Enhancers of Iron Absorption: Ascorbic Acid and other Organic Acids

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Abstract: Ascorbic acid (AA), with its reducing and chelating properties, is the most efficient enhancer of non-heme iron absorption when its stability in the food vehicle is ensured. The number of studies investigating the effect of AA on ferrous sulfate absorption far outweighs that of other iron fortificants. The promotion of iron absorption in the presence of AA is more pronounced in meals containing inhibitors of iron absorption. Meals containing low to medium levels of inhibitors require the addition of AA at a molar ratio of 2:1 (e.g., 20 mg AA: 3 mg iron). To promote absorption in the presence of high levels of inhibitors, AA needs to be added at a molar ratio in excess of 4:1, which may be impractical.

The effectiveness of AA in promoting absorption from less soluble compounds, such as ferrous fumarate and elemental iron, requires further investigation.

The instability of AA during food processing, storage, and cooking, and the possibility of unwanted sensory changes limits the number of suitable food vehicles for AA, whether used as vitamin fortificant or as an iron enhancer. Suitable vehicles include dry-blended foods, such as complementary, precooked cereal-based infant foods, powdered milk, and other dry beverage products made for reconstitution that are packaged, stored, and prepared in a way that maximizes retention of this vitamin. The consumption of natural sources of Vitamin C (fruits and vegetables) with iron-fortified dry blended foods is also recommended.

Encapsulation can mitigate some of the AA losses during processing and storage, but these interventions will also add cost. In addition, the bioavailability of encapsulated iron in the presence/absence of AA will need careful assessment in human clinical trials.

The long-term effect of high AA intake on iron status may be less than predicted from single meal studies. The hypothesis that an overall increase of dietary AA intake, or fortification of some foods commonly consumed with the main meal with AA alone, may be as effective as the fortification of the same food vehicle with AA and iron, merits further investigation. This must involve the consideration of practicalities of implementation.

To date, programs based on iron and AA fortification of infant formulas and cow's milk provide the strongest evidence for the efficacy of AA fortification. Present results suggest that the effect of organic acids, as measured by *in vitro* and *in vivo* methods, is dependent on the source of iron, the type and concentration of organic acid, pH, processing methods, and the food matrix.

The iron absorption-enhancing effect of AA is more potent than that of other organic acids due to its ability to reduce ferric to ferrous iron. Based on the limited data available, other organic acids may only be effective at ratios of acid to iron in excess of 100 molar. This would translate into the minimum presence/addition of 1 g cit-

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ric acid to a meal containing 3 mg iron. Further characterization of the effectiveness of various organic acids in promoting iron absorption is required, in particular with respect to the optimal molar ratio of organic acid to iron, and associated feasibility for food application purposes.

The suggested amount of any organic acid required to produce a nutritional benefit will result in unwanted organoleptic changes in most foods, thus limiting its application to a small number of food vehicles (e.g., condiments, beverages). However, fermented foods that already contain high levels of organic acid may be suitable iron fortification vehicles.

Key words: iron, food fortification, absorption, ascorbic acid, organic acids, iron status

1. Introduction

Increasing iron absorption from fortified foods through the addition of enhancers of iron absorption may improve the efficacy of fortification strategies, especially in diets containing inhibitors such as phytate and tannins [1]. Ascorbic acid (AA) is the most widely studied enhancer of iron absorption, with other organic acids having received much less attention. The potential importance of AA as a means of improving iron nutrition was underlined in 1970 by the U.S. Food and Nutrition Board when it suggested the promotion of "a program featuring iron, in addition to vitamin C, as a breakfast nutrient" [2]. The aim of the present review is to summarize the effects of ascorbic acid and other organic acids on iron absorption and iron status.

2. Chemistry and Function of Ascorbic Acid in Iron Nutrition

Ascorbic acid is a chemically defined compound, having the empirical formula $C_6H_8O_6$ and a molecular weight of 176.13 [3,4]. The role of AA as a reducing agent and oxygen scavenger explains some of its biological functions [5] and its use in food processing [6]. The addition of this vitamin to foods and beverages during processing or prior to packaging protects color, aroma, and nutrient content and improves iron absorption. In meat processing, ascorbic acid makes it possible to reduce both the amount of added nitrite and the residual nitrite content in the product. The addition of ascorbic acid to fresh flour improves its baking qualities, thus saving the four to eight weeks of maturation that flour would normally have to undergo after milling [7].

2.1 Role of Ascorbic Acid in Iron Absorption

The dominant form of iron in foods is ferric iron, which is much less bioavailable than ferrous iron. One of AA's main attributes is its ability to reduce ferric to ferrous iron. AA undergoes a reversible two-stage redox process with a free radical intermediate. The latter reacts preferably with itself, thus preventing the propagation of free radical reactions [8]. At the same time, AA maintains a transition metal, such as Fe(III), in its reduced form Fe(II) and can promote the reaction of these ions with hydrogen peroxide to form highly reactive hydroxyl radicals in the Fenton reaction [9]. Such pro-oxidant activities have been demonstrated in various food matrices [10, 11] and may adversely affect shelf life.

In vivo observations showing an enhancement of iron absorption in the presence of AA have been attributed to AA's chelating and reducing properties [12]. Reduction was previously thought to be an essential function of AA prior to ferrous iron uptake by the divalent metal transporter (DMT1) [13]. However, ferric iron reduction has now been shown to be due to the action of ferrireductase Dcytb (duodenal cytochrome b) located at the brush border membrane of duodenal enterocytes [14]. This suggests that AA's main role is to promote iron solubility. Recent experiments confirm that the reduction of 2 mol Fe(III) by 1 mol of AA occurs in the pH range between 2.6 and 6.0. The rate of reduction decreases with increasing pH, and above pH 6.0 AA is no longer an effective reducing agent for Fe(III). There is potential for the formation of ferric ascorbate complexes at pH above 5 but determination of the complexes formed is difficult due to complicated kinetic effects. Further, the formation of a 1:1 EDTA/Fe(III) complex can prevent the reduction of ferric iron by AA [15].

AA's role in iron chemistry suggests that AA and iron must be consumed together to promote iron absorption. Absorption studies by Cook *et al* [16] confirmed that the increase in iron absorption in the presence of AA was not observed when AA was administered several hours before an identical iron-containing meal. The necessity of consuming both nutrients together causes technological difficulties because of the instability of AA during food processing operations and storage.

2.2 Stability of Ascorbic Acid

AA is sensitive to heat, light, and oxygen and interaction with copper, iron, and tin [4]. In the dry state, ascorbic acid is reasonably stable in air, but in solution it rapidly oxidizes. On exposure to light, moisture, and heat, it may also darken. Cooking typically destroys ascorbic acid by accelerating the oxidation reaction. Low pH values enhance its stability while alkaline values are deleterious [17]. Some derivative compounds of AA (dipotassium L-ascorbate 2-sulfate, L-ascorbate 2-monophosphate, L-ascorbate 2-polyphosphate, ascorbyl palmitate) and coated AA have a better stability than AA [17–20], with losses during thermal processing and storage being smaller. Since the stability of AA in beverages and/or liquid foods depends to a great extent on the oxygen content and exposure, packaging methods and materials have an important impact on the product's shelf life. Absolute vitamin C loss in fruit juices can be diminished with proper de-aeration techniques [21]. Heat processing methods have different impacts on AA stability. After sterilization of milk at 131°C for 5 minutes, retention is 35%, while ultrahigh temperature (UHT) processing at 142°C for 4 seconds retains 57% of the original AA content [22]. After 12 months of storage, only the UHT-processed product retains part of the nutrient content (40%). AA is virtually destroyed during the baking process [18]. Spraying a formulation containing AA on baked goods after the heating period is a partial remedy for the stability problem [23]. This technology has been used widely in extruded products. Baking seems to have a more destructive effect on AA than boiling. For example, retention in a sweet potato variety, after 30 minutes of boiling, was 45.6% while the value after the same time under baking conditions was 30.3% [24]. Boiling causes losses that are on average 10% higher than with steaming [25]. Overcooking leads to a major loss of the nutrient. Some heat-sensitive vitamins, such as AA, are more stable during frying than pressure cooking and boiling, since the temperature inside the food never exceeds 100°C provided there is some liquid water left in it. In addition, frying times are usually very short and there is no leaching of water-soluble vitamins [26].

Post-cooking handling of food also has a major effect on the vitamin content. In analyses of a large number of foodstuffs, Paulus [25] concluded that a product loses half of its AA content after 12 months of sterilization/freezing, 6 weeks of pasteurization/chilling, 7 days of chilling, and after only 6 hours when kept warm. Under normal operating conditions, with hot-holding limited to less than 90 minutes, vitamin retention seems to be better in conventional cook/hot-hold food services than in cook/chill systems [27].

Iron absorption studies have shown a reduction or lack of effect from AA when the vitamin is added to meals before cooking, baking, or even warming for a prolonged time [28, 29]. Oxidative destruction of ascorbic acid occurs as a consequence of two factors, the height of the temperature and the length of exposure. In a study carried out by Sayers *et al* [28], iron absorption was increased in a dose-dependent fashion when AA was added to porridge prior to heating for 20–25 minutes at 88°C. In contrast, no enhancement in iron absorption was noted when ascorbic acid was added before baking to soy biscuits (100 mg) and wheat bread (50 mg) [28]. In another study, a reduction in ascorbic acid content and iron absorption was observed in meals heated at 75ºC for 4 hours [29]. Iron bioavailability of the heated meals was restored when the content of ascorbic acid was returned to its previous level.

3. Effect of Ascorbic Acid on Non-heme Iron Absorption in Humans

3.1 Single Foods versus Complete Diets

Historically, most information on the effect of ascorbic acid and other enhancers or inhibitors of iron absorption was generated from studies using the single meal technique in which the non-heme iron component of the meal is labeled with either a radioisotope or stable isotope of iron. These studies convincingly established the iron-enhancing properties of ascorbic acid, as first summarized by Lynch and Cook [30]. The dose-response relationship obtained from a semi-synthetic meal containing 4.1 mg iron and AA in doses ranging from 25 to 1000 mg is best described by a steep linear response up to a 7.5 molar ratio of AA to iron, followed by a less pronounced linear dose-response for molar ratios above 7.5. At the 7.5 molar ratio the increase in iron absorption was about 3-fold (Figure 1) [30].

Because of the many variables involved (including total iron, AA:Fe, AA:inhibitors, presence of other enhancers), it is difficult to predict the ideal combination of iron and AA that will maximize absorption. A great variety of single meal studies have shown a pronounced relative increase in iron absorption depending on the presence and type of inhibitors and other enhancers of iron absorption [31–33]. The two most important inhibitors of iron absorption, phytate and polyphenols, were found to inhibit iron absorption at very low levels. For example, 2 mg phytate reduced absorption by about 18% [34] and 12 mg tannic acid reduced absorption by 30% [33]. In general, studies have differed in their experimental approach, in that some added enhancers and/or inhibitors to test

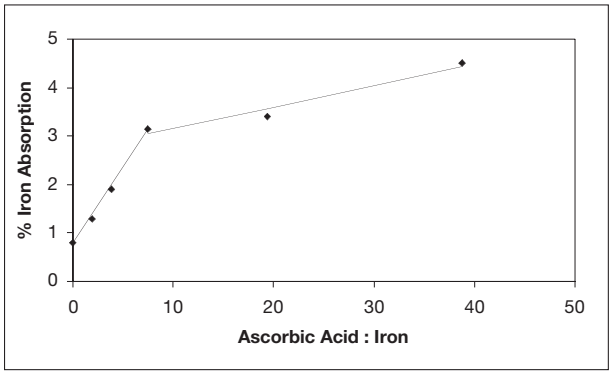

Figure 1: The effect of ascorbic acid on iron absorption from a semi-synthetic meal. (Redrawn with permission of the Annals of the New York Academy of Sciences.
Figure adapted from [32] Interaction of vitamin C and iron. Ann. N. Y. Acad. Sci. 355:32–44).

meals as purified components [33, 34] while others added AA and/or phytate-rich foods or food extracts [33, 35, 36]. The amount of AA required to completely counteract the effect of 25 mg phytates was estimated at 80 mg AA. Several hundred milligrams of AA would therefore be required to counteract the inhibition of high-phytate diets (250 mg-plus) [34]. A minimum of 50 mg AA was found to compensate for the negative effect of a bread meal containing in excess of 100 mg polyphenols [33]. The phytate content of typical western meals ranges from 10 to 100 mg and phytate levels > 250 mg are present in many meals typically consumed in developing countries [34]. A cup of black tea contains, on average, 200 mg polyphenols and the total dietary intake of polyphenols is estimated to be in excess of 1 g/day [37].

Although increasing amounts of AA progressively increase iron absorption, a less pronounced response is observed at high ratios, suggesting an optimal level of AA above which the additional nutritional benefit is negligible. For example, the addition of 30 mg AA to a bread meal containing 59 mg phytate was sufficient to double iron absorption, while adding 50 mg AA to the same meal resulted in a further 40% increase; the AA to iron molar ratio was 1.6:1 and 2.7:1, respectively [33]. A further 8% increase in iron absorption was seen by adding 150 mg AA (7.9:1 molar ratio). The enhancing effect of AA has mostly been investigated at molar ratios of AA to Fe > 1.0. A significant increase in absorption of about 50% or more was found with various test meals containing low to medium levels of inhibitors at molar ratios ranging between 1:1 and 6:1 [31, 32]. Molar ratios of 20:1 and 40:1 may be required to significantly enhance iron absorption from meals high in inhibitors such as soya bean-based products [38] and cereal-based breakfast meals, respectively [32].

There is some evidence to suggest that single meal studies may not reflect iron absorption from a complete diet [39]. So far, only a very limited number of studies have investigated the actual magnitude of the enhancing effect of AA on iron absorption from a complete diet. In one study, supplementation of all the main meals with 500 mg AA over a period of 5 weeks did not change apparent absorption and iron status of individuals consuming a diet with a predicted low (n = 13) or high iron bioavailability (n = 12). However, the small sample size in this study and the use of the balance method may not have provided sufficient statistical power to detect small changes. [40].

A recently published study determined absorption from a complete Western type diet before and after altering the diet to maximally increase or decrease ascorbic acid intake. All main meals were labeled with radio-iron for 5 consecutive days and absorption was determined by the red cell incorporation method in 12 iron-replete subjects [41]. Although an average molar ratio of AA to iron of 47:1 was achieved during the high AA period, the increase in absorption (35%) from the low AA (molar ratio 5.8) to the high AA diet (molar ratio 47.0) was not statistically significant (n = 12). It should, however, be noted that between-subject variation was large, with responses ranging from a decrease to a 3.5-fold increase in absorption with the high AA diet. In contrast, a significant 3.3-fold increase in iron absorption was found in 15 Mexican iron-deplete women consuming their habitual diet following the consumption of an AA-containing lime lemonade (50mg AA/day) with 2 main meals each day for 2 weeks [42]. The daily consumption of the lime lemonade doubled the AA intake, which was reflected by an increase in the molar ratio of AA to iron from an average of 1.2 to 2.3.

3.2 Effect of ascorbic acid on various iron fortificants

The bioavailability of iron fortificants has been investigated in the absence and presence of AA. When AA is added together with iron it preserves the solubility of the iron with increasing pH in the duodenum, and protects it from inhibitory factors. The results of studies investigating the effect of AA on iron fortificants in various food matrices are given in Table I.

Since infants and children are highly susceptible to iron deficiency, the majority of studies have focused on optimizing the bioavailability of fortified infant formulas or cereals. It has been demonstrated that differences in iron absorption from various cereals (rice, maize, wheat, oats, millet, quinoa) is related mostly to the total phytate content, which may differ for each type of cereal according to variations in processing methods [43]. A relatively poor absorption from infant cereals (0.8–3.2%) fortified with

Table I: Effect of ascorbic acid on various iron fortificants

Food matrix (fortificant/added iron)	Population	Design & No of Subjects	Baseline (no AA) Yes/No	AA:Fe (molar ratio)	Change in Absorption	Reference
Maize meal porridge (ferrous sulfate)	Adults	Cross-over n = 6 n = 6 n = 6 n = 6 n = 6	Yes	 0.8 1.6 3.2 6.4 12.7	 n.s. 3-fold 2.7-fold 4.6-fold 6.1-fold	[110]
Rice and dhal sauce (ferrous sulfate)	Adults	Cross-over n = 10 n = 11	Yes	 1.6 3.3	 n.s. 3.7-fold	[111]
Rice with potato and onion soup (ferrous sulfate)	Adults	Cross-over n = 11	Yes	 5.1	 2.9-fold	
Maize meal porridge (ferrous sulfate)	Adults	Cross-over n = 10	Yes	2.4	1.8-fold	[112]
Semi-synthetic meal (ferric chloride)	Adults	Cross-over n = 12 n = 13	Yes	 1.5 3.5 7.5 19 38 77	 1.6-fold 2.5-fold 4.1-fold 4.4-fold 6.2-fold 9.6-fold	[16]
Western type Breakfast with coffee (ferrous sulfate)	Adults	Parallel 12	Yes (n = 21)	7.2	2.5-fold	[113]
Infant milk formula (ferrous sulfate)	Adults	Cross-over n = 12 Cross-over n = 10	Yes No	 2.0 2.0 vs. 6.3	 2.7-fold 2.5-fold	[114]
Maize meal porridge (ferrous sulfate)	Adults	Cross-over n = 12 Cross-over n = 10	Yes No	1.6 1.6 vs. 3.2	5.0-fold 1.4-fold	
Infant cereal (A) (ferric ammonium citrate	Adults	Cross-over n = 10	Yes	3.2 1.5	12.9-fold	
Infant cereal (B) (ferrous sulfate)	Adults	Cross-over n = 12 n = 12	Yes	 1.1 2.4	 3.7-fold 6.2-fold	
Infant cereal (C) (iron pyrophosphate)	Adults	Cross-over n = 12 Cross-over n = 9	Yes No	1.6 0.9 vs. 3.2	4.0-fold 2.7-fold	
Maize meal porridge (FE(III)EDTA)	Adults	Cross-over n = 12 n = 11 n = 9	Yes	 1.5 3.0 6.0	 n.s. n.s. 2.0-fold	[45]
Semi-synthetic meals with isolated soy protein (ferric chloride)	Adults	Cross-over n = 11	Yes	8.0	5.7-fold .	[115]

Table I (continued): Effect of ascorbic acid on various iron fortificants

Food matrix (fortificant/added iron)	Population	Design & No of Subjects	Baseline (no AA) Yes/No	AA:Fe (molar ratio)	Change in Absorption	Reference
Sorghum porridge (ferrous sulfate)	Adults	Parallel 13 vs. 18	No	1.0 vs. 53	3.9-fold	[31]
Soy formula (ferrous sulfate)	Adults	Cross-over n = 11 n = 9 n = 12	Yes	 2.1 4.2 4.2 vs. 8.5	 n.s. 7.5-fold n.s.	[111]
Milk formula (ferrous sulfate)	Infants	Cross-over n = 17	Yes	2.1	2.7-fold	[90]
Soy bean-based infant formula (ferrous sulfate)	Adults	Cross-over n = 12 n = 11	 Yes No	 2.0 2.5 v.s. 6.3	 n.s. n.s.	[38]
Milk-based infant formula (ferrous sulfate)	Adults	Cross-over n = 12 n = 10	 Yes No	 2.0 2.0 vs. 6.3	 5.0-fold 2.2-fold	
Farina Meal • Ferrous sulfate • Electrolytic Fe • Ferric orthophosphate	Adults	Cross-over n = 12	Yes	 5.3 5.3 5.3	 3.9-fold 2.4-fold 4.0-fold	[48]
Wheat rolls (ferrous sulfate)	Adults	Cross-over n = 7 n = 8 to 10 n = 8 to 10	Yes	 3.9 3.9; 7.8 3.9; 7.8	 1.7-fold 2.2-fold; 3.6-fold 2.8-fold; 3.4-fold	[34]
With bread with added phytate (ferrous sulfate)	Adults	Parallel n = 11 n = 12 n = 14	Yes (n = 11)	 1.6 2.7 8.0	 5.2-fold 5.5-fold 5.9-fold	[33]
Chocolate Drink • Unfortified • Ferrous fumarate	Adults	Cross-over n = 11	Yes	 16.8 (0.5 mg Fe) 1.7	 n.s. n.s.	[50]
Soy-based infant formula (ferrous sulfate)	Infants	Cross-over n = 10	No	2.0 vs. 4.2	1.7-fold	[116]
Weaning foods (ferrous sulfate) • Weetabix • Wholemeal bread • Baked beans • Vegetables	Infants	Cross-over n = 9 n = 10 n = 10 n = 9	Yes No	 7.2 7.6 6.6 8.2 vs. 14.7	 3.9-fold 2.4-fold 4.0-fold n.s.	[117]
Milk (ferrous bis-glycine chelate)	Adults	Parallel n = 14	Yes	2.1	1.4-fold	[46]
Chocolate-Milk drink (ferrous sulfate)	Children (age 6–7)	Cross-over n = 10 n = 10	 Yes No	 1.3 1.3 vs. 2.7	 3.2-fold 1.4-fold	[118]
Infant cereal (ferrous fumarate)	Infants	Parallel n = 10	Yes (n = 10)	2.7; 5.6	n.s.	[51]
Peruvian School breakfast meal (ferrous sulfate)	Children (age 6–7)	Cross-over n = 9	No	0.6 vs. 1.6	1.6-fold	[119]

Table I (continued): Effect of ascorbic acid on various iron fortificants

Food matrix (fortificant/added iron)	Population	Design & No of Subjects	Baseline (no AA) Yes/No	AA:Fe (molar ratio)	Change in Absorption	Reference
Infant formula (peaprotein isolate)	Adults	Cross-over n = 10	No	2.1 vs. 4.2	1.5-fold	[120]
Breakfast cereal (reduced iron)	Adults	Cross-over n = 20	Yes	1.3 2.1	n.s. n.s.	[49]

various iron fortificants (ferric ammonium citrate, ferrous sulfate, iron pyrophosphate) can be significantly increased by adding AA, the mean increase being 3-fold with an AA to iron ratio of 1.5:1 and more than 6-fold with a ratio of 3:1. For soy-based infant formulas, a significant increase in iron absorption from 1.8% to 6.9% was achieved at a molar ratio of approximately 4:1 [44].

Although the AA effect has been tested in combination with various iron fortificants, the majority of studies employed ferrous sulfate. With the exception of Fe(III)EDTA [45], ferrous bis-glycine chelate [46], and ferrous fumarate, AA seems to increase the absorption of most iron fortificants to a similar extent [47]. A marked increase in absorption (2- to 12-fold) from meals with low to medium levels of inhibitors is often observed with a molar ratio of AA to Fe above 2:1, while meals with high levels of inhibitors, such as soy-based infant formula, may require molar ratios above 4:1 [47].

The effect of AA on less soluble fortificants seems to be less pronounced. For example, the increase in absorption of electrolytic iron from a farina meal was 2.4-fold at a molar ratio of AA to Fe of 5.3 compared to a 3.9-fold increase in absorption of ferrous sulfate from an identical meal [48]. A molar ratio of 1.3 (AA:Fe) did not result in any significant change in absorption of reduced iron from a breakfast cereal meal [49]. There was a marginal non-significant increase in absorption of ferrous fumarate from a chocolate drink and a semi-synthetic meal at ratios of AA to Fe of 1.7 and 4.4, respectively [50]. The 1.2-fold change in absorption of ferrous fumarate from an infant cereal (relatively low in phytic acid) following an increase in the molar ratio from 2.7 to 5.6 (AA:Fe) was also non-significant [51]. In this context, it should be noted that ferrous fumarate and elemental iron (mainly reduced and electrolytic iron) are being increasingly considered for addition to some infant cereals, drinking powders, complementary foods, maize, and wheat flour [47, 52]. Because of this, more research is needed to determine the effectiveness of AA as an enhancer of iron absorption from these compounds. Additional information is also needed on the AA to Fe molar ratios required to optimize iron absorption from Fe(III)EDTA and ferrous bis-glycine chelate. If AA is to be used effectively to enhance iron bioavailability, future studies should focus on suitable shelf-stable food vehicles, such as pre-cooked infant and complementary foods, as well as beverages made for reconstitution and spreads.

3.3 Interaction between ascorbic acid and other enhancers of iron absorption

There is some evidence to suggest that the enhancing effect of AA in addition to meat or fish is complementary rather than additive [16, 32, 53]. For example, the addition of 100 mg of AA to a semi-synthetic meat-free meal increased iron absorption by a factor of 4. The same amount of AA added to a meat-containing meal of similar nutrient composition increased absorption by a factor of 1.6 [16]. When fish was added to a meal of maize and papaya, containing approximately 70 mg AA, no further increase in absorption was observed [53]. The increase in iron absorption following the addition of 50 mg to a hamburger meal was about half of the increase observed with the addition of 50 mg AA to a meat-free Latin American meal [32].

3.4 Limitations of food fortification with ascorbic acid

The effectiveness of AA depends on the amount added, the stability of AA, the type and concentration of iron fortificant, and the presence of inhibitors in the diet. Ascorbic acid is not easily formulated into many finished food products because of its sensitivity to heat, water, and oxygen. The naturally high AA content of some fruits and vegetables readily degrades when exposed to air during storage [54], some post-harvest treatments [55], and cooking in the household [56]. In the dry state, ascorbic acid is reasonably stable in air, but in solution it rapidly oxidizes. On exposure to light, moisture, and heat it may also darken. Cooking typically destroys ascorbic acid by accelerating the oxidation reaction. Packaging and encapsulation can mitigate some of these losses but these interventions will also add costs.

The instability of ascorbic acid during storage, heat processing, and cooking; the possibility of unwanted senso-

ry changes in liquid products; and the cost of ascorbic acid itself or the cost of effective packaging are the major reasons for its not being used to enhance fortificant iron added to food staples such as wheat flour, maize flour, and milk, or to condiments such as salt, fish sauce, or soy sauce.

Pro-oxidant effects may also cause problems in foods containing fat [10], especially in the presence of both iron (or other catalytic metal ions) and AA. The shelf life of such foods will dictate the choice of fortificant. For example, the problem may be addressed by proper packaging to prevent oxidation or a more inert fortificant may be required. Encapsulation is another option but current encapsulation techniques are unlikely to withstand the baking process.

The interest in the field of encapsulation is huge, as is demonstrated by over 400 records on Foodline (FROSTI database) for patents referring to "encapsulation or microencapsulation". Encapsulation techniques are currently being developed to enable fortification of food products with AA [57] and/or iron [58], and to provide new forms of supplements that may be consumed with foods without causing any adverse organoleptic effects. While the bioavailability of such new fortification compounds will need careful assessment in human clinical trials, limited results with encapsulated ferrous sulfate in milk [58] compared well with findings previously obtained with non-encapsulated ferrous sulfate [59]. Similar promising results have been reported with microencapsulated ferrous fumarate given together with ascorbic acid [60]. The role of encapsulation is considered in detail in this Supplement. Fortification with both ascorbic acid and iron increases costs and careful consideration should therefore be given to the optimum combination of AA and iron for various foods. The costs of AA fortification may be too high in food vehicles where AA stability is a concern because of the large amounts that would need to be added to achieve nutritionally significant increases in iron absorption (see Table 1). No promising staple food vehicle for untargeted dual fortification has been identified because of AA's instabilities during storage and food preparation. The addition of AA may therefore be limited to use in carefully targeted shelf-stable and/or pre-cooked food products, such as infant and complementary foods, spreads and beverages made for reconstitution. An alternative strategy could be aimed at iron and AA fortification of different food vehicles that are likely to be consumed together.

4. Ascorbic Acid Safety

An upper safe limit of <1g AA per day has been proposed [61]. There exists a considerable body of literature discussing the potential adverse effects of large iron stores in the development of chronic diseases, which may at least in part be due to the absorption-enhancing effect of AA and other organic acids. Such concerns are commonly restricted to population groups with initial high iron stores and hemochromatosis patients [8, 62]. A previously published review of the literature on the effect of high ascorbic acid dosage on iron absorption in apparently healthy individuals concluded that there was no evidence to suggest that ascorbic acid increases the incidence of high iron absorption in iron-replete individuals [63]. Whether specific meal patterns and prolonged high AA intake may affect iron stores in persons who are heterozygous for the iron-loading *HFE* mutation remains to be investigated. *In vivo* pro-oxidant activities in otherwise healthy subjects ingesting AA supplements is not proven and its biological relevance highly controversial [64–67].

To comply with the upper safe limit, fortification strategies need to take into consideration the overall dietary AA intake of the target populations. Since a high percentage of the population in developing countries has an AA intake well below the recommended daily allowance (RDA) [68–70], AA fortification at a level that is compatible with various food vehicles is very unlikely to reach an upper safe limit of intake.

5. The Effect of Ascorbic Acid on Iron Status

Increased ascorbic acid intake through supplementation, fortification, or habitual consumption of ascorbic-rich foods represents an important strategy for improving the iron status of individuals or populations. Evidence for the efficacy of these different strategies is presented below, with the method of choice depending on the nutritional aim and the target group.

5.1 Dietary intake of ascorbic acid

The effect of high dietary AA intake on iron status is often difficult to assess because of the interaction with other dietary enhancers (e.g., meat) and inhibitors (e.g., polyphenols and phytate), the length and timing of dietary assessment, and the inclusion of other confounding factors such as age and the presence of disease. Consequently, associations between AA intake and iron status in cross-sectional studies are inconsistent [71, 72]. Meal-based assessments of the habitual dietary intake of iron, as well as the key enhancers and inhibitors in the diet, are rarely undertaken but are likely to provide a better estimate of dietary effects on iron status [73].

5.1.1 Western countries

Relatively few studies have examined the importance of dietary factors and patterns of intake in explaining iron status at a population level. However, two recently published surveys of people aged 65 and over, the Framingham Heart Study [74] (n = 643), and the UK National Diet and Nutrition Survey [75] reported a positive association between iron status and dietary AA or fruit intake in this age group, thus supporting a role for dietary AA in the long-term maintenance of iron status. Interestingly, there was no positive association between the serum ferritin and total AA intake following the inclusion of supplemental AA in the data analysis, which suggested that AA supplements were likely to be consumed outside the main meal times and/or that supplement users were iron replete. Another recently published UK cross-sectional study of 18-month-old children (n = 796) also found evidence of a positive association of hemoglobin levels with heme iron and AA intakes and fruit and vegetable consumption [76]. The amount of AA-fortified baby food consumed was considered too small to constitute a major source of AA in this study. The mean AA intake was 40.6 mg/day and 37.0 mg/day for boys and girls, respectively. Finally, in an Australian case control study of 12- to 36-month-old children (n = 124), only heme iron intake was found to be positively associated with iron stores. It should however be noted that the AA intakes of both the iron-replete and iron-depleted study groups were 3 times the recommended daily intake (i.e. 30 mg/day) for this age group [77].

5.1.2 Developing countries

To our knowledge, only one prospective cohort-study, involving 125 non-pregnant Mexican women, has been published to date [69]. Total AA intake was found to be positively correlated with iron status as assessed by serum ferritin and hemoglobin levels. This finding was still significant for hemoglobin after the exclusion from the analysis of the main source (pulque) of AA, which also contained ethanol. The total AA intake ranged from 22.4 to 48.8 mg/day and the average iron intake was 16.2 mg/day.

5.2 Increased intake of foods rich in ascorbic acid

An AA intake well below the RDA is a common phenomenon in developing countries [68–70]. An increase in AA intake, either by increasing the total intake of AA or by changing meal patterns, may therefore represent a sustainable way of improving the iron status in such populations. The feasibility of this approach will depend on sustained behavioral changes and access to ascorbic acid-rich foods. An intervention trial carried out in India found a significant improvement in hemoglobin levels in young working women who received ascorbic acid-rich gooseberry juice with their lunch at the workplace for 6 months [78]. The gooseberry juice provided an extra 20 mg AA per day, which increased the total AA intake to 49 mg/day; the average iron intake was 31 mg/day. In contrast, an increase in AA intake from about 56 mg/day to 113 mg/day over a period of 8 months by providing a lime-flavored AA-rich beverage did not improve the iron status of iron-deficient women in rural Mexico [79]. These women habitually consumed diets containing, on average, 11 mg non-heme iron and approximately 2900 mg phytate. The lime-flavored AA-rich beverage was consumed within 30 minutes of two main daily meals. The experimental and control groups consisted of 18 women each and 11% percent of subjects in each group were anemic at the start of the trial. The paucity of local food sources rich in AA and the lack of efficacy in this trial led to the conclusion that a food-based intervention would not be likely to improve iron status under these specific conditions.

In summary, the paucity of data on the efficacy of increasing dietary ascorbic acid intake to improve iron status underlines the need for better, controlled field trials. Careful consideration should be given to the range of iron status of the subjects investigated, the acceptability of the chosen food vehicle, and the sustainability of access to AA-rich food sources.

5.3 Ascorbic acid supplementation

A small number of studies have been published on the efficacy of AA supplementation for improving iron status in iron-depleted subjects [40, 80–82]. Two AA supplementation studies in pre-school children consuming plant-based diets demonstrated significant improvement in iron status [80, 82]. Mao *et al* [82] found that supplementation with 50 mg AA per day (total intake 80mg/day) was the most suitable dosage for improving iron status over an 8-week intervention period in children habitually consuming 7.5mg Fe/day. The AA-containing beverages were freshly prepared and consumed after lunch and supper.

Young women with low iron stores (serum ferritin 3.5–17.7 μg/L), consuming a Western type diet, did not show any statistically significant improvement in storage iron in response to AA supplementation of 1500 mg/day for 5 weeks [40]. In another study, an improvement in hemoglobin but not in serum ferritin was found in response to 5.5-weeks of ascorbic acid supplementation (1500 mg/day) in women consuming a Western type diet, whose body iron stores had been depleted by means of phlebotomy (serum ferritin < 8.5 μg/L) [81].

Negative results were obtained in two final studies. There was no change in serum ferritin in response to 8 weeks supplementation with 100 mg AA at mealtimes

(300 mg AA/day) in Irish student nurses and there was no evidence that subjects with low iron stores responded differently than subjects with high iron stores [83]. Furthermore, supplementation with 1 g of AA at meal times for 20 months did not raise the average serum ferritin level in 5 iron-replete and apparently healthy subjects [84]. Four iron-depleted subjects showed a less homogenous response, which was difficult to interpret. It was concluded that AA supplementation has no effect on iron status when the diet contains substantial amounts of meat. These findings also reflect the well-documented regulation of iron absorption, which prevents excess iron uptake in iron-replete individuals.

In summary, the results obtained thus far are difficult to interpret for a number of reasons. These include differences among studies in sample size, length of intervention, baseline iron status, AA-to-iron molar ratio, timing of supplementation, age of subjects, type of diet, presence/absence of other micronutrient deficiencies, and choice of measures of iron status endpoints. More research is required therefore to assess the effectiveness of AA supplementation as a means of improving iron status of iron-depleted subjects.

5.4 Fortification with ascorbic acid and iron

The steady drop in the prevalence of anemia among infants in both the USA and Scandinavia has been attributed in part to the widespread consumption of milk-based and soy-based infant formulas fortified with iron, as ferrous sulfate, and ascorbic acid [85, 86]. In addition, several field studies performed in Chile have demonstrated a higher efficacy and effectiveness of cow's milk fortified with iron and ascorbic acid than of cow's milk fortified only with iron in the prevention of iron deficiency in infants [87–89]. Following the observation that the enrichment of milk with ascorbic acid markedly improved iron bioavailability [90], powdered full-fat milk fortified with 15 mg of iron as ferrous sulfate, and 100 mg of ascorbic acid per 100 g of powder was developed. In an efficacy study performed in 1976, infants who were spontaneously weaned before 3 months of age were given the fortified milk or non-fortified milk for 12 months. At 15 months of age, the prevalence of anemia was 2.5% and 25.7% in the fortified and control groups, respectively [88]. Two years later, a study was started in a large group of infants to demonstrate the effectiveness of the fortified milk under the normal distribution conditions of the National Supplementary Fortification Program (NSFP). Fortified or non-fortified milk was introduced after spontaneous weaning took place. At 15 months of age, anemia was present in 5.5% of the infants in the fortified group as compared to 29.9% in the control group [89]. In 1998, the powdered cow's milk that is delivered free of cost by the NSFP to approximately 70% of Chilean infants was fortified with ascorbic acid and iron, as ferrous sulfate, in a molar ratio of 2:1 (70 mg of ascorbic acid and 10 mg of Fe/100 g). Preliminary results of the effectiveness of this intervention have demonstrated a threefold reduction of the prevalence of anemia [91].

5.5 Commercial Considerations in Ascorbic Acid Fortification

The prices of vitamins and fine chemicals in general are rather volatile. Production and demand affect the prices considerably. At present, ascorbic acid prices are ranging from US$ 7 to 12/Kg on a CIF basis. For the purpose of this discussion, we will take a value of US$ 8/Kg. Table II shows an exercise on the possible costs of fortifying a food with iron and ascorbic acid, at different molar ratios, using a fixed amount of added iron at (40mg/Kg ferrous sulfate). The cost of the food is set at US$500/Ton for impact assessment purposes. If we assume a daily consumption of this food of 200 g, then the cost/person/year for this ascorbic acid-enhanced iron fortification would range from US$ 0.03 to 0.25 for the different molar ratios described above.

Table II: Possible costs of fortifying a food with iron @40 mg/kg (as ferrous sulfate) and vitamin C, at different molar ratios

AA:Fe Molar Ratio	mg Vit.C/Kg	Iron cost/Ton	AA cost/Ton	Impact on cost @$500/ton
1:1[1]	41.46	$ 0.12	$ 0.33	0.09%
2:1	82.92	$ 0.12	$ 0.66	0.16%
3:1	124.38	$ 0.12	$ 1.00	0.22%
4:1[2]	165.84	$ 0.12	$ 1.33	0.29%
5:1	207.30	$ 0.12	$ 1.66	0.36%
6:1	248.77	$ 0.12	$ 1.99	0.42%
7:1	290.23	$ 0.12	$ 2.32	0.49%
8:1	331.69	$ 0.12	$ 2.65	0.55%
9:1	373.15	$ 0.12	$ 2.99	0.62%
10:1	414.61	$ 0.12	$ 3.32	0.69%

[1] Minimum effect
[2] Minimum required on high phytate/polyphenol meals

6. Role of other Organic Acids in Iron Nutrition

In addition to ascorbic acid, other organic acids, such as citric, lactic, malic, and tartaric acids occur naturally in foods, especially fruit and vegetables. They can be used as food additives and/or preservatives and are classified as GRAS. Table III lists some of their applications in foods,

Table III: Applications of some organic acids used as food additives (adapted from [32])

Acid	Food Application
Acetic acid	• Preservative and seasoning agent used in ketchup, mayonnaise, acid-pickled vegetables, bread, and other baked products
Citric acid	• Candy production, fruit juice, ice-cream, marmalade and jelly manufacturing, vegetable canning • Aroma improvement of dairy products such as processed cheese and buttermilk • Prevention of enzymatic browning in fruits and vegetables and as a synergistic compound for antioxidants
Fumaric acid	• Shelf-life extension of some dehydrated food products (e.g., pudding and jelly powders) • Lowering of pH • Additive promoting gel setting
Lactic acid	• Flavor improvement of beverages, and vinegar-pickled vegetables • Prevention of discoloration of fruits and vegetables • Improving egg white whippability (pH adjustment 4.8–5.1)
Malic acid	• Manufacturing of marmalades, jellies, and beverages and canning of fruits and vegetables (e.g., tomato) • Anti-spattering agents in cooking and frying fats and oils (monoesters of malic acid with fatty alcohols)
Propionic acid	• Additive in baked products for inhibition of molds
Succinic acid	• Emulsifier in the baking industry
Tartaric acid	• Acidification of wine, fruit juice drinks, sour candies, ice-cream • As a synergistic compound for antioxidants due to formation of metal complexes

including the modification of flavor, the lowering of pH, and the simplification of processing operations [92]. Of special interest is their ability to chelate iron, which serves two purposes. It prevents lipid peroxidation in foods and increases the solubility of both ferrous and ferric iron, presumably through binding with carboxyl and hydroxyl groups [92–94]. Thus far, the potential of organic acids to improve iron bioavailability has received much less attention compared to AA. Although organic acids have the advantage of being more stable and less reactive than AA in certain food applications, their function as food additives is limited by the amounts required and their organoleptic effects. Naturally occurring levels of organic acids in fruits are 400 mg tartaric acid/100 g grapes (wine), 1100 mg malic acid/100 g plums or peaches, and 2900mg citric acid/100g black currants [95]. In vegetables, the organic acid content is usually lower and ranges from 100 to 800 mg/100 g [96]. Since only a few human studies have been carried out to determine the effect of organic acids on iron absorption, the following review also includes findings from *in vitro* studies.

6.1 *In vitro* studies

The chelating properties of organic acids have mostly been studied in the presence of known inhibitors of iron absorption such as phytates and soy protein [94,97,98]. In general, these studies have found that the ability of various organic acids to enhance iron solubility depends on pH, iron source, ligand, processing methods, and the food matrix. A comparison of chelating properties found that complexes of organic acids with Fe^{+3} were more stable than those with Fe^{+2}, with the exception of AA [94]. There is also some indication that these acids maintain the Fe^{3+} in a soluble form as the pH increases to values commonly encountered in the small intestine. Synergistic effects were found in the combination of ascorbic acid with some organic acids (fumaric, lactic, and succinic acids) but not with others (citric and malic acids) at molar ratios of 1:2, 1:1, and 2:1 [94]. A further comparison between the findings of various studies is not possible because of the wide range of ratios of iron to organic acid used (1:0.6 to 1:10) and differences in the experimental set-ups.

To model luminal events some studies have employed *in vitro* digestion aimed at simulating physiological events that would improve the estimates of relative iron bioavailability. Iron solubility following simulated *in vitro* digestion of various seafoods with added iron ($FeSO_4$ and $FeCl_3$) and organic acids (ascorbate and citrate) was highest at pH 2 and lowest at pH 6 with all ligand combinations tested [99]. The molar ratio of iron to organic acid tested was 1:1.9. The results suggest that iron solubilization primarily depended upon the type of seafood (protein) and to a lesser extent upon the oxidation state of the iron salt.

The addition of phytase to whole-wheat flour reduces the level of phytate during bread preparation and thus increases the percentage of dialyzable iron. The phytase action can be maximized by adding citric acid (6.25 g/kg whole wheat flour) to the whole wheat flour. The improved effectiveness is thought to be due to the lowering of the pH by adding citric acid and to the complex formation with minerals that render the phytate molecule more susceptible to the phytase action. Compared with the untreated bread, citric acid alone and the combination of citric acid and phytase enhanced total iron dialyzability by 12- and 15-fold, respectively [100].

The two-tier Caco-2 cell line model has been offered as a potential screening tool for predicting iron uptake by

humans. The cells resemble mature enterocytes [101] and results generated so far are consistent with human absorption studies [102]. The findings of a recently published study on the dose-dependent effect of nine organic acids on iron uptake into Caco-2 cells (no inhibitors) provide some insights into the complex mechanisms involved [93]. Uptake was measured from samples containing a range of molar ratios of iron to organic acid of between 1:1 and 1:400. Of interest was the identification of a group-specific effect when organic acids were classified according to chemical structure, which suggested that differences in dose-response are most likely related to the number of hydroxyl and carboxyl groups of these acids. With few exceptions a maximum increase in Fe^{3+} uptake was achieved at molar ratios of organic acid to iron of above 250:1.

Another Caco-2 study has been published describing the effect of various commercially available fruit juices (apple, pear, white grape, red grape, prune, grapefruit, orange) with added ascorbic acid on Fe^{3+} uptake [103]. The authors reported data for gallic acid (GA), ascorbic acid (AA), iron (Fe), and corresponding molar ratios of AA:Fe, GA:Fe, and GA:AA for each of the juices tested. Iron uptake was significantly increased and most efficient in the presence of juices with the highest AA-to-Fe molar ratio and the lowest GA-to-AA molar ratio.

6.2 Human studies

Evidence supporting a role for organic acids in iron absorption can be obtained from a series of human studies carried out in South Africa in the 1980s. In the first study, a 4-fold increase in iron absorption from a $FeCl_3$ solution was observed (12.3% versus 3.1%) when added lactic acid (2 mL of acid per liter, pH 2.5) was compared with added hydrochloric acid (amount needed to adjust to the same pH). The iron-enhancing effect of lactic acid was also demonstrated following the consumption of a maize gruel meal, which contains inhibitors of iron absorption. As expected, iron absorption from the experimental meal was low but increased significantly from a mean of 0.4 % to 1.2% when lactic acid was added instead of hydrochloric acid [104]. In the second study, addition of either 1 g of citric, malic, or tartaric acid was shown to increase iron absorption 2- to 3-fold from a rice meal containing 3 mg ferrous sulfate plus intrinsic iron. Further experiments were carried out to study the effect of vegetables on iron absorption. The meals consisted of 100 g cooked vegetables with added iron ($FeSO_4$). According to food table values, vegetables associated with good iron bioavailability contained appreciable amounts of organic acids [35]. For example, the most pronounced increase in iron absorption was observed with sauerkraut (rich in lactic acid), as well as with cabbage and turnip (rich in ascorbic acid). In another study, sauerkraut was also found to promote iron absorption from a meat-containing meal [105]. In contrast, addition of 1 g oxalic acid to a cabbage meal resulted in a significant reduction of iron absorption [35]. Oxalic acid is also known to reduce bioavailability of other minerals, such as calcium [106].

A third study investigated the effects of citrus fruit juices and fruits on iron absorption from a rice meal. The total iron content of the meal was approximately 3.4 mg. Iron absorption from fruit juices containing 30 mg ascorbic acid and either 0.75 g or 4 g citric acid was higher compared to fruit juices with no added citric acid. Further experiments demonstrated that a positive association between iron absorption and various fruits was related to the ascorbic acid content and to a lesser degree to the citric acid content of individual fruits [107].

In a fourth study, addition of 1, 2, and 4 g of citric acid to 10 g of isolated soybean protein was shown to increase iron absorption significantly in a dose-related manner [38]. A 2-fold increase in absorption was found following the addition of 1 g citric acid, and a further 1.4-fold increase in absorption was found by increasing the citric acid content from 2 to 4 g. However, in one other study citric acid was found to inhibit iron absorption. Addition of 1 g citric acid to a Latin American-type meal (maize chapatis, black beans and rice) significantly decreased iron absorption [108]. The reason for these discrepant findings is unclear, since they are at variance with *in vitro* dialyzability and Caco-2 studies and with the other absorption results in humans.

Soy sauce, which is naturally rich in organic acids and simple peptides, has been shown to increase iron absorption from a rice meal (low in inhibitors) while absorption was unchanged when soy sauce was ingested with a soy flour meal (high in inhibitors) [109]. The soy sauce contained 37.5 mg citric acid and 47.5 mg lactic acid per 25 mL portion consumed with the meal. The molar ratio of organic acids (lactic plus citric acid) to iron was approximately 10:1. However, there was no change in iron absorption when the rice meal was consumed with 340 mg of lactic acid per meal instead of soy sauce, which is equivalent to about 4 times the concentration of citric and lactic acid contained in a portion of soy sauce fed with the meal. It was concluded that the promoting effect of soy sauce might be due to the presence of fermentation products other than organic acids. As already discussed, there is evidence to suggest that the molar ratio of organic acid to iron, as well as the source of the iron and the type of organic acid, affect iron absorption [93]. This may explain the negative finding following the addition of pure lactic acid at a 57:1 ratio.

7. Conclusion

Ascorbic acid, with its reducing and chelating properties, is the most efficient enhancer of iron absorption when its stability in the food vehicle is ensured. Although AA increases absorption of soluble iron fortificants to a similar extent, more research is needed to determine the effectiveness of AA as an enhancer of less soluble iron fortificants. Suitable vehicles for AA fortification include dry-blended foods such as complementary, pre-cooked cereal-based infant foods, powdered milk, and other dry beverage products made for reconstitution that are packaged, stored, and prepared in a way that maximizes retention of this vitamin. The challenge remains to develop technologies that preserve AA and prevent unwanted chemical reactions with iron in food applications (e.g., encapsulation). The hypothesis that an overall increase of dietary ascorbic acid intake, or fortification of some foods commonly consumed with the main meal with AA alone, may be as effective as the fortification of the same food vehicle with AA and iron, merits further investigation. The addition of other organic acids to foods at concentrations required for iron-enhancing properties is associated with unacceptable organoleptic changes in most foods. Promotion of food vehicles that already contain significant amounts of organic acids, such as fermented foods (e.g., sauerkraut), strongly flavored condiments, and beverages, should be further explored, especially if they are widely consumed in the target population.

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