Enhancing the Absorption of Fortification Iron

A SUSTAIN Task Force Report

Richard F. Hurrell[1], Sean Lynch[2], Thomas Bothwell[3], Héctor Cori[4], Ray Glahn[5], Eva Hertrampf[6], Zdenek Kratky[7], Dennis Miller[8], Mario Rodenstein[9], Hugo Streekstra[10], Birgit Teucher[11], Elizabeth Turner[12], C.K. Yeung[8] and Michael B. Zimmermann[1]

[1]Laboratory for Human Nutrition, Swiss Federal Institute of Technology Zurich, Switzerland
[2]Eastern Virginia Medical School, Norfolk, Virginia, USA
[3]Department of Medicine, University of Witwatersrand, Johannesburg, South Africa
[4]DSM Nutritional Products, Santiago, Chile
[5]USDA-ARS Plant Soil and Nutrition Laboratory, Ithaca, New York, USA
[6]Institute of Nutrition and Food Technology, Santiago, Chile
[7]Nestlé, New Milford, Connecticut, USA
[8]Department of Food Science, Cornell University, Ithaca, New York, USA
[9]Unilever, Englewood Cliffs, New Jersey, USA
[10]DSM Food Specialties, Delft, Netherlands
[11]Institute of Food Research, Norwich Laboratory, Norwich, UK
[12]SUSTAIN, Washington, DC, USA

Received for publication: June 25, 2004; Accepted for publication: September 3, 2004

Abstract: Iron deficiency remains a major global health problem affecting an estimated 2 billion people [1]. The World Health Organization ranked it as the seventh most important preventable risk for disease, disability, and death in 2002 [2]. Since an important factor in its causation is the poor bioavailability of iron in the cereal-based diets of many developing countries, SUSTAIN set up a Task Force, consisting of nutritional, medical, industry, and government experts to consider strategies for enhancing the absorption of fortification iron. This paper summarizes the findings of this Task Force. Detailed reviews of each strategy follow this overview.

Highly soluble compounds of iron like ferrous sulfate are desirable food fortificants but cannot be used in many food vehicles because of sensory issues. Thus, potentially less well-absorbed forms of iron commonly are used in food fortification. The bioavailability of iron fortificants can, however, be enhanced with innovative ingredient technologies. Ascorbic acid, NaFeEDTA, ferrous bisglycinate, and dephytinization all enhance the absorption of fortification iron, but add to the overall costs of fortification. While all strategies cannot be recommended for all food fortification vehicles, individual strategies can be recommended for specific foods. For ex-

The authors wish to acknowledge the exceptional efforts of the following SUSTAIN team members for their editorial and technical support in the preparation of this publication, and for their assistance with the organization of the Workshop: Betty Bugusu, Fred Grant, Martha Lassiter, Wayne Moore and Sue Wunder. Additionally, we wish to thank and acknowledge the following individuals who contributed expertise during the workshop and in the subsequent review of this manuscript: Erick Boy, Helene Carlson, Ram Chaudhari, Michael DiNovi, Alison Edwards, Chuck Gagel, Brent Hagen, Leif Hallberg, Janet Hunt, A. Patrick MacPhail, Haile Mehansho, Manuel Olivares, Paul Richardson, Steve Schorn, Geoff Smith, Wolfgang Vogl, and Carel Wreesmann.

DOI 10.1024/0300-9831.74.6.387

ample, the addition of ascorbic acid is appropriate for dry blended foods such as infant foods and other dry products made for reconstitution that are packaged, stored, and prepared in a way that maximizes retention of this vitamin. NaFeEDTA can be recommended for fortification of fish sauce and soy sauce, whereas amino acid chelates may be more useful in milk products and beverages. With further development, dephytinization may be possible for low-cost, cereal-based complementary foods in developing countries. Encapsulation of iron salts in lipid coatings, while not an iron absorption-enhancing strategy *per se*, can prevent soluble forms of iron from interacting undesirably with some food vehicles and hence broaden the application of some fortificants.

Research relevant to each of these strategies for enhancing the bioavailability or utility of iron food fortificants is reviewed. Individual strategies are evaluated in terms of enhancing effect and stability, organoleptic qualities, cost, and regulatory issues of interest to the nutrition community, industry, and consumers. Recommendations are made on potential usages and further research needs.

Effective fortification depends on the selection of technically feasible and efficacious strategies. Once suitable strategies have been identified, cost becomes very important in selecting the best approach to implement. However it is essential to calculate cost in relation to the amount of bioavailable iron delivered. An approach to the calculation of cost using a conservative estimate of the enhancing effects of the innovative technologies discussed in the supplement is given in the final section.

Key words: iron fortification, ascorbic acid, EDTA, amino acid chelates, phytate degradation, encapsulation

Iron – a Global Problem

Deleterious Effects of Iron Deficiency

Many infants, children and women of childbearing age, particularly in the poorer countries of the developing world, are iron deficient. About half of these iron-deficient individuals develop iron deficiency anemia (IDA), the most advanced form of the disease, which has several major negative impacts on health and contributes substantially to the risk of early death and disability [2].

There are five major negative health consequences of IDA. Firstly in the pregnant woman, IDA leads to sub-optimal pregnancy outcome, including lower birth weight, increased morbidity in mothers and neonates, increased infant mortality, and a greater risk of the infant developing iron deficiency after four months of age [3, 4]. Secondly, during infancy, IDA leads to delayed mental and motor development with effects on behavior and cognitive performance when the child reaches school age. The effects of early IDA on brain development may not be reversible by subsequent treatment, and failure to reach educational goals may affect earning capacity later in life [5, 6]. In children, IDA can also lead to increased frequency and duration of upper respiratory infections [7] and to increased risk for goiter due to diminished utilization of iodine for thyroid hormone production [8]. Finally, physical work capacity is impaired for all individuals as IDA negatively affects aerobic capacity related to intense physical activity and reduces endurance capacity, voluntary activity, and work productivity. This results in a lower income for the individual, the family, and the country [9, 10].

Iron deficiency is therefore a major health problem in the developing world and recently WHO [2] ranked it as seventh out of the ten major global preventable risks for disease, disability, and death that together account for 40% of the 56 million deaths that occur world-wide each year, and for one third of the global loss of healthy life years. In developing countries, underweight has been reported to be the greatest risk factor and accounts for 9.5% of the global DALYs (disability-adjusted life years, one DALY is equal to the loss of one year of healthy life). Iron deficiency is the next highest *nutritional* risk factor and accounts for 2.4% of global DALYs, preceded only by sexually transmitted diseases, diseases related to unsafe water, poor sanitation and hygiene, alcohol abuse, and indoor smoke from solid fuels. It has been estimated that if iron deficiency were eliminated worldwide, more than 35 million people would have one additional year of healthy life.

Prevention of Iron Deficiency

The elimination of iron deficiency however has not proven easy. Dietary diversification (promoting the consumption of iron-rich foods) is hindered by the difficulty in achieving behavioral change as well as by the predominance in developing countries of plant-based diets deficient in the more bioavailable heme form of iron. Iron supplementation has been mainly targeted at high-risk groups such as pregnant women and young children. Supplementation interventions however have often been compromised as the side effects of the high doses impact compliance. There are often supply or distribution problems in developing countries as well [11].

Food fortification offers a more cost-effective approach to providing additional iron to most segments of the population by mass fortification of staples such as wheat and maize flour, or condiments such as salt, fish sauce, or soy sauce. In addition, infants and young children can be specifically targeted through iron-fortified infant formulas and cereal-based complementary foods. However, because iron is such a difficult mineral to add to foods in a sufficiently bioavailable form without adverse sensory changes, not all fortified foods have provided enough absorbable iron to improve iron status. While infant formulas, infant cereals, soy sauce, and fish sauce are generally regarded as effective vehicles for food fortification, some doubt remains as to the usefulness of iron-fortified cereal flours and salt because of continuing technical barriers [12].

The first technical barrier that still remains, particularly for cereal flours and salt, is finding an iron compound that is sufficiently bioavailable but which causes no adverse sensory changes to the food vehicle. In an attempt to partially address this barrier, an earlier SUSTAIN task force [13] evaluated the usefulness of elemental iron powders for cereal flour fortification. The task force concluded that of the five elemental iron powders sold for food fortification, only electrolytic iron had been demonstrated to be a useful iron fortificant. Because this powder was judged to be only half as well absorbed as ferrous sulfate, they recommended that it should be added to foods at twice the level of ferrous sulfate. The second technical barrier to successful food fortification is overcoming the inhibitory effect of dietary components, such as phytate, phenolic compounds, and calcium, which are often present in the food vehicle itself or in the accompanying diet. For example, iron absorption may be unacceptably low from high-phytate whole cereal flours even when fortified with highly absorbable iron compounds such as ferrous sulfate [14].

SUSTAIN Task Force Review

The present Task Force was set up to assess the different strategies that are available for increasing the bioavailability of fortification iron from diets containing significant amounts of inhibitors of iron absorption, while, at the same time, not causing adverse sensory changes in the chosen vehicles. The five approaches that were considered included the following:

- Addition of ascorbic acid and other organic acids
- Addition of NaFeEDTA or other EDTA compounds
- Addition of amino acid chelates
- Degradation of phytate
- Encapsulation of highly bioavailable compounds, such as ferrous sulfate

This overview paper briefly reviews the successful iron fortification programs or trials, and then summarizes the key issues in relation to each of the enhancers of iron absorption. Relevance to finished food staples and widely consumed condiments is emphasized. These short review sections are followed by a consensus statement framed by a SUSTAIN task force consisting of academic and industry experts on the strategies to enhance the absorption of fortification iron. The Task Force based its consensus points in part on industry and scientific review papers presented at SUSTAIN's Workshop on Innovative Ingredient Technologies to Enhance Iron Absorption (Washington, D.C., March 2003). Workshop discussions of these materials among participants from industry, the science community and government also helped shape the consensus as did new information obtained in consultations with experts from these sectors subsequent to the Workshop. Five invited review papers, one on each iron-enhancing technology, are published in full after this overview.

Successful Iron Fortification Programs and Field Trials

The ultimate proof that an iron-fortified food is efficacious is the demonstration that it improves or maintains iron status in a target population. The iron-fortified food must provide an adequate amount of absorbable iron to the consumer so as to counter the deficit between normal intake of absorbable iron and the iron needs. The amount of absorbable iron provided thus depends on the amount of iron added to the fortified food and the bioavailability of that iron when the fortified food is consumed as part of a mixed diet. Because such efficacy studies are technically difficult, expensive, and usually take six months to one year to complete, few have been published. It is noteworthy however that most of the successful efficacy studies have added ascorbic acid as an enhancer of iron absorption together with the iron compound or employed an iron compound which is "protected" from absorption inhibitors. Some successful efficacy studies are described below.

Ascorbic Acid: Although ascorbic acid enhances the iron absorption of all iron fortification compounds [15], there is little direct evidence of its influence on iron status when added to iron fortified foods. The best evidence comes from two separate efficacy studies on iron-fortified powdered milk fed to infants and young children in Chile [16]. The studies were made with a similar protocol but two years apart (1972 and 1974). In the first study, three-month-old infants who had spontaneously discontinued breastfeeding were fed a reconstituted milk powder that had been fortified or not with ferrous sulfate (15 mg Fe per liter). At 15 months, the prevalence of anemia was 35% in the group receiving the non-fortified milk compared to 13% in the infants consuming the fortified milk. In the second trial, ascorbic acid was added to the milk at a 2:1 molar ratio relative to iron and, at 15 months, the prevalence of anemia was again high in the group receiving the non-fortified milk (28%) but had fallen to less than 2% in the group receiving the fortified milk. In addition, the satisfactory iron status of most American infants is thought to be due in part to the widespread use over the last few decades of commercial infant formulas (fortified with ferrous sulfate and ascorbic acid) and complementary foods (fortified with various iron compounds). Anemia surveys of American infants in the 1980s to 1990s showed significantly lower prevalences compared to the 1970s, a trend corresponding to the decline in exclusive cow milk use and simultaneous increase in fortified formula use beyond six months of age [17]. In some countries, infant formulas are also commonly added to reconstituted infant cereals. This practice could partially explain the efficacy of an electrolytic iron-fortified infant cereal in improving the iron status of infants in Chile when fed regularly over a one-year period [18]. However, the high iron content and the relatively high intake of the cereal (which provided an extra 14–17 mg per day) undoubtedly played a role.

Chelated Iron Compounds: Iron status in target populations was significantly improved when NaFeEDTA was used to fortify fish sauce in Thailand and Vietnam [19, 20], soy sauce in China [21], sugar in Guatemala [22], and curry powder in South Africa [23]. Similarly when ferrous bisglycinate was added to flavored milk in Saudi Arabia [24] and a whey drink in Brazil [25], anemia prevalence was drastically reduced in children and adolescents after three months and 12 months, respectively, of regular daily consumption. Iron is strongly chelated in both NaFeEDTA and ferrous bisglycinate and is thus protected from reacting with dietary components that inhibit iron absorption.

Encapsulated Ferrous Sulfate: One recent study [26], reported a substantial improvement in iron status of school children in Morocco consuming iron-fortified salt. The prevalence of IDA decreased from 35% at baseline to 8% at 40 weeks. The salt was fortified with ferrous sulfate encapsulated with partially hydrogenated soybean oil (1 mg Fe/g) but contained no enhancer of iron absorption. The lipid capsule helped prevent unwanted color changes and iodine losses often associated with highly soluble ferrous sulfate. The salt was provided to the family at the household level and was added primarily to bread, olives, and family meals. The children consumed 7–12 g per day, providing 7–12 mg of iron. While it has been suggested that encapsulation may in some way protect iron from reacting with absorption inhibitors, no research has been conducted on this issue.

These successful efficacy studies demonstrate that iron fortification of foods can be a useful strategy to combat iron deficiency. They indicate as well that protecting the fortification iron from absorption inhibitors such as phytate is a useful way to ensure adequate iron absorption and to improve iron status. However the addition of an absorption enhancer or "protected" iron compound may not be necessary if the amount of added iron is sufficiently high. The various strategies that can be used to enhance iron absorption are now considered separately.

Potential Strategies for Enhancing Absorption of Fortification Iron

Ascorbic Acid and other Organic Acids

Commercial Usage: Ascorbic acid is the most commonly added compound for the enhancement of iron absorption from iron-fortified foods. It is routinely added to infant formulas and infant cereals to improve iron absorption, and is also added to iron-fortified chocolate drink powders and other dietetic beverages.

When adding ascorbic acid to a finished product, its sensitivity to heat (prolonged boiling, baking, and frying temperatures), water, and oxygen must be taken into consideration. In the dry state, ascorbic acid is reasonably stable in air, but in solution and interfacing with other components, it oxidizes rapidly. Cooking typically degrades ascorbic acid by accelerating the oxidation reaction. In order to keep ascorbic acid stable, heat, oxygen, and humidity should be avoided. Storage at high ambient temperatures in oxygen and humidity-permeable packaging will lead to losses. Proper packaging and encapsulation can mitigate some of these losses, but will also add cost. Ascorbic acid is relatively stable in dry blended foods such as infant formulas, precooked cereal-legume infant foods, powdered milk, and other dry products made for reconstitution. It is less stable in liquid beverages and liquid milk, and not stable in cereal products that are baked.

Biochemical Effects: The enhancing effect of ascorbic acid has been attributed to its reducing and chelating properties during digestion of the food [27]. In the pH range 2 to 6, ferric iron in the food is reduced to ferrous iron thus preventing the formation of the more insoluble, and nonabsorbable, ferric hydroxide as the pH rises in the duodenum. Above pH 5, a ferric ascorbate chelate can be formed keeping iron in a soluble, absorbable form [28]. The same reducing and chelating properties presumably also explain the reported ability of ascorbic acid to overcome the negative effects of all major inhibitors of iron absorption including phytate, polyphenols [29], calcium, and casein from milk products [30]. Ferrous iron binds less strongly to the inhibitory compounds than ferric iron. Because of these unique properties, ascorbic acid can increase by several fold the absorption of most iron fortification compounds as well as non-heme food iron.

Isotopic Absorption Studies: In single meal isotopic studies, there appears to be a linear dose response at lower amounts of ascorbic acid addition, which eventually levels off to a plateau. When feeding an iron-fortified liquid formula meal, Cook and Monsen [31] reported a linear increase in iron absorption with additions of ascorbic acid up to a 7.5 molar ratio of ascorbic acid to iron. At this level of ascorbic acid, iron absorption was increased threefold. The magnitude of the increase in iron absorption however depends not only on the molar ratio of ascorbic acid to iron but also on the presence of other enhancers and inhibitors in the fortified food or in the meal. An ascorbic acid to iron molar ratio of 2:1 has been reported to increase iron absorption from fortification iron by 2–12 fold in adult women fed infant formula or cereal porridges [32], by three-fold in Jamaican children fed a chocolate drink [33], and by about two-fold in infants fed an infant formula [30]. On the other hand, in foods containing high levels of phytate such as soy infant formulas, a minimum 4:1 molar ratio was necessary to increase iron absorption by about three-fold [34]. Hurrell [15] has recommended that a molar ratio of 2:1 should be used to enhance the absorption of soluble iron compounds from milk products and low-phytate foods but at least 4:1 should be used for foods high in phytate or phenolics. There are not enough studies to ascertain whether ascorbic acid enhances the absorption of the more insoluble iron compounds in a similar way to the soluble compounds, although from the results of Forbes *et al* [35], any differences would not appear to be substantial.

Questions have been raised as to whether the enhancing effect of ascorbic acid on fortification iron absorption observed in single meal studies can be translated into a better efficacy of the fortification iron in improving or maintaining iron status when the fortified food is fed over a period of time. Some doubt has been cast on the ability of ascorbic acid to improve iron status by the suggestion that single meal studies over-emphasize iron absorption from a complete diet [36]. More recently, Cook and Reddy [37] were unable to demonstrate an influence of high ascorbic acid intake on iron absorption from typical meals in the United States fed over five days. In addition, high dose supplementation with 1 g of ascorbic acid with meals for 20 months by iron-replete healthy adults (n = 5) did not increase their iron stores [38]. It should, however, be emphasized that the body has a strong control over intestinal iron absorption and, although ascorbic acid renders iron available for absorption, the body will not absorb iron if it does not need it. Ascorbic acid may therefore have no measurable beneficial effect on dietary iron absorption in iron-replete individuals. It has however been shown to increase iron status in iron-depleted people in both developing [16] and industrialized countries [39].

Other Organic Acids: Other organic acids, such as citric, lactic, malic, and tartaric, also complex iron [40] but have no reducing activity. Although they have been demonstrated to enhance iron absorption in human studies, they have not been considered as an enhancer of fortification iron because the amount of compound needed to enhance iron absorption would likely change the taste of the food. Levels of organic acids are often considerably higher than the level of ascorbic acid in many fruits and vegetables [41], and their presence might help explain the beneficial effect of fruit juices [42] and some fruits and vegetables on iron absorption [42, 43]. The amounts required however are high and 1 g or more of citric, malic, or tartaric acid was necessary to increase by two- to threefold the absorption of 3 mg iron as ferrous sulfate from a rice meal [44]. A more practical utilization of far lower levels of organic acids may be needed to stabilize iron compounds in liquid products. For example, small amounts of citric acid have been found to stabilize ferrous sulfate and prevent peptide precipitation in ferrous sulfate-fortified fish sauce [45].

NaFeEDTA and other EDTA Compounds

"JECFA Guidance and Regulatory Status of NaFeEDTA". Evidence suggests that sodium iron ethylenediaminetetraacetic acid (NaFeEDTA) is a highly effective iron fortificant, being two to three times better absorbed from inhibitory meals than ferrous sulfate [46–48], and causing fewer organoleptic problems in many foods than freely water-soluble compounds. However, it has not yet been widely introduced as a fortificant.

In 1993 the Joint FAO/WHO Expert Committee on Food Additives (JECFA) evaluated NaFeEDTA for use in supervised food fortification programs in populations in which iron deficiency anemia is endemic [49] and provisionally concluded it was suitable for such an application. At the time, however, JECFA requested further animal toxicology data. When JECFA reviewed additional data on the compound in 1999, it removed the provisional qualification from its previous decision and concluded that NaFeEDTA "could be considered safe when used in supervised fortification programs [50]." JECFA is not, however, a regulatory body. In the USA or elsewhere, petitions for the use of NaFeEDTA as a direct food additive have not been submitted to regulatory authorities nor has anyone submitted a Generally Recognized As Safe (GRAS) notice to the Food and Drug Administration (FDA) in the United States regarding NaFeEDTA. Further constraining the use of NaFeEDTA are the lack of a consolidated body of evidence on its stability during processing, storage, and cooking, as well as questions relating to its possible interactions with other dietary minerals and trace elements.

Though NaFeEDTA is not currently recognized by FDA in the United States for use as a direct food additive, two other salts of EDTA, disodium EDTA (Na_2EDTA), and calcium disodium EDTA ($CaNa_2EDTA$), have long been used by the food industry as preservatives, processing aids, and color stabilizers in a variety of foods [51]. In 1974, based on animal toxicology studies, JECFA evaluated $CaNa_2EDTA$ and Na_2EDTA as food additives and allocated an acceptable daily intake (ADI) of 2.5 mg EDTA/kg body weight/day.

FDA has evaluated Na_2EDTA and $CaNa_2EDTA$ specifically for use as sequestrants in various foods, with intended use and limitations on use levels stated in the respective regulations for these additives. At present, Na_2EDTA and $CaNa_2EDTA$ have not been evaluated by FDA for use in the enhancement of mineral absorption (as when combined with an iron fortificant). It should be noted, however, that infant formula is not listed by the FDA among the approved uses of either Na_2EDTA or $CaNa_2EDTA$. Similarly the fortification of milk and cereal formulas with NaFeEDTA does not seem appropriate, since the amounts of NaFeEDTA required to deliver sufficient fortification iron would approach the ADI of 2.5 mg EDTA/kg body weight/day.

The use of iron enhancers is of more relevance to countries where the prevalence of iron deficiency is high. Interest exists in the use of NaFeEDTA as an iron source in some such settings, in part because NaFeEDTA is beneficial for enhancing iron absorption in highly inhibitory meals, such as the cereal-based diets typically consumed in developing countries. Vietnam is at present planning a national fortification program with NaFeEDTA-fortified fish sauce. China has announced its plans to use NaFeEDTA for the mass fortification of soy sauce and wheat flour in two different provinces.

Use as a Fortificant: Although EDTA can complex with virtually every metal in the periodic table, it binds most strongly to ferric iron at the pH of the gastric juice and then exchanges the ferric iron for other metals as the pH rises in the duodenum. EDTA acts as a shuttle, protecting iron in the stomach from binding to phytate and polyphenols, and then releasing iron for absorption in the duodenum [52]. Such properties make the addition of NaFeEDTA a potentially useful fortification strategy for phytate-containing food vehicles such as cereal flours, or for addition to condiments such as salt, fish sauce, and soy sauce, which are commonly consumed with phytate-containing meals. NaFeEDTA is most stable and bioavailable in slightly acidic conditions of pH less than 7 [53]. It could be especially useful as an iron fortificant for whole grain cereal products. Iron absorption by iron-replete adults from wheat bread rolls made from high extraction wheat flour was only 1% when fortified with ferrous sulfate compared to 4% when fortified with NaFeEDTA. The corresponding iron absorption values from rolls made with low extraction wheat flour were 6% and 12% [54]. On the other hand, iron absorption from ferrous sulfate and NaFeEDTA is similar from meals containing no phytate [52] or a low level of phytate such as contained in a meal of white rice and vegetables consumed with fish sauce [54].

Other EDTA complexes: Na_2EDTA or $CaNa_2EDTA$ added together with the fortification iron compound is a possible alternative to the addition of NaFeEDTA. Na_2EDTA has been demonstrated to increase absorption of iron from ferrous sulfate added to a rice meal [56] and ferrous sulfate-fortified wheat-soy complementary foods [54], even at EDTA to iron molar ratios below 1. There are, however, no efficacy studies showing that this approach works. Unfortunately, EDTA appears to enhance only the absorption of soluble iron compounds and not the more insoluble compounds, which dissolve only slowly in the gastric contents. Na_2EDTA at a 1:1 molar ratio did not increase iron absorption by adolescent girls consuming ferrous fumarate-fortified tortillas [57], or by adults consuming either a ferric pyrophosphate fortified cereal porridge [54] or an elemental iron-fortified breakfast cereal [58].

Organoleptic Effects: Systematic sensory studies have been made with soy sauce and fish sauce but not with cereal flours. In relation to cereal flours, NaFeEDTA added to wheat flour has been reported not to cause fat oxidation during a six-month storage period at 37°C, in contrast to the rancidity which occurred on addition of ferrous sulfate with Na_2EDTA [59]. There are however reports of color development in cereal-based foods [22] and questions have been raised about a possible inhibitory effect of EDTA on yeast action and thus a negative effect on bread volume [60].

Unlike ferrous sulfate and other soluble iron compounds, NaFeEDTA does not precipitate peptides during storage of fish sauce and soy sauce. The taste and color of NaFeEDTA-fortified sauces is also satisfactory. However, NaFeEDTA in liquid products can be degraded by ultraviolet (UV) rays from sunlight. While this does not appear to be a problem with soy sauce, presumably due to its dark brown color, up to 35% losses of EDTA have been reported in the lighter colored fish sauce which had been filled into clear glass bottles and left for 2–6 weeks in the open air under daily sunlight [61]. Storage in amber bottles, or storage of clear bottles indoors under artificial light resulted in little or no degradation of EDTA. As EDTA enhances iron absorption at molar ratios below 1, this level of degradation should not affect the nutritional efficacy of the fish sauce. The degradation products, ethylenediamine triacetic acid, -diacetic acid, and -monoacetic acid can further degrade into a range of compounds which include formaldehyde. However, the amounts of formaldehyde that could theoretically be formed are within the limits considered safe [62].

Interactions with other dietary minerals: The influence of EDTA on the absorption of other minerals and trace elements was discussed by INACG [52]. Considering the relative amounts of dietary minerals and the amount of NaFeEDTA in fortified foods, they concluded that EDTA would not be expected to influence the metabolism of dietary calcium and magnesium but could theoretically influence the metabolism of zinc and copper, and of the potentially toxic metals, lead, cadmium, aluminum, and mercury [52].There are few studies to confirm this although there is some evidence from rat and human studies that EDTA can increase zinc and copper absorption from meals containing phytate, but without affecting calcium metabolism [63–64].

Data on how EDTA might influence the metabolism of potentially toxic minerals is limited, but available evidence suggests that EDTA does not increase their absorption and may even reduce their retention. Studies in mice showed no influence of EDTA on lead absorption [65], while the results of two human studies indicated that the absorption of ^{203}Pb was markedly reduced by EDTA [66]. In a third unpublished study, mineral balances were carried out on women fed NaFeEDTA-fortified bread. Stool and urine samples were subsequently analyzed for heavy metals. There was no difference between ferrous sulfate and NaFeEDTA with respect to the fecal excretion of lead, cadmium, aluminum, or mercury, or with respect to the urinary excretion of cadmium, aluminum, or mercury. Urinary lead excretion was however increased [67]. In two other studies acute cadmium toxicity in mice was reduced from 90% to zero by the concomitant administration of EDTA [68], while manganese absorption and excretion were unaffected in women fed an infant cereal containing NaFeEDTA [69]. Notably, while Na_2EDTA and $CaNa_2$EDTA have been present in the U.S. diet for 30 years, there has been no evidence of heavy metal toxicity.

The most appealing reason to use EDTA for food fortification is that NaFeEDTA-fortified foods have been consistently shown to improve iron status of targeted human populations. These foods include fish sauce in Thailand [19] and Vietnam [20], sugar in Guatemala [22] curry powder in South Africa [23], and soy sauce in China [21]. To date however there are no demonstrations of efficacy in wheat or maize flours.

Amino Acid Chelates

Ferrous bisglycinate (ferrous bisglycine chelate) is the major amino acid chelate produced commercially, although ferric trisglycinate and ferric glycinate are also available. A newly developed product, ferrous bisglycinate hydrochloride, has undergone *in vitro* bioavailability testing. A new fortified juice powder based on ferrous bisglycinate hydrochloride is ready for commercial release [70]. A patented ferrous bisglycinate compound manufactured by Albion Laboratories, Clearfield, Utah, USA, has been used in most of the published studies. This chelate is reported to be formed by two glycine molecules combining with ferrous iron in a double heterocyclic ring structure [71]. Evidence would suggest that the iron is protected from absorption inhibitors by the chelate, since in most studies iron absorption from ferrous bisglycinate has been two to three times higher than from ferrous sulfate when added to cereal and milk products containing absorption inhibitors such as phytate or calcium [72–74].

GRAS Status and Organoleptic Effects: The main advantage of ferrous bisglycinate over NaFeEDTA is that it has GRAS status and can be considered a natural compound with no potential anti-physiological effects. However, it readily promotes fat oxidation in cereal foods [75] unless an antioxidant is added [76], and causes undesirable color reactions in some foods [77]. Data available in the literature on the organoleptic functionality of iron glycinate chelates are very scarce. However, several fortified commercial products seem to have good consumer acceptance. Ferrous bisglycinate is suitable for the fortification of commercial food products such as liquid milk as well as other dairy products and flavored beverages that are reconstituted from dry products, all vehicles which allow the delivery of significant amounts of bioavailable iron. Further research is needed to establish the compatibility of iron amino acid chelates with different food matrices during processing storage and food preparation.

Efficacy Studies: Four efficacy studies with ferrous bisglycinate-fortified foods all reported a marked decrease in the prevalence of anemia or iron deficiency anemia in children or adolescents. Unfortunately none of these studies had a control group receiving no iron, so there is no certainty that the improvement in iron status was due to the iron-fortified food. Three studies were carried out in Brazil. These studies investigated the influence of a ferrous glycinate-fortified liquid milk [78], sweetened bread roll [79], and whey-based beverage [25] on iron status of children. A further study made in Saudi Arabia investigated an iron-fortified flavored milk drink [24].

Phytate Degradation

Cereal grains and legume seeds are rich in phytate (myoinositol-6-phosphate), a food component which strongly inhibits the absorption of iron and other essential minerals [80]. In the digestive tract, phytate is thought to bind iron in insoluble complexes from which the iron is unavailable for absorption. While the formation of such complexes can be retarded by the addition of ascorbic acid, EDTA, or iron as ferrous bisglycinate, an alternative approach for the enhancement of iron absorption would be the removal of phytate from cereal flours by milling; or the degradation of phytate in cereal or legume foods with native (endogenous) or added (exogenous) phytases during the manufacturing process and/or food preparation [49]. Another approach would be to add an active phytase to cereal foods or condiments after processing. The active phytase would then degrade phytate in the gastrointestinal tract thus preventing the formation of iron-phytate complexes [81]. Phytases are widely used in this manner in animal husbandry to increase the liberation of phosphate from the food to enhance the bioavailability of added minerals and the conversion of feed proteins. They have not been applied as a means of improving the absorption of fortification iron from human foods.

Inhibitory Effect of Phytate on Iron Absorption: Phytate is a potent inhibitor of iron absorption even at relatively low levels and partial degradation (ca. 50%) of phytate in whole grain products would not be expected to markedly improve iron absorption [82–84]. In order to achieve the maximum increase in the absorption of fortification iron from cereal- and legume-based foods, phytate degradation should be virtually complete. Hurrell [12, 15] has recommended complete phytate degradation as the goal but, as this is not always possible, it has been recommended that the molar ratio of phytate to iron should be decreased to < 1:1 and ideally to < 0.4:1. The nutritional benefit of these low phytate levels in cereal foods is exemplified by the reports that decreasing the phytate in whole wheat by 90% during milling might be expected to double iron absorption [82] Complete dephytinization has been reported to increase iron absorption by as much as 12-fold in a single meal study (.99% to 11.54%) [49].

Methods for Reducing Dietary Phytate: Phytate can be degraded through the enzymatic action of phytase in cereal foods during the manufacturing processes with native or added phytases yielding a phytate-degraded food. Cereal- and legume-based complementary foods or soy-based infant formula are good candidates for dephytinization, as infants over six months of age often depend on these foods as the main sources of dietary iron. Breakfast cereals and breads can also be considered. Phytase enzymes are required to degrade phytate to the recommended low levels. These enzymes may be exogenous phytases (purified from bacterial, fungi, or plants) which are added during the manufacture of foods [85] or native phytases (intrinsic to the cereal) which are activated during the manufacturing process [86]. Iron absorption in babies was doubled when fed phytate-free soy formula that had been manufactured using a phytase from *Aspergillus niger* [85]. Similarly, using the same enzyme to manufacture phytate-free complementary foods based on rice, maize, oat, or wheat increased iron absorption in single meal studies in adults [from .33–1.80% to 2.79–11.54%] [49]. Low-phytate or phytate-free complementary foods can also be manufactured using traditional food processes such as soaking, germination, and fermentation [87–88]. Traditional lactic acid fermentation of cereal flours in India can reduce phytate by up to 80% [89–90], and yeast fermentation during bread manufacture, depending on the fermentation time, can decrease phytate by up to 50% in whole grain flours and almost completely in low extraction flours [91]. Iron absorption from a wheat bread roll made from low extraction flour was twice as high as iron absorption from a chapati made from the same flour [92].The bread roll preparation included a yeast fermentation step, while the chapati preparation did not. A slightly different approach commonly employed in the animal feed industry makes use of the addition of active phytases to finished products. In this type of application, the degradation occurs in the intestinal tract. This approach has also been demonstrated to increase iron absorption in humans [81].

Manufacturing Issues: The main concern of including a phytate degradation step in the manufacture of cereal-based complementary foods is that the cereal mixture should be held for 1–2 hours in an aqueous medium at 55°C and with a slightly acid pH so that the added or native phytase remains active and degrades most or all of the phytate. Complementary foods manufactured in pilot studies have been dephytinized in an aqueous slurry with an exogenous enzyme [49] or by activating the native phytases in a small amount of added whole wheat or whole rye [86]. Dephytinization has been followed by a steam injection step to precook the cereal and then by roller drying. Such precooked cereals can be fed mixed with hot water or milk (although milk itself is an inhibitor without the addition of ascorbic acid). While such dephytinized cereals should benefit the iron status of all weaning infants, infants in developing countries would be expected to benefit most. A potential problem in developing countries however is that many low-cost complementary foods, including most of those provided in food aid programs, do not include the aqueous phase necessary for dephytinization in their manufacturing process. Dry cereal and legume flours are often mixed with other ingredients and extruded and thus have to be cooked prior to consumption. Other technical issues, including technologies to prevent the growth of undesirable microorganisms during holding of the aqueous slurry at 55°C may also need to be addressed Dephytinization of these low-cost extruded cereals would be a challenge.

Practical Applications: No studies have been made to compare the effect of high-phytate complementary foods (phytate to iron molar ratio > 2) with phytate-free complementary foods (molar ratio < 0.5) (in the absence of ascorbic acid) on iron status of infants during the weaning period. A recent study by Lind *et al* [93] however has cast some doubt on the usefulness of dephytinization of Swedish baby foods. These authors compared a cereal porridge and cereal milk drink (phytate to iron molar ratios 0.9–2.6 and 1.4 respectively) with identical products after dephytinization (phytate to iron molar ratios 0.4–0.9 and 0.5 respectively). These products were fed to infants from 6–12 months of age as part of a diet including either breast milk or iron-fortified formula. Dephytinization caused no improvement in iron status. These results can perhaps be explained by the relatively low phytate levels together with the relatively high levels of ascorbic acid in the cereal porridge and cereal milk drink (ascorbic acid:iron molar ratio around 5:1).

High phytate is not such a concern in complementary foods or soy-based infant formulas sold in industrialized countries as these foods can be fortified with ascorbic acid to overcome the inhibitory effect of phytate and thus provide an adequate amount of absorbable iron [85, 94]. In developing countries however, ascorbic acid is often not stable due to the inferior packaging used for low-cost complementary foods and phytate degradation would be a better option.

Microencapsulated Ferrous Sulfate and Ferrous Fumarate

Microencapsulation is a process whereby the iron compound is encapsulated with a continuous layer or layers of coating material that separate the iron compound from the food matrix. Its main advantage is that it should allow the addition of iron compounds of high relative bioavailability to difficult food vehicles, such as cereal flours and low-grade salt, without causing the customary color and flavor changes. There is no evidence that microencapsulation will enhance the absorption of fortification iron. Bioavailability however potentially can be decreased by encapsulates. This should be strictly controlled.

Encapsulated ferrous sulfate and encapsulated ferrous fumarate, now used mainly to fortify infant formulas and cereals, have potential to be used in wheat and maize flours as well, provided that manufacturing and cooking practices do not result in unacceptable organoleptic changes to foods if the capsule material melts. Encapsulated iron compounds are suitable for most dry products, such as infant foods, dry beverage mixes, and other minimally processed foods. Other promising vehicles are condiment sachets for noodle flavorings and "sprinkle" sachets for mixing with complementary foods.

Choice of Coating Materials: Several different coating materials and processes are used to manufacture microencapsulated ingredients and variations can occur in the thickness and tightness of the coating as well as in the amount of compound still exposed on the surface of agglomerates. Microencapsulated ferrous sulfate and ferrous fumarate are usually manufactured using fluidized bed or spray chilling technology. Commonly the coatings are hydrogenated palm oil or soybean oil, although maltodextrin and celluloses have also been used. The hydrogenated oils protect against moisture but melt during heat treatments above 52–70°C, whereas maltodextrin and celluloses are usually water-soluble and do not provide adequate protection against iron oxidation in moist environments. When the ratio of coating material to iron compound is close to 1:1, there is no change in the bioavailability of ferrous sulfate in rat assays [95] and the efficacy of the encapsulated ferrous sulfate to improve iron status in school children is high [26]. Greater amounts of coating material or the inclusion of other compounds, such as waxes, into the coating however may decrease bioavailability.

Organoleptic Issues: Ferrous sulfate encapsulated with hydrogenated soybean oil prevented fat oxidation in stored infant cereals [95]. When, however, the dried cereals were reconstituted with hot water or hot milk, unacceptable colors developed. Similarly a series of different encapsulated ferrous sulfate and ferrous fumarate compounds failed to prevent color changes and iodine losses in moist, low-grade salt during storage in Morocco or the Ivory Coast [96]. Presumably sufficient iron was still on the surface of the capsule or, due to the moist conditions, leaked from within the capsule and reacted with impurities in the salt. Thus, although salt is a good potential vehicle for encapsulated iron, sensory issues still need to be resolved, particularly for low-grade salt. Further sensory testing of flours with encapsulated iron is also needed.

Future Potential of Microencapsulation: The potential of microencapsulation technology to allow use of iron compounds of high bioavailability without problematic sensory changes has not been fully evaluated. Current commercial compounds are used in infant foods and would be expected to prevent fat oxidation in stored cereal flours, although this and efficacy questions remain to be investigated. Ferrous sulfate and ferrous fumarate encapsulated with hydrogenated oils were recently recommended by PAHO [97] as useful compounds for the fortification of wheat and maize flours. Further improvements in encapsulation technology are necessary to produce microencapsulated iron compounds for the addition to low-grade salt, or alternatively the quality of the salt needs improvement.

Consensus Statement from SUSTAIN Task Force

This consensus statement on appropriate uses of iron enhancing technologies was reached after expert review of an extensive body of information. Presentations by science and industry as well as background review papers were rigorously discussed at a SUSTAIN Workshop in Washington, D.C. (March 9–12, 2003), resulting in a draft consensus statement. Subsequently, nutrition, science, medical, and industry experts collaborated to evaluate relevant literature and industry experience to address information gaps and areas needing further clarification. The implications of processing, storage, and (where applicable) cooking were evaluated for the enhancers in various food vehicles with a focus on the end product consumed rather than on intermediate products (e.g., flours) alone. In some cases regulatory issues were also addressed.

Ascorbic Acid

1. Ascorbic acid enhances the absorption of both intrinsic food iron and major iron fortificants in a dose-dependent fashion[1]. The enhancement is attributed to its reducing and chelating properties.
2. Numerous scientific studies report two- to three-fold increases in iron absorption associated with ascorbic acid use.
3. The addition of ascorbic acid to improve iron absorption is recommended for iron-fortified dry food preparations, such as pre-cooked complementary infant foods (e.g., cereal and legume-based blends), powdered milk, and other dry beverage products made for reconstitution.
4. A 2:1 molar ratio is recommended for powdered milk and low-phytate products; the ratio should be 4:1 for high-phytate products.
5. The use of ascorbic acid as an enhancing agent is limited by its instability in aqueous solutions (due to exposure to oxygen) and during prolonged heat processing methods. Proper packaging to exclude oxygen can be used to mitigate oxidation, but this approach will increase cost. Nearly all ascorbic acid is destroyed during prolonged cooking and high heat.
6. Ascorbic acid is not recommended for liquid foods, unless stability can be maximized by adequate packaging, or for foods that require baking and prolonged cooking (e.g., breads and gruels).

Other Organic Acids

1. Organic acids, such as citric, lactic, malic, and tartaric acid, are commonly used as food additives and preservatives. There is limited evidence indicating that they have an enhancing effect on iron absorption.
2. Available data suggests that organic acids may only be effective at high molar ratios (in excess of 100:1, organic acid:iron).
3. The necessary quantities will cause unacceptable flavor changes in most food vehicles, although applications in fruit drinks merit further consideration.

Chelates

Iron EDTA Chelates

1. NaFeEDTA's promoting effect on iron absorption from inhibitory meals has been shown in a number of radioisotopic studies and has been confirmed in five efficacy trials, using condiments as the vehicle. The iron in NaFeEDTA is two to three times better absorbed than is the iron in ferrous sulfate when present in high-phytate meals. The absorption of the native food iron is also enhanced to a similar extent by the EDTA. Iron absorption from NaFeEDTA and ferrous sulfate is similar in low-phytate meals.
2. NaFeEDTA is recommended for use in soy and fish sauces, and potentially with high-phytate flours and other condiments. Fish sauce should be packaged in

1 Combinations of certain enhancers are not additive. For example ascorbic acid is not an effective enhancer of iron absorption from NaFeEDTA.

amber bottles to prevent UV degradation of EDTA. Research is needed on its sensory effects on flour-based food staples.

3. NaFeEDTA is not recommended for complementary foods consumed by children under the age of three years because the amounts required to supply sufficient iron for effective fortification would approach the acceptable daily intake for EDTA (2.5 mg/kg body weight/day).
4. NaFeEDTA does not adversely affect the metabolism of nutritionally important metals such as zinc, copper, and calcium.
5. The available data indicates that EDTA does not increase the absorption of lead or cadmium in human beings or experimental animal models and may even reduce retention. In this context, the intravenous injection of EDTA has been a therapy for lead poisoning for many years. Current evidence is, however, limited and further research is desirable.
6. NaFeEDTA's widespread introduction as an iron enhancer is currently hampered by its ambiguous regulatory status.
7. There is little published evidence on the stability and organoleptic effects of NaFeEDTA during processing, storage, and cooking. Although it has been successfully used in several extended fortification trials, and maize and soy sauce have been commercially fortified, additional research, using a wider range of vehicles, including flour-based food staples, is recommended.

Amino Acid Chelates

1. In most studies iron absorption from ferrous bisglycinate has been two to three times higher than from ferrous sulfate when added to bread rolls and milk products containing absorption inhibitors such as phytate or calcium. Ferrous bisglycinate is more bioavailable than ferric trisglycinate. Ferrous bisglycinate HCl is another amino acid chelate that appears to merit further consideration and may offer favorable cost attributes. However its bioavailability needs to be established in human studies.
2. Amino acid chelates are recommended for milk and beverage products. The iron in ferrous bisglycinate, a patented product, appears to be well absorbed from milk, milk products, beverages, and high-phytate cereal products.
3. There is very little published evidence on stability and organoleptic effects of amino acid chelates during processing, storage and cooking. The data available indicates that they may promote sensory changes in some food vehicles and may promote fat oxidation in cereals in the absence of an antioxidant. Additional research is needed.
4. Further research is needed for amino acid chelates in vehicles other than milk and beverage products.

Phytate Degradation

1. Phytate is a potent inhibitor of absorption for native and fortification iron in cereals and legume-based foods. Effective enhancement of iron absorption requires near-complete degradation or removal of phytate. Under controlled experimental conditions, native and exogenous phytases effectively degrade phytate. This requires prolonged wet processing within a narrow pH range at controlled temperatures. A phytate:iron molar ratio of < 1:1 is recommended for effective enhancement of iron absorption, with < 0.4:1 being optimal.
2. Complete dephytinization has been reported to increase percentage iron absorption between 2- and 12-fold in single-meal studies. However, if dephytinization is not complete (or not at least achieving a phytate:iron molar ratio of < 1:1), then the increases in iron absorption are substantially lower or nonexistent.
3. Low-cost cereal- and legume-based complementary foods reconstituted with potable water may be the most appropriate vehicles for dephytinization in the developing world. Milk, however, without the addition of ascorbic acid, is an inhibitor of iron absorption.
4. While commercial phytase products are commonly used in feed for monogastric animals to free phosphates, and to increase mineral availability and protein conversion, their use in food products for humans has been limited.
5. Questions about technical feasibility and cost need to be resolved for food uses.

Encapsulation

1. Encapsulation has been shown to effectively overcome many of the limiting sensory and stability problems associated with adding soluble iron forms to finished food products. It may improve the potential shelf life of fortified products by preventing iron-mediated fat oxidation. It also appears to provide protection from oxidation of the soluble ferrous to the less soluble ferric form.
2. The main advantage of microencapsulation is that it should allow the addition of iron compounds of high relative bioavailability into difficult food vehicles. There is no evidence that microencapsulation will enhance the absorption of fortification iron. The effect of encapsulation on bioavailability depends on the capsule material and the capsule-to-substrate ratio. Research indicates that encapsulation of ferrous sulfate and ferrous fumarate with coatings composed of hy-

drogenated palm and soy oils, mono- and diglycerides, and maltodextrin does not compromise bioavailability, provided that the capsule-to-iron compound ratio does not exceed 1:1 and provided that the coating does not contain other additives.

3. Industry produces a large variety of encapsulated compounds. The technical properties of the capsules vary considerably. The bioavailability of encapsulated iron compounds needs to be demonstrated in animal or human studies before they are used for large-scale fortification. Appropriate standards for the encapsulation of iron compounds are needed to ensure optimal bioavailability .
4. Preferred systems for including encapsulated iron are those that are low in moisture and stored at low temperatures. Encapsulated iron compounds are suitable for most dry products such as infant foods, dry beverage mixes, and other minimally processed foods. Other promising vehicles are condiment sachets for noodle flavorings and "sprinkle" sachets for mixing with complementary foods.
5. However, food preparation that involves heat may cause melting of the capsule, which in turn may lead to undesirable organoleptic changes. Encapsulation may be a useful strategy for preventing iron-catalyzed sensory changes in cereal-based staples, provided that manufacturing and cooking practices do not result in unacceptable organoleptic changes if the capsule material melts. Encapsulation is not recommended for stored liquids because elevated moisture leads to leaching of the iron fortificant from the capsule, with accompanying sensory problems.

Other Strategies

Vitamin A has not been consistently demonstrated to improve the absorption of fortification iron. It is, however, necessary for normal iron metabolism in the body. Since vitamin A and iron deficiency are commonly found together, dual fortification with iron and vitamin A is frequently appropriate.

Conclusion

The success of iron fortification as a strategy for alleviating iron deficiency can potentially be improved by ingredients/technologies that enhance iron absorption and/or mitigate undesirable interactions between fortificant iron and food vehicles. Less soluble forms of iron are commonly used in food fortification because of shelf life and sensory concerns associated with more soluble iron salts such as ferrous sulfate in many food vehicles. Enhancing the bioavailability of widely used iron fortificants and/or preventing undesirable interactions between more soluble iron fortificants and food vehicles could benefit iron-deficient populations.

Different ingredients/technologies will be more or less appropriate for different classes of staple foods and widely consumed condiments. The practical applications of individual ingredient technologies are limited with respect to some food vehicles by issues of ingredient stability, cost, and consumer acceptance. A SUSTAIN Task Force has evaluated the limitations of each technology and recommended appropriate applications of the technologies, based on reviews of the scientific literature, as well as extensive input from and dialogue among industrial, nutrition, medical, and government experts.

Effective fortification depends primarily on the selection of technically feasible and biologically efficacious strategies. However, once suitable strategies have been identified, cost becomes very important in selecting the best approach to implementation. Manufacturers catering to the target population in developing countries can absorb only minimal increases in production costs of fortified products and governmental subsidies are usually not sustainable. It is essential to calculate cost with reference to the delivery of bioavailable iron. Smaller quantities of highly bioavailable iron are equivalent to larger amount of a less bioavailable product. Price per unit cost alone may therefore be misleading.

References

1. WHO (1992) Second Report on the World Nutrition Situation. ACC/SCN, WHO, Geneva.
2. WHO (2002) The World Health Report 2002 – Reducing risks, promoting healthy life. WHO, Geneva.
3. Preziosi, P., Prual, A., Galan, P., Daouda, H., Boureima, H. and Hercberg, S. (1997) Effect of iron supplementation on the iron status of pregnant women: consequences for newborns. Am. J. Clin. Nutr. 66, 1178–1182.
4. Ramakrishnan, U. (2001) Functional consequences of nutritional anemia during pregnancy and early childhood. In: Nutritional Anemias (Ramakrishna, U., ed.) pp. 43–68, CRC Press, Boca Raton.
5. Lozoff, B. (2000) Perinatal iron deficiency and the developing brain. Pediatr. Res. 48, 137–139.
6. Angulo-Kinzler, R.M., Peirano, P., Lin, E., Garrido, M. and Lozoff, B. (2002) Spontaneous motor activity in human infants with iron-deficiency anemia. Early Hum. Dev. 66, 67–79.
7. De Silva, A., Atukorala, S., Weerasinghe, I. and Ahluwahlia, N. (2003) Iron supplementation improves iron status and re-

duces morbidity in children with or without upper respiratory tract infections: a randomized controlled study in Colombo, Sri Lanka. Am. J. Clin. Nutr. 77, 234–241.
8. Zimmermann, M., Adou, P., Torresani, T., Zeder, C. and Hurrell, R. (2000) Persistence of goiter despite oral iodine supplementation in goitrous children with iron deficiency anemia in Côte d'Ivoire. Am. J. Clin. Nutr. 71, 88–93.
9. Haas, J.D. and Brownlie, T.T. (2001) Iron deficiency and reduced work capacity: a critical review of the research to determine a causal relationship. J. Nutr. 131, 676S–688S; discussion 688S–690S.
10. Zhu, Y.I., and Haas, J.D. (1997) Iron depletion without anemia and physical performance in young women. Am. J. Clin. Nutr. 66, 334–341.
11. Mora, J.O. (2002) Iron supplementation: overcoming technical and practical barriers. J. Nutr. 132, 853S–855S.
12. Hurrell, R.F. (2002) Fortification: overcoming technical and practical barriers. J. Nutr. 132, 806S–812S.
13. Hurrell, R.F., Bothwell, T., Cook, J.D., Dary, O., Davidsson, L., Fairweather-Tait, S., Hallberg, L., Lynch, S., Rosado, J., Walter, T. and Whittaker, P. (2002) The usefulness of elemental iron for cereal flour fortification: a SUSTAIN Task Force report. Nutr. Rev. 60, 391–406.
14. Cook, J.D., Reddy, M.B., Burri, J., Juillerat, M.A. and Hurrell, R.F. (1997) The influence of different cereal grains on iron absorption from infant cereal foods. Am. J. Clin. Nutr. 65, 964–969.
15. Hurrell, R. (2002) How to ensure adequate iron absorption from iron-fortified food. Nutr. Rev. 60, S7–15; discussion S43.
16. INACG (1986) Combating iron deficiency in Chile: A case study. ILSI – Nutrition Foundation, Washington, D.C.
17. Fomon, S. (2001) Infant feeding in the 20th century: formula and beikost. J. Nutr. 131, 409S–420S.
18. Walter, T., Dallman, P.R., Pizarro, F., Velozo, L., Pena, G., Bartholmey, S.J., Hertrampf, E., Olivares, M., Letelier, A. and Arredondo, M. (1993) Effectiveness of iron-fortified infant cereal in prevention of iron deficiency anemia. Pediatrics 91, 976–982.
19. Garby, L. and Areekul, S. (1974) Iron supplementation in Thai fish-sauce. Ann. Trop. Med. Parasitol. 68, 467–476.
20. Thuy, P.V., Berger, J., Davidsson, L., Khan, N.C., Lam, N. T., Cook, J.D., Hurrell, R.F. and Khoi, H.H. (2003) Regular consumption of NaFeEDTA-fortified fish sauce improves iron status and reduces the prevalence of anemia in anemic Vietnamese women. Am. J. Clin. Nutr. 78, 284–290.
21. Mannar, V. and Gallego, E.B. (2002) Iron fortification: country level experiences and lessons learned. J. Nutr. 132, 856S–858S.
22. Viteri, F.E., Alvarez, E., Batres, R., Torun, B., Pineda, O., Mejia, L.A. and Sylvi, J. (1995) Fortification of sugar with iron sodium ethylenediaminotetraacetate (FeNaEDTA) improves iron status in semirural Guatemalan populations. Am. J. Clin. Nutr. 61, 1153–1163.
23. Ballot, D.E., MacPhail, A.P., Bothwell, T.H., Gillooly, M. and Mayet, F.G. (1989) Fortification of curry powder with NaFe(III)EDTA in an iron-deficient population: report of a controlled iron-fortification trial. Am. J. Clin. Nutr. 49, 162–169.
24. Osman, A.K. and Al-Othaimeen, A. (2002) Experience with ferrous bis-glycine chelate as an iron fortificant in milk. Int. J. Vitam. Nutr. Res. 72, 257–263.
25. Miglioranza, L.H., Matsuo, T., Caballero-Cordoba, G.M., Dichi, J.B., Cyrino, E.S., Oliveira, I.B., Martins, M.S., Polezer, N.M. and Dichi, I. (2003) Effect of long-term fortification of whey drink with ferrous bisglycinate on anemia prevalence in children and adolescents from deprived areas in Londrina, Parana, Brazil. Nutrition 19, 419–421.
26. Zimmermann, M.B., Zeder, C., Chaouki, N., Saad, A., Torresani, T. and Hurrell, R.F. (2003) Dual fortification of salt with iodine and microencapsulated iron: a randomized, double-blind, controlled trial in Moroccan schoolchildren. Am. J. Clin. Nutr. 77, 425–432.
27. Conrad, M.E. and Schade, S.G. (1968) Ascorbic acid chelates in iron absorption: a role for hydrochloric acid and bile. Gastroenterology 55, 35–45.
28. Hsieh, Y.H. and Hsieh, Y.P. (1997) Valence state of iron in the presence of ascorbic acid and ethylenediaminetetraacetic acid. J. Agri. Food Chem. 45, 1126–1129.
29. Siegenberg, D., Baynes, R.D., Bothwell, T.H., Macfarlane, B.J., Lamparelli, R.D., Car, N.G., MacPhail, P., Schmidt, U., Tal, A. and Mayet, F. (1991) Ascorbic acid prevents the dose-dependent inhibitory effects of polyphenols and phytates on nonheme-iron absorption. Am. J. Clin. Nutr. 53, 537–541.
30. Stekel, A., Olivares, M., Pizarro, F., Chadud, P., Lopez, I. and Amar, M. (1986) Absorption of fortification iron from milk formulas in infants. Am. J. Clin. Nutr. 43, 917–922.
31. Cook, J. and Monsen, E. (1977) Vitamin C, the common cold, and iron absorption. Am. J. Clin. Nutr. 30, 235–241.
32. Derman, D.P., Bothwell, T.H., MacPhail, A.P., Torrance, J. D., Bezwoda, W.R., Charlton, R.W. and Mayet, F.G. (1980) Importance of ascorbic acid in the absorption of iron from infant foods. Scand. J. Haematol. 25, 193–201.
33. Davidsson, L., Walczyk, T., Morris, A. and Hurrell, R.F. (1998) Influence of ascorbic acid on iron absorption from an iron-fortified, chocolate-flavored milk drink in Jamaican children. Am. J. Clin. Nutr. 67, 873–877.
34. Gillooly, M., Torrance, J.D., Bothwell, T.H., MacPhail, A. P., Derman, D., Mills, W. and Mayet, F. (1984) The relative effect of ascorbic acid on iron absorption from soy-based and milk-based infant formulas. Am. J. Clin. Nutr. 40, 522–527.
35. Forbes, A.L., Adams, C.E., Arnaud, M.J., Chichester, C.O., Cook, J.D., Harrison, B.N., Hurrell, R.F., Kahn, S.G., Morris, E.R., Tanner, J.T. and Whittaker, P. (1989) Comparison of *in vitro*, animal, and clinical determinations of iron bioavailability: INACG Task Force report on iron bioavailability. Am. J. Clin. Nutr. 49, 225–238.
36. Cook, J.D., Dassenko, S.A. and Lynch, S.R. (1991) Assessment of the role of nonheme-iron availability in iron balance. Am. J. Clin. Nutr. 54, 717–722.
37. Cook, J.D. and Reddy, M.B. (2001) Effect of ascorbic acid intake on nonheme-iron absorption from a complete diet. Am. J. Clin. Nutr. 73, 93–98.
38. Cook, J.D., Watson, S.S., Simpson, K.M., Lipschitz, D.A. and Skikne, B.S. (1984) The effect of high ascorbic acid supplementation on body iron stores. Blood 64, 721–726.

39. Hunt, J.R. (2003) High- but not low-bioavailability diets enable substantial control of women's iron absorption in relation to body iron stores, with minimal adaptation within several weeks. Am. J. Clin. Nutr. 78, 1168–77.
40. Suzuki, T., Clydesdale, F.M. and Pandolf, T. (1992) Solubility of iron in model systems containing organic acids and lignin. J. Food Prog. 55, 893–898.
41. Holland, B., Welch, A.A., Unwin, I.D., Buss, D.H., Paul, A.A. and Southgate, D.A.T. (1992) McCance and Widdowson's The composition of foods, The Royal Society of Chemistry and Ministry of Agriculture, Fisheries and Food.
42. Ballot, D., Baynes, R.D., Bothwell, T.H., Gillooly, M., MacFarlane, B.J., MacPhail, A.P., Lyons, G., Derman, D.P., Bezwoda, W.R., Torrance, J.D. *et al.* (1987) The effects of fruit juices and fruits on the absorption of iron from a rice meal. Br. J. Nutr. 57, 331–343.
43. Gillooly, M., Bothwell, T.H., Torrance, J.D., MacPhail, A. P., Derman, D.P., Bezwoda, W.R., Mills, W., Charlton, R.W. and Mayet, F. (1983) The effects of organic acids, phytates and polyphenols on the absorption of iron from vegetables. Br. J. Nutr. 49, 331–342.
44. Derman, D.P., Bothwell, T.H., Torrance, J.D., Bezwoda, W.R., MacPhail, A.P., Kew, M.C., Sayers, M.H., Disler, P.B. and Charlton, R.W. (1980) Iron absorption from maize (Zea mays) and sorghum (Sorghum vulgare) beer. Br. J. Nutr. 43, 271–279.
45. Personal communication with Dr. Thomas Walczyk.
46. Layrisse, M. and Martinez-Torres, C. (1977) Fe(III)-EDTA complex as iron fortification. Am. J. Clin. Nutr. 30, 1166–1174.
47. Viteri, F.E., Garcia-Ibanez, R. and Torun, B. (1978) NaFeEDTA as an iron fortification compound in Central America. Absorption studies. Am. J. Clin. Nutr. 31, 961–971.
48. MacPhail, A.P., Bothwell, T.H., Torrance, J.D., Derman, D. P., Bezwoda, W.R., Charlton, R.W. and Mayet, F. (1981) Factors affecting the absorption of iron from Fe(III)EDTA. Br. J. Nutr. 45, 215–227.
49. Hurrell, R.F., Reddy, M.B., Juillerat, M.A. and Cook, J.D. (2003) Degradation of phytic acid in cereal porridges improves iron absorption by human subjects. Am. J. Clin. Nutr. 77, 1213–1219.
50. FAO/WHO, JECFA (1999) Fifty-third meeting. Summary and conclusions.
51. FAO/WHO, JECFA (1974) Toxicological evaluation of some food additives including anti-caking agents, antimicrobials, antioxidants, emulsifiers and thickening agents.
52. INACG (1993) Iron EDTA for food fortification, The Nutrition Foundation/ILSI, Washington, D.C.
53. Personal communication with Dr. Carel Wreesmann, Akzo-Nobel, Arnhem, Netherlands, June 25, 2004.
54. Hurrell, R.F., Reddy, M.B., Burri, J. and Cook, J.D. (2000) An evaluation of EDTA compounds for iron fortification of cereal-based foods. Br. J. Nutr. 84, 903–910.
55. Fidler, M.C., Davidsson, L., Walczyk, T. and Hurrell, R.F. (2003) Iron absorption from fish sauce and soy sauce fortified with sodium iron EDTA. Am. J. Clin. Nutr. 78, 274–278.
56. MacPhail, A.P., Patel, R.C., Bothwell, T.H. and Lamparelli, R.D. (1994) EDTA and the absorption of iron from food. Am. J. Clin. Nutr. 59, 644–648.
57. Davidsson, L., Walczyk, T., Zavaleta, N. and Hurrell, R. (2001) Improving iron absorption from a Peruvian school breakfast meal by adding ascorbic acid or Na_2EDTA. Am. J. Clin. Nutr. 73, 283–287.
58. Fairweather-Tait, S.J., Wortley, G.M., Teucher, B. and Dainty, J. (2001) Iron absorption from a breakfast cereal: effects of EDTA compounds and ascorbic acid. Int. J. Vitam. Nutr. Res. 71, 117–122.
59. Hurrell, R.F. (1997) Preventing iron deficiency through food fortification. Nutr. Rev. 55, 210–222.
60. Dary, O. (2002) Staple food fortification with iron: a multifactorial decision. Nutr. Rev. 60, S34–41; discussion S46–39.
61. Fidler, M.C., Krzystek, A., Walczyk, T. and Hurrell, R.F. (in press) Photostability of NaFeEDTA in stored fish sauce and soy sauce. J. Food Sci.
62. Restani, P. and Galli, C.L. (1991) Oral toxicity of formaldehyde and its derivates. Crit. Rev. Toxicol. 21, 315–328.
63. Hurrell, R.F., Ribas, S. and Davidsson, L. (1994) $NaFe^{3+}EDTA$ as a food fortificant: influence on zinc, calcium and copper metabolism in the rat. Br. J. Nutr. 71, 85–93.
64. Davidsson, L., Kastenmayer, P. and Hurrell, R.F. (1994) Sodium iron EDTA [NaFe(III)EDTA] as a food fortificant: the effect on the absorption and retention of zinc and calcium in women. Am. J. Clin. Nutr. 60, 231–237.
65. Garber, B.T. and Wei, E. (1974) Influence of dietary factors on the gastrointestinal absorption of lead. Toxicol. Appl. Pharmacol. 27, 685–691.
66. James, H.M., Hilburn, M.E. and Blair, J.A. (1985) Effects of meals and meal times on uptake of lead from the gastrointestinal tract in humans. Hum. Toxicol. 4, 401–407.
67. Personal communication with Dr. Lena Davidsson.
68. Andersen, O., Nielsen, J.B. and Svendsen, P. (1988) Oral cadmium chloride intoxication in mice: effects of chelation. Toxicology 52, 65–79.
69. Davidsson, L., Almgren, A. and Hurrell, R.F. (1998) Sodium iron EDTA [NaFe(III)EDTA] as a food fortificant does not influence absorption and urinary excretion of manganese in healthy adults. J. Nutr. 128, 1139–1143.
70. Personal communication with Dr. Brent Hagen, Vice President, Technical Services, Kelatron Corporation, Ogden, UT, June 2, 2004.
71. Ashmead, H.D., Graff, D.J. and Ashmead, H.H. (1985) Intestinal absorption of metal ions and chelates. Charles C. Thomas, Springfield, IL.
72. Olivares, M., Pizarro, F., Pineda, O., Name, J.J., Hertrampf, E. and Walter, T. (1997) Milk inhibits and ascorbic acid favors ferrous bis-glycine chelate bioavailability in humans. J. Nutr. 127, 1407–1411.
73. Layrisse, M., Garcia-Casal, M.N., Solano, L., Baron, M.A., Arguello, F., Llovera, D., Ramirez, J., Leets, I. and Tropper, E. (2000) Iron bioavailability in humans from breakfasts enriched with iron bis-glycine chelate, phytates and polyphenols. J. Nutr. 130, 2195–2199.
74. Bovell-Benjamin, A., Viteri, F. and Allen, L. (2000) Iron absorption from ferrous bisglycinate and ferric trisglycinate in whole maize is regulated by iron status. Am. J. Clin. Nutr. 71, 1563–1569.

75. Bovell-Benjamin, A., Allen, L., Frankel, E. and Guinard, J. X. (1999) Sensory quality and lipid oxidation of maize porridge as affected by iron amino acid chelates and EDTA. J. Food Sci. 64, 371–376.
76. Allen, L. H. (2002) Advantages and limitations of iron amino acid chelates as iron fortificants. Nutr. Rev. 60, S18–21; discussion S45.
77. Personal communication with Dr. Richard Hurrell, Swiss Federal Institute of Technology, Zurich, Switzerland, June 3, 2004.
78. Iost, C., Name, J. J., Jeppsen, R. B. and DeWayne Ashmead, H. (1998) Repleting hemoglobin in iron deficiency anemia in young children through liquid milk fortification with bioavailable iron amino acid chelate. J. Am. Coll. Nutr. 17, 187–194.
79. Giorgini, E., Fisberg, M., De Paula, R.A.C., Ferreira, A. M. A., Valle, J. and Braga, J. A. P. (2001) The use of sweet rolls fortified with iron bis-glycinate chelate in the prevention of iron deficiency anemia in preschool children. Arch. Latinoam. Nutr. 51, 48–53.
80. Hurrell, R. F. (2003) Influence of vegetable protein sources on trace element and mineral bioavailability. J. Nutr. 133, 2973S–2977S.
81. Sandberg, A. S., Hulthen, L. R. and Turk, M. (1996) Dietary Aspergillus niger phytase increases iron absorption in humans. J. Nutr. 126, 476–480.
82. Hallberg, L., Brune, M. and Rossander, L. (1989) Iron absorption in man: ascorbic acid and dose-dependent inhibition by phytate. Am. J. Clin. Nutr. 49, 140–144.
83. Hurrell, R. F., Juillerat, M. A., Reddy, M. B., Lynch, S. R., Dassenko, S. A. and Cook, J. D. (1992) Soy protein, phytate, and iron absorption in humans. Am. J. Clin. Nutr. 56, 573–578.
84. Larsson, M., Rossander-Hulthen, L., Sandstrom, B. and Sandberg, A. S. (1996) Improved zinc and iron absorption from breakfast meals containing malted oats with reduced phytate content. Br. J. Nutr. 76, 677–688.
85. Davidsson, L., Galan, P., Kastenmayer, P., Cherouvrier, F., Juillerat, M. A., Hercberg, S. and Hurrell, R. F. (1994) Iron bioavailability studied in infants – the influence of phytic acid and ascorbic-acid in infant formulas based on soy isolate. Pediatr. Res. 36, 816–822.
86. Egli, I., Davidsson, D., Juillerat, M. A., Barclay, D. and Hurrell, R. (2003) Phytic acid degradation in complementary foods using phytase naturally occurring in whole grain cereals. J. Food Sci. 68, 1855–1859.
87. Marero, L., Payumo, E., Aguinaldo, A., Matsumoto, I. and Homma, S. (1991) The antinutritional factors in weaning foods prepared from germinated legumes and cereals. Lebensm.-Wiss. u. -Technol. 24, 177–181.
88. Sharma, A. and Kapoor, A.C. (1996) Levels of antinutritional factors in pearl millet as affected by processing treatments and various types of fermentation. Plant Foods Hum. Nutr. 49, 241–252.
89. Gupta, M., Khetarpaul, N. and Chauhan, B. M. (1992) Rabadi fermentation of wheat: changes in phytic acid content and in vitro digestibility. Plant Foods Hum. Nutr. 42, 109–116.
90. Gupta, M. and Khetarpaul, N. (1993) Effect of rabadi fermentation on phytic acid and *in vitro* digestibility of barley. Nahrung 37, 141–146.
91. Daniels, D. G. and Fisher, N. (1981) Hydrolysis of the phytate of wheat flour during breadmaking. Br. J. Nutr. 46, 1–6.
92. Hurrell, R. F., Reddy, M. B., Burri, J. and Cook, J. D. (2002) Phytate degradation determines the effect of industrial processing and home cooking on iron absorption from cereal-based foods. Br. J. Nutr. 88, 117–123.
93. Lind, T., Lonnerdal, B., Persson, L. A., Stenlund, H., Tennefors, C. and Hernell, O. (2003) Effects of weaning cereals with different phytate contents on hemoglobin, iron stores, and serum zinc: a randomized intervention in infants from 6 to 12 mo of age. Am. J. Clin. Nutr. 78, 168–175.
94. Davidsson, L., Galan, P., Cherouvrier, F., Kastenmayer, P., Juillerat, M. A., Hercberg, S. and Hurrell, R. F. (1997) Bioavailability in infants of iron from infant cereals: effect of dephytinization. Am. J. Clin. Nutr. 65, 916–920.
95. Hurrell, R. F., Furniss, D. E., Burri, J., Whittaker, P., Lynch, S. R. and Cook, J. D. (1989) Iron fortification of infant cereals: a proposal for the use of ferrous fumarate or ferrous succinate. Am. J. Clin. Nutr. 49, 1274–1282.
96. Wegmüller, R., Zimmermann, M. B. and Hurrell, R. F. (2003) Dual fortification of salt with iodine and encapsulated iron compounds: stability and acceptability testing in Morocco and Côte d'Ivoire. J. Food Sci. 68, 2129–2135.
97. PAHO and WHO (2002) Guiding principles for complementary feeding of the breastfed child, PAHO, Washington, D.C.

Richard Hurrell

Swiss Federal Institute of Technology Zurich (ETH)
Laboratory of Human Nutrition
PO Box 747
CH-8803 Rüschlikon
Switzerland
Fax ++41-1-704 57 10
E-mail: richard.hurrell@ilw.agrl.ethz.ch