an iron-rich genotype (containing 65% extra iron) and a low-density genotype. Only a small amount of iron was absorbed from both cultivars, probably because of their high phytate and polyphenol content. Decrease of the content of these inhibitors in high-iron cultivars might be needed to have a positive effect on human nutrition. Genotypes of maize, barley, and rice have been identified that are low-phytic-acid mutants, with phytic acid phosphorus content decreased by up to two-thirds compared with wild type.[140] Although such reductions might improve iron absorption from diets containing small amounts of meat and ascorbic acid,[141] phytic acid content might be needed to be lowered by more than 90% to increase iron absorption from the monotonous cereal-based diets seen in many developing countries.[142]

Because of these limitations, genetic engineering might prove to be the most effective way to have a useful amount of absorbable iron in plant foods.[143,144] Iron content in rice can be increased two-to-three fold by introduction of the ferritin gene from soy bean[145] or phaseolus vulgaris.[146] Iron uptake from soils might be increased by introduction of a ferric reductase gene into plant root systems.[147] To lower the phytic acid content of rice, Lucca and colleagues[146] introduced a phytase from *Aspergillus fumigatus* that was developed to withstand food processing. Although phytase activity increased seven-fold, it proved to be unstable and was destroyed when rice was cooked. Overall, these studies suggest that iron content can be increased in staple foods by plant breeding, genetic engineering, or both.

Conclusions

Nutritional iron deficiency is still common in young women and children in developing countries where monotonous, plant-based diets provide low amounts of bioavailable iron. The high prevalence of iron deficiency in the developing world has substantial health and economic costs. However, more data are needed on the functional consequences of iron deficiency; for example, the effect of iron status on immune function and cognition in infants and children needs to be clarified. Continuing rapid advances in understanding the molecular mechanisms of iron absorption and metabolism might enable development of new strategies to combat iron deficiency. Although technical challenges limit the amount of bioavailable iron that can be added to many foods, evidence from controlled trials has shown that iron fortification can effectively control iron deficiency. Whether iron fortification can be successful in tropical areas without concurrent control of malaria and hookworm infections remains to be seen. Specific laboratory measures of iron status—eg, serum ferritin, sTfR, and zinc protoporphyrin—should be used to assess the need for fortification and for monitoring. Because of findings showing the risks of untargeted iron supplementation in young children, development of new strategies are urgently needed to provide additional dietary iron to susceptible infants and young children in developing countries who might not be reached by universal fortification programmes. New methods to enhance native iron content of plant-based staple foods are also needed. Selective plant breeding and genetic engineering are promising new approaches to improve dietary iron bioavailability; however, a major challenge is to show that they can increase iron content to nutritionally useful levels and that the additional iron is bioavailable.

Conflict of interest statement

We declare that we have no conflict of interest.

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