Improving Iron Status Through Diet

The Application of Knowledge Concerning Dietary Iron Bioavailability in Human Populations

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INTRODUCTION

More than 500 million people have iron deficiency anemia (ACC/SCN 1992, Craig 1994), and a much larger number have iron deficiency without anemia. Iron deficiency is a result of the amount of dietary iron absorbed being insufficient to meet iron requirements. This situation is more common when iron requirements increase during pregnancy and growth, when iron is lost in menses or through some parasitic infections, and when food constituents impair iron absorption.

Iron is an essential micronutrient. As an integral part of hemoglobin, it is required for the transport of oxygen and carbon dioxide in blood. Iron is also a component of several tissue enzymes, such as cytochromes that are critical for energy production, and enzymes involved in the immune system. The symptoms of iron deficiency include low concentrations of serum ferritin which reflect iron stores, progressing to anemia (defined as a low hemoglobin concentration). Iron deficiency per se, before the onset of anemia, may have adverse effects on functions such as work performance (Davies et al. 1982; Baynes and Bothwell 1990). Once anemia results, there are also impairments in cognitive performance and behavior (Idjradinata and Pollitt 1993), low birth weight due to prematurity (Scholl and Hediger 1994), and other pregnancy complications (Viteri 1994). For each 10 percent deficit in hemoglobin concentration, there is a 10 to 20 percent deficit in work performance (Edgerton et al. 1979). Although anemia can result from deficiencies of other nutrients such as vitamins B_{12}, B_{6}, B_{2}, A, and E, folic acid, copper, and protein, iron deficiency is the predominant cause.

There is a consensus that in developing countries, poor dietary quality rather than quantity is the key determinant of impaired micronutrient status, including iron deficiency (Baker and deMaeyer 1979; Allen 1991; Allen et al. 1992; Allen 1993; World Bank 1994). Populations with limited resources avoid hunger by consuming more cereals and tubers, while restricting their intake of more expensive animal products, fruits, and vegetables (Allen 1991). The total iron intake of populations that are dependent on predominantly plant-based diets may meet dietary recommendations and even exceed that of populations consuming more animal products (FAO/WHO 1988; Baynes and Bothwell 1990; Murphy et al. 1992). However, due to mechanisms discussed below, the bioavailability of iron bound to plant constituents is usually poor, so that a high prevalence of iron deficiency and anemia often co-exist with "adequate" total iron intakes. Thus, in theory there is an opportunity to reduce the prevalence of iron deficiency by improving the bioavailability of iron present in plant-based diets.

The key to the long-term prevention of iron deficiency and resulting anemia is to increase the amount of dietary iron absorbed. The most commonly used strategies to combat iron deficiency are the provision of iron supplements or the fortification of foods with iron. In some situations, treatment of parasites improves iron status by reducing intestinal blood loss and/or increasing iron absorption. Surprisingly little attention has been paid to improving the bioavailability of dietary iron by promoting culturally acceptable changes in food choices. This may be the only sustainable long-term approach at the community level, especially where dietary fortification with iron is impractical or the absorption of fortificant iron is affected very adversely by dietary constituents.
The purpose of this paper is to review current knowledge about the most important factors affecting dietary iron bioavailability. While there have been many reviews of this general topic, the focus and purpose of this review is to integrate the available information on iron bioavailability in a way that is useful for designing the most effective and practical strategies to improve the absorption of iron from specific staple foods and diets. The final decision on which interventions to choose must also depend on other factors such as economic feasibility and cost-benefit, food distribution and marketing, and cultural issues, which are outside of the scope of the present review.
MEASURING BIOAVAILABILITY OF DIETARY IRON IN HUMANS

Most of the information about iron bioavailability has been obtained in the past 30 to 40 years. Three major milestones have facilitated research on this topic. The first was the development of the intrinsic tag approach for measuring iron absorption (Moore and Dubach 1951). The second was a series of experiments using this approach to demonstrate that food constituents had a major influence on iron absorption by humans. The third milestone was the realization that iron absorption from foods could be measured by extrinsic tags, i.e., by adding isotopes to a food or meal. This development made it possible to measure iron absorption from single foods, as well as from mixed diets and iron fortificants. A fourth milestone may be the validation of a new method for measuring iron absorption from the whole diet rather than from one or two meals per day (Hultén et al. 1995). Also, there is increasing recognition that differences in iron bioavailability among diets may be obscured when iron stores are adequate and iron absorption is low (Hultén et al. 1995).

In this section the in vivo and in vitro methods that have been used to measure the bioavailability of dietary iron in humans are described briefly. Several good reviews of this topic provide further details (Van Campen 1983; Hallberg 1981b). Different terms have been used to describe and estimate “bioavailability” (Table 1, adapted from Van Campen 1983) and it is important to bear these in mind when reviewing the large number of publications on iron bioavailability.

**Table 1: Estimates of Iron Availability**

<table>
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<th>Term</th>
<th>Measurement</th>
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<tr>
<td>Apparent Absorption</td>
<td>Intake - fecal excretion</td>
</tr>
<tr>
<td>Net Retention</td>
<td>Intake - (excretion in feces + urine)</td>
</tr>
<tr>
<td>True Absorption</td>
<td>Intake - (excretion in feces and urine corrected for endogenous losses)</td>
</tr>
<tr>
<td>Bioavailability</td>
<td>Fraction of consumed iron that can be utilized</td>
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The apparent absorption and net retention of iron give very similar estimates of iron absorption, because the urinary excretion of iron is small. Likewise, the value for true absorption may be quite close to that for apparent absorption and net retention if the latter two measurements are obtained meticulously. However, bioavailability is the key measure, because it reflects how much of the iron consumed is both absorbed and truly utilized by the body.¹ When the utilization of absorbed iron is impaired, for example in deficiencies of vitamin A (Suharno et al. 1993), vitamin B₁₂ (Hillman and Finch 1985) or folic acid (Hillman and Finch

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¹ Iron bioavailability in humans is often assessed from the incorporation of isotopically-labeled dietary iron into the circulating hemoglobin pool, i.e., from the amount of labeled iron that is used for the synthesis of hemoglobin. This depends on the amount of iron absorbed, and utilized.
1985), or as a result of coffee consumption (Muñoz et al. 1986, 1988), true absorption and bioavailability may be quite different. In practice, this problem is usually ignored, and it is assumed that the efficiency of utilization of absorbed iron for hemoglobin synthesis is 80 percent in non-anemic individuals, or 100 percent in those who are iron deficient. In summary, in most instances these terms (when obtained carefully) would give similar estimates, except in circumstances when the utilization of absorbed iron is affected.

**In Vivo Methods**

These may be categorized as non-isotopic or isotopic, depending on whether iron isotopes are used.

**Non-isotopic methods**

**Chemical balance**

This technique measures iron absorption from the whole diet over a period of time. It is based on the difference between the intake and excretion of iron. The test food or diet is fed for about two weeks with a fecal marker to ensure that previous meals have been excreted from the intestinal tract, then intake and excretion are measured for at least six days (Rosado et al. 1992). Absorption is usually expressed as apparent absorption (intake minus fecal excretion), or as net retention if urinary losses of iron are also measured. This method was widely used prior to the introduction of radioisotopes (Widdowson and McCance 1942). However, it is insensitive, imprecise, and time-consuming, and cannot provide information on iron absorption from more than one diet at a time.

**Change in hemoglobin and serum ferritin concentrations**

Body iron stores are reflected in, and can be estimated from, changes in hemoglobin and serum ferritin concentrations (Cook et al. 1986). Thus, changes in serum ferritin concentrations over a period of time when subjects consume an experimental diet containing iron provide an indirect functional estimate of the amount of iron absorbed. In closely controlled efficacy trials, interventions to enhance iron absorption, such as ascorbic acid supplementation, can be reflected in a change in hemoglobin in two months if hemoglobin concentrations are initially low (Seshadri et al. 1985). A considerably longer period of time would be needed to see changes in hemoglobin or ferritin as a result of community based interventions such as sugar fortification (Viteri et al. 1995). Ferritin concentrations are more responsive to changes in

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2. The original formulas (Cook et al. 1986) for estimating iron stores are as follows: i) in individuals with iron deficiency anemia (low hemoglobin plus at least two abnormal iron status measures such as ferritin, transferrin saturation or serum iron, and erythrocyte protoporphyrin), iron stores (mg) = 15 x (mean hemoglobin [where hemoglobin = 140 g/L in women and 150 g/L in men] - observed hemoglobin); ii) for those without anemia but low serum ferritin (<12 μg/L), iron stores (mg) = 80 X index, where a value of 1 was given for each of the following: ferritin <9 μg/L, transferrin saturation <16 percent, erythrocyte protoporphyrin >70 μg/dL RBC, and transferrin saturation <10 percent, erythrocyte protoporphyrin >70 μg/dL RBC, and iii), for individuals with normal hemoglobin and ferritin values, iron stores (mg) = 400 X log serum ferritin - log 12). These formulas have been adapted for different age and sex groups, for body weight (an important adjustment in developing countries) (Viteri et al. 1995) and fewer iron status indicators (Ballot et al. 1989).
Measuring Bioavailability of Dietary Iron in Humans

iron status because an increase in iron stores would be expected in more subjects. Limitations of this approach to estimate iron absorption are that only one intervention can be tested in each subject, and change in hemoglobin or ferritin concentrations must be corrected for initial iron status. Both hemoglobin and serum ferritin values will eventually plateau once stores are repleted and absorption is sufficient to meet iron needs (Viteri et al. 1995). Changes need to be measured prior to this point if the effectiveness of different, simultaneous interventions is to be compared among population groups. The main value of this approach is that it can be used to measure the impact of interventions to improve iron absorption at the community level, whereas isotopic methods are impractical for this purpose. Simultaneous deficiencies of other micronutrients may, however, reduce hemoglobin or ferritin response to iron supplementation (Fairweather-Tait et al. 1992; Suharno et al. 1993).

Radioisotope methods

Radioisotopes of iron (usually $^{55}$Fe and $^{59}$Fe) may be used to intrinsically label individual foods, such as rice, maize, wheat, meat, etc., by biosynthetic incorporation of the radioactive iron into live plants or animals (INACG 1982). More commonly, radioactive iron is simply added to a food or to a complete meal as an extrinsic label. The underlying assumptions of the extrinsic tag method are that: i) dietary iron forms two pools in the gastrointestinal tract, heme and nonheme iron; and ii) if a small amount of a radioisotope of iron is added to the test meal as a soluble salt, it exchanges completely with the unlabeled nonheme pool in the meal. Alternatively, if a small amount of biosynthetically radiolabeled heme is added, it exchanges completely with the heme pool of the meal (Hallberg 1974). Several investigators (Bjorn-Rasmussen et al. 1972; Bjorn-Rasmussen 1973; Sayers et al. 1974) have validated this “common nonheme iron pool” concept. Thus, the most practical approach is to add an extrinsic radiotracer to a test meal, which will uniformly label all nonheme iron compounds in the constituent foods (Bjorn-Rasmussen et al. 1976). The same approach can be used to assess the bioavailability of a labeled fortificant iron, if the iron is exchangeable with dietary iron. However, because the iron in chelates such as NaFeEDTA does not exchange with the iron in food, such fortificants must be labeled intrinsically (e.g., MacPhail et al. 1981). More recently, Hultén et al. (1995) validated a new method of measuring iron absorption from the whole diet. In this method, all nonheme iron in all meals is labeled to the same specificity with an extrinsic radiolabeled iron tracer. This is clearly a better approach than labeling one or two meals per day, if the goal is to estimate daily iron absorption from the whole diet.

After the test food, meal, or iron fortificant has been labeled with an isotope, iron absorption is measured by the incorporation of the isotope into whole blood or hemoglobin (Cook et al. 1969) or by whole body counting (Anand et al. 1977). The former method involves drawing a basal blood sample before the administration of a radiolabeled food or meal, followed by another blood sample 14 days later to determine the amount of radioiron in hemoglobin or whole blood. The approach is based on the fact that most of the absorbed iron is normally incorporated into red blood cells within 10 to 14 days of ingestion. Thus, the percent of radioiron absorbed can be calculated from the radioactivity present in red cells after 14 days, assuming that 80 percent of absorbed iron is incorporated in normal subjects, and 100 percent in iron-deficient subjects (Huebers 1986; INACG 1982). Because both $^{55}$Fe and $^{59}$Fe can be used as labels, and distinguished from each other during counting, two different meals each labeled with a different isotope can be tested in a single subject over a period of 14 to 16 days. An important limitation of this method is the need to use radioactivity in human subjects. While the dose of radiation is very small, and would be
approved by most Human Subjects Committees, the radioactivity may make it more difficult to recruit participants, and radiolabeled foods should not be given to children, pregnant or lactating women, or women at risk of becoming pregnant. Because radioactivity is used, this method is unsuitable for the assessment of community-level interventions.

The whole-body counting technique has been used less often. It measures the retention of dietary radioiron in animals and in humans directly (Van Campen 1983). Subjects are fed radioiron or radiolabeled food(s) following an initial background radioactivity count. The radioactivity in subjects is counted in the whole-body counter 14 days later and the percent retention is calculated as the fraction of radioactivity retained from the original dose administered. Absorption can be calculated by adjusting for radioactive decay of the isotope given the physical half-life of the tracer. This method offers the advantage of not having to estimate blood volume or the efficiency of iron utilization, because the proportion of isotope retained in the body is measured. Theoretically this is an advantage in developing countries, where undernutrition and other micronutrient deficiencies could affect the incorporation of absorbed iron into red blood cells. However, the procedure requires a whole-body counter and is somewhat cumbersome for humans and, therefore, requires highly motivated subjects. Furthermore, most whole-body counters can measure $^{59}$Fe, but not $^{55}$Fe, so that only one test can be conducted at a time in each subject. Again, the limitations imposed by the use of radioactivity in humans apply.

Rather than measure whole-body retention of an isotope directly, some investigators have calculated absorption as the difference between intake and fecal excretion of an isotope. For example, Turnland et al. (1990) used this approach to measure the impact of milk on the absorption of a stable iron isotope from cereals, and the same procedure could be used for a radioactive isotope. As with whole-body counting, no assumptions need to be made about blood volume or the efficiency of utilization of absorbed iron. However, even when a quantitative fecal marker is fed, errors could be introduced by incomplete excretion of unabsorbed isotope and complete fecal collections need to be made for about two weeks after the isotope is consumed.

**Stable isotope methods**

Methods that use stable isotopes of iron do not expose the subjects to ionizing radiation and are, therefore, safe to use in vulnerable groups such as infants, children, and pregnant or lactating women. The principles of extrinsic labeling and the common pool concept remain the underlying basis; the only difference is the type of iron isotopes utilized. Small amounts of iron occur naturally as the stable isotopes $^{54}$Fe, $^{57}$Fe, and $^{59}$Fe in foods, but additional amounts are added to “enrich” the test meal(s) in quantities greater than their natural abundance. The sensitivity with which stable isotopes can be measured has only recently improved to the point where sufficiently small doses can be used in human studies of iron absorption. Previously the low sensitivity of measurement meant that a large dose of iron had to be fed as the stable isotope, which in itself affected the efficiency of iron absorption. The stable isotopes are measured by methods such as thermal ionization mass spectrometry (Turnland et al. 1990; Abrams et al. 1994), inductively coupled plasma mass spectrometry or fast-atom bombardment mass spectrometry (Ehrenkranz et al. 1992; Flory et al. 1993; Fomon et al. 1993; Barrett et al. 1994; Davidsson et al. 1994a, 1994b; Kastenmayer et al. 1994). Because the stable isotopes are costly (approximately $400 per subject
Measuring Bioavailability of Dietary Iron in Humans

for one dose of $^{57}$Fe plus one dose of $^{58}$Fe), their application is limited to studies with a relatively small number of subjects.

In Vitro Methods

Earlier, in vitro methods assessed iron bioavailability by determining the amount of iron dissolved from food by dilute acids or the extractability of "ionizable" iron from food by chelating agents. More recent methods are based on simulated digestion of the food or test meal with pepsin, hydrochloric acid, and sometimes other digestive enzymes, followed by determination of the dialyzable or soluble iron released. Estimates of iron availability using in vitro methods generally rank similarly to those obtained in human trials (Rao and Prabhavathi 1978; Reddy et al. 1986; Chidambaram et al. 1989; Miller and Berner 1989). They may be a useful preliminary screening tool for predicting the bioavailability of iron for humans, although they may underestimate the absorption of low bioavailability compounds (Forbes et al. 1989). Also, large differences in in vitro bioavailability may not be reflected by large in vivo differences, especially when iron-replete subjects are used (Turnlund et al. 1990).

Animal Models

The rat hemoglobin repletion assay provides a method of comparing the relative efficiency of iron sources for repleting hemoglobin in anemic rats (Forbes et al. 1989). This approach can be a useful screening tool to rank bioavailability, although the actual percent of iron absorbed is likely to differ between humans and rats. Iron is absorbed relatively better in rats than in humans, especially from sources with low bioavailability (Reddy and Cook 1991, 1994). Because rats have at least 30 times more intestinal phytase activity (Iqbal et al. 1994), and for other reasons that are not completely understood (Manju and Reddy 1994), they absorb iron from high phytate foods better than humans.
DIETARY IRON: TYPES, AND USUAL INTAKES

Forms of Iron in Diets

Iron is present in food in both inorganic (ferric and ferrous) and organic (mostly heme) forms. Heme iron is derived primarily from the hemoglobin and myoglobin of flesh foods such as meats, fish, and poultry. About 40 percent of iron from meat, fish, and poultry is in the heme form while the rest is nonheme iron. Other sources of nonheme iron include dairy products, eggs, and plant foods such as beans, cereals, nuts, fruit, and vegetables, and water from some iron pipes, tube wells, and iron containers.

After digestion, most dietary iron enters a pool of either heme or nonheme iron (Bothwell et al. 1989). The exceptions to this two-pool model are dietary iron in the form of ferritin, hemosiderin, ferric oxide and hydroxides, and contaminant iron (e.g., from soil or dirt, or derived from milling or from containers used for food preparation), which may be significantly less soluble than the common pool of nonheme iron and, therefore, only partially enter the soluble nonheme iron pool (Martinez-Torres et al. 1976; Hallberg 1981a; Derman et al. 1982).

The extent to which iron is absorbed from a meal depends on the individual’s iron status and requirements, on the sources and content of iron within the meal, and on the other meal constituents. Heme iron is highly bioavailable (15 to 35 percent), because it is absorbed intact within the porphyrin ring and is therefore not exposed to the inhibitory ligands (binders) present in the diet. In contrast, the nonheme iron in food enters an exchangeable pool, which is subject to the effects of endogenous and exogenous promotor and inhibitory ligands. Nonheme iron is, therefore, absorbed at a lower rate, between 2 and 20 percent depending on the ligands and the iron status of the individual (Bothwell et al. 1989; Craig 1994).

Usual Intakes of Heme and Nonheme Iron

The intake of dietary iron is linked to energy intake. In developed countries, a typical diet contains about 6 mg iron per 1,000 kcal (equivalent to a daily consumption of 8 to 18 mg iron by most adults) with little variation from meal to meal (U.S. Department of Health, Education, and Welfare 1973), or among persons of different economic status (Cook and Finch 1979). The daily intake of iron in many developing countries is usually higher, ranging from 15 to 30 mg (Baker and de Maeyer 1979; FAO/WHO 1988). A greater proportion of this iron is likely to be contaminant iron from soil, which may be poorly absorbed, as discussed later in the section on bioavailability of contaminant iron. Estimates of iron intake per caput (mg/day) and intake expressed as mg/1,000 kcal, respectively are: Bangladesh, 23.4 and 12; Philippines, 11 and 6; Latin America, 16.2 and 8 (in maize-eating areas). In India, per caput intake is 10 to 17 mg/day for children, 23 to 30 mg/day for adult women, and 28 to 35 mg/day for adult men (FAO/WHO 1988).
Heme iron contributes only about 10 to 15 percent of the total iron intake (1 to 3 mg/day) in diets in developed countries, but may provide a substantial amount of the total absorbed iron. Where meat is consumed extensively this can rise to close to 50 percent, e.g., Argentina and New Zealand. In contrast, heme iron intake is negligible for the majority of people in many developing countries (Huebers 1986; Bothwell et al. 1989) due to cost and cultural constraints, so that nonheme iron is the main source of dietary iron for most people in the world.
CHEMISTRY OF IRON

The difference in iron absorption from various foods or meals depends, in part, on the chemical properties of iron. This subject has been reviewed extensively elsewhere (Lee and Clydesdale 1979; Hurrell 1984; van Dokkum 1992). A brief outline of the chemical properties of iron is provided here to assist in understanding the influence of various dietary factors on iron absorption.

Valence States

There are five oxidation states of iron in foods ranging from Fe$^{6+}$ to Fe$^{3+}$ depending on its chemical environment. Iron is present naturally in foods in the ferrous (Fe$^{2+}$) or ferric (Fe$^{3+}$) form. The ferric form is reduced to ferrous in the presence of hydrochloric acid and reducing agents such as ascorbic acid.

- Reduction: $\text{Fe}^{3+} + e^{-} \rightarrow \text{Fe}^{2+}$
- Oxidation: $\text{Fe}^{2+} - e \rightarrow \text{Fe}^{3+}$, where $e$ = an electron.

Solubility of Ferrous and Ferric Iron

Both ferrous and ferric iron are readily soluble under the acidic conditions of the stomach. With the increase of pH in the small intestine, where most iron absorption takes place, ferric iron is not soluble and thus is less well absorbed.

Formation of Complexes

During digestion, nonheme iron can change its valence state and rapidly form iron-chelate complexes with dietary ligands such as ascorbic acid, phytate, tannins, and oxalate. The stability of the iron-chelate increases with the concentration of the chelating ligand. The strength of the iron-chelate bond, the solubility of the complex, and environmental factors such as pH and the presence of other competing chelators, determine whether iron is available for uptake by mucosal cells (van Dokkum 1992).

In the digestive environment of the stomach, most iron is released from the iron complexes in food (aided by the acidic pH and digestive processes) and enters the common nonheme iron pool. Here, in the presence of reducing agents such as ascorbic acid, 75 to 98 percent of the ferric iron is reduced to the ferrous form (Forth and Rummell 1973). The entry of elemental iron and iron fortificants into the common iron pool is limited by their solubility.

The common pool of ferrous and ferric iron, and ligands such as phytate, tannins, ascorbate, oxalate, etc., leaves the stomach and enters the intestine where the pH is about 7 or 8, which favors the re-formation of complexes. It is at this point that there is competition between the different ligands to form complexes with iron, and the impact of inhibitors is reinforced. Iron bioavailability is, therefore, determined by the degree of the affinity of each ligand for iron and the solubility of the iron-ligand complex. The strength
of the bond with which the ligand complexes iron may contribute to either enhancement or inhibition of iron absorption. Enhancers of iron absorption bind iron securely to maintain the stability of the bond and the solubility of the complex through the gastrointestinal tract. Thus, enhancers are those ligands that form soluble chelates with iron (especially ferric iron) and prevent its precipitation, allowing the release of iron for absorption by the mucosal cell. Amino acids, the "meat factor," ascorbic acid, and citric acid are such enhancers. On the other hand, absorption inhibitors are those ligands that chelate iron to form insoluble complexes or complexes of very high affinity, so that iron is not released from the chelate for absorption. Examples include tannins, phytate, and oxalates.
BIOAVAILABILITY OF NONHEME IRON

The absorption of iron from food is influenced by the form and content of iron, the concomitant presence of modifying dietary factors (such as inhibitors and enhancers of iron absorption), and host factors including iron status.

Dietary Factors

The solubility of nonheme iron in the small intestine is a major determinant of its absorption.

Absorption from foods, meals, and diets

This section summarizes the effects of various inhibitors and enhancers on iron absorption. Studies on the quantitative impact of changing the amount of enhancers on iron absorption from maize-, rice- and wheat-based diets will be described later in this publication. Table 2 summarizes the energy, phytate, and total and bioavailable iron content of important cereals, seeds and legumes, before and after milling. The phytate values were taken from Spiller (1993) and the WorldFood Manual (1992). The latter also provided most of the values for energy, iron, and fat. Data on the energy, iron, and fat content of dry rice were USDA values (USDA 1975). Information on fat content is provided because fortification of high fat foods (such as undergermed maize) with soluble iron will produce rancidity more rapidly. The bioavailability values for maize, wheat, and rice were obtained from data presented in the section “Relative Impact of Potential Interventions to Improve Dietary Iron Availability,” later in this publication; the value for whole sorghum from INACG (1982); and values for legumes from Lynch et al. (1984).

Effect of inhibitors

Phytate versus dietary fiber

Dietary fiber is the generic name for that component of the diet which is resistant to digestion by the endogenous secretions of the upper gastrointestinal tract. It includes polysaccharides other than starch (cellulose, β-glycans, hemicelluloses, pectins, and gums) and lignin. Dietary fiber and its components interact and bind with iron in vitro (Fernandes and Phillips 1982) and lignin and psyllium mucilage have been shown to inhibit iron absorption in man (Cook et al. 1983; Gillooly et al. 1983). More recent studies (Gillooly et al. 1984; Hallberg 1978; Brune et al. 1992) show that the inhibitory effect of bran on iron absorption is not due to its fiber content, because changing the amount of fiber in meals has little effect.

Phytates constitute about 1 to 2 percent of the weight of many cereals, nuts, seeds, and legumes. They prevent the accumulation of excessively large amounts of inorganic phosphorus during seed maturation by acting as a phosphorus store. About 75 percent of phytic acid (myoinositol hexaphosphate) is associated with the soluble fiber components of foods (Torre et al. 1991). During some types of food processing
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(fermentation, germination, baking, etc.), and to a limited extent with the action of endogenous phytases in some cereals (see “Cooking” below), phytic acid is dephosphorylated to yield lower inositol phosphates such as myoinositol bis-, tris-, and penta-phosphates (IP2, IP3, and IP5 respectively) (Torre et al. 1991). Inositol phosphates lower than IP5 do not inhibit iron absorption.

Phytic acid in foods is normally complexed with essential minerals and/or proteins. Many of its complexes are insoluble and not biologically available under physiological conditions. The majority of in vivo studies show that phytates inhibit dietary iron bioavailability, probably due to the formation of di- and tetraferric phytates, from which iron absorption is poor (Ellis et al. 1982; Bothwell et al. 1989; Torre et al. 1991). In fact, the consensus among various studies and reviews (Hallberg 1981a, 1981b; Hallberg 1987; Rossander 1987; Harland 1989; Torre et al. 1991) is that the high phytate content of most plant staples is the primary cause of inhibition of iron absorption from plant-based diets. Phytates, but not fiber per se, in whole-grain cereals, beans (legumes), nuts, and seeds reduce iron absorption in a dose-dependent manner, due to the formation of insoluble and/or indigestible complexes between iron, phytate, and proteins (Hallberg 1981; Brune et al. 1992).

The dose-dependent inhibitory effect of phytate on iron absorption has been demonstrated by adding varying amounts of sodium phytate to wheat rolls (Hallberg et al. 1989). It is important to note that the inhibitory effect of phytate on iron absorption is proportionately more pronounced when dietary phytate is low, and smaller when the dietary content is high (Figure 1, modified from Brune et al. 1992). Bothwell et al. (1989) demonstrated the dose-dependent inhibitory effect of the natural phytate in food by changing the phytate content of a bread test meal. The phytate was altered using different proportions of maize bran and phytate-free maize bran produced by a wet, acid milling process. There was a rapid decrease in iron absorption to about one-half of the basal level over the range of 30 to 60 mg phytate, and a flat dose-response curve thereafter.

It follows that reducing the phytate content of maize has little effect on iron absorption unless the final phytate content is very low (Figure 1), which has major implications for attempting to improve iron absorption using this approach (see “Effects of Food Processing” below).

While it is difficult to compare values directly due to differences in meal composition among studies, the International Nutritional Anemia Consultative Group (INACG 1982) stated that iron absorption is highest from white wheat-based (30.9 percent), intermediate from rice-based (6.5 percent) and whole wheat-based (about 5 percent) (Sayers et al. 1973), and lowest from maize-based (3.7 percent) and sorghum-based (red sorghum: 3.6 percent, white sorghum: 2.8 percent) meals. These differences appear to be due, in part, to the different levels of phytate in these foods (Hurrell 1984) and to other inhibitors such as tannins in red sorghum (INACG 1982). Iron in legumes such as soybeans, black beans, lentils, mung beans, and split peas is very poorly absorbed (less than 2 percent); the factors involved may be phytate and tannins, and others that remain to be identified (Lynch et al. 1984; Hurrell 1992).
### Table 2: Composition and Iron Bioavailability of Cereals, Seeds and Legumes

<table>
<thead>
<tr>
<th></th>
<th>Maize</th>
<th>Wheat</th>
<th>Rice</th>
<th>Sorghum</th>
<th>Millet</th>
<th>Legumes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Whole</td>
<td>Degemermed</td>
<td>Whole</td>
<td>White flour</td>
<td>Brown</td>
<td>Polished</td>
</tr>
<tr>
<td>Kcal/100g</td>
<td>362</td>
<td>364</td>
<td>339</td>
<td>364</td>
<td>360</td>
<td>363</td>
</tr>
<tr>
<td>Fat (g/100g)</td>
<td>4</td>
<td>1.2</td>
<td>1.9</td>
<td>1</td>
<td>1.9</td>
<td>0.4</td>
</tr>
<tr>
<td>Phytate (mg/100g)</td>
<td>800</td>
<td>72</td>
<td>800</td>
<td>280</td>
<td>500</td>
<td>255</td>
</tr>
<tr>
<td>Fe (mg/100g)</td>
<td>3</td>
<td>1</td>
<td>3.3</td>
<td>1.2</td>
<td>1.6</td>
<td>0.8</td>
</tr>
<tr>
<td>Phytate/Fe molar ratio (^b)</td>
<td>25</td>
<td>6</td>
<td>23</td>
<td>20</td>
<td>26</td>
<td>27</td>
</tr>
<tr>
<td>Bioavail. Fe (percent)</td>
<td>3.7</td>
<td>5</td>
<td>5</td>
<td>20(^c)</td>
<td>?</td>
<td>3</td>
</tr>
<tr>
<td>Bioavail. Fe (mg/100g)</td>
<td>0.1</td>
<td>0.05</td>
<td>0.16</td>
<td>0.24</td>
<td>?</td>
<td>0.02</td>
</tr>
<tr>
<td>Bioavail. Fe density (mg/100 Kcal)</td>
<td>0.28</td>
<td>0.14</td>
<td>0.47</td>
<td>0.66</td>
<td>?</td>
<td>0.06</td>
</tr>
</tbody>
</table>

\(^a\) Dry Weights  

\(^b\) Phytate (g)/660 ÷ Fe (mg)/56  

\(^c\) Leavened bread. No data available for bread made from unleavened white flour.
Figure 1: Effect of Phytate on Nonheme Iron Absorption from Bread

Because phytic acid is the major site for the storage of phosphorus in seeds, cereals and soybeans grown in low phosphorus soils or environments contain dramatically less phytate. The low level of phytate, however, does not adversely affect germination or plant growth (Raboy 1990). Efforts are being undertaken to select and breed seeds, including maize and soybeans, that are low in phytate (Raboy 1990). The potential for these seeds to improve the mineral status of populations depends on many factors, including the degree of improvement in mineral bioavailability (which has yet to be tested in humans), the viability of seeds and productivity of the plants, and their acceptability to the relevant populations. As a cautionary note, phytate has been shown to be protective against cancer in animals, so that the potential carcinogenic impact of a large reduction in the phytate content of the food supply is of some concern.

Tea, coffee, and polyphenols

Polyphenols such as tannins in tea, coffee, and certain vegetables, bind nonheme iron to form insoluble iron-tannate complexes that are poorly absorbed (Disler et al. 1975a, 1975b; Gillooly et al. 1983; Rossander et al. 1979). There was a drastic reduction in iron absorption (by about 60 percent) from foods when they were consumed with just one cup (200 to 250 mL) of normal-strength tea (Disler et al. 1975b; Morck et al. 1983). Iron absorption from bread was reduced to one-third, and from a vegetable soup to one-fourth, when served with tea compared with water (Disler et al. 1975a). In a Western breakfast meal,
Bioavailability of Nonheme Iron

Iron absorption was reduced 56 percent by 150 mL tea made from 2.5 g dry tea (Rossander et al. 1979). Similarly, tea (1.75 g dry tea/200 mL water) reduced iron absorption by 64 percent from a hamburger meal (Morck et al. 1983) and by 45 percent from a maize-porridge meal (5 g dry tea/150 mL water) (Derman et al. 1977). The inhibitory effect is proportional to the quantity of tea (Derman et al. 1977) or tannic acid (Siegenberg et al. 1991) consumed with the test meals.

Israeli infants (6 to 12 months) given tea (median intake of 250 mL/d) had a significantly higher rate of microcytic anemia (32.6 percent) compared with those who did not receive tea (3.5 percent) after controlling for other factors such as age, sex, and duration of breast feeding. The hemoglobin concentrations in the tea-fed infants were significantly lower than those of the non-tea-fed infants (Merhav et al. 1985).

The inhibitory effect of coffee on iron absorption in humans is less than that of tea: one cup of coffee reduced iron absorption by 39 percent when drunk either with, or one hour after, a hamburger meal (Morck et al. 1983) or a maize-porridge meal (Derman et al. 1977). Coffee may also impair the utilization of iron thus reducing its bioavailability. In a rat study, maternal coffee intake, especially during lactation, impaired the mobilization of iron from the liver reserves of pups of coffee-exposed versus control rats (Muñoz et al. 1986). This may result in reduced hemoglobin synthesis. In a follow-up, prospective human study in Costa Rica, the same investigators found that maternal coffee intake during pregnancy was negatively associated with infant hemoglobin at one month of age. This effect was independent of maternal iron status and infant birth weight (Muñoz et al. 1988).

The polyphenols in vegetables, legumes, and condiments also have a strong inhibitory effect on iron absorption (Rao and Prabhavathi 1978; Gillooly et al. 1983). Vegetables such as eggplant, spinach, green and brown lentils, and beetroot greens have a high polyphenol content and low iron bioavailability (Gillooly et al. 1983).

**Oxalates**

The low availability of iron from some green vegetables, such as spinach, is partly due to their high oxalic acid content (Oke 1969). Oxalic acid may form insoluble iron-oxalate complexes, although this may be compensated for, to some extent, by the presence of ascorbic acid. While the addition of 1 g calcium oxalate to cabbage reduced iron absorption by 61 percent, no relationship between oxalic acid content and iron absorption emerged when three vegetables containing large amounts of oxalate were examined: iron absorption was poor from spinach and beetroot greens and good from beetroot (Gillooly et al. 1983). The bioavailability of iron from these vegetables was evidently influenced by the presence of other inhibitors and enhancers.

**Calcium**

While it is usually stated that calcium impairs iron absorption, the inconsistent results across experiments suggest that calcium-iron interactions are complex. The addition of calcium phosphate reduced the absorption of nonheme iron from a semi-synthetic meal by 50 percent, whereas calcium alone did not (Monsen and Cook 1976). Calcium salts also lowered iron absorption by 55 percent from a typical
breakfast meal with low iron availability and a high calcium content (Cook et al. 1991), and by 28 percent from a high iron availability hamburger meal with a low calcium content (Cook et al. 1991). Similarly, 165 mg calcium added as calcium chloride, milk, or cheese inhibited the absorption of nonheme iron from wheat rolls by 50 to 60 percent, while 300 to 600 mg calcium reduced the absorption of heme iron as well (Hallberg et al. 1991). Adding calcium to wheat rolls before baking had the strongest inhibitory effect, possibly by inhibiting phytase activity during fermentation. The addition of 450 mL of whole milk to a test breakfast meal halved iron retention compared with a placebo (Deehr et al. 1990). The inhibitory effect of calcium appears to be dose related up to 300 mg calcium, after which there is little additional inhibition. It is likely that iron absorption can be protected by consuming calcium-rich foods and iron-rich foods at different meals. For example, about 30 to 50 percent more iron was absorbed when no milk or cheese was served with lunch or dinner to Swedish subjects fed a typical Scandinavian diet containing 1.4 mg heme and 11.9 mg nonheme iron. Over a 10-day period, on average subjects absorbed 0.4 mg more iron per day when milk or cheese (providing 937 mg calcium/day) were consumed separately from the main meals (lunch and dinner) (Gleerup et al. 1995). In other longer-term (rather than single meal) studies, investigators found no inhibitory effect of milk on iron absorption from cereal based meals (Turnland et al. 1990), from a typical French meal containing meat and cheese (Galan et al. 1991), or when milk was added to meals for six weeks (Tidehag et al. 1995). Part of the explanation may be the fact that Gleerup et al. (1995) labeled all nonheme iron in all meals (Hultén et al. 1995).

In contrast to calcium citrate and calcium phosphate, calcium carbonate (300 or 600 mg Ca) did not reduce the absorption of iron from ferrous sulfate supplements (containing 37 mg or 18 mg Fe) taken without food (Cook et al. 1991). When taken with food (a hamburger), all three calcium supplements were inhibitory. However, when 1,000 mg/day of calcium was taken by premenopausal women as a calcium carbonate supplement for 12 weeks with meals, serum ferritin concentrations were not affected during this relatively short period (Sokoll and Dawson-Hughes 1992).

It is important to consider the potentially adverse effect of calcium on iron absorption in diets where calcium salts are routinely used to prepare foods, as in areas of Mexico, and Central and South America. In the preparation of tortillas, maize is soaked with quicklime (calcium oxide). This may contribute to the poor iron absorption from these diets, although this has not been systematically shown. Nevertheless, iron absorption from maize tortillas prepared with quicklime can still be improved by adding a source of ascorbic acid to the meal (Hallberg et al. 1984). This is a more acceptable strategy than discouraging the use of quicklime.

**Dietary protein**

Several plant foods that have a high protein content, including soy beans and nuts, significantly inhibit nonheme iron absorption (Derman et al. 1987; Macfarlane et al. 1988a, 1988b, 1990). Data from in vitro studies suggest that high molecular weight peptides may be involved in this inhibitory effect (Kane and Miller 1984). Because soy protein is being used more by the food industry (for infant formulas, extended meat products, baked goods, and dairy foods), and because of its good protein quality, abundant supply, and low cost, the absorption of iron from soy and soy products deserves special mention. Although full fat soy flour, textured soy flour, and isolated soy protein all markedly reduce iron absorption, isolated soy protein had the greatest inhibitory effect mostly due to its high phytic acid content (Hurrell 1992a, 1992b).
However, even after removing virtually all the phytic acid, iron absorption from a soy-protein meal was still only one-half that of an egg white control. Thus, a residual “soy factor” is implicated, which reduces iron absorption by binding it to insoluble peptides in the duodenum (Hurrell 1992; Hurrell et al. 1992). Soy products, such as miso, in which the protein complex is broken down during processing, have significantly better iron bioavailability in vitro (Macfarlane 1990).

Effect of enhancers

Meat, fish, and poultry (MFP), and ascorbic acid, are quantitatively the most important dietary enhancers of nonheme iron absorption.

Meat, fish, and poultry

The enhancing effect of meat, fish, and poultry on iron absorption is well known. Unlike other enhancers, components of these foods increase the absorption of both heme and nonheme iron (Hurrell 1984).

The addition of either fish — or an equivalent amount of synthetic amino acids to 100 g fish — doubled iron absorption from black beans (Martinez-Torres and Layrisse 1970). Adding 80 g ground beef to a standard meal consisting of a bun, french fries, and a milk shake doubled iron absorption from 1.96 to 3.90 percent (Reddy and Cook 1991). The effect of meat, fish, and poultry relates specifically to muscle protein, and not to animal protein in general (Cook and Monsen 1976; Bjorn-Rasmussen and Hallberg 1979). The mechanism by which meat, fish, and poultry enhance iron absorption is not clear, but some “meat factor” is involved. It may involve the release of cysteine (Layrisse et al. 1984), cysteine-containing peptides (Layrisse et al. 1984) and other peptide digestion products (Kane and Miller 1984), or the interaction of nonheme iron with the carboxyl groups of amino acids (Shears et al. 1987).

Quantitatively, about 1 to 1.5 g of meat is equivalent to 1 mg ascorbic acid in its ability to promote nonheme iron absorption from semisynthetic meals or a standard hamburger meal (Monsen et al. 1978; Hallberg et al. 1984). In a study of iron absorption from a simple Latin American diet (maize, rice, and black beans), 75 g of ground beef improved iron bioavailability to the same extent as 50 mg of ascorbic acid (Hallberg and Rossander 1984). However, the quantitative relationship between the enhancing effect of ascorbic acid and that of meat, fish, or poultry may be modified depending on the amount or type of inhibitory ligands in the meal (Monsen et al. 1978).

Ascorbic acid

Ascorbic acid is a strong enhancer of nonheme iron absorption. It may exert its “enhancing” effect by promoting acid conditions within the stomach so that the dietary iron is efficiently solubilized; by reducing ferric iron to its better absorbed ferrous form; by forming chelates with iron in the stomach; and by maintaining the solubility of nonheme iron when the food enters the alkaline environment of the small intestine — which counteracts the inhibitory effect of dietary ligands such as phytates and tannins. The latter effect can be explained by the fact that ascorbic acid forms complexes with soluble food iron at a lower pH than do inhibitory ligands. The overall result is that iron from the common nonheme pool
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complexes with ascorbic acid in the stomach and passes into the intestine as an iron-ascorbate complex, thereby reducing the influence of the inhibitory ligands that bind iron in the more alkaline pH of the duodenum (Hurrell 1984).

Iron absorption from a semisynthetic meal increased three-fold after adding 75 mg ascorbic acid and four-fold after adding 100 mg ascorbic acid (Cook and Monsen 1977; Monsen 1988; Reddy and Cook 1991). Adding 15 mg ascorbic acid caused a three-fold increase in iron absorption from a rice porridge meal (Gillooly et al. 1983). Studies with maize meals have shown a six-fold increase in iron absorption with 150 g papaya containing 65 mg ascorbic acid (Layrisse et al. 1974), to a ten-fold increase with 50 or 100 mg ascorbic acid (Derman et al. 1977). The most profound effects of ascorbic acid occur when meals have a high content of “inhibitors” such as phytates and tannins, which are found in a traditional maize-based Latin American meal (Hallberg et al. 1986a, 1986b). A similar level of iron absorption enhancement was obtained using synthetic ascorbic acid versus the same amount of natural ascorbic acid consumed in foods such as cauliflower and papaya (Layrisse et al. 1974; Hallberg et al. 1986a, 1986b). This enhancing effect of ascorbic acid on iron absorption is dose-related, both from single meals such as a maize meal containing 100 g maize (Bjorn-Rasmussen and Hallberg 1974) and a semisynthetic meal containing dextrimaltose, corn oil, ovalbumin, and 4.1 mg iron. The increase in iron absorption (0.77 to 7.1 percent) was directly proportional to the amount of ascorbic acid added over the range of 25 to 1,000 mg (Cook and Monsen 1977).

Ascorbic acid can improve iron absorption even in the presence of inhibitors such as phytates in cereals and soya, tannins in tea, and calcium (Derman et al. 1977; Hallberg et al. 1989; Deehr et al. 1990; Siegenberg et al. 1991). The addition of ascorbic acid (50 or 100 mg) significantly counteracted the inhibitory effect of phytate added to wheat rolls at various levels, i.e. 0, 25, and 250 mg of phytate phosphorus (Hallberg et al. 1989); the latter constitutes 28 percent of the phytic acid molecule. Similarly, 30 mg of ascorbic acid overcame the inhibitory effects of 10 to 58 mg of phytate phosphorus, and it was concluded that more than 50 mg ascorbic acid would be required to overcome the inhibitory effects on iron absorption of any meal containing more than 100 mg tannic acid (Siegenberg et al. 1991). The inhibitory effect of tannic acid in tea consumed with a maize-meal porridge was counteracted by giving large amounts of ascorbic acid (250 or 500 mg) to iron-deficient Indian women (Derman et al. 1977). Likewise, the inhibitory effect of taking a calcium supplement (500 mg calcium as calcium citrate malate) on iron absorption from a breakfast test-meal was overcome when postmenopausal women drank 450 mL orange juice with the calcium supplement (Deehr et al. 1990).

These studies show that the greater the level and effect of inhibitors in a meal, the greater the amount of ascorbic acid required to overcome the inhibition. However, a given quantity of ascorbic acid causes a proportionately greater increase in the amount of iron absorbed from diets higher in inhibitors.

One limitation of the studies that have been conducted with ascorbic acid is that they were predominantly limited to measuring its impact on iron absorption from single meals. There is little information on the effectiveness of increasing ascorbic acid intake on iron status over the longer term, or at the population level. Long term ascorbic acid-induced increases in nonheme iron bioavailability might be less than that observed from single meals, particularly among those who are not iron-deficient. For example, 2 g/day of ascorbic acid with meals for 16 weeks did not increase serum ferritin in non-iron-deficient volunteers in the U.S. eating self-selected diets (Cook et al. 1984). This was not caused by adaptation to the high ascorbic acid intake because iron absorption from single meals was still stimulated by a dose of ascorbic
Bioavailability of Nonheme Iron

acid at the end of the 16 weeks. Similarly, 100 mg of ascorbic acid, fed with meals three times per day for eight weeks failed to increase serum ferritin (Malone et al. 1986). Cook et al. (1991) observed that nonheme iron absorption from an enhancing diet was 2.5-fold higher than that from an inhibitory diet, when each diet was fed over a two-week period. In contrast, iron absorption from a single enhancing meal was 5.9-fold higher than from an inhibitory meal. Hunt et al. (1994) reported no significant effect of ascorbic acid (500 mg, three times per day) on serum ferritin although the observed increase (from 11.4 to 12.9 μg/L with predicted poorly-available iron, and from 10.7 to 11.9 with a typical diet in a developed country) may have become significant if the treatment had been given for more than five weeks. Iron balance was not improved but this measure is relatively insensitive to changes in absorption. The level of inhibitors tested in these longer term studies was much lower (less than 500 mg phytate per day) compared with many traditional diets, especially those that are maize-based. For example, in rural Mexico adult women consume more than 4,000 mg of phytate per day, and adult men consume 5,000 mg (Black et al. 1994). As Hallberg et al. (1986b) pointed out, “a more marked longterm effect of ascorbic acid on iron balance can only be expected when the diet has a fairly high content of inhibitors.” Also, improvements in iron status would be more apparent in iron-deficient populations.

There are only two reports of the impact of ascorbic acid supplementation at the community level. In India, 54 anemic preschool children were supplemented with 100 mg synthetic ascorbic acid versus a placebo, at each of the two main meals, for two months (Seshadri et al. 1985). Usual iron and ascorbic acid intakes were low. Ascorbic acid treatment improved hemoglobin concentrations significantly, from 9.38 to 11.30 g/L on average. There was no change in controls (9.08 versus 9.18 g/L). Initially 96 percent of all the children had a microcytic hypochromic blood profile, but only 26 percent showed this post-intervention. In China, 65 children with mild anemia received 0, 25, 50, 100, or 150 mg ascorbic acid daily for eight weeks (Mao and Yao 1992). Usual intakes of iron and ascorbic acid were 7.5 mg and 30 mg respectively. Weekly iron status assessment showed the 50 mg ascorbic acid supplement to be most effective, and an improvement in iron status could be detected in six weeks. No community trials have been attempted on adults, and equally importantly, no interventions have been attempted using local, potentially sustainable food sources of ascorbic acid. Because citrus and other fruits and vegetables are also high in citric acid, these may have a stronger effect on iron absorption than synthetic ascorbic acid (see following section).

**Other organic acids including citric acid**

Organic acids such as citric acid, malic acid, tartaric acid, and lactic acid also enhance iron absorption (Derman et al. 1980; Gillooly et al. 1983; Ballot et al. 1987). The geometric mean iron absorption from a rice meal increased significantly with the addition of 1 g citric acid (by 3-fold), 1 g L-malic acid (by 2-fold), and 1 g tartaric acid (by 2.3-fold) (Gillooly et al. 1983). Using various fruit juices and synthetic combinations of organic acids, Ballot et al. (1987) demonstrated that there was a significant correlation between iron absorption from a rice meal and the citric acid content of added fruits, and that the enhancing effect of citric acid was additive to that of ascorbic acid. Ascorbic acid had less effect than whole orange juice on iron absorption from a Mexican meal, an effect attributed to citric acid and bioflavonoids (Maisterrena et al. 1977). In vitro there is a significant enhancing effect of citric acid alone, or in combination with ascorbic acid, on solubilization of iron in beans (Kojima et al. 1981) and on iron
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diffusibility (solubility) from a variety of cereals, legumes, and nuts (Hazell and Johnson 1987). The
 carboxylic and hydroxyl groups of citrate may prevent polymerization of insoluble iron hydroxides by
 forming soluble complexes with iron. Because citrate is present in high concentrations in milk, vegetables,
 and citrus fruits, its quantitative effect on improving iron absorption could be substantial. Although most
 studies on humans have shown a promoting effect of citric acid on iron absorption, in one study the
 addition of 1 g citric acid reduced the absorption of iron from a simple Latin American meal composed of
 maize, rice, and black beans to one-third of that from the meal alone (Hallberg et al. 1984).

Lactic acid has been identified as the factor promoting iron absorption from sorghum- and maize-derived
 beers (Derman 1980). However, adding large amounts of lactic acid (340 mg) to a rice meal had no effect
 on iron absorption (Baynes 1990). Succinic acid increases iron absorption from pharmaceutical iron
 preparations such as ferrous sulfate (Brise and Hallberg 1962), and iron absorption was enhanced by 35
 percent when 150 mg of succinic acid was added to a standard hamburger meal (Hallberg 1981a, 1981b).

Sugars

Sugars such as fructose and lactose (van Dokkum 1992) but not glucose or galactose (Pollack et al. 1965)
 have some enhancing effect on iron absorption. Sorbitol (12 mg sorbitol/mg iron) doubled iron absorption
 from pharmaceutical preparations of ferrous sulfate (Loria et al. 1962). Mannitol and xylose also promote
 iron absorption from oral iron preparations (Hallberg et al. 1966).

Effects of food processing

Food processing, such as heat treatment, baking, fermentation, soaking, and milling may enhance or reduce
 iron availability. Heating foods and storing cooked foods can destroy ascorbic acid and subsequently
 decrease iron absorption. Prolonged storage of canned foods may release iron from some cans and
 increase the total amount of iron absorbed in absolute terms (Henriksen et al. 1985).

Cooking

Prolonged cooking reduces iron absorption from meat (Martinez-Torres et al. 1986). When ground meat
 was fried for 8, 18, and 30 minutes, heme denaturation was 5, 38, and 45 percent respectively. Boiling
 meat for two hours reduced heme iron by 22 percent, and subsequent frying reduced it by an additional 16
 percent. Although frying meat for 10 minutes did not affect iron absorption from heme, when this was
 followed by heating in an oven at 350°F for an hour heme iron absorption fell to 44 percent of that
 expected. Cooking also reduces the amount of iron absorbed from the ferritin in meat (Martinez-Torres et
 al. 1986), presumably because the integrity of the “meat factor” is affected.
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Fermentation, soaking, and germination

In general, cereals high in phytate tend to have a higher iron content. Low extraction (white) flour contains less phytate and iron, while high extraction (brown) flour has both more phytate and more iron (Table 2). Phytase enzymes will break down inositol hexa- and penta-phosphates, which inhibit iron absorption, to smaller inositol phosphates and inorganic phosphate, which do not affect iron absorption. The amount of hexa- and penta-phosphates remaining after treatments that involve phytase, such as germination or soaking, has therefore been used as an indicator of phytase activity and is a good predictor of remaining phytate and its ability to impair iron absorption (Landberg et al. 1989).

Humans have negligible intestinal phytase activity (Iqbal et al. 1994), even if they usually consume high phytate diets (Brune et al. 1989). Cereals, however, contain an endogenous phytase. In raw (but not heated) wheat bran, endogenous phytase may break down as much as 60 percent of the phytate in the bran during transit through the stomach and upper intestine (Sandberg and Andersson 1988), but the phytate content is not reduced enough to improve iron absorption (Sandberg et al. 1996). Because the endogenous cereal phytase has a pH optimum of 5.15, it is probably inactivated in the low pH of the stomach. Thus, there has been some interest in reducing the phytate content of cereals by soaking or germination (which activate endogenous phytase), fermentation or leavening (which lower the pH thus producing a more optimal pH for phytase activity), or by adding a commercial phytase enzyme.

Fermentation can hydrolyze most of the phytate in wheat so that iron absorption is improved. For instance, iron absorption from baladi, a traditional Egyptian flat-bread made from high-extraction flour, was only one-sixth of that observed from leavened European bread made from similar flour (El Guindi 1988). Prolonged fermentation (48 hours at 23°C) of wholemeal wheat bread reduced the total phytate content to the same level as that in low phytate control rolls. It subsequently increased the absorption of iron seven-fold, to the same bioavailability as the iron in the control bread, despite the five times higher total fiber content of wholemeal flour (Brune et al. 1992). After comparing iron absorption with the phytate content of breads made from various types of flour and fermented in different ways, Brune et al. (1992) were able to demonstrate that iron absorption was related to the final phytate content. More importantly, about 90 percent of the phytate in high-phytate flours had to be degraded before iron absorption increased substantially (Figure 1).

In some developing countries, it is common for the preparation of cereals to entail soaking, and germination and/or fermentation (Chavan and Kadam, 1980a, 1980b). These techniques could be especially useful for preparing complementary foods and porridges (Svanberg and Sandberg, 1987). For example, whole sorghum grains are soaked, milled, and fermented to make ogi, a complementary food used in Nigeria (Obizoba and Atii 1990). A series of studies on the use of lactic acid fermentation to improve the nutritional quality and microbiological safety of cereal complementary foods has been conducted in Tanzania (Lorri 1993). Fermentation was enhanced by using a natural lactic acid starter. Flour containing amylase from germinated seeds was added to decrease the viscosity of the gruels so that three times more flour could be added. In vitro iron solubility from low tannin cereals (maize and white sorghum) improved from about 4 percent to 9 percent after lactic acid fermentation. Soaking the flour in water before fermentation, or adding exogenous wheat phytase, dramatically increased iron absorption to 50 percent (Svanberg et al. 1993). In contrast, the solubilization of iron in high tannin cereals was minor, which was attributed to the inhibitory effect of the tannins both on iron solubility and on phytate hydrolysis. An additional benefit of lactic acid fermentation is the inhibition of gram negative pathogenic
bacteria as well as gram positive bacteria (Svanberg et al. 1992) and subsequently less spoilage during storage. Diarrhea episodes in preschoolers were significantly reduced when fermented versus non-fermented gruels were used (Lorri and Svanberg 1993). Fermentation also increases the amount of water soluble vitamins such as riboflavin, and improves digestibility of protein in high tannin cereals such as brown sorghum, bush millet, and finger millet (Lorri 1993). It also improves the bioavailability of zinc (Sandberg 1991), and presumably that of calcium and other minerals in cereals and seeds.

Fermented soy products (tempeh, miso, and nato), as well as silken tofu precipitated with a gluconic acid derivative, show enhanced iron absorption compared with soy flour (Macfarlane 1990). This is due to the reduction in the phytate content and/or breakdown of the soy protein complex that binds iron and reduces its absorption. In vitro evidence also suggests that the fermentation process enhances iron solubility in different diets mixed with fresh versus fermented vegetables (Sandberg 1991).

Soaking under optimal conditions activates naturally occurring phytases in cereals and results in varying degrees of phytate hydrolysis, depending on the kind of cereals. Wheat and rye flour can be treated in this manner to destroy practically all their phytate and enhance in vitro iron availability (Sandberg and Svanberg 1991). For instance, soaking wheat bran (at pH 4.5-5, 55°C) hydrolyzed 95 percent of the phytate within one hour and all of it within two hours. The soluble iron content increased from less than 5 percent to over 50 percent. For wholemeal rye flour, which contains more endogenous phytase, complete hydrolysis of phytate was achieved within 30 minutes. In contrast, even 17 hours of soaking did not improve iron solubility in whole oatmeal flour because it contains less phytase.

Germination (also called malting) is a process in which whole grains are soaked and then germinated. Malting of wheat, barley, rye and oats for 30 to 44 hours at 15°C had little effect on phytate content, but when this was followed by soaking at pH 4.5-5 phytate was degraded completely, except for oats, which were relatively unaffected because they contain little endogenous phytase (Sandberg 1991). In sorghum, phytate was completely hydrolyzed during germination (soaking for 12 hours) followed by fermentation (lactic acid fermentation in water for 96 hours), so that iron solubility under simulated physiological conditions was greatly increased (Svanberg and Sandberg 1987). However, fermentation of previously germinated raw sorghum seeds stimulates the enzymes responsible for hydrolysis of cyanogenic glycosides to hydrogen cyanide. This problem can be avoided by cooking the seeds prior to fermentation (Obizoba and Atii 1990). Cooking, followed by fermentation for 72 to 96 hours, reduced the tannin content of sorghum by 50 percent (Obizaba and Atii 1990).

It is somewhat difficult to predict the overall impact of soaking, fermentation or germination, on iron solubility. Using sorghum porridge as an example, the lowest phytate concentrations were achieved by germinating whole grains and fermenting them for 96 hours (producing about 40 percent soluble iron) or fermenting whole grains for 96 hours (producing 25 percent soluble iron) (Sandberg 1991). None of the other germination or soaking strategies had a major impact on soluble iron content. Soaking, or germination followed by soaking or fermentation might be effective in reducing the phytate content of wheat, rye, barley, and white sorghum, but it appears that germination and fermentation could take several days, and is more effective if whole grains are used (because they have a higher phytase content). In addition, it may be necessary to break down over 90 percent of the hexa- and penta-phosphates (to less than 0.5 μmol/g) before iron solubility is markedly improved in vitro (Sandberg 1991). This matches the formerly discussed data of Brune et al. (1992 and Figure 1), where the phytate content of wheat rolls had to be reduced by about 90 percent to improve iron bioavailability in humans.
Adding exogenous phytase

It is possible to add commercial exogenous phytase to break down phytate in cereals. For example, the addition of 10 to 50 mg wheat phytase to wheat bran, in which the endogenous phytase is activated by heat treatment, and soaking for four hours at 55°C, reduced the phytate content and increased iron solubility, but to a lesser extent than occurred with soaking and subsequent activation of endogenous phytase (Sandberg and Svanberg 1991). Microbial phytase, isolated from bacteria such as *Aspergillus niger*, may be more effective. Microbial phytase is a 3-phytase, while cereal phytase is a 6-phytase. The microbial enzyme has two pH optima, at 2.0 and 6.0, and works in the range of pH 1 through 7.5 at 37°C, i.e., in the stomach. Microbial phytase doubled iron absorption (from 14 to 26 percent) from white wheat rolls containing added wheat bran (Sandberg et al. 1996).

Milling

Milling has a major impact on both the phytate and the iron content of cereals and seeds (INACG 1982 and Table 2).

Maize milling results in either whole or degermed meal or flour. Whole corn meal is produced by grinding with very little removal of the germ. This product is high in fat (about 4 percent, which greatly reduces shelf life) and phytate (about 800 to 1,000 mg/100 g) (Reddy et al. 1989; Spiller 1993), and contains about 3 mg iron/100 g. It is used frequently in Africa, the Caribbean, and much of Latin America. Most dry-milled maize is degermed, so that the endosperm remaining is lower in fat (about 1.2 percent) and iron (about 1 mg/100 g). The phytate content is also much lower, e.g., 72 mg/100 g in corn flakes (Spiller 1993).

During processing of wheat, the bran (14.5 percent) and germ (2.5 percent) are removed from the endosperm (83 percent) (INACG 1982). Whole wheat flour contains over 800 mg phytate (Spiller 1993) and 2 to 4 mg iron per 100 g, whereas unenriched white flour (70 to 75 percent extraction) contains only 280 mg phytate (Spiller 1993) and 1.2 mg iron per 100 g.

Brown (unpolished) rice is moderately high in phytate, containing more than 500 mg/100 g. The phytate content of polished rice grains varies depending on the method of milling. In a study in Thailand, it ranged between 11.5 and 66 mg phytate phosphorus (41 to 235 mg phytate)/100 g rice when sampled across 45 rice mills (Tuntawiroon et al. 1990). The iron content of raw brown rice is about 1.6 mg/100 g, falling to half of this value in white raw rice.

In Africa and India sorghum is prepared using a mortar and pestle, by hand-operated stone mills, or in commercial mills (INACG 1982). The outer bran is then removed by winnowing. Whole grain sorghum contains over 600 mg phytate and 4 mg iron per 100 g. In decorticated sorghum flour the phytate is somewhat reduced, to 439 mg phytate per 100 g, whereas the iron content is little affected (WorldFood 1994). Estimates of the phytate content of whole millet range from about 500 mg (Spiller 1993) to 870 mg (WorldFood 1994) phytate/100 g, falling to 70 percent of this after milling (WorldFood 1994). Decortication will also remove some of the tannins in seed coats.

Dehulling reduces the tannin content of beans, but this has little impact on iron availability (Cook et al. 1981). Unlike cereals, the phytate concentration of legumes is increased by dehulling because the phytate is located in the cotyledonous fraction (Reddy et al. 1982).
Improving Iron Status Through Diet

Summary

The amount of phytate remaining after food processing will depend on the type of cereal (and its initial content of phytate and phytase); the extent of extraction during milling; flour freshness (because phytase content falls over time); and the fermentation and germination techniques employed. Clearly, there needs to be further testing of the effectiveness of location-specific soaking/fermentation/germination strategies, which will depend on the local cereal and acceptable methods of preparation. These are especially useful for the preparation of complementary foods, but in many locations commercial flours are produced from fermented cereals that can be used by all age groups.

A new development with potential is the engineering of transgenic seeds (including maize) that are high in phytase that is active in the intestinal tract of animals (Pen et al. 1993). The animal feed industry is actively pursuing approaches to break down the phytates in feed to eliminate phosphorus supplementation, thereby protecting the environment from excessive phosphorus in animal excreta.

Bioavailability of iron used in fortification

Fortification of foods with iron can be a useful strategy to increase the iron intake of populations or subpopulation groups. Iron fortification is most prevalent in industrially processed infant foods. Iron is also added to cereals during the milling of flour or resulting fortified cereal-based products. Iron fortification of other foods, such as sugar, salt, and soy sauce, has been successful on an experimental basis. The bioavailability of the fortificant iron added to a food (vehicle) is determined by several factors: the form of iron fortificant used, the amount of added iron, the iron status of the individual, and potentially most important, the presence of other dietary constituents (Hurrell 1992). The latter can affect the absorption of the fortificant iron in the same way as they affect the absorption of the endogenous (native) iron in food.

Bioavailability of different forms of iron

There are several excellent reviews of the advantages and disadvantages of the many kinds of iron that have been used as fortificants (Hurrell 1984; Hurrell 1992). The present review is limited to a discussion of the bioavailability of fortificant iron. Iron compounds commonly used in food fortification range from those that are soluble in water (ferrous sulfate), or soluble in dilute acids (ferrous fumarate, ferric saccharate), to those that are water-insoluble and poorly soluble in dilute acids (ferric pyrophosphate, ferric orthophosphate, and elemental iron).

Hurrell (1992) has summarized the results from various studies on the relative bioavailability of iron from various fortificants added to infant formula (Table 3). The bioavailability of iron compounds used in food fortification is usually compared with that of ferrous sulfate, which is assigned a “relative bioavailability value” (RBV) of 100. Because iron absorption from most infant formulas (some of those based on soy being an exception) is reasonably good, the values in Table 3 probably represent the “best case scenario” in terms of how much added iron can be absorbed from foods.
Table 3: Relative Bioavailability of Various Iron Compounds Used in Food Fortification

<table>
<thead>
<tr>
<th>Iron Compound</th>
<th>Relative Bioavailability</th>
<th>Commonly Fortified Foods</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ferrous sulfate</td>
<td>100</td>
<td>Infant formulas</td>
</tr>
<tr>
<td>Ferrous fumarate</td>
<td>100</td>
<td>Infant cereal</td>
</tr>
<tr>
<td>Ferrous saccharate</td>
<td>74</td>
<td>Infant cereal, chocolate drink powders</td>
</tr>
<tr>
<td>Ferric pyrophosphate</td>
<td>21-74</td>
<td>Infant cereal, chocolate drink powders, rice</td>
</tr>
<tr>
<td>Ferric orthophosphate</td>
<td>25-31</td>
<td>Infant cereals</td>
</tr>
<tr>
<td>Elemental iron</td>
<td>5-90</td>
<td>Wheat flour, breakfast cereal, infant cereal</td>
</tr>
</tbody>
</table>

From Table 3, it is evident that iron fortificants that are soluble in water or dilute acids are most absorbable. However, their solubility carries the disadvantage that they cause oxidative reactions in the food used as the fortification vehicle (e.g., cereals) and subsequent undesirable organoleptic changes such as discoloration and off flavors (rancidity). While ferrous fumarate is a relatively good compound for fortification, it causes color problems in off-white foods (e.g., those containing bananas). It also promotes fat oxidation during storage and is, therefore, unsuitable for fortifying wheat or other flours. Desiccated ferrous sulfate is used in low-acid foods and infant formulas. Although the RBV of iron from ferric pyrophosphate and ferric orthophosphate is relatively poor, these forms of iron are often added to cereal products and dry milk because they interact less with food components. Ferrous lactate presents a problem when it is added to dry foods, as it absorbs moisture. Elemental iron is added to cereal products as reduced, electrolytic, or carbonyl forms. Unfortunately, published values for the RBV of elemental iron range from 5 to 90 percent, with the higher absorption values obtained when the iron particles are uniform and small in size and more soluble in dilute hydrochloric acid (Hallberg et al. 1986a). Because of the inconsistency in these characteristics, elemental iron is not currently considered a dependable source of bioavailable fortificant iron (Hallberg et al. 1986a, 1986b; Hurrell 1992). Electrolytic iron has a mean particle size of about 8 μm. It is probably the most reliable source of elemental iron for food fortification. Although its RBV is only about 50 percent it has been shown to contribute substantially to the prevention of iron deficiency anemia when used to fortify infant cereals, if high enough quantities are added and fed with ascorbic acid (Walter et al. 1993a).

The iron content of drinking water is high in some locations. Because of the universal consumption of water, adding iron to it might be a feasible approach to improve the iron status of households or population groups. This idea was tested in a Brazilian preschool. Ferrous sulfate crystals were added to a common drinking pot to increase the iron content of the water to 20 mg/L. Within five months, the children's mean hemoglobin concentration had increased from 10.6 g/L to 12.1 g/L (no control group was used) (Dutra de Oliveira et al. 1994). This would appear to be a practical approach to improving the iron status of groups drinking water from a common source, using bioavailable forms of iron such as ferrous sulfate, ferrous citrate, or ferrous fumarate. Presumably some drinking water is consumed between meals, which would reduce the potentially adverse effect of dietary constituents on absorption of its iron content.
The effect of dietary constituents on the bioavailability of fortificant iron

Dietary composition can be a more important determinant of fortificant iron absorption than the type of fortificant itself. This has been assessed by measuring the bioavailability of fortificant iron from various vehicles (foods/meals), labeling it with an extrinsic tag, and using ferrous sulfate as a reference. For example, when 5 or 10 mg iron (ferric chloride) was added to a maize meal containing no meat, the increased amount of iron absorbed was only about 0.1 mg (Layrisse et al. 1974). About 0.2 mg of the 5 mg iron (FeSO₄) added to a Thai basal meal (rice and vegetables) was absorbed, and this increased to about to 0.5 mg when the meal also contained 60 mg fish (Hallberg et al. 1978) (Figure 2). The significance of these data is that the bioavailability of fortificant iron is poor if it is consumed in the presence of absorption inhibitors and/or in the absence of absorption promoters. In this situation it is necessary to improve the bioavailability of fortificant iron using the same approaches for improving the bioavailability of native iron in the diet.

Figure 2: Increase in Iron Absorption from Different Types of Meals Fortified with Ferrous Sulfate

Assuming that the native iron content of diets in some developing countries is relatively high, that the diets are high in inhibitors, and that the iron fortificants mentioned above are as poorly absorbed as the native iron, it would probably be more effective to find sustainable approaches to improve the absorption of native iron than it would be to add fortificant iron. Alternatively, the bioavailability of the fortificant iron needs to be better than that of ferrous sulfate if it is to be consumed with diets containing substantial...
amounts of inhibitors. At present there are only three fortificants that have this potential: sodium iron ethylenediaminotetraacetate (NaFeEDTA), ferrous bisglycinate, and hemoglobin.

**NaFeEDTA as an iron fortificant**

The valuable properties of NaFeEDTA as an iron fortificant have been described in the literature for about 25 years. When mixed with food at fortification levels, not only is the iron in NaFeEDTA absorbed better than other nonheme forms of iron, but it has the advantage of making the total nonheme iron pool, including that from food, as absorbable as the iron in NaFeEDTA, i.e., 2.5 times higher than that from ferrous sulfate added to foods such as a milk-rice-sugar formula for infants (Ballot et al. 1989), or a standard meal containing beans, tortillas, bread, and coffee in Guatemala (Viteri et al. 1977). Table 4 shows the very strong inhibitory effect of cereals on the absorption of iron from added ferrous sulfate, and their smaller effect on iron added as NaFeEDTA (Martinez-Torres et al. 1979; Hurrell 1992; INACG 1993). Similarly, bran decreased iron absorption from ferrous sulfate 11-fold, but had no effect on iron absorption from NaFeEDTA (MacPhail et al. 1981). Only tannins in tea were potent enough inhibitors of iron absorption to cause a seven-fold decrease in the absorption of iron from NaFeEDTA (MacPhail et al. 1981). In summary, when the diet contains inhibitors, iron in NaFeEDTA is two to three times more available than from ferrous salts, and an absorption of 8 to 10 percent of both food iron and NaFeEDTA can be expected. However, iron absorption from NaFeEDTA is similar to that for ferrous sulfate in meals that contain substantial amounts of ascorbic acid or meat (INACG 1993). Where food is fortified with 10 mg iron as NaFeEDTA, the iron absorption of iron deficient individuals should be increased by more than 0.8 mg/day (INACG 1993).

**Table 4: Percent Absorption and Relative Bioavailability of Iron from Ferrous Sulfate and NaFeEDTA When Added to Foods**

<table>
<thead>
<tr>
<th>Food</th>
<th>Ferrous sulfate (A)</th>
<th>NaFeEDTA (B)</th>
<th>Relative Bioavailability (B/A)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Refined sugar</td>
<td>38</td>
<td>11</td>
<td>0.3</td>
</tr>
<tr>
<td>Sugar cane syrup</td>
<td>33</td>
<td>11</td>
<td>0.3</td>
</tr>
<tr>
<td>Sweet manioc</td>
<td>14</td>
<td>17</td>
<td>1.2</td>
</tr>
<tr>
<td>Wheat</td>
<td>6</td>
<td>15</td>
<td>2.3</td>
</tr>
<tr>
<td>Egypt flatbread</td>
<td>2</td>
<td>5</td>
<td>2.5</td>
</tr>
<tr>
<td>Rice</td>
<td>4</td>
<td>12</td>
<td>2.9</td>
</tr>
<tr>
<td>Maize porridge</td>
<td>4</td>
<td>7</td>
<td>2.1</td>
</tr>
<tr>
<td>Beans, plantain, rice, maize, soy</td>
<td>3</td>
<td>7</td>
<td>2.3</td>
</tr>
</tbody>
</table>

*Source: Data summarized by INACG 1993*

Because EDTA acts by forming a chelate with iron, there has been some concern that the absorbed iron would not be utilizable, and that it might be excreted in urine along with other trace minerals such as zinc. In fact, only 5 percent of the EDTA is absorbed and appears in urine, while the other 95 percent is
unabsorbed and excreted in feces (Candela et al. 1984). The absorption of a stable isotope of zinc from a bread meal increased from 21 percent with added ferrous sulfate to 34 percent with added NaFeEDTA, while urinary zinc losses were increased only slightly (Davidsson et al. 1994b). Calcium absorption was unaffected by the EDTA salt. Thus, adding NaFeEDTA might increase both iron and zinc absorption from foods.

The binding of metal ions by EDTA depends on the relative affinity of the ion to EDTA. The stability constant is highest for Fe$^{3+}$, followed by Cu$^{2+}$, Zn$^{2+}$, Fe$^{2+}$, Ca$^{2+}$, and Mg$^{2+}$. In fact, the addition of EDTA, Na$_2$EDTA or CaNa$_2$EDTA, which are widely used as food additives in many developed countries, should be equally effective in enhancing the absorption of native iron in foods that have a high iron content, even in the presence of inhibitors such as phytate (Davidsson et al. 1994b). For example, the addition of EDTA itself prevented the inhibitory effect of Egyptian flatbread, made with high-extraction wheat flour, on the absorption of ferrous sulfate that had been added as a fortificant (El Guindi et al. 1988).

The efficacy of NaFeEDTA for improving iron status has been demonstrated in several community trials. In Guatemala it was tested as a sugar fortificant (Viteri et al. 1995). At a concentration of 1 g FeNa$_2$EDTA/kg sugar, fortification increased iron stores significantly, an effect that was apparent by eight months and was still increasing 32 months after the intervention started. While details were not provided, it was reported that there were no detrimental effects of the fortified sugar on morbidity, or on plasma or urinary excretion of trace elements. NaFeEDTA also successfully improved hemoglobin concentrations when incorporated as a fortificant into curry powder in South Africa (Ballot et al. 1989) and into fish sauce in Thailand (Garby and Areekul 1974).

NaFeEDTA has not been approved as a food additive in the United States or other countries. This is based on concerns that dietary EDTA levels are already high; EDTA, Na$_2$EDTA and CaNa$_2$EDTA are used as preservatives in many countries in Europe, Asia, North and South America, Africa, and Australia. The acceptable daily intake (ADI) of EDTA is 150 mg/person/day or 2.5 mg/kg/day. In 1992 the mean overall exposure to EDTA in the United States was 15 mg/person/day, which suggests that the use of NaFeEDTA as a fortificant may be possible in the United States (INACG 1993) and especially in developing countries where usual intakes are lower. One concern is the potential for high EDTA intakes per kg body weight in young children; if a young child consumed 5 mg iron/day from NaFeEDTA, this would provide twice the current ADI for EDTA (Hurrell 1992). EDTA causes severe birth defects when fed to pregnant rats at very high levels, because at these dietary concentrations it interferes with zinc absorption and utilization; zinc supplementation of the mother during pregnancy abolishes the adverse effects (Swenerton and Hurley 1971). Nephrotoxicity can result from the very high doses used to treat metal poisoning (INACG 1993). It is doubtful that there are harmful effects of EDTA at the levels it would be consumed as a fortificant; however, a review of the ADI for EDTA, and of current legislation, is probably warranted.

**Ferrous bisglycinate as a potential iron fortificant**

The potential of ferrous bisglycinate as an iron fortificant has been recognized relatively recently. It is formed by the binding of one molecule of ferrous iron to the alpha-amino and carboxyl portion of two molecules of glycine, so that the iron is shared with two heterocyclic rings. It remains to be confirmed whether the absorption of its iron is affected by the presence of inhibitors or promoters of iron absorption. Absorption is more efficient in individuals with lower hemoglobin concentrations (Piñeda 1994; Name
1995) but further studies are needed to ensure that absorption of iron from this molecule is not excessive in non-anemic individuals, i.e., that absorption is adequately down-regulated once iron stores are replete. Ferrous bisglycinate is currently used in foods for humans in Canada, Japan, Western and Central Europe, and Brazil, and a human grade product is commercially available in the United States.

Ferrous bisglycinate providing 30 mg iron per day for four weeks improved hemoglobin and ferritin concentrations in anemic Guatemalan adolescents (Pineda et al. 1994). While the authors claim that the amino acid chelate was four times more effective than ferrous sulfate — 30 mg of the chelated iron producing the same effect as 120 mg of iron as ferrous sulfate — this claim would have been more credible had 30 mg of iron been compared from both sources. Name (1995) added ferrous bisglycinate to cow’s milk after homogenization, and provided one liter of milk per day containing 3 mg iron as ferrous bisglycinate to 6 to 24 month old children in Brazil. Anemia prevalence was halved on average by 12 months later, and reduced even further in those who did not share the milk with their family. Estimated iron absorption was 40 percent. Ferritin concentrations were not measured and there was no control group.

Further work is needed to ensure the safety and efficacy of ferrous bisglycinate in various conditions. Preliminary reports (Name 1995) claim that it is non-reactive with fat and does not promote rancidity of cereals, margarine or milk; causes no objectionable changes in taste, color, or smell; and may be cheaper than other fortificants when the poor efficiency of absorption of most iron fortificants from cereals is considered. It may be possible to add both ferrous bisglycinate and vitamin A to sugar without degrading vitamin A. There is a need to conduct and publish more peer-reviewed studies of the regulation of iron absorption and metabolism following consumption of this molecule, as well as the bioavailability of its iron in the presence of various food constituents.

**Hemoglobin and dried blood as iron fortificants**

The absorption of hemoglobin iron is not affected by other dietary constituents except calcium. Bovine hemoglobin concentrate can be made from cow’s blood at a reasonable price, and has been used with some success as an iron fortificant in Chile. The hemoglobin was incorporated into cookies given to school children, to supply 5 mg iron/day, 20 percent of which was absorbed (Walter et al. 1993b). In infant cereals, 14 percent of added hemoglobin was absorbed (Calvo et al. 1989). There are serious limitations to using hemoglobin as a source of fortificant iron, including its taste, dark color, and relatively low iron content; technical and potential contamination problems involved in production; as well as cultural and religious prohibitions on its use. However, foods high in hemoglobin such as blood pudding and other sausages may be useful sources of highly bioavailable iron in some situations.

**Bioavailability of contaminant iron**

Dietary iron often contains substantial amounts of “contaminant” iron, which usually enters food during collection and storage (dirt, soil), preparation (from cooking pots), or processing (milling). The amount of contaminant iron can be estimated from the difference between the total iron content of individual foods (calculated from food composition tables or measured prior to processing/preparation), and the actual iron content of consumed food measured by chemical analysis. Measuring the bioavailability of contaminant iron requires labeling it with a tracer. Because the exchangeability of the contaminant iron with the tracer
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varies with the source of the contaminant iron, and cannot be easily predicted, the bioavailability of contaminant iron remains largely unknown.

There is relatively little information on the bioavailability of iron from soils, but such iron is probably extremely variable. For example, the exchangeability of the contaminant iron in an in vitro digestion assay (simulating its capacity to join the nonheme iron pool in vivo) ranged from zero for iron in red soil to about 35 percent in clay from Thailand (Hallberg and Bjorn-Rasmussen 1981).

The fact remains that the proportion of the contaminant iron that is exchangeable and joins the common nonheme iron pool is still subject to the presence of the same enhancers and inhibitors affecting the absorption of the native iron present in foods. However, under optimal conditions of food preparation, such as acidic pH, and the presence of enhancers, contaminant iron could offer a useful source of bioavailable iron as illustrated by the following examples.

Cooking food in iron pots can increase iron intake considerably. For example, in China, cooking acidic foods in iron pots increased the iron intake of adults by 14.5 mg/day, and that of children by 7.4 mg/day (Liu et al. 1990). Using an in vitro method, the metallic iron that enters food in this way was found to be as available as the intrinsic iron in the food (Mistry et al. 1988). An initially iron-free diet cooked in an iron pot repleted hemoglobin in iron-depleted rats as effectively as a diet with added ferrous sulfate (Martines and Vannucchi 1986).

The more acidic the food, the higher the moisture content, and the longer the cooking time, the more iron is dissolved from iron pots (Brittin and Nossaman 1986). The Bantu tribe in Africa consumes a large amount of iron from traditional alcoholic beverages brewed in iron containers at acidic pH. Both the pH and the alcohol renders this extraneous iron more bioavailable (Charlton et al. 1973). Wheeler et al. (1994) tested the usefulness of adding pieces of iron wire to water stored in glass, cement, or clay containers. The iron entered the water from the wire only when the pH of the water fell below 3.5. At this pH, the appearance and taste of the water were acceptable after 12 and 24 hours. This level of acidity could be achieved by adding 100 mL lemon juice per liter of water, which reduces microbial growth as well. The water contained over 5 mg/L iron after 12 hours, and more than 8 mg/L after 24 hours. The metallic iron that was dissolved into the water was as bioavailable as ferrous sulfate or ascorbate, when tested in animal models. The alkaline pH of new cement pots prevented the iron wire from dissolving; whether this would happen in older pots is uncertain.

Thus, the promotion of iron pots for food preparation under acidic conditions (e.g., with tomatoes, lemons, tamarind) could be a useful public health strategy to enhance iron intake, although it would be fairly unrealistic in most situations.

Host Factors

A variety of host factors such as the iron status of the individual, enhanced requirements for growth and during pregnancy, and some types of morbidity, affect the absorption of dietary and fortificant iron.
Iron status

The absorption of nonheme iron is markedly affected by iron status as iron-deficient individuals absorb iron more efficiently than those who are iron-replete. The serum ferritin concentration has been shown to reflect body iron stores in the range of 20 to 200 μg/L; 1 μg serum ferritin per liter is roughly equivalent to 8 mg of storage iron (Baynes and Bothwell 1990). There is an inverse relationship between serum ferritin and nonheme iron absorption (Hallberg 1981b). As iron deficiency develops, body iron stores are utilized, serum ferritin concentration falls, and nonheme iron absorption is increased.

Because iron absorption is inversely related to the host’s iron status, studies of iron bioavailability should include a standard reference dose (consisting of 3 mg iron as ferrous ascorbate) and express the absorption of iron from the test meal as a ratio to absorption from the reference dose. Iron deficient individuals (i.e., those with no iron stores, but who have not yet developed anemia) usually absorb more than 40 percent of the reference dose (Hallberg 1981b). Thus, the problem of differences in iron status between subjects and between studies can be partially overcome by including a reference dose, and expressing the iron absorption from test meals as the absorption value that corresponds to a reference dose absorption of 40 percent. Hultén et al. (1995), however, showed that differences in iron absorption from diets that differ in bioavailability may be clear in iron deficient individuals but obscured when iron stores are adequate. A reference dose is less important if the purpose is to only compare iron absorption from one meal with that from a second meal in the same individual.

Other micronutrient deficiencies

Several micronutrients can affect iron absorption and/or utilization. Vitamin A deficiency, which is highly prevalent in many developing countries, impairs the utilization of iron for hemoglobin synthesis, in part by trapping iron in the liver and spleen. While iron absorption is probably not affected, the usual 80 percent or 100 percent (in the case of iron deficiency) assumptions about the efficiency of utilization of absorbed iron for hemoglobin synthesis may be overestimates. Also, in vitamin A deficiency, dietary or other interventions intended to increase iron absorption are less likely to increase hemoglobin concentration, and iron plus vitamin A may be needed for the most effective treatment of anemia (Suharno et al. 1993).

Although its epidemiology is inadequately documented, riboflavin deficiency is relatively common in parts of Latin America, Africa, and Asia. In animal studies this deficiency impaired iron absorption by altering the turnover of intestinal cells, while in humans only a change in iron utilization for hemoglobin or ferritin synthesis could be detected (Fairweather-Tait et al. 1992). While iron absorption is little affected by zinc deficiency, it can be reduced by high zinc intakes (over 50 mg/day) from supplements (Yadrick et al. 1989). Both vitamin B_{12} and folic acid deficiency can impair the utilization of iron for hemoglobin synthesis, but do not affect iron absorption. Again, the implication is that the conventional assumptions about the efficiency of iron utilization for hemoglobin synthesis will be overestimated in the presence of these deficiencies, and interventions to improve iron absorption alone may be less effective at increasing hemoglobin concentration than those that address multiple micronutrient deficiencies.
Physiological status: growth, pregnancy, and lactation

The bioavailability of nonheme dietary or supplementary iron nearly doubles during pregnancy in response to increased iron requirements (Institute of Medicine 1990). Iron bioavailability from breast milk is approximately 50 percent, compared with 10 percent from cow’s milk and infant formula (Lonnerdal 1984). Feeding solid foods reduced iron absorption from breast milk fed to adults (Oski and Landaw 1980). Feeding whole cow’s milk, compared with fruit juice, reduced the absorption of iron from a ferrous sulfate supplement given to one-year-old infants (Abrams et al. 1994). It is difficult for infants to meet their iron requirements from foods. Presumably the too early introduction of foods that inhibit iron absorption from breast milk could be a major factor in determining the age of onset of iron deficiency, although this remains to be determined. While iron absorption probably increases during the growth spurt of adolescence, especially with the onset of menstruation in females, there are few quantitative data on this subject. The dearth of data on the above issues is primarily due to the fact that radioisotopes cannot be used to study iron absorption at these stages of the life span. The recent improvements in measuring bioavailability with stable isotope techniques should permit more studies on these vulnerable population groups (Abrams et al. 1994).

Morbidity

Acute and chronic infections affect the traditional laboratory measurements of iron status independently of the individual’s iron status, and can confound the interpretation of these tests (Hallberg 1981b; Ahluwalia et al. 1995). For instance, serum ferritin increases in response to infection yet there is some evidence that iron absorption may be reduced during infection (Hallberg 1981b). This must be borne in mind in population studies where minor infections are rampant and can confound the interpretation of laboratory tests of iron status. The simultaneous measurement of indicators of infection, such as C-reactive protein or α-2 macroglobulin in serum, may help to identify individuals who are currently infected (Brown et al. 1993).

Parasitic infestation can also affect iron absorption. There is significant intestinal blood loss related to infestation with hookworm (*Ancylostoma duodenale, Necator americanus*), schistosomiasis, and heavy infestation with whip worm (*Trichuris trichiura*) (Baker and de Maeyer 1979), which can lead to iron deficiency and thereby increase the efficiency of iron absorption secondarily. Furthermore, infestation with round worms (*Ascaris lumbricoides*) and *Giardia lamblia* are commonly associated with general malabsorption and may impair iron absorption as well. Treatment of Mexican school children for *Giardia* and *Ascaris* significantly increased their hemoglobin and plasma ferritin concentrations (Mejia et al. 1989). Because reinfection with parasites occurs rapidly in unsanitary environments, frequent treatments are needed in order to see a sustained improvement in the iron status of a population.
BIOAVAILABILITY OF HEME IRON

Host Factors
Heme iron is absorbed by intestinal mucosal cells as the intact iron-porphyrin complex. In these cells it is split by a specific enzyme and, following the same pathway as nonheme iron, leaves the mucosal cells and enters the plasma (Hallberg 1981b). Compared with nonheme iron, the absorption of heme iron is much less influenced by the iron status of the subject. If a meal contains a usual amount of heme iron (5 mg or less), the subject’s iron status does not influence heme iron absorption (Hallberg 1981b). However, if the heme iron content of the test meal is as high as in blood sausage (which may contain up to 50 mg heme iron), heme absorption is inversely related to iron status (Monsen et al. 1978; Hallberg 1979). Heme iron absorption can be calculated from the equation $y = 3.3x^{0.49}$, where $y =$ percent absorption of iron and $x =$ the measured percent absorption of iron from a reference dose of ferrous sulfate (Taylor et al. 1988).

Dietary Factors
The bioavailability of heme iron is high (12 to 26 percent) and somewhat influenced by dietary composition. While iron from heme alone is poorly absorbed (Bothwell et al. 1989), when supplied as hemoglobin (Bothwell et al. 1989) or in meat (Hallberg et al. 1979) absorption is good. It is likely that the proteins in meat and hemoglobin prevent the formation of poorly absorbed heme complexes (Bothwell et al. 1989). Heme iron absorption is not affected by dietary constituents such as phytate, tannins, and ascorbic acid. However, consuming calcium-rich foods (milk, cheese) can impair heme as well as nonheme iron absorption from a meal (Gleerup et al. 1995). As discussed above, baking and prolonged frying can reduce heme iron absorption by 40 percent (Bothwell et al. 1989).
RELATIVE IMPACT OF POTENTIAL INTERVENTIONS TO IMPROVE DIETARY IRON AVAILABILITY

This section summarizes the quantitative impact of studies that have tested the impact of dietary interventions on iron bioavailability. The aim is to synthesize the available information on test meals in a manner that is useful for planning effective interventions. The studies are grouped by dietary staple, i.e., maize-, rice- and wheat-based meals, and by type of intervention within each of these categories, i.e., increasing ascorbic acid intake with synthetic ascorbic acid or with fruits/vegetables; adding meat/fish/poultry; adding tea, etc. The data for maize-based meals are presented in detail in Table 5, while those for rice-based meals and wheat-based meals are presented in Tables 7 and 9, respectively. Figure 3 summarizes the results graphically. Each point on Figure 3 is the absorption ratio averaged from all studies conducted at each level of ascorbic acid intake. For example, average absorption ratios are provided for studies comparing iron absorption from rice, or wheat, or maize, in the absence or presence of 50 mg ascorbic acid.

The columns in Tables 5, 7, and 9 describe, from left to right: the authors and test meals; the intervention (amount and form of ascorbic acid [AA] added, or amount and form of meat/fish/poultry [MFP] added, or amount of citrate added, or amount of tea added); the number (N) of subjects; iron status (NL = normal, ID = iron deficient, IDA = iron deficient and anemic); the amount of intrinsic and added nonheme iron and the amount of heme iron in the meal; the absorption of the reference dose of iron; the actual absorption of nonheme iron as well as the absorption adjusted to correspond to a 40 percent iron absorption from the reference dose; and the absorption ratio (the ratio of adjusted absorption with and without the modifier). An absorption ratio of 2.0, for example, means that the intervention doubled nonheme iron absorption. In some studies, the investigators evaluated absorption before and after the intervention in the same subject, thus they did not adjust the data to a reference dose; this is recorded as “not applicable” (N/A) in the tables. The data points in Figure 3 are the mean absorption ratios for all studies, at each level of ascorbic acid intake.

Maize-based Meals

Studies on maize-based meals are presented in Table 5 and Figure 3. Maize and maize-based diets usually contain more phytate than wheat- (except where wheat is consumed only as whole wheat) or rice-based diets. In the majority of interventions, ascorbic acid (AA) was added to improve nonheme iron absorption. In many studies, nonheme iron was also added to the meal to increase total iron intake. This was done to improve the measurement precision, assuming that the added iron has the same bioavailability as intrinsic nonheme iron in the maize (based on the common pool concept). From Table 5, it can be seen that the actual absorption of intrinsic iron from maize averaged 3.7 percent (range 1.2 to 8.5 percent, studies C1a, CIIa, CIIIa, CIVa, CVa, Dla, Ela, EIIa, EIVa, Fila, Glia, and Hla) in subjects with adequate iron status, and 5.3 percent (6.8 percent in study AI and 3.8 percent in study BI) in those who were iron-deficient or anemic.
The first four studies (A-D) tested the effect of adding up to 200 mg of ascorbic acid per meal to maize alone, prepared as a porridge. The increase in adjusted nonheme iron absorption was about 130 percent with 12.5 mg added ascorbic acid (CIb), 210 to 300 percent with 25 mg (CIIb, EIIb), 500 percent with 50 mg (All, BII, CIIIb, EIIIb), and 800 percent with 65 to 100 mg (BIII, BIV, CIVb, DIIb, EIIb). Above 100 mg ascorbic acid there was relatively little additional benefit (CVb, EIVb). Adding the ascorbic acid as papaya (from which 66 mg ascorbic acid increased absorption by 630 percent, DIIc), or as cauliflower (an absorption increase of 300 percent with 65 mg ascorbic acid, EIIb) was at least as effective as using crystalline ascorbic acid. When the maize was treated with calcium and consumed as tortillas with rice and beans (a typical Latin American meal, Study E), absorption of iron was slightly lower than from maize alone (mean 2.6 percent, range 1.2 to 3.6 percent in studies Ela through EIVa) with no added ascorbic acid. The relative effect of adding ascorbic acid was reduced but still substantial, namely a 210 percent increase with 25 mg (EIIb) and a 240 percent increase with 50 mg (EIIIb).

Adding 100 g of fish as well as 66 mg ascorbic acid as papaya did not improve nonheme iron absorption more than the papaya alone (FIIc). Adding 75 g of ground beef to the Latin American meal had the same effect on nonheme iron absorption as adding about 50 mg ascorbic acid (GIIb). (The iron absorbed from heme was not estimated in these studies because only nonheme iron was labeled.) Citrate actually reduced iron absorption from the Latin American meal by 50 percent (HIb) and 5 g tea plus milk reduced absorption from maize porridge by 40 percent (Study I, Ib).

The information in Table 5 can also be used to estimate the amount of nonheme iron that would be absorbed in each case. The reported intrinsic iron content (column 5) ranged from 0.5 to 4.7 mg iron/100 g dry maize. For the purpose of these calculations, a value of 2.0 mg/100 g dry maize, based on United States Department of Agriculture data, has been used. Based on the values for the bioavailability of nonheme iron in Table 5 and Figure 3, Table 6 shows the amounts of nonheme iron that would be absorbed from 40 to 50 g dry maize prepared as a porridge, or from a Latin American meal. It is assumed that the porridge contains 1 mg iron, and the absorption of 4.5 percent, with zero ascorbic acid is increased 500 percent with 50 mg, and 800 percent with 100 mg ascorbic acid. Iron absorption from the Latin American meal is 2.6 percent (Study E), increasing to 240 percent with 50 mg ascorbic acid.
Table 5: Strategies to Increase Iron Absorption from Maize-based Meals

<table>
<thead>
<tr>
<th>Author/Test Meal</th>
<th>Intervention</th>
<th>N</th>
<th>Iron Status</th>
<th>Diet Iron (mg)</th>
<th>Reference Dose Abs (percent)</th>
<th>Nonheme Iron Abs (percent)</th>
<th>Abs Ratio with/without modifier</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Native</td>
<td>Added</td>
<td>Nonheme</td>
<td>Heme</td>
<td></td>
</tr>
<tr>
<td><strong>MAIZE MEALS</strong></td>
<td><strong>+ ASCORBIC ACID (AA)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. Sayers et al. 1973, S. Africa.</td>
<td>I. 0 mg AA</td>
<td>77</td>
<td>IDA</td>
<td>1.8</td>
<td>0.0</td>
<td>0.0</td>
<td>55.8</td>
</tr>
<tr>
<td></td>
<td>II. 50 mg AA (added prior to cooking; 68 percent AA left in reduced form after cooking)</td>
<td></td>
<td>IDA</td>
<td>1.8</td>
<td>0.0</td>
<td>0.0</td>
<td>48.9</td>
</tr>
<tr>
<td></td>
<td>B. Derman et al. 1977, S. Africa.</td>
<td>I. 0 mg AA</td>
<td>22</td>
<td>ID</td>
<td>2.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>II. 50 mg AA</td>
<td></td>
<td>ID</td>
<td>2.0</td>
<td>0.0</td>
<td>0.0</td>
<td>42.1</td>
</tr>
<tr>
<td></td>
<td>III. 100 mg AA</td>
<td></td>
<td>ID</td>
<td>2.0</td>
<td>0.0</td>
<td>0.0</td>
<td>44.2</td>
</tr>
<tr>
<td></td>
<td>IV. 100 mg AA</td>
<td></td>
<td>IDA</td>
<td>2.0</td>
<td>0.0</td>
<td>0.0</td>
<td>51.2</td>
</tr>
<tr>
<td>C. Bjorn-Rasmussen and Hallberg 1974, Sweden.</td>
<td>Ia. 0 mg AA</td>
<td>6</td>
<td>NL</td>
<td>0.5</td>
<td>4.5</td>
<td>0.0</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>Ib. 12.5 mg AA</td>
<td></td>
<td>NL</td>
<td>0.5</td>
<td>4.5</td>
<td>0.0</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>IIa. 0 mg AA</td>
<td></td>
<td>NL</td>
<td>0.5</td>
<td>4.5</td>
<td>0.0</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>IIb. 25 mg AA</td>
<td></td>
<td>NL</td>
<td>0.5</td>
<td>4.5</td>
<td>0.0</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>IIIa. 0 mg AA</td>
<td></td>
<td>NL</td>
<td>0.5</td>
<td>4.5</td>
<td>0.0</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>IIIb. 50 mg AA</td>
<td></td>
<td>NL</td>
<td>0.5</td>
<td>4.5</td>
<td>0.0</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>IVa. 0 mg AA</td>
<td></td>
<td>NL</td>
<td>0.5</td>
<td>4.5</td>
<td>0.0</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>IVb. 100 mg AA</td>
<td></td>
<td>NL</td>
<td>0.5</td>
<td>4.5</td>
<td>0.0</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>Va. 0 mg AA</td>
<td></td>
<td>NL</td>
<td>0.5</td>
<td>4.5</td>
<td>0.0</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>Vb. 200 mg AA</td>
<td></td>
<td>NL</td>
<td>0.5</td>
<td>4.5</td>
<td>0.0</td>
<td>N/A</td>
</tr>
<tr>
<td>D. Layrisse et al. 1974, Venezuela.</td>
<td>Ia. 0 mg AA</td>
<td>13</td>
<td>NL</td>
<td>?</td>
<td>?</td>
<td>0.0</td>
<td>19.9</td>
</tr>
<tr>
<td></td>
<td>Ib. 70 mg AA</td>
<td></td>
<td>NL</td>
<td>?</td>
<td>?</td>
<td>0.0</td>
<td>7.9</td>
</tr>
<tr>
<td></td>
<td>Ic. 66 mg AA as 150 g papaya</td>
<td></td>
<td>NL</td>
<td>?</td>
<td>?</td>
<td>0.0</td>
<td>8.8</td>
</tr>
</tbody>
</table>
### MAIZE MEALS + ASCORBIC ACID (AA)

<table>
<thead>
<tr>
<th>Author/Test Meal</th>
<th>Intervention</th>
<th>N</th>
<th>Iron Status</th>
<th>Diet Iron (mg)</th>
<th>Reference Dose Abs (percent)</th>
<th>Nonheme Iron Abs (percent)</th>
<th>Abs Ratio with/without modifier</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. Hallberg et al. 1984, 1986, Sweden.</td>
<td>Ia. 0 mg AA</td>
<td>10</td>
<td>NL</td>
<td>4.4</td>
<td>0.0</td>
<td>0.0</td>
<td>30.4</td>
</tr>
<tr>
<td></td>
<td>lb. 65 mg AA as 25 g boiled cauliflower</td>
<td></td>
<td></td>
<td>5.4</td>
<td>0.0</td>
<td>0.0</td>
<td>7.7</td>
</tr>
<tr>
<td></td>
<td>IIa. 0 mg AA</td>
<td>10</td>
<td></td>
<td>4.3</td>
<td>0.0</td>
<td>0.0</td>
<td>34.7</td>
</tr>
<tr>
<td></td>
<td>IIb. 25 mg AA</td>
<td>10</td>
<td></td>
<td>4.3</td>
<td>0.0</td>
<td>0.0</td>
<td>36.0</td>
</tr>
<tr>
<td></td>
<td>IIIa. 0 mg AA</td>
<td>10</td>
<td></td>
<td>4.3</td>
<td>0.0</td>
<td>0.0</td>
<td>3.4</td>
</tr>
<tr>
<td></td>
<td>IIIb. 50 mg AA</td>
<td>9</td>
<td></td>
<td>4.3</td>
<td>0.0</td>
<td>0.0</td>
<td>37.3</td>
</tr>
<tr>
<td></td>
<td>IVa. 0 mg AA</td>
<td></td>
<td></td>
<td>4.3</td>
<td>0.0</td>
<td>0.0</td>
<td>8.4</td>
</tr>
<tr>
<td></td>
<td>IVb. 500 mg AA</td>
<td></td>
<td></td>
<td>4.3</td>
<td>0.0</td>
<td>0.0</td>
<td></td>
</tr>
</tbody>
</table>

### MAIZE MEALS + MFP ± AA

<table>
<thead>
<tr>
<th>Author/Test Meal</th>
<th>Intervention</th>
<th>N</th>
<th>Iron Status</th>
<th>Diet Iron (mg)</th>
<th>Reference Dose Abs (percent)</th>
<th>Nonheme Iron Abs (percent)</th>
<th>Abs Ratio with/without modifier</th>
</tr>
</thead>
<tbody>
<tr>
<td>F. Layrisse et al. 1974, Venezuela.</td>
<td>Ia. 0 mg AA</td>
<td>14</td>
<td>NL</td>
<td>?</td>
<td>?</td>
<td>?</td>
<td>36.9</td>
</tr>
<tr>
<td></td>
<td>lb. 66 mg AA (150 g papaya)</td>
<td></td>
<td></td>
<td>24.7</td>
<td>26.8</td>
<td>5.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>lc. 66 mg AA (150 g papaya) + 100 g MFP (fish)</td>
<td></td>
<td></td>
<td>23.0</td>
<td>24.9</td>
<td>5.0</td>
<td></td>
</tr>
<tr>
<td>G. Hallberg et al. 1984, Sweden.</td>
<td>Ia. 0 mg MFP</td>
<td>9</td>
<td>NL</td>
<td>4.7</td>
<td>0.6</td>
<td>0.0</td>
<td>38.9</td>
</tr>
<tr>
<td></td>
<td>lb. 100 g MFP (75 g ground beef, grilled)</td>
<td></td>
<td></td>
<td>5.3</td>
<td>0.0</td>
<td>0.7</td>
<td>8.0</td>
</tr>
<tr>
<td>Author/Test Meal</td>
<td>Intervention</td>
<td>N</td>
<td>Iron Status</td>
<td>Diet Iron (mg)</td>
<td>Reference Dose Abs (percent)</td>
<td>Nonheme Iron Abs (percent)</td>
<td>Abs Ratio with/without modifier</td>
</tr>
<tr>
<td>------------------</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Native</td>
<td>Added</td>
<td>Actual</td>
<td>Adj*</td>
</tr>
<tr>
<td><strong>MAIZE MEALS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+ CITRATE</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H. Hallberg et al.</td>
<td>0 g citrate</td>
<td>10</td>
<td>NL</td>
<td>4.3</td>
<td>0.0</td>
<td>0.0</td>
<td>36.9</td>
</tr>
<tr>
<td></td>
<td>1 g dry citrate (mixed with black beans)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td><strong>MAIZE MEALS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- TEA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I. Derman et al.</td>
<td>Without tea</td>
<td>22</td>
<td>ID</td>
<td>2.0</td>
<td>0.0</td>
<td>0.0</td>
<td>50.5</td>
</tr>
<tr>
<td></td>
<td>With tea (5 g leaves to make 150 mL, plus 10 mL milk)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations used in the table:

- Adj = nonheme iron absorption (actual) adjusted to correspond to iron absorption of 40 percent from reference dose. In most cases this was calculated as: Adj = actual nonheme iron absorption (percent) X 40 / reference dose absorption (percent). When adjusted values were reported by authors, these values are used and indicated by R.

- AA = ascorbic acid in crystalline form unless otherwise noted.

- Abs Ratio = absorption ratio with/without modifier, calculated using adjusted nonheme iron absorption values when available, or else with actual absorption values. When absorption ratio was reported by authors those values were used and indicated by R.

- Iron Status: iron status of the study subjects; NL = normal, ID = iron deficient, IDA = iron deficient anemic.

- N = number
Figure 3: Effect of Ascorbic Acid on Nonheme Iron Absorption from Cereal-based Meals

Table 6: Effect of Ascorbic Acid on the Amount of Nonheme Iron Absorbed from Maize and a Latin American Meal

<table>
<thead>
<tr>
<th>Amount of ascorbate added (mg)</th>
<th>Amount of nonheme iron absorbed (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>50 g dry maize as porridge*</td>
<td>0.045</td>
</tr>
<tr>
<td>80 g dry maize + 31 g dry beans + 50 g cooked rice**</td>
<td>0.112</td>
</tr>
</tbody>
</table>

* Using 2 mg Fe/100 g maize
**Meal contained 4.3 mg intrinsic iron (Study E)

These calculations suggest that about 0.27 mg iron can be absorbed from a Latin American meal containing only maize, beans, and rice if 50 mg of ascorbic acid is added. This amount of ascorbic acid would be found in approximately two tomatoes, one lemon or orange, or one cup of cooked cauliflower. The daily requirement for absorbed iron is about 1 mg/day for adult men and children and 2 mg/day for menstruating women and adolescents during peak growth.

In summary, the addition of food containing a reasonable amount of ascorbic acid (25 to 50 mg) to maize or to maize-based meals containing no meat, fish, or poultry can be expected to increase iron absorption by at least 400 percent. Two such meals would provide about one-half the iron requirement of men and one-quarter that of women. Thus, while increasing ascorbic acid can make an important contribution to iron absorption, it is likely that iron from meat, fish, and poultry will also be needed to prevent negative iron balance where the intrinsic iron content of the diet is low. However, individuals with depleted iron
stores will generally absorb a higher percentage of food iron (see “The FAO/WHO Model” below). It is also clear that simultaneous consumption of ascorbic acid would be important for improving the absorption of iron added to maize as a fortificant, unless it is a chelate such as NaFeEDTA.

Rice-based Meals

Data from studies on rice-based meals are presented in Table 7 and Figure 3. Doses of ascorbic acid ranged from 1.4 to 100 mg. Approximately 2 to 4 mg of iron were added because the intrinsic iron content of dry rice is only about 3 mg/100 g. No study evaluated the effect of synthetic ascorbic acid on iron absorption from rice alone — vitamin C was supplied as fruit and vegetables and, in almost all studies, the rice was served as part of a Southeast Asian meal. Because the foods in some of these meals already contained ascorbic acid, the data are more difficult to interpret than those from the studies on maize. In the two studies (C and D) that measured absorption from rice alone, 15 to 30 mg of ascorbic acid as fruit juices or potatoes increased absorption by 260 to 600 percent. It is difficult to say whether there was a dose-response, because each level of ascorbic acid was given as a different food. Assuming that the effects of synthetic and food sources of ascorbic acid are similar, it can be seen from Figure 3 that adding 15 to 40 mg of ascorbic acid approximately doubled nonheme iron absorption from the various rice-based meals. With 50 to 100 mg, absorption was increased by 200 to 300 percent. In general, the effect of ascorbic acid on iron absorption from the meals was remarkably similar across the range of ascorbic acid tested.

The intrinsic iron content of the meals ranged from about 0.4 mg for 200 g rice pudding (study C) to 2 to 3 mg for Southeast Asian meals containing rice, vegetables, chili paste, fish sauce, and coconut milk or coconut cream (study F). (Study E reported values of 8 to 10 mg native nonheme iron per meal, but much of this was probably contaminant iron; values estimated from food composition tables, and using the same or very similar diets in later studies [Hallberg et al. 1984; Hallberg et al. 1986], were between 1.3 and 2.3 mg per meal.) Given that on average about 3.2 percent of the iron in rice was absorbed in the absence of added ascorbic acid (studies Ala, AIlla, AlIV, Bla, CI, DI), 200 g of rice pudding would provide only about 0.013 mg of absorbed iron. Adding ascorbic acid up to 100 mg would increase this to about 0.05 mg of absorbed iron at most. Assuming that the Southeast Asian meals provided about 2 mg nonheme iron, of which 8 percent was absorbed in the absence of added ascorbic acid (study Fla), about 0.16 mg iron would be absorbed. Adding ascorbic acid increased the amount of iron absorbed to about 0.2 mg/meal (25 mg, study FII) and 0.6 mg/meal (75 mg ascorbic acid from papaya, study Flb).

Iron absorption doubled with the addition of 36 mg citric acid to rice (study II), and tripled with addition of 1 g (III). However, adding citric acid may not be useful if ascorbic acid is already present (study I, Ib). The absorption of nonheme iron from a Southeast Asian meal including pork was similar to that from a Western meal if the rice was well-polished. If the rice in this meal contained more phytate because of less rigorous milling, iron absorption was inversely related to the rice phytate content. The effect of the phytate could be overcome by adding ascorbic acid (50 mg) as green collard (Table 7) (Tuntawiroon et al. 1990).

In summary, the bioavailability of iron from white rice or Southeast Asian rice-based meals is about 3 percent and 8 percent, respectively. The addition of 15 to 100 mg ascorbic acid to rice-based meals increases the absorption of nonheme iron by about 200 to 300 percent in most studies. Although a dosee- response is not evident (Figure 3), this may be obscured by the complexity of the meals and the fact that
### Table 7: Strategies to Increase Iron Absorption from Rice-Based Meals

<table>
<thead>
<tr>
<th>Author/Test Meal</th>
<th>Intervention</th>
<th>N</th>
<th>Iron Status</th>
<th>Diet Iron (mg)</th>
<th>Reference Dose Abs (percent)</th>
<th>Nonheme Iron Abs (percent)</th>
<th>Abs Ratio with/without modifier</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Native</td>
<td>Added</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>RICE MEALS</strong></td>
<td>+ ASCORBIC ACID (AA)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. Sayers et al. 1974, S. Africa.</td>
<td>I. 0 mg AA</td>
<td>8</td>
<td>IDA</td>
<td>1.4 4.0 0.0</td>
<td>41.6</td>
<td>3.2</td>
<td>3.1</td>
</tr>
<tr>
<td></td>
<td>II. 35 mg AA</td>
<td>12</td>
<td>IDA</td>
<td>1.1 4.0 0.0</td>
<td>44.7</td>
<td>6.6</td>
<td>5.9</td>
</tr>
<tr>
<td></td>
<td>IIIa. 0 mg AA</td>
<td>8</td>
<td>IDA</td>
<td>1.3 4.0 0.0</td>
<td>41.6</td>
<td>3.2</td>
<td>3.1</td>
</tr>
<tr>
<td></td>
<td>IIIb. 60 mg AA</td>
<td>10</td>
<td>IDA</td>
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<td>11.9</td>
<td>11.4</td>
<td>3.7</td>
</tr>
<tr>
<td></td>
<td>IVa. 0 mg AA</td>
<td>12</td>
<td>IDA</td>
<td>3.6 4.0 0.0</td>
<td>44.5</td>
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<tr>
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<td>IVb. 100 mg AA</td>
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<td>IDA</td>
<td>2.3 4.0 0.0</td>
<td>12.2</td>
<td>11.0</td>
<td>2.9</td>
</tr>
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<td>IDA+</td>
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<td>51.1</td>
<td>4.4</td>
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</tr>
<tr>
<td></td>
<td>Ib. 35 mg AA</td>
<td>10</td>
<td>IDA</td>
<td>3.0 4.0 0.0</td>
<td>6.0</td>
<td>4.7</td>
<td>1.4</td>
</tr>
<tr>
<td>C. Ballot et al. 1987, S. Africa.</td>
<td>Adding fruit juice (100 mL)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>I. 0 mg AA</td>
<td>129</td>
<td>ID</td>
<td>0.4 3.0 0.0</td>
<td>41.2</td>
<td>1.9</td>
<td>2.5R</td>
</tr>
<tr>
<td></td>
<td>II. 1.4 mg AA as grape juice.</td>
<td>10</td>
<td>ID</td>
<td>0.4 3.0 0.0</td>
<td>41.0</td>
<td>4.8</td>
<td>4.0R</td>
</tr>
<tr>
<td></td>
<td>III. 1.7 mg AA as apple juice</td>
<td>14</td>
<td>ID</td>
<td>0.4 3.0 0.0</td>
<td>42.8</td>
<td>3.8</td>
<td>3.5R</td>
</tr>
<tr>
<td></td>
<td>IV. 5.2 mg AA as pineapple juice</td>
<td>8</td>
<td>ID</td>
<td>0.4 3.0 0.0</td>
<td>36.6</td>
<td>9.7</td>
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</tr>
<tr>
<td></td>
<td>V. 15 mg AA as guava nectar</td>
<td>14</td>
<td>ID</td>
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<td>42.8</td>
<td>9.9</td>
<td>9.2R</td>
</tr>
<tr>
<td></td>
<td>VI. 24 mg AA as apple juice</td>
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<td>48.9</td>
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<td>11.1R</td>
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<td>ID</td>
<td>0.4 3.0 0.0</td>
<td>51.0</td>
<td>16.6</td>
<td>12.3R</td>
</tr>
<tr>
<td></td>
<td>VIII. 30 mg AA as pear juice</td>
<td>8</td>
<td>ID</td>
<td>0.4 3.0 0.0</td>
<td>36.6</td>
<td>13.8</td>
<td>15.0R</td>
</tr>
<tr>
<td>Author/Test Meal</td>
<td>Intervention</td>
<td>N</td>
<td>Diet Iron (mg)</td>
<td>Reference Dose Abs (percent)</td>
<td>Nonheme Iron Abs (percent)</td>
<td>Abs Ratio with/without modifier</td>
<td></td>
</tr>
<tr>
<td>------------------</td>
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<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Nonheme</td>
<td>Heme</td>
<td></td>
<td>Actual</td>
<td>Adj*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Native</td>
<td>Added</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RICE MEALS</td>
<td>+ ASCORBIC ACID (AA)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D. Gilooly et al. 1983, S. Africa.</td>
<td>I. 0 mg AA</td>
<td>25</td>
<td>ID</td>
<td>?</td>
<td>3.0</td>
<td>0.0</td>
<td>32.0</td>
</tr>
<tr>
<td></td>
<td>II. 15 mg AA as potatoes</td>
<td>9</td>
<td>ID</td>
<td>?</td>
<td>3.0</td>
<td>0.0</td>
<td>24.2</td>
</tr>
<tr>
<td>E. Hallberg et al. 1974, Bangkok.</td>
<td>Ia. Basal diet</td>
<td>7</td>
<td>NL</td>
<td>8.4</td>
<td>1.9</td>
<td>0.0</td>
<td>24.6</td>
</tr>
<tr>
<td></td>
<td>Ib. Basal diet fortified with FeSO₄</td>
<td></td>
<td>+ID</td>
<td>8.4</td>
<td>3.4</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ila. Basal diet + fruit mix (40 g banana, 40 g papaya + 20 g orange)</td>
<td>7</td>
<td>NL</td>
<td>9.6</td>
<td>1.9</td>
<td>0.0</td>
<td>28.8</td>
</tr>
<tr>
<td></td>
<td>IIb. Basal Fe-fortified diet + fruit mix (as in Ila).</td>
<td></td>
<td>+ID</td>
<td>9.6</td>
<td>3.4</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>RICE MEALS</td>
<td>+ MFP ± AA</td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>F. Hallberg et al. 1986, Bangkok.</td>
<td>Ia. Basal meal + 0 mg AA</td>
<td>14</td>
<td>NL</td>
<td>2.3</td>
<td>0.0</td>
<td>0.0</td>
<td>30.6</td>
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<tr>
<td></td>
<td>Ib. Basal meal + 75 mg AA as 150 g fresh papaya</td>
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</tr>
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<td></td>
<td>II. Basal meal + 25 mg AA</td>
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<td>NL</td>
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<td>0.0</td>
<td>31.7</td>
</tr>
<tr>
<td></td>
<td>III. Basal meal + 50 mg AA</td>
<td>16</td>
<td>NL</td>
<td>1.8</td>
<td>0.0</td>
<td>0.0</td>
<td>16.0</td>
</tr>
</tbody>
</table>

Relative Impact of Potential Interventions to Improve Dietary Iron Availability
<table>
<thead>
<tr>
<th>Author/Test Meal</th>
<th>Intervention</th>
<th>N</th>
<th>Iron Status</th>
<th>Diet Fe (mg)</th>
<th>Reference Dose Abs (percent)</th>
<th>Nonheme Iron Abs (percent)</th>
<th>Abs Ratio with/without modifier</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Nonheme</td>
<td>Heme</td>
<td>Native</td>
</tr>
<tr>
<td><strong>RICE MEALS</strong></td>
<td>+ MFP ± AA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G. Hallberg et al. 1974, Bangkok.</td>
<td>Ia. Basal diet</td>
<td>7</td>
<td>NL</td>
<td>8.4</td>
<td>1.9</td>
<td>0.0</td>
<td>24.6</td>
</tr>
<tr>
<td></td>
<td>Ib. Basal diet fortified with FeSO₄</td>
<td>+ID</td>
<td>8.4</td>
<td>3.4</td>
<td>0.0</td>
<td>24.6</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>Ila. Basal diet + meat (80 g lean beef ground cooked in 50 g coconut milk)</td>
<td>NL</td>
<td>8.4</td>
<td>1.9</td>
<td>?</td>
<td>20.0</td>
<td>1.4</td>
</tr>
<tr>
<td></td>
<td>Ilb. Basal Fe-fortified diet + meat (as in Ila)</td>
<td>+ID</td>
<td>8.4</td>
<td>3.4</td>
<td>?</td>
<td>20.0</td>
<td>1.9</td>
</tr>
<tr>
<td></td>
<td>Ila. Basal diet + fruit mix (see Ila) + meat (as in IIa)</td>
<td>NL</td>
<td>9.6</td>
<td>1.9</td>
<td>0.0</td>
<td>20.9</td>
<td>1.9</td>
</tr>
<tr>
<td></td>
<td>Iib. Basal Fe-fortified diet + fruit mix + meat.</td>
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<td>9.6</td>
<td>3.4</td>
<td>0.0</td>
<td>20.9</td>
<td>1.9</td>
</tr>
<tr>
<td><strong>RICE MEALS</strong></td>
<td>+ CITRATE</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H. Hallberg et al. 1984, Bangkok.</td>
<td>Ia. Basal meal + fish (50 g)</td>
<td>25</td>
<td>NL</td>
<td>1.3</td>
<td>0.0</td>
<td>0.0</td>
<td>34.8</td>
</tr>
<tr>
<td></td>
<td>Ib. Basal meal + fish (50 g) + 50 mg AA</td>
<td>25</td>
<td>NL</td>
<td>1.3</td>
<td>0.0</td>
<td>0.0</td>
<td>34.8</td>
</tr>
<tr>
<td>Author/Test Meal</td>
<td>Intervention</td>
<td>N</td>
<td>Iron Status</td>
<td>Diet Iron (mg)</td>
<td>Reference Dose Abs (percent)</td>
<td>Nonheme Iron Abs (percent)</td>
<td>Abs Ratio with/without modifier</td>
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<td>Nonheme</td>
<td>Heme</td>
<td>Actual</td>
<td>Adj*</td>
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<td></td>
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<td>Native</td>
<td>Added</td>
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<td><strong>RICE MEALS</strong></td>
<td><strong>- PHYTATE ± AA</strong></td>
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<td>13</td>
<td>ID</td>
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<td>11.4</td>
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<tr>
<td></td>
<td>200 g boiled rice pudding (with sugar, margarine)</td>
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<td></td>
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<tr>
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<td>Ia. 100 mL H₂O with 33 mg AA</td>
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<td>17.0</td>
</tr>
<tr>
<td></td>
<td>lb. 100 mL H₂O with 33 mg AA + 750 mg citric acid</td>
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<td></td>
<td></td>
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<td>1.5</td>
</tr>
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<td>Ila. 100 mL H₂O with 28 mg AA</td>
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<td>9.8</td>
</tr>
<tr>
<td></td>
<td>llb. 100 mL fresh orange juice (30 mg AA)</td>
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<td>1.4</td>
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<td>IIIa. 100 mL commercial orange juice</td>
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<td>13.9</td>
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<td>IIIb. 100 mL commercial orange juice with 4 g citric acid</td>
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<td>ID</td>
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<td>3.0</td>
<td>0.0</td>
<td>16.6</td>
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<td>215 g boiled, polished rice pudding (with sugar, margarine)</td>
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<td></td>
<td>Ia. 0 mg citric acid</td>
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<td>lb. 36 mg citric acid</td>
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<td>Ila. 0 g citric acid</td>
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<td>1.9</td>
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<td>IIb. 1 g citric acid</td>
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<td>2.8R</td>
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<td>8.5R</td>
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<td>3.0</td>
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</tbody>
</table>

* Phytate content was altered in the meals by using varying proportions of polished to unpolished rice.

Abbreviations used in the table:

**Adj** = nonheme iron absorption (actual) adjusted to correspond to iron absorption of 40 percent from reference dose. In most cases this was calculated as: Adj = actual nonheme iron absorption (percent) x 40 / reference dose absorption (percent). When adjusted values were reported by authors, these values are used and indicated by R.

**AA** = ascorbic acid in crystalline form unless otherwise noted.

**Abs Ratio** = absorption ratio with/without modifier, calculated using adjusted nonheme iron absorption values when available, or else with actual absorption values. When absorption ratio was reported by authors those values were used and indicated by R.

**Iron Status**: iron status of the study subjects; **NL** = normal, **ID** = iron deficient, **IDA** = iron deficient anemic.

**N** = number
Table 8: Absorption of 3 mg Iron as FeSO₄·7H₂O in Fasting Women Given 200 g Rice with Fresh Fruit

<table>
<thead>
<tr>
<th>Fruit</th>
<th>Weight</th>
<th>Ascorbic Acid (mg)</th>
<th>Citric Acid (mg)</th>
<th>Absorption Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Banana</td>
<td>100</td>
<td>10</td>
<td>150</td>
<td>1.8</td>
</tr>
<tr>
<td>Mango</td>
<td>100</td>
<td>35</td>
<td>-</td>
<td>2.0</td>
</tr>
<tr>
<td>Pear</td>
<td>100</td>
<td>4</td>
<td>240</td>
<td>2.0</td>
</tr>
<tr>
<td>Cantaloupe</td>
<td>100</td>
<td>25</td>
<td>-</td>
<td>2.6</td>
</tr>
<tr>
<td>Guava, fresh</td>
<td>100</td>
<td>180</td>
<td>462</td>
<td>5.0</td>
</tr>
<tr>
<td>Pawpaw</td>
<td>50</td>
<td>77</td>
<td>165</td>
<td>5.6</td>
</tr>
<tr>
<td>Pawpaw</td>
<td>100</td>
<td>154</td>
<td>329</td>
<td>6.9</td>
</tr>
<tr>
<td>Guava, tinned</td>
<td>100</td>
<td>262</td>
<td>393</td>
<td>11.7</td>
</tr>
</tbody>
</table>

* Adapted from Ballot et al. 1987

b No or negative effect on iron absorption for grapes (peeled or unpeeled), avocado, peach, apple, strawberry, plum, and rhubarb most of them already contained some ascorbic acid. The iron content of the meals tested was low (0.4 mg in 200 g rice pudding and less than 2 mg from the Asian meals) so that the amount of absorbed iron was poor (under 0.2 mg/meal) even with the addition of ascorbic acid. Ascorbic acid would be proportionately more effective when the rice has a higher phytate content or is fortified with non-chelate iron.

**Wheat-based Meals**

The results of studies on wheat-based meals are presented in Table 9 and Figure 3. In addition, Figure 4 shows the strong inhibitory effect on iron absorption of phytate added to wheat. The absorption of native or fortificant iron from an Egyptian flat bread, which had a high content of phytate because it was made from relatively high extraction flour and without leavening, was only about 2 percent (El Guindi et al. 1988). In contrast, iron absorption (adjusted) was reported to be between 6 and 20 percent from leavened white bread (studies D and E) and 5 percent from leavened whole wheat bread containing more phytate (study AI). Absorption was increased by 220 to 270 percent by adding 50 mg ascorbic acid, and by 270 to 350 percent by adding 150 to 250 mg ascorbic acid (Hallberg et al. 1989; Siegenberg et al 1991). The amount of iron absorbed from two buns made with unfortified wheat flour was about 0.03 mg with no added ascorbic acid, increasing to about 0.06 mg with 50 mg added ascorbic acid. Figure 5 shows that adding 30 to 150 mg ascorbic acid doubles or triples iron absorption from wheat.

In summary, reasonable amounts of ascorbic acid can overcome the inhibitory effects of phytate in wheat even when the phytate content is high, but the iron content of wheat is low (approximately 0.5 mg/100 g flour). Thus, the addition of ascorbic acid to wheat or wheat-based meals would only have a major impact on the total amount of iron absorbed if the wheat were fortified with non-chelate iron.
Table 9: Strategies to Increase Iron Absorption (Abs) from Wheat Based Meals

<table>
<thead>
<tr>
<th>Author/Test Meal</th>
<th>Intervention</th>
<th>N</th>
<th>Iron Status</th>
<th>Diet Iron (mg)</th>
<th>Reference Dose Abs (percent)</th>
<th>Nonheme Iron Abs (percent)</th>
<th>Abs Ratio with/without modifier</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Native</td>
<td>Heme</td>
<td>Nonheme</td>
<td>Heme</td>
<td>Actual</td>
</tr>
<tr>
<td><strong>WHEAT MEALS + Ascorbic Acid (AA)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Whole wheat bread (80 g) made with whole wheat flour (100 percent extraction)</td>
<td>I. 0 mg AA</td>
<td>5</td>
<td>IDA</td>
<td>3.7</td>
<td>0.0</td>
<td>0.0</td>
<td>74.2</td>
</tr>
<tr>
<td></td>
<td>II. 50 mg AA (added prior to cooking; only 25 percent AA in reduced form left after cooking)</td>
<td>5</td>
<td>IDA</td>
<td>3.1</td>
<td>0.0</td>
<td>0.0</td>
<td>53.4</td>
</tr>
<tr>
<td>B. Hallberg et al. 1979, Sweden.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breakfast meal: 20 g white wheat unfortified flour made into 2 wheat rolls; with 12 g margarine and orange marmalade (10 g), 15 g cheese, 8 g coffee (150 mL)</td>
<td>I. Breakfast meal + 0 mg AA</td>
<td>21</td>
<td>NI+ID</td>
<td>0.5</td>
<td>2.3</td>
<td>0.0</td>
<td>52.0</td>
</tr>
<tr>
<td></td>
<td>II. Breakfast meal + 70 mg AA as orange juice</td>
<td>12</td>
<td>NI+ID</td>
<td>0.8</td>
<td>2.3</td>
<td>0.0</td>
<td>N/A</td>
</tr>
</tbody>
</table>
## WHEAT MEALS + Ascorbic Acid (AA)

<table>
<thead>
<tr>
<th>Author/Test Meal</th>
<th>Intervention</th>
<th>N</th>
<th>Iron Status</th>
<th>Diet Iron (mg)</th>
<th>Reference Dose Abs (percent)</th>
<th>Nonheme Iron Abs (percent)</th>
<th>Abs Ratio with/without modifier</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. Hallberg et al. 1979, Sweden.</td>
<td>I. Breakfast meal + 0 mg AA</td>
<td>12</td>
<td>NI+ID</td>
<td>0.5</td>
<td>2.3</td>
<td>0.0</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>II. Breakfast meal + 70 mg AA as orange juice</td>
<td>12</td>
<td>NI+ID</td>
<td>0.8</td>
<td>2.3</td>
<td>0.0</td>
<td>N/A</td>
</tr>
<tr>
<td>D. Siegenberg et al. 1991, S. Africa.</td>
<td>Ia. Bread meal + 10 mg phytate-P</td>
<td>16</td>
<td>IDA</td>
<td>?</td>
<td>?</td>
<td>0.0</td>
<td>78.3</td>
</tr>
<tr>
<td></td>
<td>Ib. Bread meal + 14 mg phytate-P</td>
<td>16</td>
<td>IDA</td>
<td>?</td>
<td>?</td>
<td>0.0</td>
<td>60.2</td>
</tr>
<tr>
<td></td>
<td>II. Bread meal + 22 mg phytate-P</td>
<td>14</td>
<td>IDA</td>
<td>?</td>
<td>?</td>
<td>0.0</td>
<td>57.1</td>
</tr>
<tr>
<td></td>
<td>III. Bread meal + 34 mg phytate-P</td>
<td>16</td>
<td>IDA</td>
<td>?</td>
<td>?</td>
<td>0.0</td>
<td>57.3</td>
</tr>
<tr>
<td></td>
<td>IV. Bread meal + 58 mg phytate-P</td>
<td>11</td>
<td>IDA</td>
<td>?</td>
<td>?</td>
<td>0.0</td>
<td>57.3</td>
</tr>
<tr>
<td>Author/Test Meal</td>
<td>Intervention</td>
<td>N</td>
<td>Iron Status</td>
<td>Diet Iron (mg)</td>
<td>Reference Dose Abs (percent)</td>
<td>Nonheme Iron Abs (percent)</td>
<td>Abs Ratio with/without modifier</td>
</tr>
<tr>
<td>------------------</td>
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<td>-------------------------------</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Native</td>
<td>Added</td>
<td></td>
<td>Actual</td>
</tr>
<tr>
<td>WHEAT MEALS + Ascorbic Acid (AA)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E. Hallberg et al. 1989, Sweden.</td>
<td>Ia. Basal meal</td>
<td>9</td>
<td>NI+ID</td>
<td>0.4</td>
<td>3.7</td>
<td>0.0</td>
<td>28.2</td>
</tr>
<tr>
<td>Basal meal: 2 wheat buns made with 80 g unfortified white wheat flour (60 percent extraction), sugar, salt and yeast.</td>
<td>Ib. Basal meal + 2 mg phytate-P</td>
<td>9</td>
<td>NI+ID</td>
<td>0.4</td>
<td>3.7</td>
<td>0.0</td>
<td>37.9</td>
</tr>
<tr>
<td></td>
<td>IIA. Basal meal</td>
<td>9</td>
<td>NI+ID</td>
<td>0.4</td>
<td>3.7</td>
<td>0.0</td>
<td>39.0</td>
</tr>
<tr>
<td></td>
<td>IIb. Basal meal + 5 mg phytate-P</td>
<td>6</td>
<td>NI+ID</td>
<td>0.4</td>
<td>3.7</td>
<td>0.0</td>
<td>35.2</td>
</tr>
<tr>
<td></td>
<td>IIIa. Basal meal</td>
<td>9</td>
<td>NI+ID</td>
<td>0.4</td>
<td>3.7</td>
<td>0.0</td>
<td>42.6</td>
</tr>
<tr>
<td></td>
<td>IIIb. Basal meal + 25 mg phytate-P</td>
<td>10</td>
<td>NI+ID</td>
<td>0.4</td>
<td>3.7</td>
<td>0.0</td>
<td>31.2</td>
</tr>
<tr>
<td></td>
<td>IVa. Basal meal</td>
<td>9</td>
<td>NI+ID</td>
<td>0.4</td>
<td>3.7</td>
<td>0.0</td>
<td>30.0</td>
</tr>
<tr>
<td></td>
<td>IVb. Basal meal + 50 mg phytate-P</td>
<td>10</td>
<td>NI+ID</td>
<td>0.4</td>
<td>3.7</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Vla. Basal meal</td>
<td>10</td>
<td>NI+ID</td>
<td>0.4</td>
<td>3.7</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Vlb. Basal meal + 100 mg phytate-P</td>
<td>10</td>
<td>NI+ID</td>
<td>0.4</td>
<td>3.7</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>VIa. Basal meal</td>
<td>10</td>
<td>NI+ID</td>
<td>0.4</td>
<td>3.7</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>VIb. Basal meal + 250 mg phytate-P</td>
<td>10</td>
<td>NI+ID</td>
<td>0.4</td>
<td>3.7</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>Author/Test Meal</td>
<td>Intervention</td>
<td>N</td>
<td>Iron Status</td>
<td>Diet Iron (mg)</td>
<td>Reference Dose Abs (percent)</td>
<td>Nonheme Iron Abs (percent)</td>
<td>Abs Ratio with/without modifier</td>
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<td>-------------------------------</td>
<td>---------------------------</td>
<td>--------------------------------</td>
</tr>
<tr>
<td><strong>WHEAT MEALS</strong></td>
<td>- Phytate ± AA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>F. Hallberg et al. 1989, Sweden.</td>
<td>Basal meal: 2 wheat buns made with 80 g unfortified white wheat flour (60 percent extraction), sugar, salt and yeast.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ia. Basal meal + 25 mg phytate-P</td>
<td>10</td>
<td>NI+ID</td>
<td>0.4</td>
<td>3.7</td>
<td>0.0</td>
<td>36.3</td>
</tr>
<tr>
<td></td>
<td>Ib. Basal meal + 25 mg phytate-P + 50 mg AA</td>
<td>8</td>
<td>NI+ID</td>
<td>0.4</td>
<td>3.7</td>
<td>0.0</td>
<td>40.6</td>
</tr>
<tr>
<td></td>
<td>Ila. Basal meal + 25 mg phytate-P</td>
<td>8</td>
<td>NI+ID</td>
<td>0.4</td>
<td>3.7</td>
<td>0.0</td>
<td>36.4</td>
</tr>
<tr>
<td></td>
<td>IIb. Basal meal + 25 mg phytate-P + 100 mg AA</td>
<td>8</td>
<td>NI+ID</td>
<td>0.4</td>
<td>3.7</td>
<td>0.0</td>
<td>30.7</td>
</tr>
<tr>
<td></td>
<td>IIIa. Basal meal + 250 mg phytate-P</td>
<td>10</td>
<td>NI+ID</td>
<td>0.4</td>
<td>3.7</td>
<td>0.0</td>
<td>30.7</td>
</tr>
<tr>
<td></td>
<td>IIIb. Basal meal + 250 mg phytate-P + 50 mg AA</td>
<td>10</td>
<td>NI+ID</td>
<td>0.4</td>
<td>3.7</td>
<td>0.0</td>
<td>30.7</td>
</tr>
<tr>
<td></td>
<td>IVA. Basal meal + 250 mg phytate-P</td>
<td>10</td>
<td>NI+ID</td>
<td>0.4</td>
<td>3.7</td>
<td>0.0</td>
<td>30.7</td>
</tr>
<tr>
<td></td>
<td>IVb. Basal meal + 250 mg phytate-P + 100 mg AA</td>
<td>10</td>
<td>NI+ID</td>
<td>0.4</td>
<td>3.7</td>
<td>0.0</td>
<td>30.7</td>
</tr>
<tr>
<td><strong>WHEAT MEALS</strong></td>
<td>- Phytate ± AA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G. Siegenberg et al. 1991, S. Africa.</td>
<td>Basal meal: Bread (80 g, made with wheat flour, salt, sugar, yeast) with margarine and 15 g mashed potatoes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ia. Basal meal + 58 mg phytate-P</td>
<td>11</td>
<td>IDA</td>
<td>?</td>
<td>3.0</td>
<td>0.0</td>
<td>46.7</td>
</tr>
<tr>
<td></td>
<td>Ib. Basal meal + 58 mg phytate-P + 30 mg AA</td>
<td>12</td>
<td>IDA</td>
<td>?</td>
<td>3.0</td>
<td>0.0</td>
<td>52.7</td>
</tr>
<tr>
<td></td>
<td>Ila. Basal meal + 58 mg phytate-P</td>
<td>14</td>
<td>IDA</td>
<td>?</td>
<td>3.0</td>
<td>0.0</td>
<td>76.3</td>
</tr>
<tr>
<td></td>
<td>IIb. Basal meal + 58 mg phytate-P + 50 mg AA</td>
<td>14</td>
<td>IDA</td>
<td>?</td>
<td>3.0</td>
<td>0.0</td>
<td>76.3</td>
</tr>
<tr>
<td></td>
<td>IIIa. Basal meal + 58 mg phytate-P</td>
<td>14</td>
<td>IDA</td>
<td>?</td>
<td>3.0</td>
<td>0.0</td>
<td>76.3</td>
</tr>
<tr>
<td></td>
<td>IIIb. Basal meal + 58 mg phytate-P + 150 mg AA</td>
<td>14</td>
<td>IDA</td>
<td>?</td>
<td>3.0</td>
<td>0.0</td>
<td>76.3</td>
</tr>
<tr>
<td>Author/Test Meal</td>
<td>Intervention</td>
<td>N</td>
<td>Iron Status</td>
<td>Diet Iron (mg)</td>
<td>Reference Dose Abs (percent)</td>
<td>Nonheme Iron Abs (percent)</td>
<td>Abs Ratio with/without modifier</td>
</tr>
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<tr>
<td></td>
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<td>Native</td>
<td>Added</td>
<td>Nonheme</td>
<td>Heme</td>
<td>Actual</td>
</tr>
<tr>
<td>WHEAT MEALS</td>
<td>Phytate ± AA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H. Hallberg et al. 1989, Sweden. Basal meal: 2 wheat buns made with 80 g unfortified white wheat flour (60 percent extraction), sugar, salt and yeast</td>
<td>Ia. Basal meal + 25 mg phytate-P</td>
<td>8</td>
<td>NI+ID</td>
<td>0.4</td>
<td>3.7</td>
<td>0.0</td>
<td>28.5</td>
</tr>
<tr>
<td></td>
<td>Ib. Basal meal + 25 mg phytate-P + 50 g meat</td>
<td>10</td>
<td>NI+ID</td>
<td>0.4</td>
<td>3.7</td>
<td>0.0</td>
<td>35.9</td>
</tr>
<tr>
<td></td>
<td>IIa. Basal meal+250 mg phytate-P</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>IIb. Basal meal+250 mg phytate-P + 50 g meat</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Phytate content was altered in the meals by using varying proportions of polished to unpolished rice

Abbreviations used in the table:

Adj = nonheme iron absorption (actual) adjusted to correspond to iron absorption of 40 percent from reference dose. In most cases this was calculated as: Adj = actual nonheme iron absorption (percent) x 40 / reference dose absorption (percent). When adjusted values were reported by authors, these values are used and indicated by R

AA = ascorbic acid in crystalline form unless otherwise noted

Abs Ratio = absorption ratio with/without modifier, calculated using adjusted nonheme iron absorption values when available, or else with actual absorption values. When absorption ratio was reported by authors those values were used and indicated by R

Iron Status: iron status of the study subjects; NL = normal, ID = iron deficient, IDA= iron deficient anemic

N = number
Improving Iron Status Through Diet

Figure 4: Effect of Phytate on Nonheme Iron Absorption from Wheat-based Meals

Source: Modified from Hallberg et al. 1989

Figure 5: Effect of Ascorbic Acid on Nonheme Iron Absorption from Wheat Meals with Varying Phytic Acid Content.

Source: Modified from Siegenberg et al. 1991
MODELS FOR PREDICTING DIETARY IRON BIOSAVAILABILITY

Dietary iron bioavailability can be estimated by taking into account the form of iron (heme or nonheme), the presence of dietary modifiers, and the iron status of the individual. This concept was first introduced by Monsen and coworkers (1978). The Monsen algorithm is used almost exclusively in developed countries, and may be invalid for developing countries. FAO/WHO has proposed a different approach, and Murphy et al. (1988) have combined elements of both models for use in developing countries.

Monsen’s Model

The model developed by Monsen et al. (1978) takes into account an individual’s iron stores (estimated as 0, 250, 500, or 1,000 mg) and, for each meal of the day, the intake of heme and nonheme iron, and enhancers (meat/fish/poultry protein and ascorbic acid). Heme iron bioavailability is assumed to be 25 percent, and, depending on the iron stores of the individual, nonheme iron absorption is estimated to be low, medium, or high based on the amount of enhancers such as meat, fish, poultry, and ascorbic acid in each meal. Thus, to compute the amount of bioavailable iron, it is necessary to have an idea of each individual’s iron stores, which are usually “guessed” although they could be estimated from plasma ferritin and hemoglobin concentrations (Cook et al. 1986; Viteri et al. 1995), as well as from dietary information coded by meals. The greatest limitation of Monsen’s model for estimating dietary iron bioavailability in developing countries is that it does not take into account the inhibitory effects of dietary ligands such as phytate, tea, coffee, etc., and thus substantially overestimates iron bioavailability where the intake of these factors is high. Furthermore, this model assumes that the enhancing effect of 1 g of meat, fish, or poultry is the same as that of 1 mg of ascorbic acid, and that their effects add up to a maximum of 15 percent. However, the type and amount of inhibitory ligands in a meal may modify this relationship. Further work is needed to establish this quantitatively.

The FAO/WHO Model

The Food and Agriculture Organization (FAO) and the World Health Organization (WHO) suggest the following practical classification of usual diets for the purpose of estimating iron bioavailability in developing countries (FAO/WHO 1988). The estimates were based on the absorption of iron from typical meals in Asia (Hallberg et al. 1983), India (Narasinga Rao et al. 1983), Latin America (Acosta et al. 1984), and Western countries (Hallberg et al. 1982b).

- **Low bioavailability diet (iron absorption about 5 percent):** a simple, monotonous diet containing cereals, roots, and/or tubers and negligible quantities of meat, fish or ascorbic acid-rich foods. This diet contains a preponderance of foods that inhibit iron absorption (maize, beans, whole wheat flour,
Improving Iron Status Through Diet

sorghum, etc.) and is dominant in many developing countries, particularly among lower socioeconomic groups.

- **Intermediate bioavailability diet (iron absorption about 10 percent):** diets consisting mainly of cereals, roots, and/or tubers and negligible quantities of food of animal origin and/or ascorbic acid, both of which promote iron availability. A low bioavailability diet can be raised to this level by increasing the intake of foods which enhance iron absorption, such as ascorbic acid-rich foods, meat, and fish. Similarly, a high bioavailability diet can be reduced to this intermediate level by the regular consumption of meals containing higher amounts of inhibitors of iron absorption, such as tea or coffee.

- **High bioavailability diet (iron absorption about 15 percent):** a diversified diet containing generous quantities of meat, poultry, fish, and/or foods containing high amounts of ascorbic acid. This type of diet is typical for most segments of the populations in industrialized countries.

The FAO/WHO approach recognizes the importance of considering the presence of inhibitors such as phytate and tannins, although the descriptions of the diets are strictly qualitative and the quantities of inhibitors and enhancers are not stated. The 5, 10, and 15 percent absorption values refer to non-anemic individuals with no iron stores. In iron deficiency, each value is assumed to be increased by 50 percent, i.e., to 7.5, 15, and 22.5 percent absorption for the low, intermediate, and high bioavailability diets, respectively (FAO/WHO 1988).

Layrisse et al. (1990) measured iron absorption from seven diets consumed by low, middle, and high socioeconomic groups in Venezuela, and evaluated the iron status of the populations. The diets were classified according to the low, intermediate, and high bioavailability diet categories described by FAO/WHO, to obtain more quantitative information on the likely content of enhancers and inhibitors in each category. The **low bioavailability diets** contained more than 400 mg phytate, more than 500 mg polyphenols, less than 50 g meat, and less than 30 mg ascorbic acid. Nonheme iron absorption from the main meal was 4 percent, and less than 1 mg of nonheme plus heme iron was absorbed from the total diet each day. The **intermediate bioavailability diets** contained more than 400 mg phytate, variable amounts of polyphenols, 50 to 100 g meat, and 30 to 50 mg ascorbic acid. Nonheme iron absorption from the main meal was 8 percent, and 1.0 to 1.7 mg of nonheme plus heme iron was absorbed from the total diet each day. The **high bioavailability diets** contained less than 400 mg phytate, variable amounts of polyphenols, more than 100 g meat, and over 50 mg ascorbic acid. Nonheme iron absorption from the main meal was 15 percent, and more than 1.8 mg of nonheme plus heme iron was absorbed from the total diet each day. The investigators commented that further studies are needed on the iron absorption from diets consumed by other populations in relation to their prevalence of iron deficiency.

**Adaptation of the Monsen and FAO/WHO Models by Murphy et al.**

The model developed by Murphy et al. (1992) is a modification of that proposed by FAO/WHO (1988) and includes components of the Monsen model. It is a useful approach for assessing iron absorption from diets in less-developed countries and was developed to estimate the bioavailability of iron from the diets of preschool children in Mexico, Egypt, and Kenya (Murphy et al. 1992). Based on the literature, it was assumed that heme iron is 25 percent available, and constitutes 40 percent of the iron in meat, poultry, and
fish. Rather than calculate iron bioavailability from each individual meal (as advised for the Monsen model), it was calculated for each entire day of food intake data. Then, rather than assuming that iron stores were 500 mg (the mean value assumed by Monsen et al. for Western women), predicted bioavailability was increased to assume no iron stores (but no overt anemia) in the population.

Table 10: Estimated Percent Bioavailability of Nonheme Iron for Iron-Deficient, Non-Anemic Individuals with Different Intakes of Meat/Fish/Poultry and Ascorbic Acid

<table>
<thead>
<tr>
<th>Ascorbic acid (mg/1,000 Kcal)</th>
<th>Meat, fish, and poultry protein&lt;sup&gt;a&lt;/sup&gt; (g)</th>
<th>less than 9 g</th>
<th>9 to 27 g</th>
<th>more than 27 g</th>
</tr>
</thead>
<tbody>
<tr>
<td>more than 5</td>
<td></td>
<td>5%</td>
<td>10%</td>
<td>15%</td>
</tr>
<tr>
<td>35-105</td>
<td></td>
<td>10%</td>
<td>15%</td>
<td>15%</td>
</tr>
<tr>
<td>less than 105</td>
<td></td>
<td>15%</td>
<td>15%</td>
<td>15%</td>
</tr>
</tbody>
</table>

<sup>a</sup> Meat, fish, and poultry protein x 5 = weight of meat, fish, or poultry

Source: Murphy et al. 1992

The 5, 10, and 15 percent figures in Table 10 were based on FAO/WHO’s separation of typical meals in regions of the world into the low, medium, or high bioavailability categories, as described in the previous section. If anemia is present, absorption would be increased by 50 percent, to 7.5, 15, and 22.5 percent (FAO/WHO 1988). The predicted impact of meat, fish, and poultry protein, and ascorbic acid, are based on Monsen’s cutoff points, expressed per 1,000 kcal by assuming that one meal provides one-third of a woman’s usual energy intake. This enables the same algorithm to be used across age and gender groups. In addition, a “tea factor” was used, ranging from 1 if no tea was consumed, to 0.40 for at least 600 mL tea/day. The final algorithm was: available iron = heme iron x 0.25 + (nonheme iron x availability factor x tea factor).

The advantages of this model are that it suggests quantitative cutoffs for ascorbic acid and meat/fish/poultry protein, and enables the joint effects of these food constituents to be considered. It also permits correction for the substantial effects of tea and coffee on iron absorption. Iron bioavailability can be estimated for populations that are nonanemic but iron deficient, and those that are anemic. However, the model still suffers from some limitations. The lowest category of ascorbic acid intake is substantial (35 mg/1,000 kcal) and the intermediate category covers a wide range (35 to 105 mg/1,000 kcal) so that the impact of adding 25 or 50 mg increments of ascorbic acid cannot always be predicted.

As applied by Murphy et al. (1992), the impacts of ascorbic acid, meat/fish/poultry, and tea were calculated on total daily food intakes (due to lack of meal-based data in some data sets) rather than meal-by-meal. A meal-based approach is theoretically more correct, because the ascorbic acid enhances nonheme iron absorption only when consumed with the iron. The model can be used for this purpose in its present form. However, from a practical point of view, the day-based approach may be adequate. This is an important consideration because many food intake data do not identify foods consumed by meal. Analysis of existing food intake from adults and children in rural Mexico produced an estimate of 0.93 mg
available iron per day with the day-based approach, and 1.01 mg/day with the meal-based approach (Murphy et al. 1996). The difference was mainly caused by the fact that the day-based algorithm assumed that coffee intake was distributed across all eating events, while in reality coffee was not consumed with the main meal of the day (which had the highest nonheme iron content). When the algorithm was adjusted to assume that coffee affected the absorption of iron from only one main meal a day, the estimates obtained with the day-based and meal-based approaches were virtually identical. Thus, the magnitude of the error involved with using total daily food intake data is probably small in most situations compared with the errors inherent in the general assumptions of the model. It will, however, depend on the distribution of enhancers, inhibitors, and nonheme iron across the meals (Carpenter and Mahoney 1992), which is likely to be less variable if the food intake data are collected from a larger number of individuals and for more days per individual.

The model contains no coffee factor because it was developed for children, but this might range from 1 (no coffee) to 0.6 (Derman et al. 1977; Morck et al. 1983). The impact of specific incremental amounts of phytate on iron absorption is not considered. This could be a limitation for estimating the bioavailability of iron from diets based on foods that contain very high amounts of phytate (such as whole maize or legumes), or the impact of processing to reduce phytate levels in food e.g., milling, fermentation, etc.

Suggestions for Improving Existing Models

The information provided by the many studies reviewed in this document should be used to further refine the existing bioavailability models. For example, there is a semi log-dose relationship between ascorbic acid intake and nonheme iron absorption from cereals and cereal-based meals, and the impact is greater for maize-based meals than those based on polished rice or milled wheat (Figure 3). The regression equation for the impact of ascorbic acid on iron absorption from the studies on maize is $y = 1.193 + 0.04 x$ ($r^2 = 0.57$), where $y$ is the ratio of nonheme iron absorption after ascorbic acid addition to nonheme iron absorption prior to intervention, and $x$ is the amount of ascorbic acid added. Similar equations could be developed for other cereals.

An additional refinement would be to include existing estimates of the magnitude of inhibition of iron absorption by phytate (Bothwell et al. 1989; Hallberg et al. 1989; Brune et al. 1992). As illustrated in Figure 1, iron absorption is strongly inhibited by even small amounts of phytate. Absorption from bread containing 4 mg iron was reduced by about 60 percent across the range of 1 to 35 mg phytate. Above this phytate intake, the slope leveled off so that absorption fell gradually to 20 percent with 700 mg phytate (Brune et al. 1992). The same effects would be anticipated with bread made from other cereals. It is also evident that the relative stimulatory effect of ascorbic acid on iron absorption is much smaller when a meal contains 207 mg of phytic acid (Siegenberg et al. 1991) or 893 mg of phytic acid (Hallberg et al. 1989) than when it contains 89 mg of phytic acid (Hallberg et al. 1989) (Figure 5).

There is a need to incorporate existing data into new models of iron bioavailability for developing countries. The validity of these models must then be tested using stable or radioactive iron isotopes.
PREDICTING THE RELATIVE IMPACT OF DIFFERENT DIETARY INTERVENTIONS TO IMPROVE IRON ABSORPTION

Potential dietary modifications include: increasing the intake of absorption enhancers (ascorbic acid, meat, poultry, and fish) or reducing the intake of inhibitors (phytate, tannins, and other polyphenols, calcium, and tea or coffee). It is important to remember that these dietary factors affect the absorption of fortificant iron (unless an iron chelate such as EDTA, or hemoglobin is used) in the same way that they affect the absorption of intrinsic iron in the diet. This implies that dietary modifications may be necessary even when a food fortification program is implemented.

Calculating the Impact of Dietary Interventions on Iron Absorption

Estimating the percent bioavailability of iron in diets

As described in the previous section, currently the best method for estimating iron bioavailability from dietary data in developing countries is that proposed by FAO/WHO (1988) as modified by Murphy et al. (1992). This requires information on the intake of iron, ascorbic acid, and protein from meat, fish, and poultry. The “tea factor” of Murphy et al. (1988) and/or a “coffee factor” (such as that suggested in section VIII.C), can be used to account for the inhibitory effects of usual tea or coffee intake. The model the adaptation of the Monsen and FAO/WHO models by Murphy et al.), can be used to account for the inhibitory effects of usual tea or coffee intake. The model could also be modified based on the suggestions in the previous section. It should be possible to develop simplified methods for assessing the intakes of the relevant food constituents focusing on staples, meat/fish/poultry, ascorbic-rich foods, coffee and tea, and other sources of nonheme iron.

If food intake data exist or can be collected, intakes of heme and nonheme iron, meat/fish/poultry protein, and ascorbic acid can be calculated with computer software programs or from food composition tables. The WorldFood Assessment System, which uses the International Minilist nutrient data base (IML), is recommended because it is a user-friendly software program and foods do not have to be coded for computer entry. The IML contains information on foods from six countries (Egypt, Kenya, Mexico, Senegal, India, and Indonesia) and can be modified to include data on additional foods. The output includes the nutrients and food components that affect iron bioavailability including phytate, although this is not used in the algorithm, and the calculated amount of bioavailable iron using the algorithm of Murphy et al. It also calculates the prevalence of inadequate intakes, discussed in the next section. One day of

3. The WorldFood System, including the International Minilist and all related documents, may be ordered for $US50 for researchers in developing countries or $250 for those in developed countries by contacting the Office of Technology Licensing, 2150 Shattuck Ave., Suite 510, University of California, Berkeley, CA 94704-1315. Phone: (510) 643-7201. Fax: (510) 642-4566.
Improving iron Status Through Diet

food intake data per individual in each category of interest (e.g., menstruating women, adolescents, children, etc.) will be sufficient to obtain an estimate of the average intake of bioavailable iron for each group. As discussed, food intake data should be coded and analyzed by meals in order to predict iron absorption more accurately, but applying the algorithm to total daily intakes will probably provide a reasonable approximation. If more heme iron is consumed on some days of the week than others, this must be considered when designing the food intake survey or analyzing existing data, as must seasonal variability in intake of ascorbic acid.

When there are data on usual dietary patterns, but not quantitative information on food intake, a cruder estimate of percent iron absorption could be obtained using the approach of Murphy et al.⁴ and/or by comparison with the information on the absorption of iron from regional foods and meals described in Tables 5, 7, and 9 (e.g., a typical Latin American meal or a Southeast Asian meal). An estimate of total iron intake will also be needed in order to calculate the amount of absorbable iron consumed.

“Bioavailable nutrient density” is a concept described by Hallberg et al. (1981c) which refers to the amount of available iron per unit of energy (e.g., per 1,000 kcal) in meals or diets, for individuals with borderline iron deficiency. Such individuals have a reference dose absorption of 40 percent and no iron stores, but have not yet developed anemia. (Note that the “adjusted” values for iron absorption in Tables 5, 7, and 9 would apply to such individuals). This concept recognizes that there is a practical limit to the amount of food that individuals can consume at meals and across days. It is useful for comparing different staple foods (as in Table 2) and meals. For example, the mean bioavailable nutrient density from a variety of breakfasts was 0.3 ± 0.15 (SD) mg iron/1,000 kcal, while in meals containing substantial amounts of meat and vegetables rich in ascorbic acid the corresponding value was 1.17 ± 0.43 (Hallberg 1992). The calculated bioavailable iron density of a good Swedish diet was 0.9 (Hallberg et al. 1981c). These data can be used to calculate the bioavailable iron density needed at specific levels of energy intake in order to cover iron requirements (Hallberg 1981c). Thus, this concept addresses the feasibility of obtaining sufficient available iron from meals or foods differing in energy content and for population groups that differ in energy intake (e.g., children versus adults, active versus sedentary populations, etc.).

Estimating the adequacy of bioavailable iron intakes

The calculated estimates of absorbable iron intake can be compared with the recommended amounts of absorbed iron for different population groups, in order to assess the prevalence of inadequate intakes. An approach for menstruating women has been described by FAO/WHO (1988) (Appendix 1). The amount of iron absorbed (mg/day) is compared with the probability (ranging from 5 to 50 percent) of developing iron

4. The following calculations illustrate how the data in Table 10 can be used. In the worst situation, where intakes of both ascorbic acid and animal protein (below 9 g/1000 kcal or about 90 g of meat, fish, or poultry/day) are low, the meat/fish/poultry protein will provide approximately 0.4 mg of absorbable iron. To meet the remaining requirement for 0.56 mg of absorbable iron (for adult men), an additional 11 mg of nonheme iron (bioavailability of 5 percent) will need to be consumed, for a total intake of 14 mg iron/day. To meet the recommendation of 2 mg absorbed iron for women, 24 mg of additional nonheme iron will be needed, or a total iron intake of 28 mg/day. Without iron fortification, this would be difficult for men (although they would probably be consuming more than 2,000 kcal) and virtually impossible for women. If ascorbic acid intake were increased to 35 to 105 mg/1,000 kcal, and meat/fish/poultry protein intake kept at 18 g/day, the bioavailability of nonheme iron would increase to 10 percent. In this case, the meat/fish/poultry protein would provide 0.57 mg absorbable iron and the total dietary iron would need to be 6.9 mg for men and 16.3 for women. This would be relatively easy to achieve for men and possible for women in some locations.
Predicting the Relative Impact of Different Dietary Interventions to Improve Iron Absorption

deficiency, or overt iron deficiency anemia. (Estimates of the probability of deficiency are based on the
distribution of iron requirements of menstruating women). Table 11 in Appendix 1 also shows, for diets in
the FAO/WHO categories of low, intermediate and high bioavailability, the total amount of iron that the
diets must contain compared with the risk of iron deficiency, and of anemia. The values in parentheses
probably cannot be met unless the diet is fortified with iron. Similar values for menstruating adult women
have also been presented graphically by Hallberg et al. (1992) (Figure 6 in Appendix 2).

If at least two days of food intake data are available for each individual, so that the intraindividual
variability of food (and available iron) intake can be estimated, the group prevalence of inadequate intakes
of absorbed iron can be estimated using the probability approach (National Research Council 1986). (If
only one day of food intake data per individual is available, estimates of day-to-day variability in intake for
that or a similar population group may be available in the literature). Murphy et al. (1992) describe in
detail how they used this approach to compute the probability that the observed available iron intake (mean
of all days of each individual's diet data) of preschoolers in three countries was below each child's
requirement, assuming that the requirement was normally distributed. The individual probabilities were
then averaged to estimate the predicted prevalence of intakes that were inadequate to maintain normal iron
stores or inadequate to prevent anemia in each population group. This approach avoids the use of cutoff
points to estimate the prevalence of inadequacy, but does require a computer to perform the analysis. The
most recent version of the WorldFood software program computes the prevalence of inadequate iron
intakes (see footnote 3).

**Predicting the impact of increasing ascorbic acid intake**

For both scientific and practical reasons, it appears that ascorbic acid intake can/should only be increased
in the range of 25 to 100 mg/meal. This is based on evidence that intakes above 100 mg/meal have
relatively little additional enhancing effect (Figure 3), and the difficulty of increasing intake even to 50 mg
in most meals. For example, 50 mg of ascorbic acid is approximately the amount contained in one lemon,
one orange, or two tomatoes. The impact of adding ascorbic acid to various diets can be calculated with the
algorithm of Murphy et al. (1992). The usefulness of this model is limited by the fact that the estimated
iron bioavailability does not change over the range of 35 mg to 105 mg/1,000 kcal. Alternative approaches
include the regression equation for maize presented in section VIII.D, and the summary data presented in
Tables 5, 7, 9, and Figure 3. The impact of ascorbic acid on the amount of iron absorbed will depend on
the amount already consumed in each meal (with less impact expected if intake is already high), and the
amount of nonheme iron in the meal. As the intake of phytate or tannins in meals increases, more ascorbic
acid will be needed to counteract their inhibitory effects on the percentage of iron absorbed, although the
percent increase in iron absorption per unit ascorbic acid added will be greater (Hallberg 1993).

**Predicting the impact of increasing the intake of meat, fish, and poultry**

The impact of increasing meat, fish, or poultry intake can also be predicted using Table 10. Theoretically,
this approach will be the most effective for increasing the total amount of iron absorbed. These animal
products stimulate the absorption of nonheme iron, and most importantly contain substantial amounts of
iron, of which 40 percent is highly (25 percent) bioavailable heme iron. A 100 g increase in meat intake

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will provide 1 mg more total iron and 0.25 mg more absorbable iron, in addition to its effects on nonheme iron absorption.

**Predicting the impact of reducing phytate intake**

From Figure 1, which shows the impact of phytate on the absorption of nonheme iron from bread, it is apparent that the percent availability of iron from breads (and presumably cereals in general) is less than or equal to 5 percent unless phytate intake is reduced to below 35 mg per meal. In the presence of added ascorbic acid, less phytate would need to be removed to see an improvement in iron absorption.

**Predicting the effect of increasing bioavailability on the prevalence of inadequate iron intakes and iron status in population groups**

From the increase in the amount of iron absorbed, as predicted by one or more of the above approaches, it is possible to estimate the potential impact of this improvement on the iron status of a population. Hallberg et al. (1994) provide the following example of how this can be estimated from Figure 6 in Appendix 2. In a population with a 32 percent prevalence of iron deficiency (low serum ferritin), the requirement for absorbed iron for menstruating women is estimated to be 1.60 mg/day. If iron absorption can be increased by 0.4 mg/day, the prevalence of iron deficiency would be halved, i.e., it would fall from 32 percent to 16 percent. As an alternative, the probability analysis described by Murphy et al. (1992) can be used to calculate how an increase in the amount of iron absorbed would lower the prevalence of intakes that are inadequate to prevent iron deficiency or anemia in different age groups.

It is also possible to calculate the effect of an incremental increase in absorbed iron on changes in serum ferritin and hemoglobin, and its subsequent impact on the prevalence of iron deficiency and anemia, in a population group. Returning to the example in the preceding paragraph (Hallberg et al. 1994 and Figure 6 in Appendix 2), even with a 0.4 mg/day increase in iron absorption (from 1.6 to 2.0 mg/day), it will take 300 days to increase hemoglobin concentration by 10 g/L in women with mild iron deficiency. This is because a 10 g/L hemoglobin deficit represents an iron deficit of about 120 mg in a 55 kg person; hemoglobin contains 3.4 mg iron/g and there is 70 mL blood per kg body weight. In populations with marked anemia it would take even longer to recover the iron deficit.

A theoretical sample size of about 25 women, (and in practice a considerably larger number of women to account for different sources of variation) would need to be followed for a year to detect an increase in hemoglobin of 10 g/L, if 30 percent of them are anemic at the onset (Hallberg 1992). The impact on serum ferritin of changing iron absorption would be easier to detect than change in hemoglobin because ferritin will increase in more subjects.

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5. The intra-individual SD of hemoglobin concentration, measured twice, is 7.7 g/L (Hallberg 1992).
Choosing Approaches Most Likely to Succeed

The approaches most likely to succeed are those predicted to have the most significant impact on iron bioavailability and status of the target population (which can be estimated as described in the previous section). The cultural acceptability, feasibility, and likely cost-benefit of each approach are equally important and must be locally assessed. Fortification is not usually an option unless staples are centrally processed.

Predicting the likely success of strategies based on dietary staples

To some extent the most successful approaches can be predicted from the predominant dietary staple consumed, if that food provides a substantial proportion of the target population’s energy requirements.

Diets high in phytate and/or tannins, and high in iron: whole maize, sorghum, millet, legumes

Diets featuring maize, sorghum, millet, and legumes are consumed in many Latin American and African countries. Legumes are also important in Asian diets. The most effective approach would be to lower the phytate or tannin content without losing the iron (fermentation, germination or leavening — which would also increase the availability of zinc, calcium, and other minerals), or to enhance the absorption of the iron present (e.g., by increasing the intake of ascorbic acid). Iron fortification can usually only be considered if foods are centrally processed. Most iron fortificants added to these foods (e.g., to whole cornmeal) will be bound by the phytate or tannins, so that iron chelates such as NaFeEDTA should be more effective, and are also advisable given the high lipid content of these foods. While increasing ascorbic acid intake would have a substantial impact on the absorption of iron from traditional iron fortificants (e.g., ferrous sulfate or reduced iron), it would also increase rancidity because of the high lipid content of these foods.

Diets moderately high in phytate and moderately high in iron: whole wheat and brown rice

Whole wheat and brown rice are staples consumed by lower socioeconomic status groups in Africa (whole wheat) and Asia (brown rice). Milling reduces phytate content by about two thirds in whole wheat and by about 50 percent in brown rice (Table 2). Iron is removed in the same proportion so that the phytate to iron molar ratio is relatively unchanged by milling. However, removal of the phytate will substantially improve the absorption of nonheme iron from other foods in the meal. Leavening and fermentation would reduce the phytate content without lowering the iron and, because the foods contain a significant amount of iron, this could slowly improve iron status over time. The phytate content of the unprocessed grains is high enough to severely reduce the effectiveness of traditional iron fortificants unless ascorbic acid intake is substantial.
Improving Iron Status Through Diet

Diets moderately high in phytate and low in iron: white flour, white rice

Wheat flour and white rice have a high phytate content and are low in dietary iron. White rice is the staple food in many Asian countries, and in regions of Latin America, particularly in Brazil, while unleavened bread products containing high amounts of phytate are widely consumed in the Middle East and North Africa. Strategies to reduce phytate content, such as leavening of bread, will improve iron absorption, but have relatively little effect on iron status because dietary iron is already low in these foods. Increased intake of enhancers such as ascorbic acid will not affect population iron status substantially because so little iron is already present in the diet. Strategies to increase meat, fish, and poultry intake or to implement iron fortification should be considered. The absorption of iron from traditional iron fortificants such as elemental iron may be sufficient to be effective, and fortificants that do not bind to phytate, such as NaFeEDTA, may have significantly more impact on population status. Increasing ascorbic acid intake would improve the availability of the iron from fortificants, such as ferrous sulfate and reduced iron, without a major risk of rancidity developing in low lipid foods such as white flour and white rice.

Diets low in phytate and low in iron: degermed maize

Neither increasing the intake of enhancers (such as ascorbic acid) nor reducing the intake of inhibitors (e.g., tea or coffee) with meals based on degermed maize is likely to have much effect on iron status. Iron fortification should be considered. Traditional iron fortificants containing soluble iron should be effective for this purpose. Degermed maize has a greatly reduced lipid content so that soluble iron fortificants are less likely to cause rancidity. Degermed maize is not widely consumed. In Venezuela it is made into the maize bread arepa, which has twice the iron bioavailability of Mexican tortillas (Acosta et al. 1984).

Strategies for increasing ascorbic acid intake

In order to increase ascorbic acid intake, it is necessary to identify locally available foods that are rich sources of the vitamin. Potential sources are fruit juices, fruits, and some vegetables. For each potential food source, information must be collected for target population groups on factors such as its acceptability; cost; availability; seasonality; and amount usually consumed, versus the amount that would need to be added to produce a significant increase in iron absorption; the meals at which it is consumed and the nonheme iron content of those meals; ease of preparation; loss of ascorbic acid during storage and preparation; and attitudes and beliefs concerning its taste and other desirable or undesirable attributes. The information can be obtained from analysis of existing dietary data, as well as key informants, pilot testing, and community application of a survey.

Decreasing phytic acid intake

Where seeds or cereals are often fermented or germinated in the home or prior to commercial preparation of a flour, decreasing phytic acid intake would seem to be a highly appropriate way to improve iron absorption without losing the iron in the food. Fermentation and germination will be more difficult strategies in locations where whole grains are ground locally to make a fairly solid food, such as tortillas from whole maize. Soaking maize might degrade some phytate by activating endogenous phytase. Milling
substantially reduces the phytic acid content of cereals and seeds, but not legumes. Because iron is also lost, in most cases milling does not improve the amount of iron that can be absorbed from the cereal or seeds (degerming maize being an exception to this, Table 2). For some foods, such as wheat, milling may not reduce phytate content sufficiently to produce an adequate improvement in iron absorption, so subsequent leavening is still important.

**Reducing tea or coffee consumption with meals**

In cultures where tea and coffee are habitually consumed with meals, efforts need to be made to increase awareness about the detrimental effects of this practice on iron absorption. Further investigation and education programs will be needed to identify suitable replacements — preferably juices high in ascorbic acid. Because this strategy requires people to eliminate traditional beverages, or to avoid drinking them within about two hours of a meal, success may be difficult to achieve. However, it may work in the population groups at greatest risk of iron deficiency; caretakers could be educated about the adverse effects of giving coffee or certain types of tea to infants and children, and women might be persuaded to change their coffee- or tea-drinking practices during pregnancy.

**Strategies to increase meat, fish, or poultry intake**

As explained above, increasing meat, fish or poultry intake is theoretically the most effective way to increase the amount of iron consumed for those population groups who do not avoid these products for religious or other reasons. Unfortunately, iron is poorly available from eggs.

The intake of animal products is usually constrained by their cost. As a result, more attention needs to be directed toward increasing home or community production of small animals such as rabbits, guinea pigs, and poultry. Small livestock, aquaculture, and other relevant animal production or agriculture programs have rarely considered increasing local consumption of animal products to be an important goal. Increasing the consumption of these products would improve the intake of iron, zinc, vitamin A, riboflavin, vitamin B₁₂, and other nutrients often lacking simultaneously in the same population. This would in turn improve the health, development, and function of these populations. Unfortunately, it may be difficult to achieve this if the animal products are not consumed by the household but sold for cash. Another problem constraining the availability of animal products is the lack of refrigeration. Projects to install refrigeration in community stores or other buildings might help in this area.

**Strategies that consider other micronutrients**

Iron deficiency in developing countries is often accompanied by other micronutrient deficiencies. Whenever possible, strategies to improve iron absorption should also be evaluated in terms of their potential to increase the intake or bioavailability of other nutrients. Examples include the content of pro-vitamin A carotenoids in ascorbic-rich foods; improvements in the absorption of zinc, calcium, and copper, and in the content of some B vitamins by fermentation or germination; beneficial effects of fermentation on the viscosity and safety of cereals fed to infants; and higher intakes of retinol, vitamin B₁₂, riboflavin, and absorbable zinc when more animal products are consumed.
Multiple strategies

In reality there may be several equally beneficial, or mutually beneficial, strategies for increasing the amount of iron absorbed from diets. For example, iron absorption from Latin American meals that contained high amounts of phytate and low amounts of enhancing factors was increased two- to three-fold by adding to the meal either synthetic ascorbic acid or a similar amount of ascorbic acid as cauliflower, ground beef, or ferrous sulfate (Hallberg and Rossander 1984). Likewise, ascorbic acid intake may need to be increased so that fortificant iron is better absorbed from staples that have a relatively high phytic acid content. Because the approaches that can be used to increase iron absorption are generally cumulative, multiple strategies should be considered whenever possible.
REFERENCES


References


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References


Table 11: Daily Iron Requirements and Dietary Iron Intakes Required to Reduce the Risk of Developing Iron Deficiency and Overt Iron Deficiency Anemia in a Randomly Selected Menstruating Woman Weighing 55 kg

<table>
<thead>
<tr>
<th>Probability of developing iron deficiency</th>
<th>Probability of developing overt iron deficiency anemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>50%</td>
<td>25%</td>
</tr>
<tr>
<td>Requirement of absorbed iron (mg/day)²</td>
<td>1.25</td>
</tr>
<tr>
<td>Requirement of dietary iron (mg/day):</td>
<td></td>
</tr>
<tr>
<td>Intermediate bioavailability</td>
<td>13</td>
</tr>
<tr>
<td>High bioavailability</td>
<td>8</td>
</tr>
</tbody>
</table>

¹ Requirements for absorbed iron have been reduced on the assumption that menstrual iron losses in women with borderline anemia are less because of an 18 g/L decrease in hemoglobin concentration from the mean of 138 g/L to the cut-off value of 120 g/L.

² Values in parentheses cannot be attained by consuming an unfortified diet.

Source: FAO/WHO 1988
APPENDIX 2

Figure 6: Relationship Between the Amount of Iron Absorbed and Prevalence of Empty Iron Stores

Effect of increasing the absorption of iron on expected prevalence of iron deficiency in adult menstruating women. The curve is an enlargement of the distribution of iron requirements in women: this curve shows that the lower the initial prevalence of iron deficiency, the less is the absolute reduction in prevalence with increasing iron absorption.

Source: Hallberg et al. 1994