Iron bioavailability from food fortification to precision nutrition. A review

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ABSTRACT

Iron deficiency anaemia is a Worldwide Public Health problem and the fortification of food with iron is the most cost-effective prevention strategy. The correct combination of iron form and food vehicle is crucial, as well as the dietary context of consumption. Combinations of iron with an enhancer of its bioavailability and avoidance of interaction with iron inhibitors are recommended. New iron fortificants, innovative complexes, coatings and nanoparticulates, and biofortification are the main research lines. Ultimately, human assays are necessary before industrial production. In this regard, precision nutrition helps to identify the vulnerable groups that, according to genotype, dietary habits, physical activity and, most recently, metagenomic profile, may benefit from a specific iron-fortified food. This review addresses the modifiers of iron bioavailability and the main aspects to take into account in the development of iron-fortified food to prevent iron deficiency.

Industrial Relevance:

The potential target population that would benefit from iron-fortified foods is that at risk of iron deficiency. However, there are also segments of population at risk of iron overload.

Iron fortification involves complex technological issues, but the economic impact is very high.

Research on “omics” sciences delivers scientific results applicable to the design and production of iron-fortified food.

Keywords

Iron bioavailability

Food fortification

Iron deficiency

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1. Iron deficiency and populations at risk

Iron deficiency is still the most common and widespread nutritional disorder in the world. Although the estimated 1995–2011 trends in distributions of haemoglobin concentration show that global anaemia prevalence decreased (Stevens et al., 2013), the numbers are still staggering. In 2011, the World Health Organization (WHO) estimated that around 800 million children and women are anaemic, mainly owing to iron deficiency (World Health Organization, 2015). Even though mean blood haemoglobin concentrations and prevalence of anaemia varied substantially across regions (**Table 1**), iron deficiency is the only nutrient deficiency that is also significantly prevalent in both developing and developed countries. Globally, almost half of the children aged between 6 and 59 months had anaemia, and one in three women of reproductive age was anaemic (World Health Organization, 2015). These data show that iron deficiency and iron deficiency anaemia affect more people than any other condition, and therefore constitute a public health condition of epidemic proportions.

The economic costs of iron deficiency anaemia resulting from annual physical productivity losses have been calculated to be around US$ 2.32 per capita, or 0.57% of gross domestic product in low- and middle-income countries (Horton & Ross, 2003). Therefore, iron deficiency and anaemia not only reduce the work capacity of individuals and entire populations but could also bring serious economic consequences and obstacles to national development.

Because of all this, reduction of the global burden of iron deficiency and iron deficiency anaemia is generally considered within the scope of public health nutrition (Pasricha & Drakesmith, 2016). In fact, in 2012 the World Health Assembly Resolution 65.6 endorsed a “Comprehensive implementation plan on maternal, infant and young child nutrition”, which specified six Global Nutrition Targets for 2025 (World Health Organization, 2012), the second target being to “achieve a 50% reduction of anaemia in women of reproductive age” (World Health Organization, 2014). In this regard, public health strategies to prevent and control anaemia include several options that are currently available for providing iron, but among them iron food fortification seems to present the best risk–benefit balance (Prentice et al., 2017).

In contrast, iron excess is another area of concern since it has been related to several diseases, such as cirrhosis, cardiovascular disease, type 2 diabetes and cancer (Toxqui et al., 2010; Vaquero, García-Quismondo, del Cañizo, & Sánchez-Muniz, 2017). Therefore,

subjects at risk of iron overload owing to genetic or acquired diseases should be protected in situations where mass fortification policies are applied.

2. Functions of iron and consequences of deficiency

Iron is a metal that is highly abundant in the earth's crust and essential for human life. By the end of the nineteenth century it was acknowledged as a micronutrient needed for recovery from anaemia. Quantitatively, the main biological function of iron is oxygen transport, as it forms part of the haem nucleus in the proteins haemoglobin and myoglobin, but small quantities of iron participate in more than 200 enzymatic systems that are essential for cellular functions (Skikne & Hershko, 2012), including energy utilization by cells, DNA, RNA and protein synthesis, and numerous redox reactions by virtue of exchanges between the ferrous (Fe^{2+}) and ferric (Fe^{3+}) states. Iron participates in enzyme systems such as those involved in cholesterol catabolism (Toxqui et al., 2010; Vaquero, García-Quismondo, Cañizo, & Sánchez-Muniz, 2017), collagen metabolism (Vasta, Higgin, Kersteen, & Raines, 2013; Vasta & Raines, 2016), vitamin D activation (Schlingmann et al., 2011), and neurotransmitter metabolism (Hare & Double, 2016). Therefore, iron is essential for oxygen transport and storage and for many other metabolic functions related to growth, immunity, muscular activity, bone strength and the nervous system.

Iron deficiency is frequently asymptomatic and thus may often go undiagnosed. Nonspecific symptoms ascribed to low delivery of oxygen to body tissues and decreased activity of iron-containing enzymes are weakness, fatigue, tiredness, low work capacity, diminished cognitive efficiency and difficulty in concentrating (Camaschella, 2015). In this regard, it is very well known that an insufficient iron status in the body may negatively affect work performance (Haas & Brownlie, 2001). In situations of more severe iron deficiency or anaemia there may also be clinical manifestations such as glossitis, angular stomatitis, koilonychia or spoon nails, hair fall and itches (Remacha, 2011). In children and adolescents, lower levels of iron have been linked to growth retardation, a worse motor and cognitive development (Halterman et al., 2001; Grantham-McGregor & Ani, 2001; McCann & Ames, 2007; Carter et al., 2010) and social inattention and decreased school performance (Allali et al., 2017). During pregnancy, iron deficiency anaemia has been associated with increased risk of low birth weight, prematurity (Scholl, 2001; Rasmussen, 2001) and maternal and child mortality (Brabin, Hakimi, & Pelletier, 2001; Brabin, Premji, & Verhoeff, 2001). Also, a link between iron deficiency and increased risk of bone resorption has been hypothesized in women at childbearing age (Blanco-Rojo et al., 2013; Toxqui et

al., 2014a; Toxqui & Vaquero, 2015; Wright et al., 2013). In the elderly, anaemia has been related to decreased quality of life and physical functioning (Thein et al., 2009). Moreover, iron deficiency anaemia has been shown to be an independent risk factor for fatality in patients undergoing surgical procedures (Musallam et al., 2011) and it has been associated with increased all-cause mortality in the general population (Martinsson et al., 2014).

3. Iron absorption and homeostasis

As stated earlier, iron is essential for life, but its ability to easily gain or lose electrons also facilitates the generation of highly reactive oxygen species, which would damage essential biological components such as lipids, proteins and DNA (Gozzelino & Arosio, 2016; Paul & Lill, 2015). Therefore, it requires a sophisticated regulation that serves to cover the body's demands but also prevents excessive accumulation of iron. Body iron status is maintained by a complex process that regulates the balance between iron absorption in the duodenum, iron recycling by macrophages and iron storage, mainly in the liver, since there is no physiological pathway for iron excretion (Rybinska & Cairo, 2017). In this regard, hepcidin is shown to play a key role. This hormone, discovered at the beginning of this century, exerts a negative control on iron homeostasis by inhibition of iron absorption and iron mobilization from tissue stores (Ganz & Nemeth, 2012). Hepcidin acts by binding to and inducing the degradation of ferroportin, an exporter transmembrane protein, inhibiting the release of iron from target cells (hepatocytes, macrophages and enterocytes) (Nemeth et al., 2004). Moreover, recent findings demonstrate that hepcidin may also regulate body iron uptake by decreasing expression of the genes involved in iron absorption (Bergamaschi et al., 2017).

Iron in food is present in two forms: non-haem and haem iron. Non-haem iron present in food is of animal and plant origin, while haem iron is only provided by animal food. Iron is absorbed mainly in the duodenum, but the absorption mechanisms of these forms are different, as presented in Figure 1.

3.1. Non-haem iron absorption

Non-haem iron complexes present in food are degraded during digestion in the gastrointestinal tract owing to the action of pepsin and hydrochloric acid. Once released from food components, most non-haem iron is present in the ferric form (Fe^{3+}), which has low solubility and bioavailability (Han, 2011). However, there are numerous dietary components capable of reducing Fe^{3+} to Fe^{2+}, including ascorbic acid, and amino acids

such as cysteine and histidine, and moreover the main reducing activity is carried out by duodenal cytochrome b reductase (Dcytb), a haemoprotein located on the apical membrane of the enterocyte that uses ascorbate to facilitate ferrireduction (Sharp & Srai, 2007).

3.1.1. Ferrous iron absorption

The soluble Fe^{2+} form is transported into the enterocyte via the divalent metal transporter-1 (DMT-1), which is a proton symporter requiring low pH for efficient function for metal transport (Figure 1). This carrier is not iron-specific and there is competition between iron and other divalent metals, such as calcium and zinc (Kordas & Stoltzfus, 2004).

3.1.2. Ferric iron absorption

Some authors have proposed that Fe^{3+} might be absorbed by intestinal enterocytes via a mechanism that is distinct from DMT-1, although this mechanism has not been totally elucidated (Simovich et al., 2003). It is proposed that luminal Fe^{3+}, by interaction with mucins and subsequent association with β3-integrin and mobilferrin, first crosses the luminal membrane and is internalized; then this Fe^{3+}-protein complex combines with flavin-monooxygenase (flavin-MO) and β2-microglobulin (β2-m) to form a paraferritin complex where Fe^{3+} is reduced to Fe^{2+} (Sharp, 2010; Simovich et al., 2003), which can finally be exported to the cytosol by DMT-1.

3.1.3. Ferritin absorption

It has been hypothesized that ferritin may also be a good source of iron. Three mechanisms have been proposed: ferritin that is taken up by a specific receptor in the luminal membrane; an endocytosis mechanism; or that ferritin provides Fe^{3+} after protease hydrolysis. It should be noted that ferritin is a macromolecule in which a large quantity of iron is stored (Theil, 2013; Theil et al., 2012), both in animals and plants, thus intense activity exists in this field (Latunde-Dada et al., 2014).

3.2. Haem iron absorption

With regard to the haem absorption route, there is a specific haem-carrier protein (HCP-1) at the brush border, which explains why this iron form is almost unaffected by dietary factors and mostly absorbed intact. This carrier is also a proton-coupled folate transporter, often called HCP-1/PCFT, that displays higher affinity for folate than haem (Laftah et al., 2009). Once internalized, haem iron is released by haem oxygenase (HO) and then

follows the same pathways as non-haem iron (Shayeghi et al., 2005) or, as suggested recently, is exported intact through the basolateral membrane by FLVCR1 to the plasma, where it is captured by haemopexin and delivered in the form of haem-haemopexin. This second export mechanism would be a minor pathway and it involves the CD91 receptor of the haem-haemopexin complex (Staroń et al., 2017).

3.3. Intracellular storage and iron export

The absorbed iron has two fates, depending on the body's requirements. If the body stores are replete, a significant amount of newly absorbed iron will be stored in the enterocytes as ferritin (Fe^{3+} form). Because duodenal enterocyte turnover is very rapid (their lifespan is approximately 3–4 d), most of the ferritin contained inside will be lost by cellular desquamation. On the other hand, if the iron needs of the body are high, most of the iron inside the enterocyte will be transported across the basolateral membrane by ferroportin (FPN), and, after oxidation to Fe^{3+} by hephaestin, a transmembrane-bound ceruloplasmin homologue, be carried as diferric-transferrin and transported in the bloodstream (Anderson, Frazer, McKie, & Vulpe, 2002; Garrick & Garrick, 2009; Lane et al., 2015). When body iron stores are high, ferroportin is inactivated by hepcidin, as this hormone downregulates iron absorption and mobilization from tissues (Gharibzahedi & Jafari, 2017a).

4. Iron bioavailability and dietary factors

The term bioavailability of food components emerged from the phenomenon of the appearance in plasma of orally administered drugs used in pharmacology. In the case of mineral bioavailability, and specifically that of iron, bioavailability was initially synonymous with absorption and it was determined by *in vitro* solubility. The more soluble the iron compound, the greater its potential absorption and thus its bioavailability. This solubility approach is related to the definition of iron availability, or dialysability, if the digestion process is simulated using a semipermeable membrane and transport through it is measured (Vaquero, 1992).

In parallel with the development of new techniques, either *in vitro* or *in vivo*, the concept of bioavailability has developed. Therefore, iron bioavailability is currently defined as the proportion of iron ingested that is absorbed by the intestine and used through normal metabolic pathways or stored. It is expressed as a percentage of intake and is known to be influenced by dietary and host factors (Aggett, 2010), as shown in Figure 2. This wider approach to iron bioavailability includes the following steps: release from its matrix;

absorption into the systemic circulation; distribution to tissues; metabolic utilization or storage in the body. From the standpoint of Food Science and Technology, the first two are the main ones to consider.

As explained in section 3, the pathway for intestinal haem absorption is independent from those of non-haem iron, and haem iron is almost unaffected by interactions with other food components. However, the haem form only constitutes about 10–15% of total dietary iron, thus non-haem is the main iron source. There are concerns related to the possible health effects of increasing haem intake to protect from iron deficiency since excess haem intake has been related to colon cancer, increased oxidative status, etc. (Turner & Lloyd, 2017). Therefore, everything that can be said about enhancers and inhibitors of iron absorption refers to non-haem iron.

4.1. Dietary components that increase or decrease iron bioavailability

First of all, the amount of iron ingested is a prerequisite for having bioavailable iron. This seems an obvious statement, but we must emphasize that no matter how powerful an enhancer or inhibitor may be, if it is consumed in a meal where iron is absent, its effect on iron absorption will be null. In this regard, it is also important to remember that interactions in the digestive tract occur within a couple of hours after ingestion. Therefore, the concept of bioavailability as the “proportion” of the iron ingested that is utilized or stored for body functions has to take into account the amount of iron ingested, the meal composition and the time between meals.

Another important factor is the form of iron. As indicated above, the pathway for intestinal haem absorption is independent from that of non-haem, and haem iron is almost unaffected by interactions with other food components. Animal foods such as meat and fish are recognized as good sources of highly available iron partly by means of their haem iron content, which is approximately 40% of total iron (Monsen et al., 1978). Clearly, a mixed diet supplies mainly non-haem iron, which interacts with dietary enhancers and inhibitors, and it has been estimated that iron bioavailability from a Western diet containing up to 90% of non-haem iron is approximately 5–15% (FAO/WHO, 2004).

Ferrous iron has generally been assumed to be better absorbed than ferric iron, but in fact both ions can be efficiently absorbed, provided that they reach the mucosa in a soluble form (Figure 1). The limiting point is solubility, as ferric salts can precipitate when pH rises from the stomach to the duodenal area. This precipitation can be prevented by complexation of iron with compounds that form absorbable chelates that remain soluble at

increasing pH (Van Dokkum, 2003). In addition, dietary compounds that reduce iron from ferric to ferrous generally increase bioavailability.

Ascorbic acid and animal tissue are the main enhancers, whereas phytic acid and polyphenols are the main inhibitors. Details of enhancers and inhibitors are presented in Table 2.

4.1.1. Enhancers

Ascorbic acid (AA) is recognized as the most powerful iron enhancer. AA acts by forming a chelate with iron at the low pH of the stomach, which is maintained in the intestine. This prevents interaction of iron with other ligands, such as phytates, that bind iron at a higher pH (Siegenberg et al., 1991). Some authors suggest that the AA–iron complex is absorbed intact in the duodenum and others indicate that the binding mechanism predominates over the ferrireductase activity of AA (Teucher, Olivares, & Cori, 2004). Interestingly, AA is a cofactor of Dcytb, the main enzyme that provides Fe^{2+} from Fe^{3+} to the enterocyte luminal membrane (Latunde-Dada et al., 2002).

The AA/Fe molar ratio is decisive in the final effect on iron absorption. Our research group studied the influence of consumption of an iron-fortified fruit juice, compared to a placebo fruit juice, in iron-deficient women (Blanco-Rojo et al., 2011b). The AA/Fe ratio was 1.7:1, and a clear increase in iron status was obtained from the first month of the assay. In this regard, several authors proposed a 2:1 ratio for low phytate content foods (Hurrell et al., 2004; Lynch & Stoltzfus, 2003; Teucher et al., 2004) and up to 4:1 for high phytate content food (Hurrell et al., 2004). However, it should be taken into account that AA is sensitive to temperature and air exposure and that food processing may decrease the concentration of AA by oxidation to dehydroascorbic acid (Teucher et al., 2004).

Animal tissue, from meat, fish or poultry, exerts an enhancing effect on iron absorption. This is a well-known factor, though the precise mechanism of action is still a matter of investigation. Non-haem iron absorption in infants increased if 25 g of lean beef was included in an 80 g vegetable purée (Engelmann et al., 1998). Similar results were obtained in young women: the addition of pork meat (50 g or 75 g) to a meal presumed to have low iron bioavailability (containing 220 mg of phytic acid and 7.4 mg of ascorbic acid) increased iron absorption significantly (Bæch et al., 2003a). Another study showed that iron absorption doubled with the addition of 60 g of lean fish to a simple meal consisting of rice, boiled vegetables and curry (Hallberg et al., 1978). Most of the studies were performed using low-fat meat or fish, but Navas-Carretero et al. (2008) tested the possible

effect of the addition of salmon fish to a high-phytate food. The experiment was aimed at reproducing a meal, red-kidney beans (80 g) with or without salmon (100 g), which was spiked with stable isotopes and given to iron-deficient women. The fish contained about 20% protein, expected to favour bioavailability, and 20% fat, part of which was n3 unsaturated fat that in high doses was reported to induce alterations in cellular membranes and haemolysis (Pérez-Granados, Vaquero, & Navarro, 1995). Results showed that the portion of salmon was able to increase iron absorption from the bean meal significantly, suggesting that an adequate combination of enhancers and inhibitors determines the final effect on iron bioavailability (Navas-Carretero et al., 2008).

Cooking temperature does not affect the enhancing effect of animal tissue (Bæch et al., 2003b), although it may decrease the amount of haem iron by degradation into non-haem (Cross et al., 2012; Lombardi-Boccia, Martinez-Dominguez, & Aguzzi, 2002), with a possible counteracting effect on iron absorption.

There are many doubts about the nature of the iron enhancer in meat and fish, but candidates include peptides rich in cysteine residues and carbohydrate fractions formed during digestion of proteins and glycosaminoglycans of the extracellular matrix of muscle tissues (Armah et al., 2008; Belluzzi et al., 2007; Huh, Hotchkiss, Brouillette, & Glahn, 2004).

4.1.2. Inhibitors

Phytates, myo-inositol phosphates, present in whole-grain cereals and legumes, inhibit non-haem iron absorption, depending on the number of phosphates linked to the inositol ring. Inositol hexaphosphate and inositol pentaphosphate are the most potent iron inhibitors, and the effect is dose-dependent (Hurrell & Egli, 2010). Phytate:iron molar ratios higher than 1 reduce iron absorption (Hallberg, Brune, & Rossander, 1989; Navas-Carretero et al., 2008).

Since the interaction is due to complexation of iron with the negatively charged phosphate groups of phytate, the use of commercial phytases for dephosphorylation of phytate has been promoted (Nielsen, Tetens, & Meyer, 2013). These may be produced biotechnologically, but there are still many problems for the application of this technology and its real repercussions, particularly in malnourished populations with iron deficiency.

Polyphenols are a heterogeneous group of plant compounds widely known for their interaction with iron and other metals. There is enormous interest in the development of

applications with this family of compounds (Khan et al., 2018). The strength of iron chelation depends on the polyphenol structure. It is suggested that at least two hydroxyl groups in the ortho- position are necessary, such as in catechins and gallates. A good example of an iron inhibitor is black tea, and, to a lesser extent, green tea, coffee, chocolate, wine, herbs and spices, and seeds (Hurrell et al., 2010; Navas-Carretero et al., 2007b; Petry, Boy, Wirth, & Hurrell, 2015). Nevertheless, polyphenols generally exert antioxidant effects, and may protect against chronic diseases, such as diabetes, cardiovascular diseases and several cancer types (Aprotosoaie et al., 2016; Lall et al., 2015; Moyano et al., 2016; Tangney & Rasmussen, 2013). Therefore, it is important to define the vulnerable population groups and the proper combinations of foods and ingredients to prevent iron deficiency anaemia.

Calcium and other divalent metals interact with iron for DMT-1 transport. Higher consumption of calcium or dairy products is associated with lower iron status. In a randomized controlled trial, consumption of a milk product fortified with iron that provided 100% of the recommended daily iron intake did not improve iron status during four months in iron-deficient women. The effect was attributed to the presence of calcium and partly also casein, which behaved as inhibitors, and the absence of iron enhancers in the product (Toxqui et al., 2013). Zinc in excess has also been reported to reduce iron absorption, and vice versa, an interaction that may be particularly relevant in pregnancy and infancy (Iyengar, Pullakhandam, & Nair, 2010; Pérès et al., 1998).

Several reports indicate that, compared to linoleic acid and polyunsaturated fatty acids, saturated fat increases iron bioavailability (Lukaski et al., 2001; Miret, Saiz, & Mitjavila, 2003; Pérez-Granados et al., 1995). The mechanism may act mostly after absorption (Vaquero, García-Quismondo, del Cañizo, & Sánchez-Muniz, 2017). It seems that an n6:n3 fatty acids unbalance induces metabolic alterations, including lipid peroxidation and changes in erythrocyte membrane composition, leading to haemolysis, iron depletion and anaemia (Pérez-Granados et al., 1995; Vaquero, Veldhuizen & Sarriá, 2001).

5. Dietary reference values for iron

Iron provided by the diet should be enough to meet physiological requirements and cover iron losses, ensuring a good iron status (Vaquero, 2011). The methodology for calculating dietary reference values for iron is complex, since it is necessary to determine both the requirements for each population group, according to physiological needs and iron losses; and a factor or ratio of intestinal iron absorption, which depends on the diet

consumed, as explained in section 4, and on the iron status and genetic background of the subject (Institute of Medicine, 2001).

Physiological iron needs depend on the individual's life stage. In infants, children and adolescents the mineral is required for haemoglobin mass increase, synthesis of new tissues and an increase in storage iron to build a reserve (Berglund & Domellöf, 2014; Domellöf et al., 2014; Mesías, Seiquer, & Navarro, 2013). Throughout the course of pregnancy, iron demands increase due to foetal growth and the expansion of plasma and blood volumes in the mother (McMahon, 2010; Vricella, 2017).

Iron losses in all population groups include loss of the mineral in faeces (physiologically regulated), and minor losses in urine and sweat and from exfoliation of skin cells. In women of child-bearing age, menstrual blood loss may constitute a relatively high iron loss (Hunt, Zito, & Johnson, 2009). In this regard, although there are several studies that show a relationship between the duration of the menstrual period and the volume of menstrual losses and serum ferritin, it is considered that the distribution is biased and difficult to estimate (Blanco-Rojo et al., 2014; Harvey et al., 2005; Toxqui et al., 2014b).

With regard to iron absorption, it is well known that it is highly dependent on the physiological situation and the iron status of the individual. In healthy subjects, intestinal absorption of dietary iron is inversely related to serum ferritin concentrations, particularly at concentrations below 60 µg/L (Ganz, 2013). Also, in pregnant women the increased need for iron is shown to be met by increases in the efficiency of iron absorption (Fisher & Nemeth, 2017). Moreover, genetic variants related to iron metabolism could increase iron absorption (Sarria et al., 2007; Ye et al., 2015). On the other hand, in situations of infection iron absorption decreases as part of the responses of the immune system to avoid proliferation of infection and sepsis (Cassat & Skaar, 2013).

Therefore, both the complexity and the variability of the various factors involved in iron homeostasis would partly explain why there is no consensus in the Recommended Dietary Allowance (RDA) of iron given by several countries and organizations, as is shown in Table 3. In summary, the highest recommended values for iron are for women of childbearing age (except for Brazil) and pregnant women, and the FAO/WHO recommendations point out the importance of dietary iron bioavailability (Brasil, 2005; European Food Safety Authority, 2015; Institute of Medicine, 2001; FAO/WHO, 2002; Nordic Council of Ministers, 2014; Moreiras, Carbajal, Cabrera, & Cuadrado, 2016; Department of Health UK, 1991).

On the other hand, the redox activity of iron makes it potentially toxic if it is present in excess. The main adverse effects that have been described are: acute toxicity, iron–zinc interactions, gastrointestinal discomfort, secondary iron overload (Institute of Medicine, 2002), and chronic diseases (Vaquero, García-Quismondo, del Cañizo, & Sánchez-Muniz, 2017; Institute of Medicine, 2002). However, not all the countries or organizations that have given a RDA have determined a Tolerable Upper Intake Level (UL). The European Food Safety Authority (EFSA) considered that the risk of systemic iron overload from dietary sources is negligible with a normal intestinal function, and the adverse effects described are secondary to an overdose of medical iron (European Food Safety Authority, 2015). Only the Institute of Medicine (IOM) has established a UL for iron in adults (45 mg/day for males and females aged 14 years and older, including pregnant and lactating women) and for infants and children (40 mg/day) (Institute of Medicine, 2001).

6. The challenge of iron fortification

Iron is the most difficult micronutrient to add to produce fortified food. A variety of iron salts have been tested and incorporated into different dietary matrices with the aim of producing iron-fortified foods according to the needs of the target population. Ferrous sulfate is widely accepted as the reference salt in studies of bioavailability; it is cheap and efficiently absorbed, but its main disadvantages are that it is quite unstable, its oxidation depending on temperature and air exposure, and it may produce adverse organoleptic changes (Huma et al., 2007). Oxidation may alter other food components and modify the colour of the vehicle's food, with the result of lower product quality, adverse sensory properties and consequently poor acceptance. The technological challenge is to achieve an iron form that, when added in sufficient quantity, provides enough bioavailability to improve iron status, and that is not rejected owing to sensory changes or adverse gastrointestinal effects. Moreover, although iron fortification is a more cost-effective strategy than iron supplementation (e.g. pharmaceutical iron) for the prevention of iron deficiency anaemia in populations at risk, the cost may be unaffordable for vulnerable individuals (Hurrell et al., 2004).

Iron fortification can be classified into three types: mass fortification, target fortification, and market-driven fortification. The first one is intended to protect the general population from deficiency and is common in developing countries, the second is limited to special population groups, and the third is the result of a manufacturer initiative intended to commercialize an enriched food with added value that would satisfy consumer choice. This

last type is usual in developed countries and can be a practical option in the improvement of public health or segments of population, e.g. athletes, vegetarians, etc.

The efficacy of iron fortification in the improvement of iron status depends on many factors, such as the vehicle selected, the iron compound and the iron status of the target population group.

6.1. Iron fortificants

Numerous fortificants are available for iron fortification. The biggest challenge is to identify a form of iron compound that is adequately absorbed, is stable, and does not alter the appearance or taste of the food vehicle.

Table 4 shows some of the iron fortificants that are widely used, together with newly proposed fortificants. Aspects such as solubility should be balanced with others such as feasibility of application in the real situation and desired bioavailability. In this regard, it may be preferable to choose a compound with lower iron bioavailability but that can be added to the food in greater quantity without organoleptic side effects.

Research on new iron fortificants is very active. The EFSA concluded that the following iron sources are of no safety concern: ferrous bisglycinate (European Food Safety Authority, 2006); ferric sodium EDTA, as long as it does not lead to an exposure to EDTA above 1.9 mg EDTA/kg body weight/day (European Food Safety Authority, 2010); iron (II) taurate (European Food Safety Authority, 2009); and iron L-pidolate (European Food Safety Authority, 2007).

In contrast, haem iron has not been proved to be safe, and in fact there are concerns about its use, as excess intake of haem has been related to colorectal and prostate cancer (Bylsma & Alexander, 2015; Fonseca-Nunes, Jakszyn, & Agudo, 2013; Span et al., 2016) Therefore, until all doubts are cleared, its use in fortification should be discarded.

As shown in Figure 3, strategies to increase bioavailability include reduction of particle size and encapsulation (Genevois, de Escalada Pla, & Flores, 2016). Micronized ferric pyrophosphate, commercialized in liquid and dried forms, has been tested in the last decade. Being insoluble in water, this fortificant does not produce colour changes in the food matrix or cause sensory changes. Reduction of its mean particle size from 8 to 4 microns increased iron absorption by 2–4 times in adults (Hurrell et al., 2004).

6.2. Food vehicle

In the design of iron-fortified food it is necessary to select the appropriate combination of fortificant and vehicle, taking into account the individuals that will benefit from its consumption. In this regard, powder infant formulas represent a successful example. Generally, they contain ferrous sulfate, which is 100% soluble when the formula is reconstituted, and ascorbic acid, which guarantees sufficient iron bioavailability (Shamah-Levy et al., 2008; Villalpando et al., 2006). Cereal foods, for the weaning stage or adulthood, are good candidates for fortification as they are staple foods in many populations around the world and can be handled in solid form to manufacture the iron-fortified cereal-based food. The iron compounds recommended by the WHO to fortify cereals are ferrous sulfate, ferrous fumarate, ferric pyrophosphate and electrolytic iron (Diego Quintaes, Barberá, & Cilla, 2017). Another aspect to be taken into account is cooking or industrial thermal processing, because these treatments may decrease iron bioavailability by modification of the fortificant itself or the accompanying food components (Lee, Clydesdale, & Tannenbaum, 1979).

Food matrix composition is decisive to ensure the efficacy of iron fortification (Figure 3). In this line, our research group studied the effects of consumption of a fruit juice fortified with microencapsulated iron pyrophosphate coated with lecithin on iron status in a target population. We performed a 16-week randomized double-blind placebo-controlled trial in iron-deficient young women (Blanco-Rojo et al., 2011b). Women were randomly assigned to the iron-fortified group or the placebo group. Both fortified and placebo juices were manufactured in 500 mL cartons and with two different flavours (orange and peach–apple) to achieve compliance. The iron-fortified juice provided 18 mg of iron (100% of the RDA per day, 500 mL/day). It was found that the iron-fortified juice induced an improvement in iron status, with significant increases in haemoglobin, haematocrit, mean corpuscular volume, serum ferritin, and transferrin saturation; and decreases in serum transferrin and soluble transferrin receptor. Therefore, it was concluded that consumption of this iron form in this particular food matrix was efficacious in improving iron bioavailability in iron-deficient women (Blanco-Rojo et al., 2011b).

However, the same iron form (microencapsulated iron pyrophosphate), using the same amount per day and given to a group of iron-deficient women (n=109) with characteristics very similar to those of the previous investigation, was not efficacious when added to a dairy product (Toxqui et al., 2013). These contrary results were explained by the different food matrices. The iron-fortified fruit juice contained a high quantity of ascorbic acid, which

favours iron absorption, whereas the dairy product contained 600 mg of calcium per 15 mg of iron, known to be an inhibitor of iron bioavailability if consumed in a meal that does not contain absorption enhancers.

In this regard, other findings by our research group reinforce the importance of the food vehicle. Encapsulated iron pyrophosphate was demonstrated to be bioavailable when added to a meat pâté (Navas-Carretero et al., 2007a; Navas-Carretero, Pérez-Granados, Sarriá, & Vaquero, 2009), but not if included in cocoa powder (Navas-Carretero et al., 2007b). Although encapsulation was intended to protect the iron from interactions with other food components, thus maintaining bioavailability, experimental results in animal and human models demonstrated that, no matter how good the iron fortificant may be, its intake in combination with enhancers and inhibitors determines the final effect (Shilpashree, Arora, Sharma, & Singh, 2015).

6.3. New strategies for iron fortification

New iron fortificants are under development. Nanotechnology engineering provides nanosized iron that can be highly absorbed by physiological routes (Gharibzahedi & Jafari, 2017b). However, because excess free iron in biological systems is harmful, maximum safety conditions are required before industrial production (Mahler et al., 2012). We observed in an animal model that Fe (III) oxide nanoparticulates were absorbed by the ferric pathway and did not cause adverse haematological or organ effects (Chamorro et al., 2015), suggesting that it is feasible to produce customized iron forms and fractions with the objective of controlling iron solubility and absorption as desired.

Ferritin nanoparticles are another option. Biologically, ferritin is a large protein capable of storing high amounts of iron and, as mentioned in section 3, it may be a good source of iron, although the exact mechanism of absorption is unknown. Thus synthetic ferritin-mimetic nanoparticulates have been developed and studied in isolated duodenal loops or cultured cells (Aslam et al., 2014; Latunde-Dada et al., 2014).

With regard to, the food vehicles to be fortified with iron, apart from infant formula and staple foods, seasonings (i.e. table salt, soy sauce, fish sauce, bouillon and curry powder) have been assayed owing to their extensive use in the various target populations (Degerud, Manger, Strand, & Dierkes, 2015). The steps in the industrial production of micronutrient-fortified condiments and seasonings have been reported (Mejia, Aguilera-Gutiérrez, Martin-Cabrejas, & Mejia, 2015). Reports indicate that consumption of

NaFeEDTA in a sauce (World Health Organization, 2006) decreased the prevalence of anaemia in India and China. Electrolytic elemental iron added to curry powder has also been assayed (Karn, Chavasit, Kongkachuichai, & Tangsuphoom, 2011). Likewise, the fortification of bouillon cubes seems promising (Cercamondi et al., 2016). Moreover, combinations with probiotics have been proposed to increase bioavailability (Hoppe, Onning, Berggren, & Hulthen, 2015).

Biofortification, i.e. breeding and genetic modification of plants to obtain a final plant food with a higher iron content, is another approach for which there are high expectations. Efforts have been made with micronutrient biofortification in staple foods, but the final objectives are still far from being accomplished and all requirements related to safety, cost-benefit and low environmental impact should be fulfilled before implementation (Finkelstein, Haas, & Mehta, 2017; Petry et al., 2015; Ramzani et al., 2016).

7. Current trends in iron research. "Omics" and Precision Nutrition

Since ancient times it has been observed that individuals respond differently to diet and environmental factors. But it was not until the end of the twentieth century, when the sequencing of the human genome was completed, that a new research field emerged, Nutritional Genomics, which aimed at discovering how genetic variations could modulate the effect of certain diets or nutrients on different phenotypes (nutrigenetics). At the same time, this discipline also pursued the study of how dietary components may influence gene expression and metabolic routes (nutrigenomics) (Ordovas & Corella, 2004). Current technological advances have allowed the discovery of other "omics" that also interact with dietary factors to influence individual responses and metabolism. Thus, nutriepigenetics refers to the effect of certain nutrients or diets in reversing epigenetic alterations, relating to gene expression without concomitant changes in the DNA coding sequence, which might have a significant impact on preventing and treating chronic human diseases (Choi et al., 2013). Another concept is metagenomics, which is related to the interaction between gut microbiota, diet and various metabolic processes (Ferguson et al., 2016).

These "omics-based" investigations have also reached the field of iron research. Several studies have shown how iron metabolism can be modulated by genetic variations affecting the corresponding proteins. Among them, the best-known and most widespread genetic variants are two related to susceptibility to iron overload located in the *HFE* gene, C282Y and H63D mutations (Merryweather-Clarke et al., 2000). These polymorphisms result in diminished expression of hepcidin, which leads to a failure to downregulate the

efflux of iron absorbed from enterocytes when iron stores are replete or excessive, therefore causing iron accumulation and potential tissue damage, a disorder also known as haemochromatosis (Fleming et al., 2005). Less common mutations in haemojuvelin, hepcidin, transferrin-2 and ferroportin genes also occur and, through a deficiency of or resistance to hepcidin, also result in unregulated iron absorption and efflux from the enterocyte, leading to iron overload and tissue damage (Brissot et al., 2017). However, these variants could be considered as an advantage for certain populations, such as women at childbearing age, which have high susceptibility to iron deficiency due to menstrual losses. Indeed, high iron status was observed in heterozygous young women, for either C282Y or H63D, compared with women lacking these mutations (Blanco-Rojo et al., 2011a; Blanco-Rojo et al., 2014; Gordeuk & Brannon, 2017). On the other hand, other mutations have been related to iron deficiency. Several *TMPRSS6* mutations may increase systemic hepcidin levels in humans, leading to iron-deficiency anaemia (Beutler et al., 2010). Also, mutations in the transferrin (*TF*) gene, the calcium channel gene (*CACNA2D3)* and the *HIST1H2BJ* gene have been associated with low iron status in iron-deficient young women (Baeza-Richer et al., 2015; Baeza-Richer et al., 2013; Blanco-Rojo et al., 2011a).

Moreover, the best documented examples of clinically significant nutrigenetic interactions are those concerning the *HFE* mutation C282Y. Iron absorption from isotopically labelled iron dosages and test meals was repeatedly reported to be higher in subjects who were homozygous or heterozygous for the C282Y mutation than in healthy control individuals (Hunt & Zeng, 2004; Hutchinson et al., 2008; Roe et al., 2005). Also, both longitudinal and cross-sectional iron intervention studies showed interactions between dietary components, *HFE* genotype and iron status (Greenwood et al., 2005; Kaltwasser et al., 1998; Milward et al., 2008; Scotet et al., 2003). However, investigations about other genes involved in iron homeostasis and diet interactions are scarce, particularly those related to iron deficiency anaemia, and the results are less clear. Sarria et al. (2007) found no influence of mutation G277S in the *TF* gene on iron absorption in iron-deficient women. Cheng et al. (2014) observed that women with the minor allele of the *TMPRSS6* rs855791 polymorphism presented higher serum iron and transferrin saturation compared to T subjects after a 12–month trial with a higher-protein, higher-haem iron weight loss diet. Moreover, in a placebo-controlled nutritional intervention with an iron-fortified fruit juice in iron-deficient women, Blanco-Rojo et al. (2010) showed that the iron-

fortified juice markedly increased iron status, except in women that presented the minor allele of SNP rs3811647 located in the *TF* gene.

With regard to epigenomics, cells have several mechanisms that specifically regulate the expression of iron metabolism-related genes. These include the modulation of general cellular mechanisms that give rise to alternative transcript variants (such as alternative transcription initiation, polyadenylation and splicing) or of more specific systems that control the stability of the mRNAs and proteins (Silva & Faustino, 2015). In this regard, it was recently suggested that non-coding microRNAs (or miRNAs) may be implicated in iron metabolism regulation, as well as in the development of iron-related disorders (Davis & Clarke, 2013). These miRNAs normally repress gene expression and exert their function by binding to the target mRNA and consequent translation inhibition and/or degradation (Eulalio, Huntzinger, & Izaurralde, 2008). Several miRNAs involved in iron metabolism were widely reviewed by Davis and Clarke (2013) and Silva and Faustino (2015), who described miRNAs implicated in dietary iron absorption, cellular iron uptake and systemic control of iron homeostasis.

In addition, it has been suggested that a bidirectional relationship exists between the host iron intake and gut microbiota (Deschemin et al., 2016). It is well known that the amount of iron ingested (by diet or supplements) directly influences the composition of the microbiota (Bullen, Rogers, Spalding, & Ward, 2005). Accumulating evidence suggests that unabsorbed iron can stimulate growth and virulence of bacterial pathogens in the intestinal environment (Dostal et al., 2014; Jaeggi et al., 2015), although it has also been proposed that the iron status of the host and susceptibility to gut inflammation could play a role in the changes observed in gut microbiota composition (Buhnik-Rosenblau, Moshe-Belizowski, Danin-Poleg, & Meyron-Holtz, 2012). On the other hand, a recent study in mice demonstrated that microbial colonization of the gut has an impact on the intestinal proteins involved in iron metabolism, so gut microbiota may affect the iron status of the host by interacting with iron transport by enterocytes and subsequent exportation and storage (Deschemin et al., 2016).

The scientific background in this field should be considered when a fortification programme is planned. In this regard, iron fortification altered gut microbiota of Kenyan infants, increasing pathogen abundance and causing intestinal inflammation (Jaeggi et al., 2015). Other reports indicate that iron fortification, independently of the iron source used, can increase diarrhoea incidence in low-income countries (Paganini & Zimmermann,

2017). The strategy in these cases has to achieve a balance between the iron level and the provision of other micronutrients together with medical care. The use of probiotics in iron fortification is also being explored. Hoppe et al. (2015) observed that in a group of menstruating women the addition of *Lactobacillus plantarum* increased iron absorption from an iron-fortified fruit drink by approximately 50%. In another study (Paganini et al., 2017), galacto-oligosaccharides added to a low dose of iron fortificant reduced most of the negative intestinal effects in malnourished infants. Thus, probiotics included in the fortified food may alleviate possible adverse effects on the gut microbiome (Paganini et al., 2017) and perhaps may have additional benefits, such as reduction of the iron cost and health effects.

All these advances in "omics" sciences, and their reported effect on iron homeostasis, may add a new level in iron dietary recommendations and may involve the customization of iron-fortified products to increase benefits for individuals, thus expanding into more effective public health strategies on iron fortification. Although further investigations on the interactions in "omics" sciences are needed, we are on the path of change, moving from traditional recommendations to Precision Nutrition (Ferguson et al., 2016).

8. Conclusions

In the design of iron-fortified food it is important to delineate the main factors involved. Clearly there is a social demand, as iron deficiency is by far the most widespread nutritional deficiency, so the number of potential consumers is enormous. In this context, at present recommendations tailored for specific groups according to precision nutrition concepts are increasing. The selection of the iron to be used as fortificant and innovation in searching for new fortificants are the main manufacturing aspects. If a traditional compound such as iron sulfate or elemental iron can be used, possibly the cost of its production is the lowest, but cost calculations should be related to a reference bioavailability value, because very cheap iron fortificants may need double or triple the amount to have the same bioavailability as other forms. Moreover, safety has to be proved firstly *in vitro* or in animal models, and, if positive results are obtained, later the iron-fortified food should be tested in human subjects. The target population has to be protected against iron deficiency and also against overload and side effects.

The interactions between the iron fortificant, the food vehicle and the consumer deserve a multidisciplinary approach. Further studies should be done on these aspects, with

participation of experts such as food scientists, technologists, manufacturers, nutritionists, geneticists, biotechnologists and public health professionals.

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Figure 1. Mechanisms of iron absorption in duodenal enterocytes. Continuous thick arrows indicate main mechanisms; dashed arrows indicate minor routes or doubts about the mechanism. Dcytb, duodenal cytochrome b reductase; DMT-1, divalent metal transporter-1; FPN, ferroportin; HCP-1, haem-carrier protein; Flavin-MO, flavin monooxygenase; β2-m; β2-microglobulin; HO, haem oxygenase.

Figure 2. Host and environmental factors, including dietary factors, modulating iron bioavailability.

Figure 3. Strategies to improve iron bioavailability of fortified food.

Table 1. Global and United Nations regional mean blood haemoglobin concentration and prevalence of anaemia by population group for 2011 (World Health Organization, 2015).

United Nations region	Children aged 6–59 months		All women of reproductive age (15–49 years)	
	Mean (95%CI) blood haemoglobin concentration (g/L)	Percentage (95% CI) of population with anaemia	Mean (95%CI) blood haemoglobin concentration (g/L)	Percentage (95% CI) of population with anaemia
Africa	105 (103 to 106)	60.2 (57.0 to 63.1)	123 (121 to 125)	37.6 (32.4 to 43.0)
Latin America and the Caribbean	117 (114 to 120)	29.1 (22.5 to 36.9)	130 (126 to 134)	19.1 (13.1 to 29.4)
Northern America	124 (122 to125)	7.0 (4.9 to 12.3)	131 (130 to 133)	12.4 (9.3 to 17.1)
Asia	112 (109 to 115)	42.0 (34.1 to 49.9)	124 (122 to 127)	31.9 (24.6 to 40.6)
Europe	120 (116 to 123)	19.3 (10.9 to 30.7)	129 (126 to 131)	20.1 (13.8 to 28.3)
Oceania	117 (112 to 122)	26.2 (14.5 to 41.6)	128 (123 to 132)	20.0 (12.0 to 35.5)
Global	111 (110 to 113)	42.6 (37.7 to 47.4)	125 (124 to 127)	29.4 (24.5 to 35.0)

Table 2. Main enhancers and inhibitors of iron bioavailability.

	Food or food group	Components	Mechanism	Notes	References
Enhancers	Citric fruits, vegetables, citrus fruit juices	Ascorbic acid	Formation of soluble iron-ascorbate complexes which remain soluble in the intestine; and reduction of Fe^{3+} to Fe^{2+}. Both favour absorption	Ascorbic acid is the most potent enhancer. But it is thermolabile	(Beck et al., 2011; Blanco-Rojo et al., 2011b; Hurrell, Reddy, Juillerat, & Cook, 2003)
	Meat, fish, poultry	Animal tissue	Binding of iron to digestion products mainly from proteins resulting in soluble complexes	Not affected by thermal processes	(Bæch et al., 2003b; Hurrell, Reddy, Juillerat, & Cook, 2006; Navas-Carretero et al., 2008; Navas-Carretero et al., 2009)
Inhibitors	Whole grain cereals, nuts, legumes	Phytic acid	Formation of insoluble iron phytate complexes in the gut	The most important inhibitor associated with high fibre diets	(Brune et al., 1992; Layrisse et al., 2000)
	Coffee, tea, cocoa	Polyphenols	Formation of insoluble complexes with iron in the gut	Polyphenols in tea are the strongest known inhibitors	(Navas-Carretero et al., 2007b)

	Cow's milk, dairy products	Milk protein	Whole casein and α_{s}-casein phosphopeptides strongly bind iron preventing its absorption	Whey proteins are not inhibitors	(Kibangou et al., 2005; Szymlek-Gay et al., 2012)
		Calcium	Disruption of the enterocyte transport mechanism to blood. Absorption of haem and non-haem iron is reduced	Results from *in vitro* assays and from epidemiological studies in humans	(Toxqui et al., 2013; Walczyk et al., 2014)

Table 3. Recommended Dietary Intake of iron (mg/day) by age and gender among different agencies and countries selected.

	Spain (2015)[1]		United Kingdom (1991)[2]		Nordic CM (2014)[3]		Brazil (2005)[4]		IOM (2001)[5]		FAO/WHO* (2004)[6]		EFSA (2015)[7]	
Age														
0–12 months	7		7.8		8		0.27		0.27		6-19		11	
1–3 years	7		6.9		8		9		11		4-12		7	
4–6 years	9		6.1		8		6		7		4-13		7	
7–9 years	9		8.7		9		9		10		4-18		11	
	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
10–12 years	12	18	11.3	14.8	11	11	14	14	8	8	10-38	9-65	11	13
13–19 years	15	18	11.3	14.8	11	15	14	14	11	18	9-38	9-65	11	16
20–50 years	10	18	8.7	14.8	9	15	14	14	8	18	9-27	20-59	11	16
> 51 years	10	10	8.7	8.7	9	9	14	14	8	8	9-27	8-23	11	11
Pregnancy		18		14.8		-		14		27		-		16
Lactation		18		14.8		15		27		10		10-30		16

* Recommended Nutrient Intake for a bioavailability of dietary iron between 5 and 15%.

[1]Moreiras, Carbajal, Cabrera, & Cuadrado, 2016; [2]Department of Health UK, 1991; [3]Nordic Council of Ministers, 2014; [4]Brasil, 2005; [5]Institute of Medicine, 2001; [6]FAO/WHO, 2004; [7]European Food Safety Authority, 2015.

Table 4. Characteristics of iron fortificants.

Compound	Fe content (%)	Solubility	Relative bioavailability*	Main food vehicles	Selected references
Traditional compounds					
Ferrous sulfate. $7H_2O$	20	Water soluble	100	Infant foods, dry milk, cereals	(Shamah-Levy et al., 2008; Villalpando et al., 2006)
Ferrous gluconate	12	Water soluble	85-95	Cereals, fruit juice	(Navas-Carretero et al., 2007b; Shamah-Levy et al., 2008; Villalpando et al., 2006)
Ferrous lactate	19	Water soluble	106	Cereals, dairy products	(Kapsokefalou, Alexandropoulou, Komaitis, & Politis, 2005; Kloots, Op den Kamp, & Abrahamse, 2004)
Ferrous bisglycinate	20	Water soluble	>100	Cereals, fluid milk	(Layrisse et al., 2000)
Ferric ammonium citrate	17	Water soluble	51	Cereals, fluid milk	(Diego Quintaes, Barberá, & Cilla, 2017)
Sodium iron EDTA	13	Water soluble	>100	Cereals, sugar, fish sauce, soy sauce	(European Food Safety Authority, 2010)
Iron (II) taurate	18	Water soluble	-	Soft drinks	(European Food Safety Authority, 2009)
Iron L-pidolate	18	Water soluble	-	-	(European Food Safety Authority, 2007)
Ferrous fumarate	33	Poorly water soluble, soluble in dilute acid	100	Cereals, cocoa products	(Davidsson et al., 2000; Hurrell, 2010; Navas-Carretero et al., 2007b)
Ferric pyrophosphate	25	Water insoluble, poorly soluble in dilute acid	21-74	Cereals, fluid milk	(Wegmuller et al., 2004)
Elemental electrolytic iron	97-99	Water insoluble, poorly soluble in dilute acid	75	Cereals, breakfast cereals, curry powder	(Hoppe, Hulthen, & Hallberg, 2006; Karn et al., 2011)

Encapsulated iron				
Ferrous sulfate	Coating dependent	100	Cereals, cocoa products, salt	(Genevois et al., 2016)
Ferrous fumarate	Coating dependent	100	Cereals, salt	(Diego Quintaes, Barberá, & Cilla, 2017)
Ferric pyrophosphate	Poorly soluble	92	Fruit juice, bouillon cubes	(Blanco-Rojo et al., 2011b; Navas-Carretero et al., 2007a; Navas-Carretero et al., 2009; Navas-Carretero et al., 2007b; Toxqui et al., 2013)
Nanoparticulate iron				
Iron oxide	Coating dependent	-	-	(Chamorro et al., 2015)
Iron oxyhydroxides	Coating dependent	-	-	(Pereira et al., 2014)
Ferritin mimetic iron	Coating dependent	-	-	(Aslam et al., 2014; Latunde-Dada et al., 2014)

* To ferrous sulfate, $7H_2O$. Adapted from WHO, 2006; Hurrell, 2010; Diego Quintaes, Barberá, & Cilla, 2017.

Highlights:

• Iron is the most challenging micronutrient to add to produce fortified food

• The choices of iron form, food vehicle and target population are decisive

• Other food components and meal composition may greatly modify iron bioavailability

• Safety issues are related to the iron fortified food and also to consumer protection

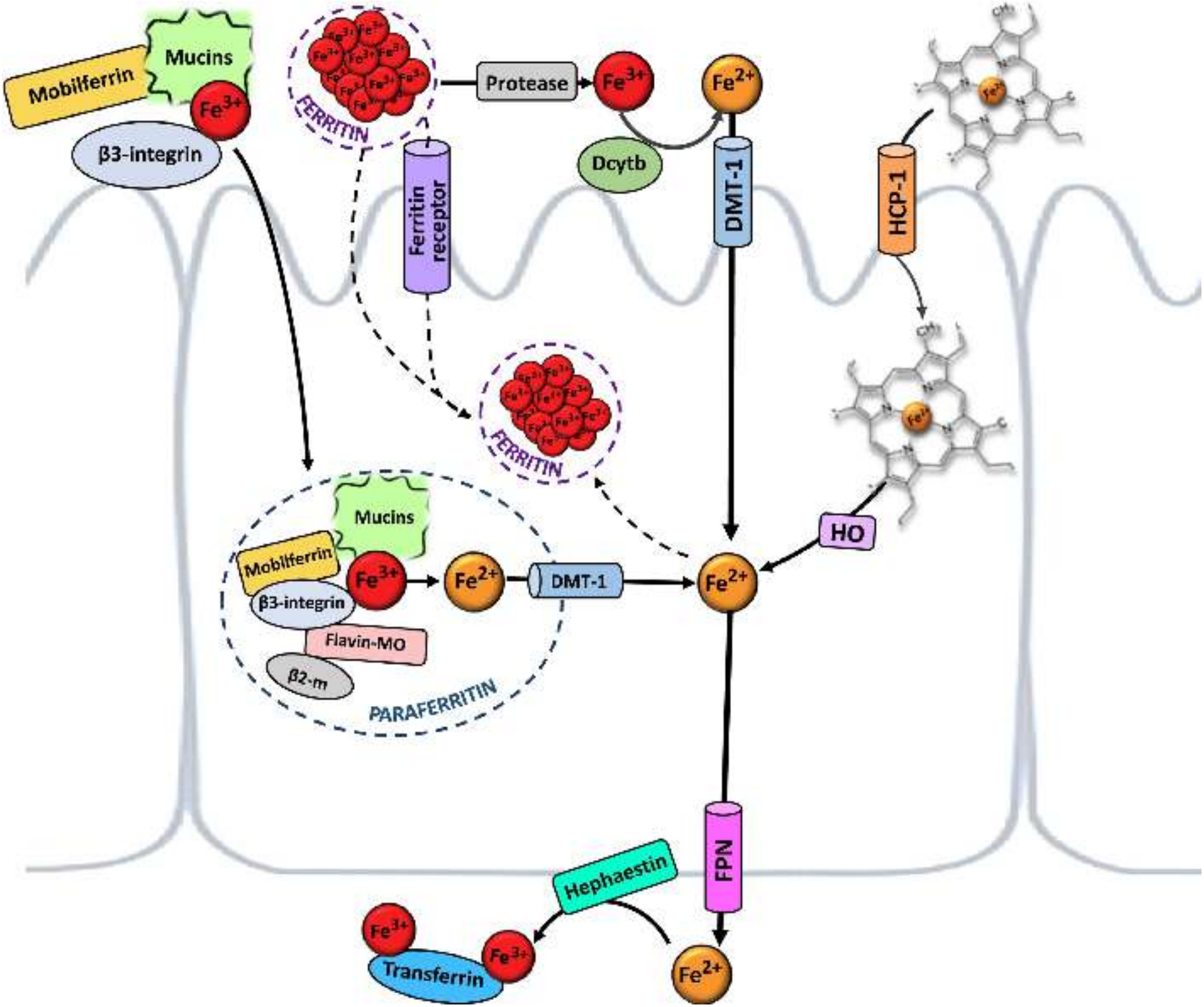
Mobilferrin
Mucins
Fe3+
β3-integrin
FERRITIN
Protease
Fe3+
Fe2+
Dcytb
DMT-1
Ferritin receptor
HCP-1
FERRITIN
Mucins
Mobilferrin
Fe3+
Fe2+
DMT-1
β3-integrin
Flavin-MO
β2-m
PARAFERRITIN
HO
Fe2+
FPN
Hephaestin
Fe3+
Transferrin
Fe3+
Fe2+

Figure 1

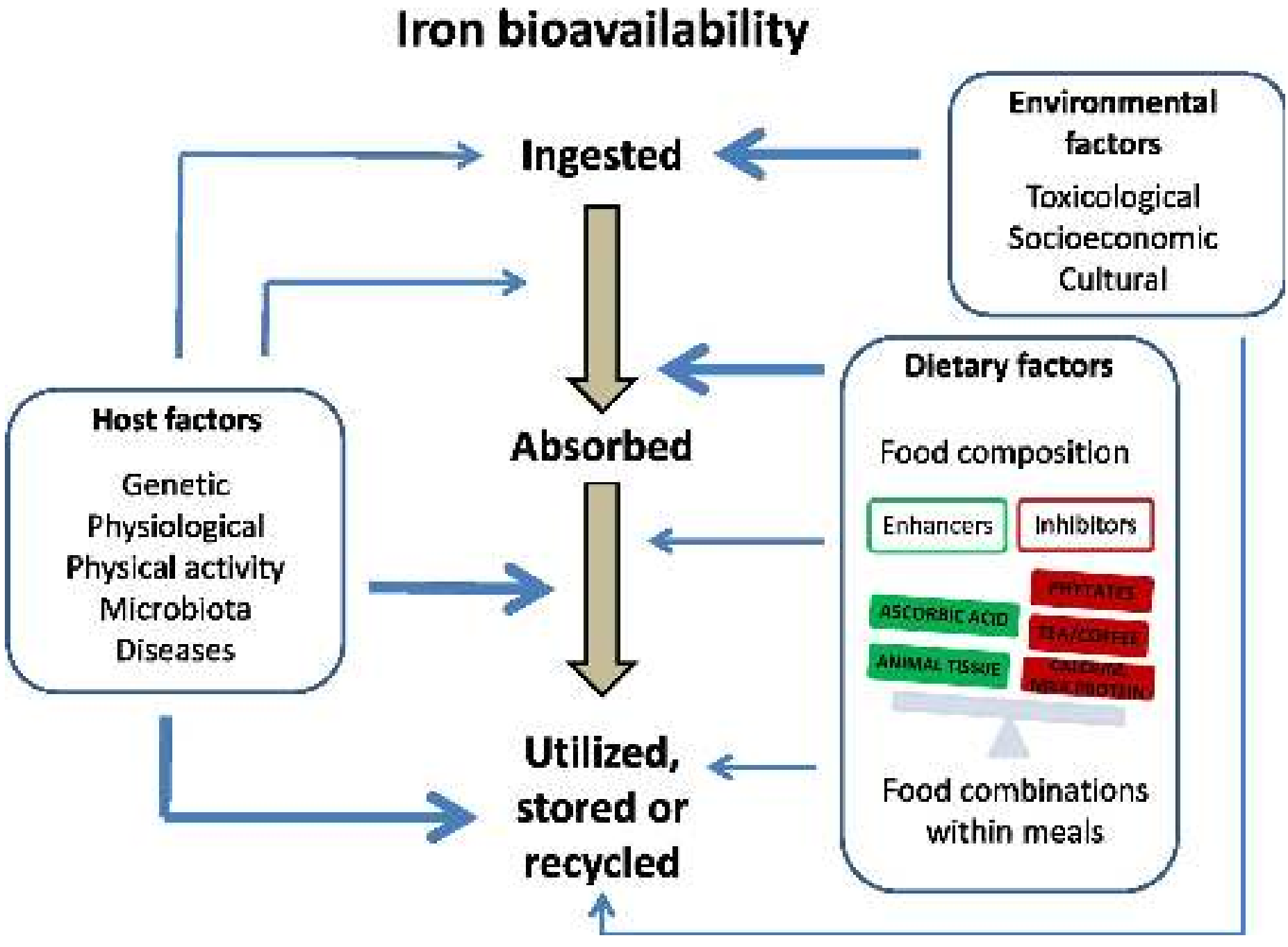
Iron bioavailability
Ingested
Absorbed
Utilized, stored or recycled
Environmental factors
Toxicological
Socioeconomic
Cultural
Dietary factors
Food composition
Enhancers
Inhibitors
ASCORBIC ACID
ANIMAL TISSUE
Food combinations within meals
Host factors
Genetic
Physiological
Physical activity
Microbiota
Diseases

Figure 2

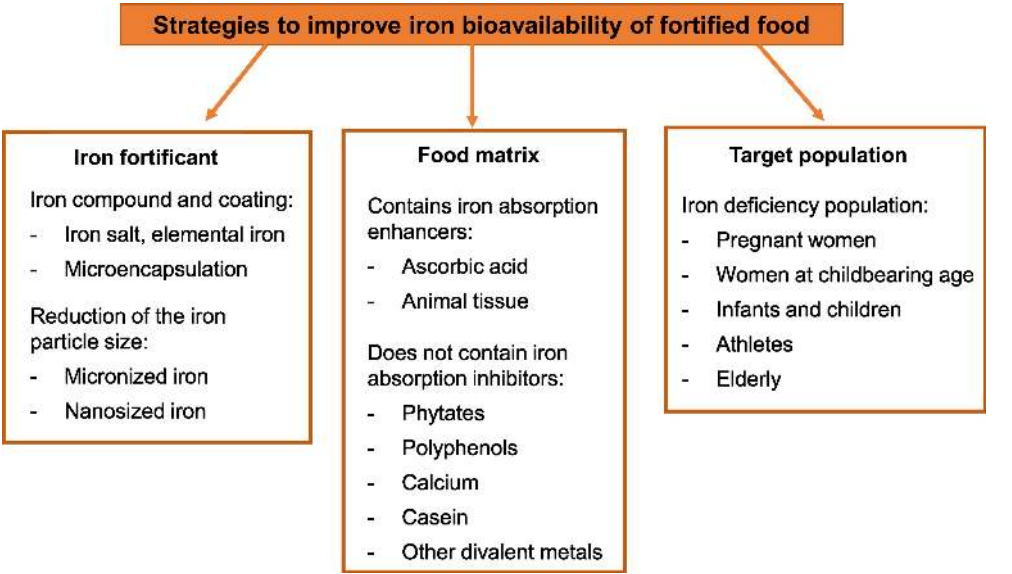
Strategies to improve iron bioavailability of fortified food
Iron fortificant
Iron compound and coating:
- Iron salt, elemental iron
- Microencapsulation
Reduction of the iron particle size:
- Micronized iron
- Nanosized iron
Food matrix
Contains iron absorption enhancers:
- Ascorbic acid
- Animal tissue
Does not contain iron absorption inhibitors:
- Phytates
- Polyphenols
- Calcium
- Casein
- Other divalent metals
Target population
Iron deficiency population:
- Pregnant women
- Women at childbearing age
- Infants and children
- Athletes
- Elderly

Figure 3