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Nutritional Iron Deficiency: The Role of Oral Iron Supplementation

J.I. Lachowicz*[,a], V.M. Nurchi[a], D. Fanni[b], C. Gerosa[b], M. Peana[c] and M.A. Zoroddu[c]

[a]*Dipartimento di Scienze Chimiche e Geologiche, University of Cagliari, Cittadella Universitaria, I-09042 Monserrato, Italy;* [b]*Dipartimento di Citomorfologia, Sez. Anatomia Patologica, University of Cagliari, Via Ospedale 46, I-09124 Cagliari, Italy;* [c]*Dipartimento di Chimica e Farmacia, University of Sassari, Via Vienna 2, I-07100 Sassari, Italy*

Abstract: Nutritional iron deficiency represents a relevant health problem mainly in developing countries. Children and pregnant women represent the main target of this disease, and the low amount of bio-available iron mostly depends on plant-based diets. Iron deficiency may have serious consequences, with severe impairment of the immune function leading to infectious diseases. The brain development in embryos and fetuses during gestation can be greatly affected by iron deficiency of the mother with heavy outcomes on the cognition status of children. A better understanding of molecular pathways involved in iron absorption and metabolism are the basis for new strategies for developing a therapy for iron deficiency. Different therapeutic strategies are summarized, and iron fortification appears the best tool.

Keywords: Iron deficiency, iron metabolism, iron absorption, iron supplement, iron-fortified food.

1. INTRODUCTION

Iron deficiency represents an important clinical problem for pediatricians as well as for gynecologists and geriatrics, as young children, women, and older people are the groups with the highest possibility of iron deficiency. Iron-deficiency anemia is characterized by a lower-than-normal red blood cell count, or when a correct count does not include an adequate amount of hemoglobin (the iron-protein devoted to the transport of oxygen from lungs to other tissues). Iron-deficiency anemia usually develops over time, when the intestines do not absorb enough iron to carry to bone marrow hematopoietic cells and build healthy red blood cells. This leads to fatigue, chest pain, shortness of breath, and other minor symptoms. Severe iron-deficiency anemia can retard growth and development in children, lead to heart problems, infections, and a number of other complications.

Iron deficiency is considered one of the most important risk factors of death and disability among some two billion people, mainly children and young adults in developing countries [1]. In infancy, insufficient iron intake has severe complications in brain development, resulting not only in diminished school performance [2], but also in cognitive limitations that, in many cases, persist into adulthood [3]. The fundamental role of iron in the immune system is confirmed by the higher frequency of infections in children affected by iron-deficiency-related anemia, in whom T-lymphocytes are about 20% reduced, and cell-mediated immune response is decreased [4].

This paper reviews the most important phases of iron metabolism in humans. We report the complex pathogenesis of anemia related to iron deficiency, and analyze the clinical consequences of iron deficiency. The final part of this review is dedicated to iron deficiency therapy. We directed particular attention to iron supplementation and fortification of foods, promising, effective strategies that could ameliorate the lack of dietary iron and reduce iron deficiency, particularly in developing countries.

*Address correspondence to this author at the Dipartimento di Scienze Chimiche e Geologiche, University of Cagliari, Cittadella Universitaria, I-09042 Monserrato, Italy; Tel: +39 070 675 4471; Fax: +39 070 675 4478; E-mail: lachowicz@unica.it

2. IRON IN HUMAN METABOLISM

Iron Daily Needs

The "recommended daily allowance" (RDA) for adult males is estimated at around 10 mg of iron per day. Considering an assimilation of 10%, this amount results in a net uptake of 1.0 mg iron per day, an amount adequate to restore daily iron losses [5], and fulfill the daily necessities [6]. RDA values differ greatly by gender, and change markedly with age. The RDA values for males and females are reported in (Table **1**) as a function of age, and are relative to US and UK regulations [7], together with the values in pregnancy and lactation.

A male weighting 70 kg accumulates about 4 g of iron in his body during his growth. About 2.5 g of iron is found in hemoglobin and about 1 g in liver cells as ferritin or hemosiderin. While men absorb and expel approximately 0.8 mg of iron per day, women absorb an almost double amount (1.4 mg per day) to cope with menstrual losses [8]. As such the RDA of iron for women aged 19 and >50 years has been set at 14.8 and 8.7 mg/day, respectively [7].

The iron requirements for pregnant women is 2-6 times greater than those of men [9]. Women in their fecund age need a nutritional supply of 15 mg iron per day. Since a typical Western-type 1000 kcal diet contains about 6 mg iron, many young women often do not consume enough iron in their diets [10].

During pregnancy, iron consumption increases significantly. The body transfers approximately 15% of a mother's

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Table 1. Recommended daily allowances of iron, expressed as mg/day, for males and for females as a function of age, relative to UK [7] and US regulations [8], with values for pregnancy and lactation

United Kingdom [7]				United States [8]				
Age	**Males**	**Females**	**Pregnancy**	**Age**	**Males**	**Females**	**Pregnancy**	**Lactation**
				0-6 month	0.27	0.27		
<1 year	5.4	5.4		7-12 month	11	11		
1-3	6.9	6.9		1-3 years	7	7		
4-6	6.1	6.1		4-8 years	10	10		
7-10	8.7	8.7		9-13 years	8	8		
11-14	11.3	14.8		14-18 years	11	15	27	10
15-18	11.3	14.8	14.8	19-30 years	8	18	27	9
19-50	8.7	14.8	14.8	31-50 years	8	18	27	9
>50	8.7	8.7	14.8	50-70 years	8	8		
				>70 years	8	8		

bodily iron content to the fetus and placenta during pregnancy. She further loses 1 mg of basal iron daily through cell desquamation, primarily in the gastrointestinal tract. Throughout gestation, 450 mg of iron are necessary for the development of red cell mass, while 270-300 mg iron are consumed by the fetus and 25-50 mg by the placenta [11]. The maternal RDA rises sharply during the 3rd trimester when the fetus requires 1.6 up to 2.0 mg iron per day [12]. As a consequence, consumption of 30 mg iron per day by pregnant women is recommended during the last three months [9]. In areas of the world where women are particularly at risk of iron deficiency, pregnant women require a supplement of 60 mg iron per day in the second half of gestation, as it is presently carried out in Indonesia [13].

The net iron requirement during pregnancy has been estimated to be approximately 1 g (corresponding to the amount contained in 4 units of blood), mostly during the last two trimesters [6]. The recommended quantity of iron supplementation in mass programs did not change from the above-cited suggestions, apart from the pregnancy dose, which was changed to 60 mg/day. The effectiveness of iron absorption is enhanced in the cases of severe iron deficiency. An amount of 60 mg/day should constitute a convenient supply of iron to women who do not present a severe anemia, when there is time for a prolonged treatment. A greater dosage (120 mg/day) is suggested when supplementation in gestation is short [14].

A particular consideration should be given to adolescent girls, for whom a weekly supplement of 60 mg iron for 3-4 months (minimum) per year is suggested [13]. Newborns and infants grow quickly and, according to their body weight, have higher iron needs [15]; iron stores formed due to the high intrauterine iron supply are used in the first 4-6 months of postnatal life. In the following months, at normal nutritional iron intakes, children are at high risk for iron deficiency [16]. The Committee on Nutrition of the American Academy of Pediatrics suggests an intake of 1 mg of iron per kg of body weight per day between the ages of 4 months and 3 years [17]. The American Academy of Pediatrics for Premature Babies suggests the delivery of 2 mg of iron per kg per day as a supplement to breast milk during the first year of life. Pre-term newborns are at higher risk for iron deficiency compared to at-term newborns. 3-4 mg iron/(kg x day) has been recommended for pre-term newborns weighing less than 1.5 Kg at birth [18, 19]. A 6-l2 month old infant normally absorbs approximately 0.1 mg of iron per kg of body weight per day [20]. During the first months of development, infants absorb approximately 0.5 mg of iron per day, which exceeds the amount normally lost by the body [8].

Iron Absorption

Iron bioavailability is low and depends on the chemical form of ingested iron and the presence of dietary components that may enhance or inhibit absorption [21]. Heme (organic) and non-heme (inorganic) iron each have a definite transporter [22]. The heme iron transporter (HCP) picks up heme iron, which withstands endocytosis and is released in the reduced form Fe^{2+} within the endosome (or lysosome). Non-heme iron consists of both ferrous and ferric forms. Ascorbic acid reduces Fe^{3+} to Fe^{2+} in the intestinal lumen; membrane ferri-reductases, which also comprise duodenal cytochrome B (DCYTB), perform the same reduction. Dietary ferric non-heme iron, which is the principal form of nutritional iron in the majority of populations, has to be reduced before absorption by cytochrome B reductase 1 (CYBRD1) [23]. The precise role of CYBRD1 is still unknown. Intestinal ferro-reductases, which contain cytochrome B, ferric/cupric reductase19 and Steap2, may reduce iron [24]. DMT1 transports ferrous iron into enterocytes, accompanied by proton gradient from the external to the internal side of intestinal cells. Dietary iron in the heme form derives mainly from meat

sources. Heme is transported by an intestinal heme transporter, the heme carrier protein 1 (HCP1) [25]. Recent research suggests that HCP1 functions as an intestinal folate transporter and renames it proton-coupled folate transporter (PCFT) [22]. Other data indicate PCFT/HCP1 as an intestinal heme transporter [26].

The acidic environment at the apical membrane supplies the protonic electrochemical gradient necessary for the transport of Fe^{2+} into the enterocyte through DMT1, the divalent metal-ion transporter. At the basolateral membrane, ferroportin 1, and hephaestin transport iron to transferrin in the circulation. Hepcidin, produced by liver cells, binds ferroportin 1 and provokes its internalization and degradation. As a consequence, it decreases iron transfer into the blood [1]. Vesicles from the basolateral surface export iron out of the cells and delivered to other intracellular compartments. Such vesicles can fuse with vesicular structures containing nutritional iron, which is absorbed mainly by the upper small bowel [27]. The behavior of iron inside enterocytes is not completely clear. Recently, an iron chaperone with the task of delivering iron to ferritin has been recognized, even if its role in the intestinal epithelium is thus far unidentified [28].

Hepcidin, a peptide hormone produced by liver, regulates absorption of iron. Erythropoiesis in bone marrow modulates the body's iron needs. Hepcidin inhibits the efflux of iron from enterocytes and is released from the main storage deposits in the body. It exerts an autocrine action on hepatocytes and an endocrine action on macrophages, including Kupffer cells. Hepcidin sequesters iron by ferritin and induces the mucosal block of metal absorption. It can lead to the loss of iron ions and enterocytes are removed into the intestinal lumen.

Duodenal enterocytes carry out the absorption of dietary iron utilizing transporters at the apical membrane. A cell-surface reductase exploits the absorption of oxidized forms of iron. Cytosolic iron transport with the enterocytes may involve chaperone proteins delivering iron to ferritin. FPN and ATP7A transport iron across the basolateral membrane and iron deficiency increases markedly with the amount of basolateral ATP7A [29].

No more than 5-10% of iron from food is absorbed when iron stores in the human body are approximately 500 mg. When body iron stores become lower than 100 mg, non-heme iron absorption increases to 15-20% [10].

The amount of iron ions absorbed depends highly on diet, particularly on the quantity of compounds that can increase or inhibit the absorption of nutritional iron. For example, coffee and tea consumed during a meal, or soon after, inhibit iron absorption [30]. The availability of heme iron is strongly diminished by divalent metal ions, as calcium, which decrease intestinal absorption [31, 32]. Supplementary calcium and dairy products have inhibitory effects on non-heme iron absorption [33, 34]. A number of papers show evidence of the inhibition of iron absorption by calcium [27, 35], even if some disagreement exists between numerous results. Calcium, both from food and supplement ingestion, inhibits the absorption of both heme and non-heme iron. Iron absorption in the presence of calcium carbonate is considerably lower than in the presence of calcium acetate [36]. Calcium carbonate is able to neutralize the acid of the stomach, whereas calcium acetate does not produce any pH variation; for this reason, in the first situation, iron is expected in the ferric state, and is hence less available [36].

Supplements with calcium inhibit the absorption of non-heme iron from food and supplements [36]. Citrate is an iron-chelating agent that is proven to inhibit mucosal iron uptake [36]. Inhibition of heme iron absorption by tannins and phytates is low [37]. Myo-inositol hexaphosphate (phytic acid), present in vegetal foods (nuts, wholegrain cereals, seeds, and legumes) strongly inhibits non-heme iron absorption [38], as well as polyphenols found in tea [39] and coffee [40]. It is thought that the active components are the galloyl groups.

Some vitamins play a relevant role in iron absorption. In populations with low vitamin intake, iron supplementation may have a limited efficacy [41]. Therefore, vitamin A needs to be supplemented together with iron [13]. Vitamin C also plays a role in iron bioavailability, as it reduces Fe^{III} to Fe^{II} before entering the duodenum like the brush border ferric-iron-reductase (DCYTB) [42], or other reducing agents [1]. Although vitamin C neutralizes inhibitory effects on iron absorption by other dietary components, at least 80 mg of vitamin C are required to overcome the effect of 25 mg of phytates [43]. When consumed with a meal, ascorbic acid strongly enhances iron absorption from non-meat foods [30]. The ability of vitamin C to increase iron absorption is high. 25 mg of vitamin C double iron bioavailability, while 1000 mg increases availability 10 times [44, 45]. Absorption is not only influence by the quality of food, but by food treatment, as well. Iron bioavailability increases when cereals and legumes undergo germination and fermentation that reduce the amount of phytates [30].

Iron Transport in Blood

Hephaestin (HP), a multi-copper ferroxidase localized at the basolateral membrane of enterocytes, close to ferroportin (FPN), oxidizes ferrous iron before leaving enterocytes [46, 47]. Apotransferrin (Apo-TF) binds ferric ions in the *lamina propria* and transport them to the liver in portal blood.

Liver Uptake of Iron Ions

Iron bound to transferrin (Fe-TF) can move into hepatocytes via TFR1 or TFR2 cycling by endocytosis. Iron transported by transferrin, reduced in the endosome, is then transported into the cytosol; the effective mechanism of this transport is not entirely known. In conditions of iron overload, iron ions compete with copper ions for the same ion transporter, and can be also take up to hepatocytes via ZIP14 or DMT1 [48]. Joint up- and down-regulation of different factors of iron uptake determine the absorption of iron into hepatocytes through different molecular pathways. Iron uptake by means of ZIP14 can be inhibited by the human hemochromatosis protein (HFE) [49]. ZIP14, a typical zinc transporter, intervenes in the transport into the cells of non-transferrin-bound iron (NTBI) [50]. Iron in the hepatocyte is stored in ferritin for use in the synthesis of iron- proteins. Export of iron from hepatocytes occurs via FPN.

Distribution of Iron to Tissues

The highest consumption of iron occurs in erythroid precursors of bone marrow, which need 20–25 mg daily in order to mature into erythrocytes. Erythroid cells receive iron only from transferrin bounded to TFR1 on the cell surface. The complex formed, Fe-TF/TFR1, is internalized into endosomes. Here, Steap3 reduces Fe^{3+} to Fe^{2+}, which DMT1 then transports into the cytosol. Erythroid cells in the stage of growth are the major iron-user cells in the body, and obtain iron totally through the Fe-TF/TFR1 cycle. Endosomal Steap3 favors the reduction of iron before its DMT1- mediated transport into the cytosol. Iron acquired by maturing erythroid cells goes to mitochondria, where it is utilized for the biosynthesis of heme and the assemblage of Fe-S cluster protein [51]. Mitochondrial iron transporter (Mitoferrin), necessary for mitochondrial iron assimilation, is an iron transporter situated on the inner membrane of mitochondria [52]. The iron chaperone Frataxin (FXN) transports iron to the site of the Fe-S cluster formation and the heme synthesis [53]. Eventually, Fe-S and heme are included into proteins containing complexes I-IV of the electron transport chain. The synthesis of heme in growing erythroid cells consumes about 70% of all bodily iron.

Macrophages located in the spleen are extremely important in iron metabolism: they phagocyte senescent red blood cells and recover their iron. The phagolysosomes break down hemoglobin in red cells and further metabolize the remaining heme to release iron. The free iron can be exported, accumulated, or used by the cell. The iron attendance by macrophages has been carefully studied [54]. Macrophages of the reticuloendothelial system can assume iron from Fe-TF, but the major part of their iron is received by phagocytosis of matured erythrocytes.

Iron liberated from the protoporphyrin ring by heme oxygenase is used by the cell, accumulated in ferritin, or released from the cell by FPN. Natural resistance associated macrophage protein 1 (Nramp1), a transporter of divalent metal ions, is placed to the late phagolysosome of macrophages, where it promotes recycling of iron ions [55]. RE macrophages constitute a great store of iron, which contain about half of the total iron in the body [54].

A consensus on the transport of iron from blood to the brain is lacking. The function of Fe-TF versus central nervous system cells, regarding the presence of DMT1 in brain capillary endothelial cells, is still unknown [56-59]. It is thought that neurons obtain iron by means of TFR1, whereas glial cells might uptake iron ions via DMT1. Ferritin might be more abundant in glial cells than in neurons, suggesting a major role for glial cells in iron uptake and redistribution to other cell types in the brain. Plasma ferritin might also represent a relevant source of brain iron [60].

All these data imply that DMT1 probably plays a major role in flow of iron ions from the central nervous system to blood across the blood brain barrier (BBB), whereas movement of iron from blood through the BBB towards neurons and glial cells might require TFR1 [61].

3. IRON DEFICIENCY

Etiology

Iron deficiency constitutes one of the main global risk factors for disability and death for some 2 billion people [1]. Anemia represents one of the most important clinical consequences of iron deficiency, both in industrialized and in developing countries. According to the WHO, 39% of children below 5 years, 48% of children between 5-14, 42% of all women, and 52% of pregnant women in developing countries are anemic [62]. Iron deficiency is the major cause of anemia in developing countries, accounting for about 50% of total cases [63]. Surprisingly, iron deficiency is also a common health problem in industrialized countries with 21% of British females between 11-18 being deficient [21]. In the USA, 29% of pregnant women in the third trimester have been found to be affected by iron deficiency anemia [64].

How to Treat Iron Deficiency

Iron deficient anemia can usually be effectively managed. The type of treatment derives from the cause of iron deficiency as well as its severity. Treatments consist of dietary changes, drugs, and surgery. The most severe cases may involve hospital treatments with blood transfusions and intravenous iron therapy. In many circumstances, iron deficiency may be treated by dietary modifications or diversification, which is the most sustainable approach [1]. When dietary modifications are not sufficient to solve the problem, two other strategies may be utilized to increase ingestion of iron and its bioavailability: iron supplements (i.e. the supply of iron, often in high doses without food), and iron enriched foods [65]. Ferrous iron salts, including ferrous sulfate and ferrous gluconate, are generally preferred as oral supplements due to their low cost and high bioavailability. The chemical species of iron found in supplements is extremely important because it determines the therapeutic effects and the toxicity. For example, ferrous species in oral iron supplements are more bioavailable than ferric species, but are less tolerated and cause significant gastrointestinal disturbances [66, 67]. Standard therapy for iron-deficiency anemia, even in pregnant women, is the ingestion of a 300-mg tablet of ferrous sulfate 3-4 times per day. Iron salt absorption is better on an empty stomach, but side effects, including epigastric pain and nausea, may occur. The side effects of ferric salts are more severe, probably due to the comparatively slower absorption of ferric iron compared to ferrous iron. Various ferrous and ferric impurities have been identified in ferrous sulfate and ferrous fumarate based drugs [68]. In mammals, iron is mainly absorbed by the epithelium of the proximal small intestine, mainly the duodenum, which absorbs ferrous iron better than ferric [42].

Even if oral iron is the preferred treatment for iron deficiency anemia, intravenous administration of iron is indispensable in some clinical settings, particularly when severe anemia affects pregnant women [69]. While oral iron supplements are efficacious, their effectiveness is highly dependent on patient compliance. Poor compliance, dependent on the length of the treatment and on related gastrointestinal side-effects, is the most frequent cause of the inefficacy of oral supplements [70]. The patient's iron stores would not be reloaded by oral treatments if they do not continue oral iron ingestion for 3-6 months after reestablishing standard hematological values [69]. Iron stores are reloaded with greater efficacy with parenteral iron [71], though there is often no other advantage to parenteral administration [71]. Parenteral iron is the only effective therapy to supply a sufficient

amount of iron for erythropoiesis in cases of severe anemia [72, 73]. Moreover, in cases of severe anemia in pregnant women, oral treatments do not stimulate erythropoiesis rapidly and consistently for clinical use [74]. Simply taking iron tablets incorrectly with meal is sufficient to cause the failure of the therapeutic strategy, as food reduces the absorption of therapeutic iron by approximately two-thirds [75]. Guidelines published in 1997 (now extensively accepted) encourage the regular use of intravenous treatments rather than oral iron supplements [76].

When choosing a strategy for iron deficiency therapy, physicians must remember that the amount of iron administered, not its form, is the main cause of any undesirable side effects [77-79]. Cost is also a factor since the intravenous treatments are far more costly than oral supplementation [36]. Weekly iron supplement treatment should be considered as an alternative to daily treatments. This diminishes costs and limits the effects of other micronutrients (such as zinc) on iron absorption [80, 81]. It also decreases the risk of iron overload, enhances the compliance of patients, and ultimately reduces the participation of health operators. Results indicate that the variations of hemoglobin in weekly patients are similar to those in patients treated daily. Taking vitamin A together with iron supplements improves counts more than taking iron alone.

Daily iron supplements may negatively affect the bodily zinc status, so zinc must be included in supplement treatments. Thus, a supplement regimen of iron, vitamin A, and zinc is more effective than unaided iron supplementation in ameliorating the status of micronutrients. Ideally, a supplement for children should include 15 mg iron, 15 mg zinc, and 350 μg vitamin A [13].

Iron supplementation strategies have to consider numerous factors: the iron complex formation with food ligands and physiological factors on one side, and social factors, cost, and achievability on the other [82]. Readily available iron supplementation that does not create gastrointestinal discomfort during digestion is greatly needed [36].

Total iron content, type of iron, and the occurrence of particular dietary enhancing and inhibiting factors influence iron bioavailability and availability for use and storage [83]. As a result of heme iron and an unknown enhancing factor in meat, diets containing meat favor iron bioavailability more than meat-free diets with an equal amount of iron [84]. On this basis, a greater percentage of iron is absorbed or conserved from diets containing meat [85]. Many iron supplements of varying quality are presently used. The quality of a supplement together with its marketing determines its success with consumers. Iron supplement programs for pregnant women normally suggest reasonably priced, easily transported and stored tablets. UNICEF manufactured a tablet containing 60 mg iron (as $FeSO_4$) with 250 μg folic acid (later increased to 400 μg). A liquid supplement, which can be directly dropped into the mouth, is necessary for children younger than 2 years, even if a powder or tablets can be easily crushed and mixed with infant foods. The higher price of liquid formulations must be weighed against their easier utilization, which determines a greater compliance by users [14].

Iron Fortification

Iron fortified food is one of the main strategies for preventing iron deficiency and is the most sustainable, convenient, and cost-effective tool for its management [86]. However, many problems exist related to the ability of iron ions to react with a number of components of foods. Iron produces changes in color, flavor, and oxidation of fats [87]. In order to avoid such undesired sensory changes in fortified food, the less soluble iron compound should be used instead of the most bioavailable water-soluble iron compounds [88]. The fortification of wheat flour is a rather simple procedure. A number of foods have been effectively fortified with iron, including salt, sugar, curry powder, and fish sauce. Milk, dried milk, and various milk-derived foods have been fortified with iron in South America. Iron-fortified baby food is a great tool to effectively control iron deficiency anemia [30].

A variety of bioavailable iron compounds, inorganic and organic, are used to fortify drinking water that can be accessible at low price with little effort to communities. Drinking water represents a valuable and economical tool to provide iron in the diet [89].

Babies accumulate approximately 250 mg of iron throughout gestation, which are then utilized during breastfeeding, since breast milk can only supply 0.15 mg of the daily absorbed iron against a daily necessity of 0.55 mg [90]. A premature utilization of cows' milk can also contribute to iron deficiency in early childhood [91]. An Indian study on the control and prevention of anemia with iron-fortified foods presented an iron intake of 10-15 mg per day using iron-fortified salt (NaCl) [92]. Further 5 mg per day were supplied with fortified sugar, and 8-10 mg iron per day with fortified rice [93]. Four studies on infants utilizing iron-fortified foods have shown the absence of significant side effects; iron appreciably protects infants from infectious diseases, and particularly from respiratory tract infections [94]. In the following, the characteristics of the most-used iron supplements are presented. The chemical structures of the anions of these salts are shown in (Fig. **1**).

Literature results indicate that ferrous sulfate, ferrous gluconate, and ferrous fumarate are equal as regards iron availability. According to FDA requirements, less than 2% of ferric iron should be contained in ferrous fumarate [95]. Ascorbic acid added to ferrous sulfate increases iron availability. The availability of the polysaccharide-iron complex was found to be considerably lower than that of all the other iron compounds [36].

Food utilization can differ considerably, day to day, as well as by person. Furthermore, large quantities of ferrous salts may modify the color and taste of food, while iron-phosphates are inadequately absorbed. However, consumption of iron-fortified milk (12 mg/L) is adequate to preserve a normal state of iron that cannot be improved by further iron administration (6 mg iron/kg body weight for 3 months) [82]. A micronized, soluble ferric pyrophosphate [82], ferrous bisglycinate, and ferrous complex with the amino acid glycine, are three iron fortificants of great utility in liquid products. The bioavailability of ferric orthophosphates is lower than that that of ferrous sulfate, ranging between 5% and 60%. Nevertheless, they are largely used in the USA to

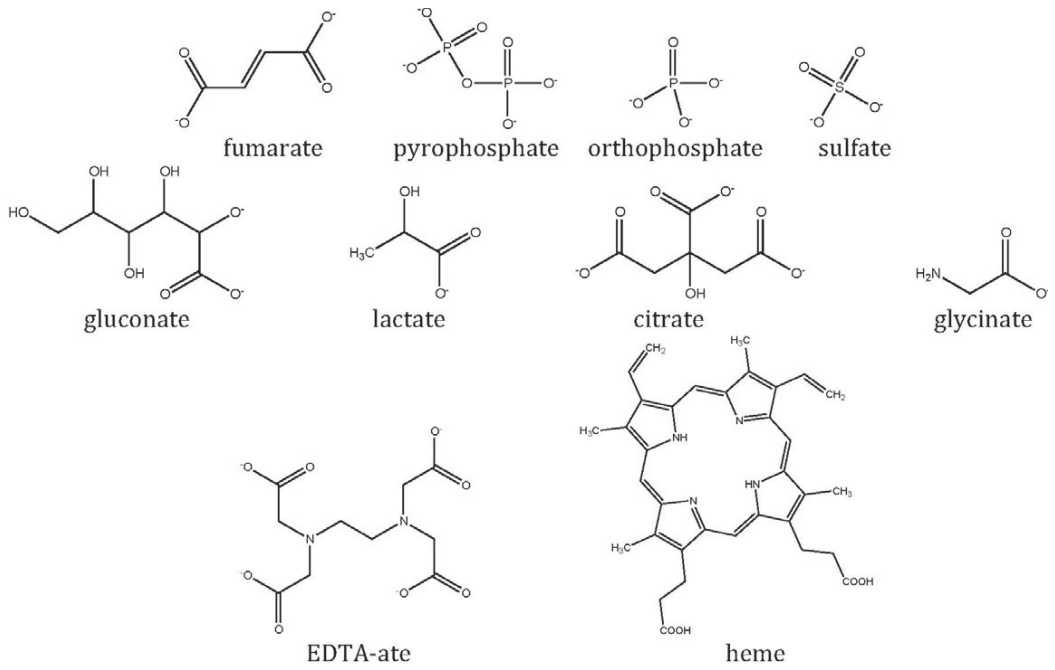

Fig. (1). Chemical structures of the anions of iron salts in use as supplements and/or in fortified foods.

fortify flour and cereal foodstuffs because they are scarcely reactive with these foods [96].

Fish sauce [97] and sugar [98] have been fortified by using NaFeEDTA. Sodium chloride (NaCl) is the almost ideal medium to be fortified with essential elements. Manufactured in centralized units, its use is homogeneous among different populations. It is fortified with different iron compounds, such as NaFeEDTA, ferrous sulfate, ferric orthophosphate in conjunction with color stabilizers [92]. NaFeEDTA, in particular, has been used to fortify different foods in various countries, resulting in a noteworthy enhancement of iron conditions: sugar (Guatemala) [99], curry powder (South Africa) [100], soy sauce (China) [101], fish sauce (Vietnam), and maize flour (Kenya) [102]. The absorption of NaFeEDTA from foods containing high amounts of phytates is 2–3 times than that of ferrous sulfate [103]; however its use as a food additive, approved at 0.2 mg Fe/day per kg bodyweight, is restricted to infants and children [104]. Unlike other iron compounds, NaFeEDTA does not favor the oxidation of fat in stock cereals nor the precipitation of peptides in fish and soy sauces.

Ferric pyrophosphate in a micronized, milled form presented positive features in the fortification of color-sensitive foods, such as salt (Africa) [105], and rice (India) [106].

Ferrous sulfate is the basic iron compound for treating iron deficiency anemia [107]. When supplemented in anchovies, it resulted in an iron intake greater than 30 mg per day [108]. Ferrous sulfate, characterized by a high bioavailability, is used in bread and in bakery foods with a short shelf-life [109] and in chapattis, a traditional bread from India and Pakistan [110]. The low cost and the high bioavailability favor the choice of ferrous salts (sulfates and gluconates) as oral supplements. 300-mg tablets of ferrous sulfate, containing 60 mg of iron, consumed 3-4 times per day, constitute the typical therapy for treating iron deficiency anemia in adults. An empty stomach facilitates iron absorption but may cause nausea and epigastria [1]. Anhydrous ferrous sulfate can be added to wheat flour just after milling for immediate consumption, but as flour is stored for a long time in the majority of developing countries, the less reactive elemental iron, in powder, is used extensively, despite its poorer bioavailability [1, 111, 112].

Tests on the efficiency of different forms of elemental iron conducted in Thailand suggest that both electrolytic iron and hydrogen-reduced iron are useful for fortification, even if their bioavailability is lower than that of ferrous sulfate (50% – 79%) [113].

The study of ferrous gluconate on iron absorption in normal subjects and in hypertensive patients under chronic methyldopa therapy revealed possible modifications in methyldopa absorption, metabolism, and blood pressure [107].

Ferrous lactate and ferrous gluconate were successfully used in milk and soybean food fortification, but high cost limited their use [96]. Even infant formulas were effectively supplemented with ferrous salts (sulfates, gluconates, lactates and ascorbates). Iron ammonium citrate demonstrated a limited bioavailability when added to wheat flour [114] and chapattis [110].

A micronized dispersible mixture of ferric pyrophosphate [115], ferrous bisglycinate and amino acid chelate [116], is an iron fortificant particularly useful for liquid products.

Heme containing foods (mainly red meats) include iron in an easily absorbed form and moreover aid iron absorption from other foods in which it is less bioavailable [30]. About 32% and 21% iron is absorbed from hemoglobin and myoglobin, respectively [117, 118]. The availability of heme iron in fortified milk was 19%, 2-6 times higher than that detected with inorganic iron compounds [119]. Nevertheless,

the availability of heme iron strongly depends on the food matrix. At alkaline and neutral pH, purified hemin forms poorly-absorbed, high-molecular-weight aggregates; the addition of proteins can reduce the formation of these aggregates [120]. Concurrent consumption of meat facilitates the absorption of heme iron [121]. Meat eating plays an important role in the balance of iron and enhances non-heme iron absorption [122]. Chocolate cookies fortified with heme iron were used in a national program in Chile [123]. 30–70% of the iron contained in meat is heme iron, about 15–35% of which is absorbed by the body. The absorption rate of non-heme iron is generally less than 10%, but can be increased by meat consumption and by ascorbic acid, while phytates, polyphenols, and calcium exert an inhibitory role [87, 124].

An extensively used parenteral iron preparation for intravenous administration based on iron dextran had been demonstrated to be extremely useful in the treatment of iron deficiency [125]. However, iron dextran has been removed from the market because of the high number of undesirable reactions, so a proper alternative for the treatment of iron deficiency is required [126]. The nonionic iron (III)-hydroxide is bound in a polymeric form to dextran. Iron ions interact strongly with endogenous iron binding proteins, resulting in denaturation products responsible for the toxicological effects [126].

Elemental iron as a fine powder (e.g. electrolytic iron) presents an acceptable bioavailability that allowed their use to fortify flour and bakery foods [127].

Plant breeding or genetic engineering are some of the most recent approach for biofortifying foods. Selective breeding may increase iron content in basic foods. The high diversity in iron content between wheat (25–56 mg/kg) and rice (7–23 mg/kg) is almost eliminated by the milling treatments [1].

4. CONCLUSION

Nutritional iron deficiency, whether accompanied by nutritional anemia or not, still represents a common health problem in developing countries, where plant-based diets provide low amounts of bioavailable iron. Young and pregnant women and children are the main sufferers of iron deficiency. Iron deficiency may have significant consequences on the health of affected subjects, with severe weakening of the immune function resulting in susceptibility to infectious diseases. Moreover, iron deficiency could play a relevant role in brain development during gestation, with consequences on the later cognition of children. Increased understanding of molecular pathways involved in iron absorption and metabolism here reported should be the basis for new strategies of iron deficiency therapy in affected subjects.

Among different therapeutic strategies here summarized, iron fortification is the best tool for halting iron deficiency, though technical problems limit the quantity of iron that may be put into different foods. The consequences of iron fortification on the status of other trace elements, including zinc and copper, should be taken into consideration when planning iron fortification strategies, given the competition for absorption and transport of iron ions and other trace metal ions.

ABBREVIATIONS

Apo-Tf	=	Apo-transferrin
ATP7A	=	Copper-transporting ATPase 1
BBB	=	Blood brain barrier
DCYTB	=	Duodenal cytochrome B
DMT1	=	Divalent metal transporter 1
FDA	=	Food and Drug Administration
Fe-TP	=	Iron transferrin
FPN	=	Feroportin
FXN	=	Frataxin
HCP	=	Heme iron transporter
HFE	=	Hemochromatosis protein
HP	=	Hephaestin
NaFeEDTA	=	Sodium iron etilendiaminetetra acetate
Nramp1	=	Natural resistance associated macrophage protein 1
PCFT	=	Proton coupled folate transporter
RDA	=	Recommended daily allowance
RE macrophages	=	Reticuloendothelial macrophages
Steap	=	Six-transmembrane epithelial anti gen of prostate
TFR	=	Transferrin receptor
ZIP14	=	Zinc transporter 14

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflict of interest.

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