MICRONUTRIENT INTERACTIONS

IMPACT ON CHILD HEALTH AND NUTRITION

WASHINGTON, DC, JULY 29–30, 1996

Sponsored by:
U.S. Agency for International Development
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Foreword

Micronutrient deficiencies result from inadequate dietary intake, impaired absorption, limited bioavailability, excessive losses, increased requirements, or a combination of these factors. Although the functional consequences of micronutrient deficiencies are largely known, micronutrient interactions at the metabolic level, as well as within the diet, are less clearly established. Micronutrient interactions are important because they affect the bioavailability (absorption and use) of nutrients. The level of bioavailable micronutrients can often be improved by changing the food preparation methods used commercially and in the home. Improved processing methods, however, may not increase the level sufficiently to meet daily micronutrient requirements. Therefore, the consumption of appropriately mixed foods, fortified complementary foods, or micronutrient supplementation may be necessary.

On July 29–30, 1996, the workshop *Micronutrient Interactions: Impact on Child Health and Nutrition* was held in Washington, DC, under the joint support of the United States Agency for International Development (USAID)/Opportunities for Micronutrient Interventions (OMNI) Research project, and the Food and Agriculture Organization (FAO). At this workshop, there were eleven presentations on the impact of micronutrient interactions on nutrient bioavailability and nutritional status, food processing techniques that can improve the micronutrient content and bioavailabilty in complementary foods, and past experiences with complementary feeding programs in developing countries. In addition, 45 scientists; nutrition program managers; and representatives of the pharmaceutical and food industry, nongovernmental organizations, and international donor agencies met to discuss and answer the following questions:

- What food processing methods have been tried to improve the micronutrient content of home-based complementary foods? What additional information is needed for improving the micronutrient content of home-based complementary foods?
- What micronutrient interactions are important in developing fortified complementary foods and supplements for infants and young children? What micronutrients should be added to complementary foods and supplements and at what level?
- What are the barriers to implementing a successful complementary feeding and supplementation program in developing countries? What additional information or efforts are needed to promote the consumption of complementary foods and use of supplements in developing countries?

USAID, through its OMNI Research project, and in cooperation with FAO, is pleased to provide this proceedings and a summary of the discussions that occurred during this workshop. It is hoped that this document will help to identify and resolve issues critical to the promotion and implementation of complementary and supplementation programs in developing countries.

Opening Remarks

David Oot

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We are all committed to bettering the lives of children, which ties us together to deliberate the questions before us. The U.S. Agency for International Development (USAID)and the Food and Agriculture Organization of the United Nations (FAO) are jointly sponsoring this 2-day discussion on micronutrient interactions, which has immediate relevance to the lives of children throughout the world.

USAID and FAO have an outstanding track record of working to improve child survival and health. These efforts are grounded in the understanding that breast-feeding is critical for infant health. USAID has long promoted breast-feeding as an essential component of child survival programs.

Exclusive breast-feeding is the best nutrition for an infant up to 6 months of age. At that point, however, nutrition science tells us that complementary foods should be added to infants' diets to complement the nutrients received from breast milk. This is particularly important in developing countries, where the mother's diet, and therefore her breast milk, may be less than optimal.

Traditional infant foods are often based on unrefined cereals and legumes that are low in bioavailable micronutrients, such as iron and zinc. Processing these foods can improve the bioavailability of micronutrients. Fortification of locally available foods can also improve the level of micronutrients in food; however, not all consumers may be able to afford fortified foods. Nevertheless, fortification of marketed infant foods or the use of micronutrient supplements can enhance the nutritional status of socioeconomically better-off children even more.

Two important questions related to micronutrients are: Which micronutrients should be added to complementary foods or included in pharmaceutical supplements? What are the optimal amounts to add? To adequately answer these questions, we must understand the extent and direction of interactions that may occur between various micronutrients when mixed together in a food or a supplement.

Present here today are a group of experts who were selected for their knowledge of the scientific issues as well as their practical knowledge of conditions in countries where fortified foods and supplements would be beneficial to children's health. In addition to identifying the optimal mix of micronutrients for complementary foods and supplement preparations, participants have been asked to help answer several other questions:

- Which food-processing methods optimize the micronutrient contribution of home-based and commercially available complementary foods?
- What barriers limit the implementation of successful complementary food programs in developing countries?
- What additional information is needed to implement successful programs designed to provide nutrient-rich complementary foods and supplements?

Together we can make an important contribution that will in a very short time provide nutritional benefits to children around the world.

Iron-zinc-copper Interactions

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Introduction

Iron deficiency is the most common nutrient deficiency in both less-developed and industrialized countries worldwide (Lönnerdal and Dewey 1995). The consequences of iron deficiency anemia are well recognized, ranging from effects on energy metabolism and immune function to effects on cognitive function and motor development (Walter 1993, Dallman et al. 1980). Infants, children, and fertile women are particularly vulnerable populations, and programs have been implemented to treat and prevent iron deficiency. Iron supplements, fortification of common staple foods with iron, and changes in food preparation methods (e.g., fermentation and germination) are used, with the choice of approach dependent on setting, population, and practical considerations. There are currently major efforts worldwide to combat iron deficiency, and the areas and number of people reached are rapidly increasing.

The causes for iron deficiency are well-known. A low iron content of the diet is common as is a low bioavailability of the iron in the diets consumed. This is compounded by rapid growth (and tissue synthesis) in infants and children, menstrual losses of iron in fertile women, and fetal demands for iron in pregnant women. The low bioavailability of dietary iron is often caused by a high intake of phytate in cereals, rice, and legumes, which inhibits iron absorption, and a low proportion of meat in the diet, which provides absorbable iron in the form of heme iron (Hallberg 1981). Much less recognized is that the factors limiting iron nutrition have similar effects on zinc nutrition.

Infants, children, and fertile women also have high requirements for zinc, and the factors inhibiting iron absorption often have an even more pronounced negative effect on zinc absorption. Similar to the situation for iron, meat is the best dietary source of zinc. Because the usual meat intake of many populations is very low, their zinc intake is also very low. A lack of data on zinc nutrition and the difficulties in assessing zinc status, particularly marginal zinc status, have led to zinc deficiency not being recognized as a major nutritional problem in less-developed countries (King 1990). However, when attempts are made to assess both iron and zinc status, the prevalence of zinc deficiency appears to be similar to that of iron deficiency.

In a recent study of pregnant women in Lima, Peru, we found that 60% of the women were anemic and about 55% had low zinc status as assessed by lower than normal serum zinc concentrations (N. Zavaleta, B. Lönnerdal, K.H. Brown, unpublished observations, 1994). Further, in a study of 1-year-old Swedish infants, we found that 35% had low serum ferritin concentrations and 29% had low serum zinc concentrations (L.A. Persson, M. Lundstrom, B. Lönnerdal,

O. Hernell, unpublished observations, 1995). These infants had a high intake of unrefined cereals, which may have affected iron and zinc absorption negatively. Thus, there may be good reasons to provide zinc along with iron.

A potential problem of supplementing both iron and zinc is that supplemental iron may inhibit zinc absorption, which then has to be taken into account when making programmatic decisions on modality and dosages. Furthermore, iron may affect copper absorption and zinc can inhibit copper absorption, making it possible that combined iron and zinc supplementation or fortification may interfere with copper absorption and status even if copper intake normally is not an area of concern. This paper will discuss the potential effects of adding iron, zinc, or both on iron, zinc, and copper nutrition and possible ways to minimize potential interactions.

Types of Trace Element Interactions

There are two fundamentally different types of trace element interactions that can occur in biological systems (Lönnerdal and Keen 1983). In the first type, two (or more) trace elements share the same absorptive pathways. This means that high concentrations of one element may interfere with the absorption of another element. The conceptual framework for this type of interaction was provided by Hill and Matrone (1970), who postulated that elements that have a similar coordination number and a similar configuration in water solution can compete for absorptive pathways. Through experiments in animals, they demonstrated that essential elements such as zinc and copper can interact and that an excess of one element can induce a deficiency of the other. They also demonstrated that a toxic element like cadmium can inhibit the absorption of an essential element like zinc. This type of interaction is well recognized and even used in some clinical applications, such as the treatment of Wilson's patients with high doses of zinc (Brewer et al. 1983).

The second type of interaction occurs when a deficiency of one element affects the metabolism of another element, as shown by the example of copper deficiency that causes iron-deficiency anemia (Cohen et al. 1985). Because copper is an essential component of ceruloplasmin, or as it more accurately should be called, ferroxidase I, copper deficiency causes a dramatic decrease in ferroxidase activity, which in turn prevents the mobilization of iron from stores (by being oxidized from +2 to +3) and its incorporation into hemoglobin. Another example is the homeostatic up-regulation of iron absorption that occurs during iron deficiency. This up-regulation also dramatically increases the absorption both of essential elements, such as manganese, and toxic elements, such as cadmium (Flanagan et al. 1980).

From a programmatic viewpoint, the first type of interaction is the most important to consider; that is, if supplements of one (or several) elements are to be used, can this create adverse effects on the utilization of other essential elements? However, the second type of interaction should also be considered because a preexisting impairment in status of one trace element (which is not being studied) may have a pronounced effect on the outcome of a study and therefore affect the evaluation of outcomes.

Trace Element Deficiencies in a Global Perspective

Iron deficiency is common in most parts of the world. There are few reports on iron deficiency affecting zinc absorption or metabolism, but this issue has not been studied extensively. The effects of iron deficiency on copper absorption and metabolism have not been studied extensively either, but the limited data available do not indicate a pronounced effect. As suggested above, zinc deficiency may be common in the same populations that are prone to iron deficiency. Zinc deficiency does not appear to have any pronounced effect on iron absorption or metabolism, but some reports suggest that copper absorption may increase when zinc status is impaired, even if the deficiency is only marginal (Polberger et al. 1996). There are limited data supporting a high prevalence of copper deficiency in less-developed countries; however, infants fed milk-based diets may have compromised copper status as a result of the low copper concentration of milk and dairy products (Levy et al. 1985). Marginal copper status can affect iron absorption and status and, consequently, the outcome of iron supplementation and fortification programs. Less is known about the effects of low copper status on zinc absorption and status.

Trace Element Ratios

Because high concentrations of one element may interfere with the absorption of another element, it is important to consider the ratios between trace elements. For example, breast milk contains iron and zinc at a ratio of approximately 1:4 whereas iron-fortified milk (or formula) may have a ratio of 12:1 (Lönnerdal et al. 1983). When considering competition for absorptive pathways, it should be recognized that the iron concentration of the diet in fortification programs may be increased 50–60 times (or more) while the zinc concentration remains unchanged.

Although not as pronounced, the ratio of zinc to copper can also be raised considerably by zinc fortification. To date, this is largely confined to infant formulas, in which the zinc-copper ratio can be as high as 70:1 whereas this ratio is approximately 5:1 in breast milk (Lönnerdal et al. 1983). It can be expected that zinc supplements will become more commonly used and that more diets will be fortified with zinc. Thus, it is evident that zinc nutriture should be monitored when iron is added to the diet and that copper nutriture should be monitored when iron or zinc is added to the diet.

Iron-zinc Interactions

Although iron and zinc do not form similar coordination complexes in water solution and were not believed to directly compete for absorptive sites, Solomons and Jacob (1981) showed that high levels of iron can interfere with zinc uptake as measured by changes in serum zinc after dosing (area under the curve). The postprandial rise in serum zinc after an oral dose of 25 mg zinc in water solution was given to fasting subjects was lower when 25 mg of iron (iron:zinc, 1:1) was given with the zinc dose than when zinc was given alone. Increasing the iron dose to 50 (2:1 ratio) and 75 mg (3:1 ratio) reduced zinc uptake even further. The dose of zinc given was high so that a measurable increase in plasma zinc would occur, and it has been argued that pharmacological amounts of zinc and iron were used. However, when supplements of iron and zinc are given to treat deficiencies, the doses used are not very different from those used by Solomons and Jacob (1981). In a subsequent study we used a lower amount of zinc, which was more similar to the zinc content of a regular meal (2.6 mg), and zinc radioisotope and whole-body counting to measure zinc absorption (Sandström et al. 1985). At this zinc level, there was no difference in zinc absorption when the iron-zinc ratio was changed from 1:1 to 2.5:1; whereas zinc absorption decreased from 60% to 38% when a molar ratio of 25:1 was used. It is not yet known whether the difference in results between our study and the one by Solomons and Jacob is due to the lower amounts of zinc and iron used or whether plasma zinc uptake yields different results from whole-body counting.

The interaction between iron and zinc in water solution is relevant to the situation when combined iron and zinc supplements are given in a fasting state. To explore whether the same interaction occurs when iron is given with meals, either as a supplement or as iron-fortified food, we gave human subjects the same quantities of iron and zinc as described above in the form of a single meal (Sandström et al. 1985). There were no significant differences among groups when iron-zinc molar ratios of 1:1, 2.5:1, and 25:1 were used. Thus, there appears to be no interaction between these two elements when they are given in a meal. To investigate whether the difference in results between giving iron and zinc in a water solution and in a meal is due to the absence or presence of ligands binding zinc or iron, we added histidine to the water solution containing iron and zinc. In the presence of histidine, there were no significant differences observed in zinc absorption among groups receiving iron-zinc ratios of 1:1, 2.5:1, and 25:1. We therefore suggest that when a dietary ligand that can chelate zinc (e.g., histidine) is present, zinc will be absorbed via a pathway that is not affected by the iron concentration in the gut. However, when no dietary ligands are present, iron and zinc may compete for common binding sites at the mucosal surface, and a high level of one element interferes with the uptake of the other. This is consistent with our earlier observation that infants given daily iron drops (30 mg) had zinc status (as assessed by plasma zinc) after 3 months of treatment similar to that of infants given placebo (Yip et al. 1985). The iron was given apart from meals and no interaction between iron and zinc could occur. That the interaction is likely to occur at the level of absorption is supported by our observation that prior iron-loading of human subjects with 50 mg/day for 2 weeks had no effect on zinc absorption (Sandström et al. 1985). Supporting this observation, Fairweather-Tait et al. (1995) recently reported that iron-fortified foods had no effects on zinc absorption in infants, and similar obervations were made by Davidsson et al. (1995) in adults.

To investigate whether high levels of zinc can interfere with iron absorption, we gave iron and zinc at different levels, in either water solution or part of a meal, to human subjects and measured iron absorption by a radioisotope method (Rossander-Hulthén et al. 1991). No effect on iron absorption was found for either method of administration even when a 300:1 molar excess of zinc to iron was used. Thus, it appears that iron uptake is not affected by excess zinc whereas excess iron can affect zinc uptake when iron and zinc are given together in a water solution and in a fasting state.

Iron-copper Interactions

An interaction between iron and copper is usually believed to occur by the mechanism described above, that is, by copper deficiency causing low ferroxidase activity and inducing iron-deficiency anemia. There is little support for copper deficiency being a common nutritional problem in less-developed countries. However, this may be due to a lack of data rather than to studies demonstrating an absence of impaired copper nutrition. Infants and children consuming predominantly milk-based diets may have marginal copper status even if the impairment in copper status is not severe enough to cause anemia. Another concern is that high iron intakes may interfere with copper absorption.

In a balance study, infants fed formula having a low iron concentration (2.5 mg/L) were shown to have higher copper absorption than infants fed the same formula but with a higher iron concentration (10.2 mg/L) (Haschke et al. 1986). In addition, Barclay et al. (1991) found lower copper status (as measured by red cell superoxide dismutase) in premature infants given iron supplements. We subsequently showed that infants fed a formula containing 7 mg iron/L had a lower serum copper concentration than did infants fed the same formula containing 4 mg iron/L (Lönnerdal and Hernell 1994). Iron-deficient children given iron supplements for 2 weeks were reported to have decreased serum copper and ceruloplasmin concentrations (Morais et al. 1994). In the last two studies, serum copper concentrations were still within the normal range. Thus, it appears that the level of iron intake affects copper absorption and status even when the differences in iron-copper ratios are relatively small.

Similar to what was described above for the interaction between iron and zinc, it is likely that iron and copper need to be ingested together for an effect to occur. In our study on infants given daily iron drops apart from meals for 3 months (Yip et al. 1985), we found no adverse effects on copper status as assessed by serum copper concentrations.

Zinc-copper Interactions

A negative effect of high concentrations of zinc on the copper status of experimental animals was described by Hill and Matrone (1970). High concentrations of zinc had been thought to induce synthesis of metallothionein, a protein that has a high capacity to bind copper as well as zinc (Hall et al. 1979). Thus, a so-called mucosal block for copper absorption was created. Recent work, however, indicates that the level of zinc required to induce metallothionein is very high and may be considered unphysiological (Oestreicher and Cousins 1985). Therefore, this induction may not occur at the normal range of zinc intakes even if zinc supplements are given. It is obvious, however, that high levels of zinc interfere with copper absorption in the small intestine.

In a study by Wapnir and Lee (1993), the removal rate of copper from perfused rat jejunum was significantly lower than normal when dietary zinc amounts were high and even lower when the zinc was given together with histidine, a chelator of zinc known to enhance its absorption. The pronounced negative effect of high concentrations of zinc on copper absorption has been recognized in human subjects and is used in the treatment of Wilson's disease, a genetic disorder of abnormally high copper absorption (Brewer et al. 1983).

Modest increases in zinc intake may affect copper absorption in humans. The two elements are likely to be, at least in part, transported by the same pathway in the enterocyte, and although metallothionein may not be induced, they may compete for a common carrier. In a study in which infant rhesus monkeys were fed formula with two different concentrations of zinc (1 and 4 mg/L), monkeys with a lower zinc intake had a significantly higher plasma copper concentration (Polberger et al. 1996). Plasma copper concentrations were, however, within the normal range in both groups, and animals with a lower zinc intake may have had enhanced copper absorption rather than those with the higher level having had decreasing copper absorption. In any case, the results demonstrate that zinc and copper interact, even at fairly small changes in their ratio.

In a recent study of infant rhesus monkeys fed soy formula, we found that when the phytate content of the formula was reduced, zinc absorption increased significantly whereas serum copper decreased (Lönnerdal et al. 1996). Although serum copper did not decrease precipitously in our infant monkey model, it should be recognized that marginal copper deficiency does not have a pronounced effect on serum copper or other indices (Turnlund et al. 1990). Thus, the relatively minor but significant decrease in serum copper may indicate a more severe depletion of copper at the tissue level. It is evident that more sensitive indicators of copper deficiency are needed.

Iron-zinc-copper Interactions

It is evident from the discussion above that both iron and zinc may be chosen for micronutrient interventions, either as a supplement or a fortificant. It can also be envisioned that in many locations the provision of iron and zinc will allow for the treatment of existing deficiencies and not only be a preventive measure. Therefore, relatively generous doses of iron and zinc may be used. For infants, iron is often given at a dosage of 5 mg/kg/day and zinc at about 10–15 mg/day. A 6-kg infant for example may consequently receive 45 mg of iron plus zinc, which is considerably higher than the customary copper intake of 0.5–1 mg/day (or less) at this age. This dramatic change in the ratio of iron plus zinc to copper may have a detrimental effect on copper absorption and status.

Few studies have evaluated the effects of combined iron and zinc supplements on copper nutrition in humans. However, one report on adult women given 50 mg of iron and 50 mg of zinc daily for 10 weeks showed decreased red cell superoxide dismutase activities, suggesting that this interaction should be studied further (Yadrick et al. 1989).

Conclusions and Recommendations

The three essential trace elements iron, zinc, and copper are known to interact with each other. However, most studies have been performed in rodent models, have used pronounced differences in element ratios for the different test groups, and have been of relatively short duration. From a programmatic view it is more relevant to evaluate the effects of moderately increased intakes of these elements for extended time periods in vulnerable groups. Although interactions are more likely to occur when the elements are given as a supplement, the modality (i.e., giving them with a meal or between meals) may affect outcome drastically. Although somewhat more difficult from a practical point of view (compliance, toxicity risk, etc.), giving the elements alternating days (e.g., one weekly vs. the other daily) needs to be explored in various settings.

Providing iron and zinc to vulnerable groups can have pronounced positive effects on several outcomes, but this should not overshadow the potential negative effects on other outcomes. This is particularly important when it comes to morbidity; giving modest amounts of zinc may have a positive effect on immune function (Schlesinger et al. 1992) whereas somewhat higher amounts may interfere with copper and iron absorption, creating suboptimal copper and iron status, which in turn may have a negative effect on immune function (Sandström et al. 1994). Thus, the apparent lack of effect of zinc on morbidity may discourage efforts to provide zinc to groups that do need it.

A careful approach with graded dosages of micronutrients provided alone or in combination, as supplements or food fortificants, and with careful monitoring of not just the micronutrient being studied is necessary to avoid misinterpretations that could have long-ranging negative impact on programs worldwide.

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Iron–ascorbic Acid and Iron-calcium Interactions and Their Relevance in Complementary Feeding

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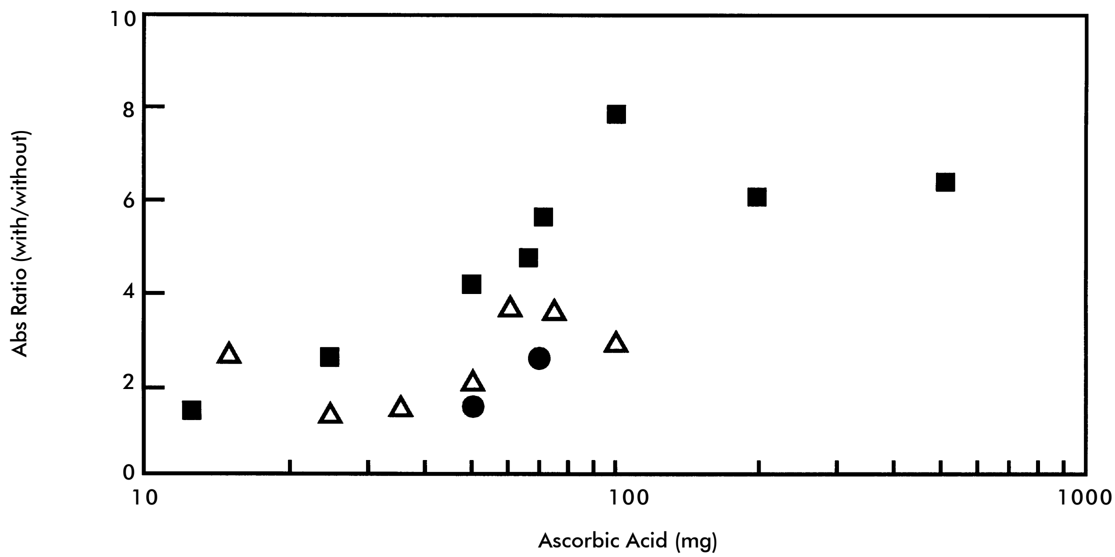
Ascorbic acid and calcium have opposite effects on iron absorption; ascorbic acid improves the absorption of naturally occurring or fortificant nonheme iron in foods whereas calcium inhibits the absorption of both heme and nonheme iron. The effects of ascorbic acid and calcium on iron absorption will be considered separately, followed by a discussion of the practical implications of these interactions for complementary feeding.

Iron and Ascorbic Acid

Ascorbic acid is a strong enhancer of nonheme-iron absorption. The mechanisms for this absorption enhancement include the reduction of dietary ferric iron to its better-absorbed ferrous form and the formation of an iron–ascorbic acid chelate in the acid milieu of the stomach. The chelate is formed at a lower pH than that at which iron complexes with phytate, tannins, and other inhibitors of iron absorption. Thus, ferrous iron is preferentially bound to ascorbic acid in the stomach and remains in this complex even in the more alkaline pH of the intestine. The overall effect is to reduce the influence of inhibitory ligands that would otherwise bind iron in the duodenum (Hurrell 1984). Although ascorbic acid does not enhance iron absorption from most ferrous iron supplements in the absence of meals, it increases the bioavailability of ferrous iron added as a fortificant to foods, especially when those foods contain a large amount of iron absorption inhibitors. This is true whether the fortificant iron is a simple ferrous salt or a chelate such as NaFeEDTA. Even less-bioavailable iron salts, such as ferric orthophosphate and ferric pyrophosphate, are reasonably well-absorbed in the presence of ascorbic acid.

We recently reviewed studies in which ascorbic acid was added to meals based on maize, wheat, and rice (Allen and Ahluwahlia 1996). Figure 1 presents, for each study, the mean ratio of iron absorption in the presence vs absence of ascorbic acid at each concentration of ascorbic acid. Absorption of the reference dose of iron in each study was used to correct for differences in the efficiency of iron absorption caused by differences in iron status among subjects. It is evident from Figure 1 that iron absorption from meals approximately doubles when 25 mg ascorbic acid is added and increases three- to sixfold when 50 mg is added. The relation between iron absorption and ascorbic acid appears to be linear up to at least 100 mg ascorbic acid per meal.

Figure 1. Effect of ascorbic acid on nonheme iron absorption from cereal based diets. △ rice, ● wheat, ■ maize.



Because ascorbic acid releases nonheme iron bound to inhibitors, the most marked effects are seen when it is added to foods with a high content of phytates, such as maize, whole wheat, and soya, or to foods high in polyphenols such as tannic acid (Siegenberg et al. 1991, Derman et al. 1977). This relation was illustrated for a variety of diets by Hallberg et al. (1986). The stimulatory effect of 50 mg ascorbic acid was less when the ascorbic acid was added to a breakfast (wheat roll, cheese, and coffee) or a hamburger meal, intermediate when it was added to a Thai rice meal, and highest when it was added to a Latin American meal (high in phytate from maize and polyphenols from beans). However, even though the enhancing effect of ascorbic acid on iron absorption is relatively greater in the presence of inhibitors, as the content of inhibitors increases, more ascorbic acid is needed to overcome the inhibitory effects.

Protein from meat, fish, and poultry also enhances iron absorption. In general, 1 mg ascorbic acid is equivalent to about 1–1.5 g meat in its ability to enhance nonheme iron absorption (Hallberg and Rossander 1984). The stimulatory effect of the natural ascorbic acid in foods is similar to that of synthetic ascorbic acid. For example, when ascorbic acid was added in the form of vegetable salad, cauliflower, or orange juice to a hamburger meal, the relative increase in iron absorption was very similar to that caused by consuming 25–500 mg synthetic ascorbic acid per meal (Hallberg et al. 1986).

Effects of Long-term Ascorbic Acid Supplementation on Body Iron Stores

Most investigators have measured the effect of ascorbic acid on iron absorption from single meals. Fewer, less-conclusive data exist on the effect of a longer-term higher ascorbic acid intake on iron status. It is generally believed that constant ingestion of ascorbic acid will have less effect on iron absorption than would be predicted from studies on meals. For example, Cook et al. (1984) gave 2 g ascorbic acid per day with self-selected meals consumed by 17 adult volunteers for 4 months. Mean serum ferritin values were 46 and 43 µg/L before and after the study, respectively. The study was continued for an additional 20 months in 4 iron-deficient and 5 iron-replete subjects. There was a significant increase in serum ferritin only in the iron-deficient subjects, from a mean of 6 µg/L initially to 35 µg/L after 2 years, although the size of the response differed greatly among the subjects.

In other longer-term trials, 300 mg ascorbic acid daily distributed among meals for 2 months failed to increase serum ferritin (Malone et al. 1986). Similarly, Hunt et al. (1994) provided 1500 mg ascorbic acid daily with meals for 5 weeks and noted no increase in ferritin even though the subjects had initially low serum ferritin values (3.5–17.7 μg/L). Thus, although the lack of long-term improvement in some of these studies may have been partly due to the inclusion of iron-replete subjects whose efficiency of iron absorption was relatively low, this does not seem to be the whole explanation. Another possibility is that the diets contained relatively low amounts of iron-absorption inhibitors and contained meat so that the additional enhancing effect of ascorbic acid would have been relatively low. It was also demonstrated that ascorbic acid supplementation has less effect on iron absorption when measured over 2 weeks than when measured after a single meal (Cook et al. 1991a). Additional research is needed on the longer-term benefits of habitually increasing the ascorbic acid intake of iron-deficient individuals who consume meals that usually contain large amounts of inhibitors of iron absorption.

Ascorbic Acid and Iron Absorption from Infant Diets

Earlier investigations of the effect of ascorbic acid on iron absorption from foods fed to infants were confined to studies of animals or adult subjects because of the need to use iron radioisotopes. Derman et al. (1980) studied 121 multiparous South African women and observed that unless ascorbic acid was added, the amount of iron absorbed from 100 g of an infant milk formula containing iron (as ferrous sulfate) at 12.7 mg/L was relatively small (about 7%); the amount absorbed increased to 20% when ascorbic acid at 80 mg/L was added and to 56% when 254 mg/L was added. Absorption from an enriched food supplement fortified with 4.4 mg iron as ferrous sulfate was increased from 2.3% to 11.5% when 22.8 mg ascorbic acid was added, but no further enhancement was noted when 44 mg ascorbic acid was added. When maize meal porridge was consumed with the supplement, iron absorption was 1.8%, increasing to 21.3% with 22.8 mg added ascorbic acid. Overall, ascorbic acid significantly improved iron absorption from infant cereal, about threefold with an iron–ascorbic acid molar ratio of 1:2 and more than sixfold when the molar ratio was 1:6 (10 mg ascorbic acid per mg iron), regardless of the form of iron used as the fortificant.

More recently, stable iron tracers have been used to measure iron absorption in infants and children; the sensitivity with which the isotopes can now be measured allows reasonably small doses of the stable iron to be fed. Abrams et al. (1994) used this approach in a study of 1-year-old infants to show that the efficiency of absorption of 11 mg of iron added to whole milk averaged 1.5% (n=3) compared with 7.0% (n=4) when 5.75 mg was consumed with juice.

Infant formulas and cereals are commonly fortified with iron. It is important to add ascorbic acid to milk powder to ensure adequate absorption of the fortificant iron, even when it is present as a bioavailable salt such as ferrous sulfate. Milk powder provided to Chilean infants has been fortified with iron as ferrous sulfate at about 15 mg iron/100 g. However, even iron-deficient infants absorbed only 3–4% of the iron from this product, and when iron was provided with milk to infants from the age of 3 months, 10% of them had anemia at 15 months. Adding 100 mg ascorbic acid per 100 mg of the iron-fortified milk powder (equivalent to 1 L of reconstituted formula) increased iron absorption to 11–12% and had the beneficial effect of virtually eliminating anemia (Stekel 1984). In general, iron absorption from various milk formulas was improved with an iron–ascorbic acid molar ratio of 1:2 or higher, and the maximum enhancing effect was seen with a 1:4 molar ratio (Stekel et al. 1986).

Fairweather-Tait et al. (1995) used stable isotopes to study iron bioavailability from four common weaning foods fed to 9-month-old infants. Each food was fed to the 10 infants; on three occasions it was given with a fruit juice enriched with 50 mg ascorbic acid, and on three occasions it was given with an ascorbic acid–free drink. Bioavailability was 3.0% (1.2–9.5%) from a proprietary dehydrated vegetable product, 3.0% (1.1–21.2%) from a whole-wheat breakfast cereal, 3.1% (1.2–15.4%) from whole-meal bread, and 4.3% (1.7–10.3%) from baked beans. Eating the meals with 50 mg ascorbic acid doubled iron absorption from all foods except the dehydrated vegetables, which were already fortified with ascorbic acid. Thus, increasing ascorbic acid intake to about 50 mg per meal doubled the amount of iron that the infants absorbed if the meal was initially low in ascorbic acid. The enhancing effect is most beneficial when meals have low amounts of ascorbic acid and high amounts of nonheme iron.

The bioavailability of iron from infant formulas made from soy isolate is significantly less than that from formulas made from cow milk (Gillooly et al. 1984). Davidsson et al. (1994 and elsewhere in this report) used stable isotopes of iron to compare the effects of dephytinizing soy formula and adding ascorbic acid on iron absorption in three groups of infants aged 13–30 weeks. The formula contained about 16 mg iron (300 μmol) and 110 mg ascorbic acid (624 μmol) per liter. Decreasing the native phytate content of the soy formula by 83% increased iron absorption only slightly, from 5.8% to 6.8%; even low concentrations of phytate markedly inhibit iron absorption (Hallberg et al. 1989). Removing all of the phytate increased iron absorption from 3.9% to 8.7%. By doubling the ascorbic acid content of the formula (to 220 mg ascorbic acid/L), iron absorption was improved from 5.7% (iron–ascorbic acid molar ratio 1:2.1) to 9.5% (molar ratio 1:4.2). Thus iron absorption from soy-based formulas could be increased to a similar extent by either removing virtually all of the phytic acid or doubling the ascorbic acid content.

Community Trials of Ascorbic Acid Supplementation

Theoretically, iron absorption from diets could be enhanced if ascorbic acid (synthetic or from food sources) is given to infants or young children to consume at meal times. The efficacy of this approach has been inadequately investigated. When 54 anemic Indian preschoolers (age not reported) were supplied with 100 mg ascorbic acid with each of two meals a day for 2 months, mean hemoglobin increased significantly, from 9.4 to 11.3 g/L (Seshadri et al. 1985). In China, children were given 0, 25, 50, 100, or 150 mg ascorbic acid daily for 2 months. Their usual diet provided 7.5 mg iron and 30 mg ascorbic acid per day. The 50 mg ascorbic acid supplement produced an increase in ferritin that was significant within 6 weeks (Mao and Yao 1992).

Because there are always negative aspects to reliance on supplements, and because supplementation with iron might be less expensive and more effective than supplementation with ascorbic acid, routine provision of ascorbic acid supplements is unlikely to be a practical solution in most situations. However, further work is needed on whether it is feasible and effective to improve iron status of infants and young children by increasing their ascorbic acid intake from natural sources. Providing infants consistently with a substantial amount of ascorbic acid in unfortified foods and juices may be difficult; for example, orange juice contains about 50 mg/100 mL, and most fruits and vegetables contain considerably less.

Iron-calcium Interactions

An inhibitory effect of calcium on iron absorption was recognized nearly 60 years ago. In the past 10–15 years many investigators have focused on this issue, trying to define the practical importance of the interaction and the mechanism by which it occurs. The results of these studies are often conflicting because several factors influence the interaction between calcium and iron absorption, including the molar ratio of calcium to iron, the forms of calcium and iron, whether these minerals are consumed in the presence of food, and the iron status of the subjects.

The mechanism by which calcium impairs iron absorption remains controversial (Hallberg et al. 1992a). It probably does not involve reduction of intestinal pH or the formation of insoluble iron salts such as carbonates. It is not clear whether calcium competes for low-molecular-weight ligands that enhance iron absorption. Calcium equally inhibits the absorption of nonheme and heme iron (Hallberg et al. 1991), which enter the mucosal cell by different pathways and leave in the same form, suggesting that calcium inhibits the intracellular transport of iron (Hallberg et al. 1992b). Calcium may compete for iron-binding sites in mobilferrin, a protein in the duodenal mucosa that may assist iron transport through the cell (Conrad and Umbreit 1993). Mobilferrin can bind calcium although it has a greater affinity for iron. Calcium may also inhibit the release of iron from mucosal cells into the circulation (Wienk et al. 1996).

The inhibitory effect of calcium on iron absorption is independent of the amount of phytate in a meal (Hallberg et al. 1992b) and does not interfere with the enhancing effect of meat (Hallberg et al. 1992b) or ascorbic acid (Hallberg et al. 1992a). It is important to recognize that adding calcium to wheat flour before leavening causes a further substantial decrease in iron absorption because calcium inhibits phytase activity in yeast (Hallberg et al. 1991). As little as 40 mg calcium added to 80 g flour inhibited phytate degradation during leavening by 50%. This means that calcium fortification of wheat flour may reduce the beneficial effects of fermentation on the bioavailability of iron and other minerals bound to phytate.

Amount of Calcium

Hallberg et al. (1991) added between 40 and 600 mg of calcium, as calcium chloride or dairy products, to wheat rolls containing 3.8 mg iron. Iron absorption fell relatively linearly to about 40% at 300–600 mg calcium. There was no effect of adding 40 mg calcium (calcium-iron molar ratio 14:1), and at the upper end adding 600 mg rather than 300 mg had relatively little additional inhibitory effect. However, it is not only the calcium-iron molar ratio that is important, because there was no effect of adding 3 mg calcium on the absorption of a trace amount (0.01 mg) of iron, a calcium-iron molar ratio of 420:1 (Hallberg et al. 1992a).

Form of Calcium

The form of calcium also influences its inhibitory effect on iron absorption. A review by Whiting (1995) revealed that 500–600 mg calcium added to meals typically reduced iron absorption by about 40% when added as cheese and milk; 40–50% when added as calcium phosphate, carbonate, or hydroxyapatite; and 60–70% when added as more soluble calcium citrate and calcium citrate malate supplements (Hallberg et al. 1992b, Cook et al. 1991b, Deehr et al. 1990, Dawson-Hughes et al. 1986). Calcium carbonate had no effect on iron absorption in at least one report (Seligman et al. 1983). Although these observations may indicate that more soluble forms of calcium have a greater effect on iron absorption because the inhibition occurs within the mucosal cell, this remains to be proven. In hemoglobin-repletion studies in rats, calcium carbonate had a strong, dose-related inhibitory effect on iron absorption whereas calcium sulfate was far less inhibitory (Prather and Miller 1992).

It is well-established that iron is absorbed more efficiently from human milk than from cow milk. Hallberg et al. (1992c) provided evidence that the lower bioavailability of cow-milk iron is probably due to cow milk's higher calcium content. When calcium was added to human milk to give the same concentration as is found in cow milk, absorption of iron from the two types of milk was similar; the high fractional absorption of iron in breast milk is due to its low iron content.

Influence of Food on the Calcium-iron Interaction

Adding calcium has no inhibitory effect on iron absorption when these nutrients are consumed together (300 mg calcium as calcium carbonate consumed with 37 mg iron as ferrous sulfate, or 600 mg calcium with 18 mg iron in the same forms) in the absence of food (Cook et al. 1991b). However, at the latter dose, when calcium was given as citrate and phosphate in the absence of foods, iron absorption was reduced by 49% and 62%, respectively. When eaten with a meal, all forms of calcium—carbonate, citrate, and phosphate—inhibited iron absorption. The relative inhibition was worse when the meal contained iron of low bioavailability.

Timing of Calcium Administration.

The inhibitory effect of calcium on iron absorption is seen only when the calcium is consumed close to the time of iron consumption. When calcium was given to rats or dogs even 1 hour after a meal, its inhibitory effect on iron absorption disappeared (Kochanowski et al. 1990). Dairy products consumed in a breakfast had no effect on iron absorption from a hamburger meal eaten 2 or 4 hours later (Gleerup et al. 1993). Meals containing lower iron (breakfast and supper) and meals containing higher iron (lunch and early dinner) with and without milk and cheese (providing 700 mg of a total daily calcium intake of 937 mg) were consumed for 10 days (Gleerup et al. 1995); as expected, about 30–50% more iron (0.44 mg iron/day) was absorbed when no milk or cheese was consumed with the higher-iron meals. However, in many situations it is impractical to separate the consumption of calcium and iron sources.

Influence of Iron Status

Differences in iron status among subjects and among studies explain some of the inconsistencies in the reported effects of calcium on iron absorption. Iron absorption is influenced independently by both iron status (inversely) and bioavailability. In iron-replete individuals, iron absorption is relatively low and differences in bioavailability among diets are more difficult to detect. In contrast, the effect of dietary factors on iron absorption is more pronounced in iron-deficient individuals.

Plotting data on iron absorption against iron status from the study of Gleerup et al. (1995), where main meals were consumed with and without milk, Hultén et al. (1995) predicted that milk would reduce iron absorption by about 25% in individuals with no iron stores but have no detectable effect when iron stores are adequate (i.e., at or above a serum ferritin concentration of 50-60 μg/L in women). Likewise, Cook et al. (1991b) observed that the inhibitory effect of calcium on iron absorption was more pronounced in individuals with low iron reserves (mean serum ferritin 24 μg/L) than in those with higher serum ferritin concentration.

Longer-term Studies

There have been few long-term studies of the effect of calcium intake on iron status. When premenopausal women were fed diets adequate in iron (15 mg/day) and ascorbic acid (>200 mg/day) for 12 weeks, the addition of 1000 mg of calcium as calcium carbonate with meals produced no changes in serum ferritin concentrations (Sokoll and Dawson-Hughes 1992). However, the study was relatively short and compensations in the efficiency of iron absorption may have occurred in the high-calcium group; iron absorption was not measured directly. In the longest experiment, which lasted 6 months, daily supplements of 1200 mg calcium given at meal times had no significant effect on hemoglobin, serum ferritin, erythrocyte zinc protoporphyrin, or serum transferrin receptor concentrations in 11 nonanemic adults (Minihane et al. 1997).

There is clearly a need for additional studies of the long-term effects of high calcium intakes on iron status. Such studies should include infants and young children with high iron requirements and low iron intakes and stores. There are virtually no studies of the influence of calcium on iron absorption from complementary foods. Neither is there clear evidence that supplemental calcium during the period of complementary feeding improves growth or bone mineralization (Prentice and Bates, 1994).

Required Intakes of Iron, Ascorbic Acid, and Calcium from Complementary Foods

In a recent review for the World Health Organization and the United Nations Children's Emergency Fund, we calculated the amount of nutrients needed from complementary foods for partially breast-fed infants aged 6–24 months (Brown et al. 1997). These calculations were based on the difference between average reported intakes of these nutrients by infants who continued to be partially breast-fed and their estimated nutrient requirements. The calculations revealed that complementary foods (i.e., foods other than breast milk) had to supply almost all the iron and half the calcium requirement of infants aged 6–24 months. However, on average, ascorbic acid requirements could still be met from the breast milk. The only foods that could theoretically supply enough iron were liver, fish, or beef, all of which would have to be consumed in large quantities. At ages 6–12 months, only dried milk contained a density of calcium high enough to provide the amount required in complementary foods, whereas cheese, fresh milk, and fish (canned or dried with bones) could serve this purpose at ages 12–24 months.

Practical Implications for the Complementary Feeding of Infants

Practical implications for complementary feeding, based on data presented here, are as follows:

- Because iron deficiency commonly develops during the period of complementary feeding when diets are typically very low in iron, increasing the ascorbic acid content of complementary foods can be an important strategy for improving iron absorption. Ascorbic acid should be given with or included in the meals that contain most iron or consumed within 1 hour of meals.
- Fortification with 50 mg ascorbic acid can approximately double the amount of iron absorbed from meals by infants. At an iron–ascorbic acid molar ratio of 1:2 or more, iron absorption is improved; a ratio of 1:4 was optimal in some studies.
- Ascorbic acid produces the greatest increase in iron absorption when complementary foods are high in inhibitors

such as phytates (e.g., soy and other legumes, maize, low-extraction wheat, and relatively unpolished rice) or polyphenols (e.g., sorghum and millet). The effect on iron status will be greatest if the foods also contain substantial amounts of iron (e.g., whole maize and whole wheat, legumes, sorghum, and millet) or if a source of fortificant iron is present. Less effect will be seen when diets are based on degermed maize, rice, refined wheat, or milk (which are low in both phytate and iron) unless the food is fortified with iron.

- Ascorbic acid could be added to complementary foods as fruits, fruit juices, or vegetables. However, it may be difficult to supply 25–50 mg ascorbic acid consistently in meals fed to infants. Approximately 100 mL orange juice or larger quantities of most fruits and vegetables are needed to supply 50 mg ascorbic acid.
- If ascorbic acid is added as a fortificant, its degradation during storage may be quite rapid, especially if the water content of the food is above 10%. One approach to this problem, used for corn-soy blend for example, has been to coat the synthetic ascorbic acid added to cereals with ethyl cellulose or other stable compounds. This adds to the cost of the ascorbic acid, which is the most expensive micronutrient in the fortificant mixture, and stability during long-term storage in suboptimal packaging may still be poor.
- The habitual intake of calcium from complementary foods may be quite high when diets contain a large amount dairy products or low when dairy products are consumed rarely. In Mexico and Peru usual diets of infants lacked about 350 mg calcium/d for infants aged 3–12 months and 200 mg/d at age 12–24 months. Fortification with these amounts of calcium would be expected to approximately halve iron absorption, although this remains to be confirmed. However, the addition of calcium to an iron-fortified food should still permit useful amounts of iron to be absorbed.
- Until there are more data on the effect on iron absorption of adding different amounts of calcium to infant foods or providing infants with calcium supplements, the total amount of calcium consumed from breast milk, the usual diet, and other sources should not exceed requirements.
- If a calcium supplement alone is provided, it should be given at a different time from high-iron meals if possible. If calcium and iron are supplied together in a micronutrient supplement to be consumed apart from food, there may be no inhibitory effect of the calcium on absorption of the iron.

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Iron Bioavailability from Weaning Foods: The Effect of Phytic Acid

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Introduction

Young infants are vulnerable nutritionally during the weaning period (Weaver 1994, Rowland 1986). The gradual in crease in energy and nutrients provided by semisolid foods, at the expense of human milk or infant formula, requires access to appropriate weaning foods with high energy and nutrient density as well as high nutrient bioavailability. Weaning practices vary among countries and also among regions and social groups within the same country depending on socioeconomic factors and food availability (Underwood and Hofvander 1982). The appropriate time for the introduction of weaning foods is not well defined, but it might be assumed that solid foods should be given when the infant signals, by evidence of an unassuaged appetite, that human milk or infant formula is no longer sufficient to provide nutritional needs (Birkbeck 1992). One prerequisite for the introduction of weaning foods must be the ability of the infant to handle them, that is, introduction depends on the degree of development of the infant's digestive and absorptive processes and the development of the local defense systems (immunological and nonimmunological) (Milla 1986). The time for the introduction of weaning foods should therefore be carefully chosen on the basis of physiological considerations as well as nutritional factors (Birkbeck 1992, Underwood and Hofvander 1982, Fomon et al. 1979).

The view that infants should not be introduced to solid foods before the age of 3–4 months of age is shared by the American Academy of Pediatrics (AAP) as well as the European Society for Pediatric Gastroenterology and Nutrition (ESPGAN) (ESPGAN Committee on Nutrition 1982, AAP Committee on Nutrition 1980). Early introduction (<3 months) of solid foods in less-developed countries is generally not considered appropriate (Rowland 1986).

Weaning Foods and Iron Nutrition

Iron-deficiency anemia is still a major nutritional problem in vulnerable population groups in the world (DeMaeyer and Adiels-Tegman 1985, Bothwell et al. 1979). Iron-deficiency anemia during infancy is of particular importance because it can lead to negative changes in psychomotor and mental development, which may be irreversible (Walter 1992). After exhausting placentally transferred iron, infants depend on dietary iron for their rapidly expanding blood volume and replacement of iron losses (Dallman 1992). Full-term breast-fed infants generally have an adequate iron status during the first 4–6 months of life but after this time, when the iron stores have been depleted, additional dietary iron needs to be supplied via the diet (Dallman 1992). Thus adequate bioavailability of iron in weaning foods must be ensured during this period to prevent iron deficiency (Dallman 1986).

Weaning cereals traditionally have been one of the first semisolid foods to be presented to infants (Herveda and Newman 1992, Underwood and Hofvander 1982) because cereals mix well with milk to give a simply prepared semisolid food, are easy to feed, and are generally well accepted by infants. The nutritional composition and the bioavailability of nutrients in this type of food are therefore important. Commercial weaning cereals are usually prepared from precooked, roller-dried cereal flours, based on low-extraction-rate flours, and are fed with cow milk or infant formula based on cow milk to complement the imbalanced amino acid pattern of cereal. Alternatively, the complementary protein can be from vegetable protein, for example, soy (Igbedioh 1991, Hofvander and Underwood 1987).

Most industrially produced weaning cereals contain low levels of dietary fiber. However, when high-extraction cereal flours are more readily available than refined cereal raw materials and vegetable proteins are added instead of cow milk, weaning cereals contain higher amounts of dietary fiber and related components, such as the strong metal chelator phytic acid (myo-inositol hexaphosphate). Before the introduction of vegetable weaning foods (cereals and legumes), only babies fed infant formula based on soy would have been exposed to phytic acid in their diet.

Whether dietary fiber should be present in the diet during early life—and, if so, in what quantity—has long been discussed. Dietary fiber is not considered to have an essential function in the diet early in life (AAP Committee on Nutrition 1981) but can be regarded as important in getting infants gradually accustomed to table foods (Clark and Laing 1990). The optimal level of dietary fiber in the diet of infants is not known, but a maximum level of 5% crude fiber was suggested (Food and Agricultural Organization 1985). Earlier observations in developing countries revealed negative effects on the intake of energy and nutrients related to high dietary fiber intake from home-made infant cereals, primarily resulting from the increased bulk and consequent reduced density of energy and nutrients in the diet (Poskitt 1987, Brandtzaeg et al. 1981, Ljungqvist et al. 1981, Burkitt et al. 1980). Very limited information is available on the effect on mineral bioavailability of the increased intake of phytic acid from weaning foods with higher dietary fiber contents and the influence on mineral status early in life.

Iron Bioavailability from Weaning Foods: The Effect of Phytic Acid

Iron bioavailability from cereal products is usually low because of the presence of phytic acid, which is the major phosphorus storage compound in grain. Phytic acid has a strong inhibitory effect on mineral absorption in adults, particularly on the absorption of iron (Hurrell et al. 1992, Brune et al. 1992, Hallberg et al. 1989). However, phytic acid in foods can be degraded by activating the native phytase during traditional food processing (e.g., during fermentation) or by adding exogenous phytase during food manufacture. Studies in adult humans showed that the negative effect of phytic acid on iron bioavailability can be overcome by phytic acid degradation by activating the native phytase in wheat bran, wheat flour, and rye (Brune et al. 1992, Hallberg et al. 1987) or by adding exogenous phytase to soy isolate (Hurrell et al. 1992). Hurrell et al. (1992) found that even small amounts of residual phytic acid in soy isolate were inhibitory and that almost complete dephytinization (98%) was needed before a meaningful increase in iron absorption was observed. However, even after complete degradation of phytic acid, the soy protein itself still inhibits iron absorption (Lynch et al. 1994).

There are few reported studies of iron bioavailability in infants. However, the recent development of stable-isotope techniques to measure iron bioavailability by analysis of the incorporation of stable isotopes of iron (^{58}Fe and ^{57}Fe) into erythrocytes about 14 days after administration has made it possible to study iron bioavailability in infants without introducing any risk to infants. Thus, more data on iron bioavailability during infancy will probably be available in the near future. The studies in infants referred to in this review have used the stable-isotope technique to measure iron bioavailability unless otherwise stated. In some of the studies, 90% of initially absorbed iron is assumed to be incorporated into erythrocytes (Davidsson et al. 1994, 1996, 1997).

The strong negative effect of phytic acid on iron bioavailability in infants was recently demonstrated in a study where soy formulas containing about 300 µmol iron/L (about 16.8 mg/L) were evaluated before and after dephytinization (Davidsson et al. 1994). A statistically significant effect on iron bioavailability in infants was found after the degradation of 83% and 100% of the phytic acid present in the soy formulas. The geometric mean bioavailability of iron increased from 5.5% to 6.8% ($p<0.05$) after degradation of 83% of the phytic acid whereas 100% dephytinization had a more pronounced effect: geometric mean bioavailability of iron increased from 3.9% to 8.7% ($p<0.001$).

Few data have been reported on the bioavailability in infants of iron from weaning foods, although it was recently shown that infant cereals based on wheat and soy (containing relatively high amounts of phytic acid) are inhibitory (Davidsson et al. 1996). Bioavailability of iron from the infant cereals was low and in some cases below the detection limit: range 1.0–5.4% (8.0% dietary fiber, 0.77% phytic acid; n=8) vs. <0.9–9.1% (1.8% dietary fiber, 0.30% phytic acid, n=6), respectively (Davidsson et al. 1996). The iron content of the labeled test meals was 86–301 µmol/100 g dry weaning cereal (4.8–16.8 mg/100 g). No difference in iron bioavailability was found between the two weaning cereals, thus the phytic acid in the low-dietary-fiber cereal was high enough to inhibit iron bioavailability; no further inhibition was observed from the weaning cereal with the higher phytic acid content.

Recently, we investigated the iron bioavailability from an infant cereal based on low-extraction wheat flour and cow milk, before (0.08% phytic acid) and after dephytinization (0.01% phytic acid) (Davidsson et al. 1997). No difference in iron bioavailability was observed between the two cereal products; the geometric means were 8.7% and 8.5%, respectively (Davidsson et al. 1997). The lack of effect of the enzymatic degradation of phytic acid in this infant cereal can be attributed to the relatively high content of ascorbic acid, which counteracted the inhibitory effect of phytic acid on iron bioavailability. Ascorbic acid is a potent enhancer of iron absorption that can overcome the inhibiting effect of phytic acid when present in sufficient amounts. This effect was demonstrated previously both in infants (Davidsson et al. 1994) and adults (Siegenberg et al. 1991, Hallberg et al. 1989, 1986). Ascorbic acid was added to the infant cereal based on wheat and cow milk at a molar ratio 2:1 relative to iron (Davidsson et al. 1997), resulting in a relatively high iron bioavailability from both the phytic acid–containing infant cereal and the dephytinized cereal. In our earlier study with infant cereals containing high-extraction wheat flour and soy flour (Davidsson et al. 1996), the level of phytic acid was much higher; although approximately the same amount of ascorbic acid was added as in the later study, the molar ratio of ascorbic acid to phytic acid was only 0.4:1 in the wheat-soy-based weaning cereal containing 0.77% phytic acid compared with 3.3:1 and 26.8:1 in the wheat–cow milk–based weaning cereals with 0.08% and 0.01% phytic acid, respectively. Thus, the ability of ascorbic acid to overcome the inhibitory effect of phytic acid clearly depends on the amount present of both phytic acid and ascorbic acid. Whether the addition of ascorbic acid to infant cereals containing relatively high phytic acid amounts could be a useful approach to ensure adequate iron bioavailability is not known. Phytic acid degradation, either via activation of the native phytase in the raw materials or the addition of phytase, appears to be an interesting approach for improving iron bioavailability from weaning foods based on high-extraction cereal flours.

Iron Fortification of Weaning Foods

Most industrially produced infant foods are iron fortified, and the importance of this fortification on the decline of the frequency of iron-deficiency anemia has been recognized (Dallman 1990). However, the effect of iron fortification of other infant foods (e.g., weaning cereals) on iron nutrition was questioned (Fomon 1987, Dallman 1986), but a more recent study demonstrated that an elemental iron-fortified rice cereal was useful in preventing iron-deficiency anemia (Walter et al. 1993). Infant formulas are usually fortified with highly bioavailable iron compounds such as ferrous sulfate, whereas cereal products are much more difficult to fortify with iron because of problems related to changes of organoleptic properties, such as rancidity, color changes, and off-flavors during storage, after addition of soluble iron compounds (Hurrell 1992). Consequently, iron is often added as less-soluble (and less-bioavailable) compounds to cereals (e.g., ferric pyrophosphate is used in Europe and electrolytic elemental iron is used in the United States). The relative bioavailability of different iron-fortification compounds compared with ferrous sulfate has not been investigated systematically in infants.

In adults fed a wheat-milk-based weaning cereal, the relative absorption of iron as ferrous sulfate was 1.0 vs. 1.0 for ferrous fumarate, 0.7 for ferric saccharate, and 0.4 for ferric pyrophosphate (Hurrell et al. 1989). No conclusive data on the bioavailability of electrolytic iron in adults or infants are available because of difficulties in producing a labeled compound with particle size and solubility characteristics similar to those of the commercial compound. Iron bioavailability from electrolytic iron was reported to be higher than that of ferrous sulfate in infants (Rios et al. 1975) and adults (Forbes et al. 1989). Both studies used electrolytic iron labeled with a radioactive iron isotope. The data from these studies are difficult to interpret because the characteristics of the labeled compound are different from those of commercial electrolytic iron and the results could be an overestimate of iron bioavailability from the labeled compound (Hurrell 1992).

Only one study reported iron bioavailability in infants after adding ^{58}Fe as ferrous sulfate or ferrous fumarate to a protein-enriched infant rice cereal (Fomon et al. 1989). The geometric mean incorporation of ^{58}Fe into erythrocytes was 4.4% (range 0.6–19.0 %) from the meal containing ferrous sulfate and 4.0% (range 2.1–7.8 %) from the meal containing ferrous fumarate, which was fed to another group of infants. No statistically significant difference was found between the two iron compounds. However, the interpretation of the data is somewhat complicated because of the differences in the total iron content of the test meals (19.7 and 82.3 µmol [1.1 and 4.6 mg]), and no conclusions can be drawn about iron bioavailability from the two different iron compounds used.

Iron Bioavailability from Weaning Foods Fortified with Ferrous Sulfate

In our infant studies (Davidsson et al. 1996, 1997) we measured iron bioavailability from weaning cereals after adding iron as ferrous sulfate. This study design was influenced by the technical problems and high cost involved in preparing compounds labeled with a stable isotope of iron in the form normally added to weaning cereals. The results can thus be regarded as representative of the weaning cereals normally fortified with ferrous fumarate; iron bioavailability from infant cereals fortified with elemental iron or ferric pyrophosphate would be expected to be lower.

In a study by Fomon et al. (1989) iron bioavailability from iron-fortified weaning foods were evaluated in infants by measuring the incorporation of ^{58}Fe added as ferrous sulfate into erythrocytes. The results demonstrated geometric mean incorporation of 5.4% (range 2.7–15.4%) from rice cereal containing fruits, 2.5% (range 0.4–4.7%) from a vegetable-beef product, and 4.8% (range 1.2–12.5%) from grape juice. The total iron content of the test meals was 50.1, 57.3, and 50.1 µmol (2.8, 3.2, and 2.8 mg), respectively. No statistically significant differences in erythrocyte incorporation of ^{58}Fe were found among the different foods tested. No information on the phytic acid content of the test meals was given.

A study of iron bioavailability from weaning foods containing added ferrous sulfate (Fairweather-Tait et al. 1995) reported geometric mean (range) iron bioavailability of 3.0% (1.4–9.5%) (vegetable dinner), 3.0% (1.1–21.2%) (whole-wheat breakfast cereal), 3.1% (1.2–15.4%) (whole-meal bread), and 4.3% (1.7–10.3%) (baked beans) in infants. The bioavailability of iron increased twofold from all meals except the vegetable dinner (which already contained added ascorbic acid) when 284 µmol (50 mg) ascorbic acid was added to the fruit drink consumed with the test meals (Fairweather-Tait et al. 1995). The total iron content of the test meals was similar (37.6–44.8 µmol iron [2.1–2.5 mg] from added ferrous sulfate). However, only two of the foods tested are normally fortified with iron—the whole-wheat breakfast cereal and the vegetable dinner. Because the whole-meal bread and baked beans used in this study do not normally contain added iron, the fractional bioavailability from these foods could be expected to be higher than observed in this study because percentage iron absorption is dose related. Furthermore, the whole-wheat breakfast cereal is normally fortified with ferrous sulfate whereas the vegetable dinner contains added ferric pyrophosphate. Iron bioavailability from the commercial vegetable dinner can thus be expected to be lower than found in this study. The content of phytic acid in the test meals is not given.

Conclusions

Our recent data on iron bioavailability from weaning cereals with a low phytic acid content clearly indicate that iron-fortified weaning cereals containing added ascorbic acid can supply iron with a relatively high bioavailability if the iron compound added to the weaning cereals is as bioavailable as ferrous sulfate. The mean fractional iron bioavailability from the infant cereals based on low-extraction wheat flour and cow milk, about 8.5%, was roughly equivalent to iron bioavailability observed earlier in infants consuming cow-milk-based infant formula (Kastenmayer et al. 1994) or completely dephytinized soy formula (Davidsson et al. 1994) as measured with the same stable isotope technique and containing similar amounts of iron as ferrous sulfate. Thus the addition of ascorbic acid in high enough quantities seems to overcome the inhibitory effect of phytic acid from infant cereals with low phytic acid content (<0.1% phytic acid).

Optimizing the bioavailability of iron from weaning foods is a major factor in increasing the efficiency in supplying iron via weaning foods, such as cereals, to young infants. This can be done by removing inhibitory ligands, such as phytic acid, or adding enhancers, such as ascorbic acid. In addition, the use of alternative iron compounds with high iron bioavailability, such as ferrous fumarate, in weaning foods should be investigated. Optimizing the bioavailability of iron from infant cereals would increase the effect of the iron fortification of these products on the iron status of the infants.

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The Influence of Vitamin A on Iron Status and Possible Consequences for Micronutrient Deficiency Alleviation Programs

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Prevalence of Iron and Vitamin A Deficiency

Vitamin A and iron deficiency are two of the most common nutritional disorders in populations living in developing countries (ACC/SCN 1992). According to a 1992 estimate, more than 124 million children worldwide are vitamin A deficient (Humphrey et al. 1992). The prevalence of anemia, which is mainly caused by iron deficiency, in pregnant women in Southeast Asia and Africa is estimated to be 74% and 52%, respectively (World Health Organization 1992). Vitamin A deficiency is not restricted to children and iron deficiency is not restricted to pregnant women. Figure 1 shows the results of several cross-sectional studies on anemia in Indonesian population groups. Although the investigated subjects did not represent the whole Indonesian population, the figure illustrates the increased risk for preschoolers, adolescents, and pregnant women to become iron deficient.

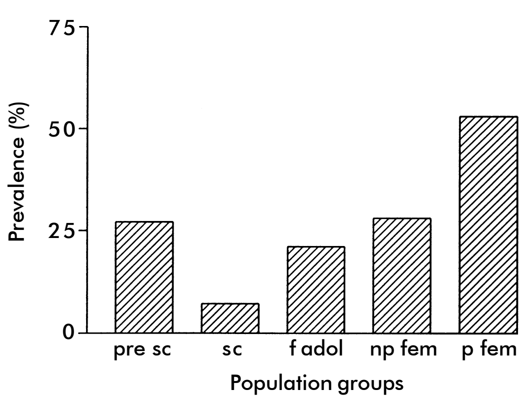

Figure 1. Prevalence of anemia among selected population groups from Indonesia. Pre sc, preschoolers (*n*=575); sc, primary school children (*n*-123); f adol, female adolescents (*n*=805); np fem, non-pregnant women (*n*=462); and p fem, pregnant women (*n*=390). From Schultink and Gross 1996.

Table 1 presents information on the prevalence of vitamin A deficiency in Indonesian population groups. Except for the national survey data (Muhilal et al. 1994), these data also do not represent the whole Indonesian population. However, the data show that a deficient or marginal vitamin A status is quite common. The situation for the female adolescents from Jakarta is striking because they can be considered to be a relatively privileged group. Even when no overt clinical signs for iron and vitamin A deficiency exist, both deficiencies may still be highly prevalent in different population groups in countries such as Indonesia.

Table 1. Prevalence of vitamin A deficiency among selected population groups in Indonesia

Population Group	*n*	Criterion	Prevalence (%)	Reference
National survey: <5 years old	18,508	Bitot's spots	0.34	Muhilal et al. 1994
Rural pregnant women	318	Retinol <0.70 μmol/L	31	Suharno et al. 1992
Rural lactating women	165	Retinol <0.70 μmol/L	33	de Pee et al. 1995
Urban female adolescents	282	Retinol <0.70 μmol/L	30	Angeles 1996
Urban anemic preschoolers	85	Retinol <0.70 μmol/L	81	Merzenich 1994

Causes and Consequences of Iron and Vitamin A Deficiency

The high prevalence of vitamin A and iron deficiency result from inadequate diets and infectious diseases. Dietary inadequacy is aggravated during periods of increased requirements, such as growth in childhood, adolescence, and pregnancy. A dietary aspect important for iron as well as for vitamin A in most populations in developing countries is nutrient bioavailability.

The bioavailability of dietary iron is influenced by the quantity of heme- and nonheme iron in the diet and by the balance between absorption enhancers and inhibitors, which mainly affect the absorption of nonheme iron (British Nutrition Foundation 1995, Hallberg 1981). Generally, the diet in developing countries includes a small quantity of heme iron (from animal foods) and a large quantity of absorption inhibitors such as phytate and polyphenols. Important sources of phytate are cereals and legumes, including the staple foods rice and corn. Polyphenols are present in green vegetables and legumes. The major absorption enhancers for iron are ascorbic acid and meat, and their quantity is low in the daily diet of most populations in developing countries.

The diet also provides vitamin A in two forms: retinol in animal products and provitamin A carotenoids, such as β-carotene, in vegetables and fruits. Similarly to iron, retinol from animal food sources has a greater potency than does provitamin A from vegetable foods. A recent study showed that although β-carotene is present in relatively high amounts in green leafy vegetables, the bioavailability is lower than was originally estimated (de Pee et al. 1995).

The consequences of iron and vitamin A deficiency are serious. Iron deficiency during pregnancy is associated with low-birth-weight babies, premature delivery, and even perinatal and fetal death (Scholl and Hediger 1994). During childhood, iron deficiency leads to impaired cognitive performance and motor development (Lozoff et al. 1991) and decreased linear growth rate (Angeles et al. 1993, Aukett et al. 1986). Iron-deficient adults suffer from reduced work capacity (Scholz et al. 1997, Basta et al. 1979). Iron deficiency also negatively influences the normal defense systems against infection (Srikantia et al. 1976, Joyson et al. 1972). Iron-deficient individuals not only absorb iron more efficiently but also other divalent metals such as lead and cadmium (Masawe et al. 1987). In environments with a high degree of lead pollution, for example, from automobile fumes in cities such as Bangkok, Jakarta, or Manilla, iron-deficient children are more susceptible to lead poisoning.

Vitamin A deficiency has long been known to be the main cause of childhood blindness in developing countries (Sommer 1982). However, even subclinical vitamin A deficiency has broader consequences in terms of childhood morbidity and mortality. Several reports over the past decade have shown that vitamin A deficiency is also associated with increased mortality and an increased severity of infectious diseases (Beaton et al. 1993, Sommer et al. 1986, Sommer et al.1984).

The Association Between Iron and Vitamin A

The metabolism of iron and vitamin A is associated in that vitamin A status influences iron metabolism (Bloem 1995). In the first half of this century, several studies suggested that a lack of vitamin A in humans and experimental animals may result in anemia (Wagner 1940, Frank 1934, Blackfan and Wolbach 1933, Sure et al. 1929, Koessler et al. 1926, Wolbach and Howe 1925, Findlay and MacKenzie, 1922). More recently, cross-sectional studies in children (Wolde-Gabriel et al. 1993, Bloem et al.1989, Mejia et al. 1977) and pregnant women (Suharno et al.1992) showed associations between retinol and indicators of iron status. These cross-sectional studies were followed-up by intervention studies that showed that increased intake of vitamin A may lead to an improvement in iron status (Table 2).

The information in Table 2 shows that vitamin A supplementation affected iron status and that iron supplemented in combination with vitamin A had a greater effect than iron supplemented alone. The studies showed that vitamin A influenced circulating iron (hemoglobin and transferrin saturation), but no study showed that vitamin A had an effect on storage iron (indicated by ferritin concentration). Of interest is the conclusion that vitamin A had an effect over a wide range of average initial retinol concentrations (0.5–1.1 μmol/L serum). Children can be considered to have a low vitamin A status when their retinol concentration is less than 0.7 μmol/L, but vitamin A supplementation also had positive effects when concentrations were higher than 0.7 μmol/L. However, not every study reported improved iron status after vitamin A supplementation. Hemoglobin concentration of anemic lactating women with baseline retinol concentrations of 0.8-0.9 μmol/L did not increase after vitamin A (given as β-carotene) supplementation (de Pee et al. 1995).

The exact mechanism of how vitamin A influences iron status still remains unclear. Vitamin A deficiency was suggested to inhibit the reuse of iron stored in liver (Bloem 1995). Studies in rats indicated that marginal vitamin A deficiency led to impaired erythropoiesis but did not impair intestinal absorption of dietary iron (Roodenburg et al. 1994). The mechanism for reuse of stored iron, however, would not explain the effect observed in the study of Suharno et al. (1993), where pregnant women had virtually no liver stores of iron and still experienced an extra effect of vitamin A. Turnham (1993) proposed that vitamin A supplementation would suppress infection and consequently stimulate the production of transferrin and retinol binding protein. However, this proposed mechanism still needs to be proven.

It can be concluded that a combined supplementation with vitamin A may have a positive influence on iron status even when the vitamin A status of a population on average does not seem to be deficient. No study reported an influence of iron supplementation on vitamin A status. The positive influence of vitamin A on iron status was reported not only for supplements but also for vitamin A–fortified foods such as sugar (Mejia and Arroyave 1982) and food additives such as monosodium glutamate (Muhilal et al. 1988) consumed by children.

Table 2. Effects of vitamin A supplementation on iron status of different population groups as reported by selected studies

Subjects	Supplement	Main Finding	Reference
Vitamin A–deficient children. Initial retinol about 0.60 µmol/L	110 mg vitamin A once	Increase in hemoglobin and transferrin saturation but not ferritin.	Bloem et al. 1990.
Anemic children. Initial retinol about 0.50 µmol/L	110 mg vitamin A once	Increase in serum iron and transferrin saturation.	Bloem et al. 1989
Anemic children. Initial retinol about 1 µmol/L	3 mg vitamin A, or 3 mg iron/kg wt, or 3 mg vitamin A + iron for 2 months	Vitamin A + iron supplement gave the largest increase in hemoglobin and transferrin saturation. Ferritin was not influenced by vitamin A.	Mejia and Chew 1988
Anemic pregnant women. Initial retinol about 1.1 µmol/L	60 mg iron + 2.4 mg vitamin A, or 60 mg iron, or 2.4 mg vitamin A for 8 weeks	Vitamin A + iron supplement gave largest changes in hemoglobin and transferrin saturation. Ferritin changes similar to iron-only supplement.	Suharno et al. 1993

Although the association between vitamin A and iron is known, thus far it has not been reflected in large-scale programs to alleviate micronutrient deficiency.

Program Implications

Three approaches can be used to increase iron and vitamin A absorption in addition to undertaking measures to decrease the prevalence of infectious diseases.

An Improvement of the Naturally Available Diet

Interventions to improve absorption from the diet are particularly important because such an improvement would be sustainable. However, these are long-term strategies to combat the problem of high prevalence of iron and vitamin A deficiency. A diet providing enough absorbable iron would contain some meat or fish, ascorbic acid, and not too much cereal with a high-fiber content. To cover vitamin A requirements a diet should contain animal foods such as egg or liver, yellow fruits, green vegetables, and sufficient fat. For many people in developing countries, especially for subgroups of a population such as small children, it currently is and will remain difficult to consume such an adequate diet. The changes in economic status and behavior needed to effect adequate food intake may only come about in several years; other options are needed until then.

The Fortification of Foods

Food fortification is an attractive option for increasing the intake of iron and vitamin A of target populations because no modification in dietary habits or active participation of the population is required. Furthermore, fortification is relatively inexpensive. Many studies and field trials have been carried out on the fortification of staple foods and other foodstuffs (Nestel 1993). A good example of a double fortification of sugar with iron (130 mg/kg) and vitamin A (15 mg/kg) was recently published (Viteri et al. 1995). However, thus far in most developing countries no fortified foods are available or consumed on a large scale, except possibly for the relatively widely available fortified weaning foods (food additional to breast milk, marketed as a powder) for children older than 4–6 months. These foods usually contain about 7–10 mg iron and 1000–2000 IU of vitamin A per 100 g, but most, although of excellent quality, find a limited usage by populations in developing countries because of their relatively high price. For example, in a small rural town in Indonesia, a mother would have to pay an amount of money equal to one daily wage to be able to provide her child with a package of fortified porridge powder that would be sufficient for 2.5 days.

Although multiple fortification of foods would be an excellent option, organizational and technical problems and financial hindrances may prevent a widespread application in the near future. Published results on fortified sugar suggest that multiple fortification of sugar might be a worthwhile option: sugar is a widely consumed food, is not produced at many different sites, and is relatively inexpensive in most countries. An optimal dosage for combined iron–vitamin A fortification of specific foods for different target groups may still need to be investigated.

Pharmaceutical Multimicronutrient Supplements

Most countries already have supplementation programs, such as the distribution of vitamin A capsules to young children. Such supplementation programs can be successful if the coverage rate of the target population is high enough. In Indonesia the prevalence of xerophthalmia has decreased markedly during the past decades, probably partially a result of a program to distribute vitamin A capsules. Iron supplementation programs also exist in many countries but are mainly targeted to pregnant women. Contrary to the situation for vitamin A, iron supplementation programs have not been successful in reducing the prevalence of iron deficiency because of such factors as availability of supplements, coverage of health services, and compliance of target groups (Schultink 1996).

Despite potential advantages of a combined iron–vitamin A supplementation, as shown by several studies in children and pregnant women, no large-scale multisupplementation programs have been carried out. Probable reasons for this are the increased cost of multisupplementation compared with single supplementations, management problems, and distribution problems. If iron cannot be distributed effectively, a combination of iron and vitamin A cannot be expected to have much impact. To address these potential problems for multisupplementation, distribution and targeting alternatives need to be considered. Recent studies showed that the effects on iron status of weekly iron supplementation were similar to those of daily supplementation (Ridwan et al. 1996, Schultink et al. 1995, Gross et al.1994, Liu et al. 1994). Weekly supplementation might remove some of the programmatic hindrances for multimicronutrient supplements.

At the SEAMEO Center in Jakarta, Indonesia, a study investigated different supplementation schedules with iron and vitamin A for adolescent girls (Angeles 1996). One group (n=90) received 60 mg iron, 2500 IU of vitamin A, 60 mg vitamin C, and 250 µg folic acid daily; another group (n=90) received 60 mg iron, 15000 IU vitamin A, 60 mg vitamin C, and 500 µg folic acid once per week; and a control group (n=90) only received placebos. Total duration of the supplementation was 3 months, and tablet ingestion was supervised. All subjects received a deworming treatment at the start of the study, and there were no differences in age, height, and weight among the groups. At baseline for the whole group, the prevalence of low serum retinol (<0.70 µmol/L) was 31%, the prevalence of anemia (Hb<120 g/L) was 21%, and the prevalence of low ferritin (<20 µg/L) was 37%. The initial concentrations of plasma retinol, ferritin, and hemoglobin were similar for the treatment groups: an average of about 0.84 µmol/L (24 µg/dL), 31 µg/L, and 126 g/L, respectively. The changes in blood variables are shown in Figure 2. The weekly and daily supplemented groups showed similar significant increases ($p<0.01$) in hemoglobin and retinol concentrations compared with the control group. Both supplemented groups showed significant increases in ferritin, but the increase was larger in the daily than in the weekly supplemented group. However, the ferritin concentration of the weekly supplemented group reached about 42 µg/L, which can be considered equivalent to an adequate iron store even for pregnancy.

Considering the effect that 12 tablets had on iron and vitamin A status, one can conclude that the effectiveness of the supplementation was optimal.

General Conclusion

On the basis of available information, an increased intake of both iron and vitamin A improves iron status more than iron alone does when populations are iron deficient and at risk of vitamin A deficiency. This conclusion should be considered when programs to alleviate micronutrient deficiency are designed.

On a middle to long-term basis the best way to achieve such an increased intake would be through a combined fortification of foods or food additives. Examples of such combined fortification exist (e.g., sugar fortification) but more information is needed about suitable levels of fortification, suitable foods that are affordable and acceptable to large parts of the target population, and the most effective chemical form of each micronutrient for selected foods.

Short-term supplementation of target groups could be the most effective way to improve iron and vitamin A status. To reduce cost and to improve compliance with supplement intake, supplementation on a weekly basis should be considered, especially for target groups such as preschool children, adolescent girls, and nonpregnant women. Among Indonesian adolescents a weekly supplement of 60 mg iron and 20,000 IU vitamin A was efficient in improving iron and vitamin A status. Suitable distribution and management systems for such supplementation programs would need to be investigated, especially when set up for new target groups. Furthermore, the effectiveness of the programs would need to be closely monitored because small-scale field trials may not have the same effects as large-scale programs.

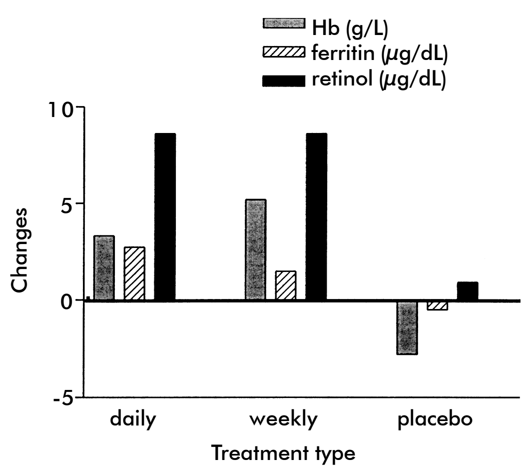

Figure 2. Changes in hemoglobin, ferritin, and retinol concentrations of female adolescents who were supplemented for 3 months. The three supplementation schedules were 60 mg iron + 2500 IU vitamin A + vitamin C and folic acid daily, 60 mg iron + 20000 IU vitamin A + vitamin C and folic acid weekly, and placebo.

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Effects of Riboflavin Deficiency on the Handling of Iron

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Background

By virtue of the redox activity of the active forms of riboflavin, this vitamin plays an essential role in many of the oxidation-reduction steps of metabolism. A diet poor in riboflavin during infancy and childhood is associated ostensibly with poor growth, but other subclinical effects of dietary inadequacy are poorly defined (Bates 1987). The poor growth characteristic of riboflavin deficiency is due in part to inefficient use of dietary energy; carefully controlled studies in animals show that weight gain per gram of diet consumed is lower if the diet is riboflavin deficient. This is conventionally explained in terms of the role for flavins in the oxidative steps of metabolism, but research carried out over the past two decades suggests very strongly that this may not be the only explanation. This paper discusses the importance of an adequate intake of riboflavin to the normal handling of iron and also argues that disturbances to normal gastrointestinal development arising from riboflavin deficiency have implications for the handling of other nutrients and may be responsible in part for the growth effects of riboflavin deficiency.

An association between hematological status and riboflavin intakes in humans was recognized and reported on by Foy and Kondi in the 1950s (1958, 1953). Studies of riboflavin deficiency in nonhuman primates provided further opportunity to characterize the anemia of riboflavin deficiency and to evaluate the specificity of the hematological and biochemical features (Foy and Kondi 1968, Foy et al. 1964). Jamdar and colleagues (1968) showed a slight decrease in plasma iron turnover rate in riboflavin-deficient rats compared with pair-fed controls, which might reflect a defect in iron used for hematopoiesis. There was a suggestion of a reduced life span of circulating erythrocytes. This effect of riboflavin deficiency was also reported in elderly people (Powers and Thurnham 1980).

Riboflavin deficiency appears to affect hematological variables without diminishing circulating iron concentrations and thus differs from simple iron deficiency. The effect on hematological variables could be via a reduction in the availability of stored or circulating iron, diminishing the rate of synthesis of globin or porphyrin, or both. Riboflavin could affect either of these processes by reducing the activity of key flavin-dependent enzymes.

Riboflavin and Ferritin Iron Mobilization

Intracellular iron is found predominantly bound to the protein ferritin. Iron is retained in the protein shell of ferritin in the ferric form and requires reduction to be released (Frieden and Osaki 1974). Reduced flavins are plausible candidates for the reduction, and therefore mobilization, of ferritin iron (Sirivech et al. 1974). FMN:NADH-linked oxidoreductase activity was described in cytosolic fractions of cells (Crichton et al. 1975), and a mitochondrial ubiquinone-linked ferriductase was also studied (Ulvik 1983). In support of a possible role for flavins in this context, iron mobilization from ferritin is reported to be impaired in some tissue preparations from riboflavin-deficient rats (Zaman and Verwilghen 1977, Sirivech et al. 1977), although the absence of appropriate controls limits interpretation of the data reported.

A number of potential sites exist for ferritin involvement in iron metabolism, including iron absorption, hepatic mobilization, and heme synthesis (Figure 1).

We have investigated the effects of feeding a diet deficient in riboflavin on ferritin iron-mobilizing activity in vitro in a number of different tissue preparations from rats. Of particular interest is a marked reduction seen in iron-mobilizing activity in duodenal mucosal homogenates prepared from riboflavin-deficient animals compared with controls (Powers 1986). The buildup of hepatic iron stores, characteristic of the weaning period in rats, is also severely im-

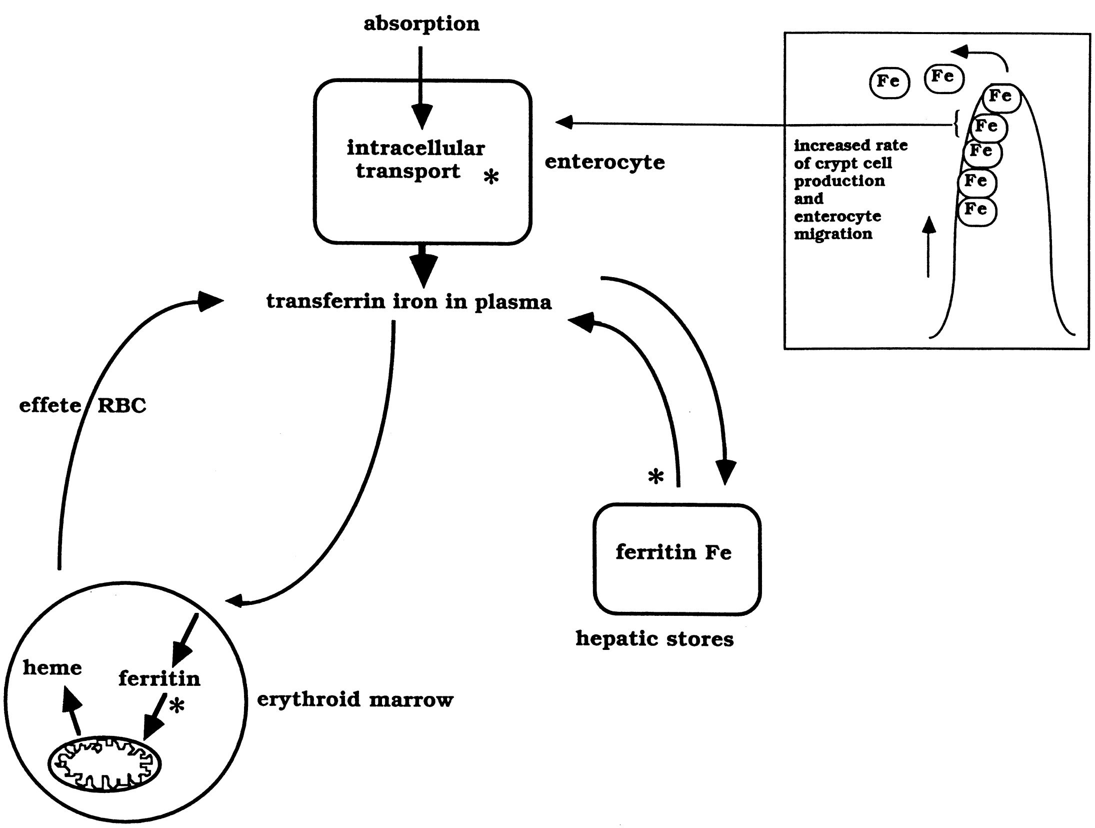

Figure 1. Potential sites of involvement of riboflavin in iron handling. * FMNH-linked ferritin iron mobilization. Inset represents the observed increase in crypt cell production rate and enterocyte migration arising from riboflavin deficiency. The effect may be to increase the rate of enterocyte loss, explaining the increased rate of gastrointestinal loss of iron in riboflavin deficiency. Crypt cell hyperproliferation and increased enterocyte migration rate would be expected to lead to a less mature enterocyte population on the villus, with reduced absorptive activity.

paired by feeding a riboflavin-deficient diet from weaning (Butler and Topham 1993, Powers et al. 1991, Adelekan and Thurnham 1986a, Powers 1985). These results suggest that FMN-oxidoreductase activity might be involved in iron absorption and that riboflavin deficiency could therefore impair this process.

The inhibitory effect of riboflavin deficiency on iron mobilization from ferritin in vitro is not restricted to the duodenal mucosa but has also been shown in mitochondrial preparations from liver (Powers et al. 1983a) and from placenta (Powers 1987). FMN is not limiting in the incubation system used for measuring iron mobilization, suggesting that tissue depletion of riboflavin leads to a reduced stability of FMN-dependent enzyme and a subsequent loss of activity.

The importance of riboflavin to the activity of ferritin iron-mobilizing activity in vivo remains unclear. Similarly the role for ferritin in gastrointestinal iron absorption has not been satisfactorily characterized.

Human Studies

Support for the contention that riboflavin status influences the handling of iron comes from a few studies conducted on schoolchildren (Charoenlarp et al. 1980, Buzina et al. 1979), pregnant women (Decker et al. 1977), and adult men and lactating women in The Gambia (Fairweather-Tait et al. 1992, Powers et al. 1985, Powers et al. 1983b). As is true for many of the poorer countries of the world, the diet in The Gambia is generally lacking in meat and dairy products and consequently riboflavin intakes are low. Biochemical riboflavin deficiency is endemic and some of the sections of the population have poor iron status (Bates et al. 1981, 1982). Placebo-controlled randomized supplementation studies carried out on children, adult males, and pregnant and lactating women showed that correcting riboflavin deficiency could improve the haematological response to iron. The effect of riboflavin was most evident in the men who had the poorest iron status at the beginning of the study (Powers and Bates 1987) (Figure 2). These results suggest that correcting a riboflavin deficiency can improve the supply of iron to the erythroid marrow; the iron could come from iron stores or directly from the diet.

Riboflavin Deficiency Impairs Iron Absorption

Iron absorption studies conducted in animals fed a riboflavin-deficient diet support the suggestion that riboflavin deficiency inhibits this process (Table 1). Riboflavin deficiency in young rats was associated with a delay and a reduction in the plasma appearance of a gastric dose of radiolabeled iron (Adelekan and Thurnham 1986b). Retention of ^{59}Fe over the 15 days after a test was also reduced after young rats were fed a diet deficient in riboflavin (Powers et al. 1991, Powers et al. 1988). In addition to these absorption studies, there is evidence from experiments using duodenal brush border membrane vesicles that riboflavin deficiency interferes with iron uptake across the brush border membrane of enterocytes (Butler and Topham 1993).

The influence of riboflavin status on the handling of iron is not limited to effects at the level of absorption. There is convincing evidence from animal studies that the daily rate of gastrointestinal loss of absorbed iron is accelerated in riboflavin deficiency (Powers et al. 1993, 1991, 1988).

The observed effect of riboflavin deficiency on iron absorption in rats has not yet

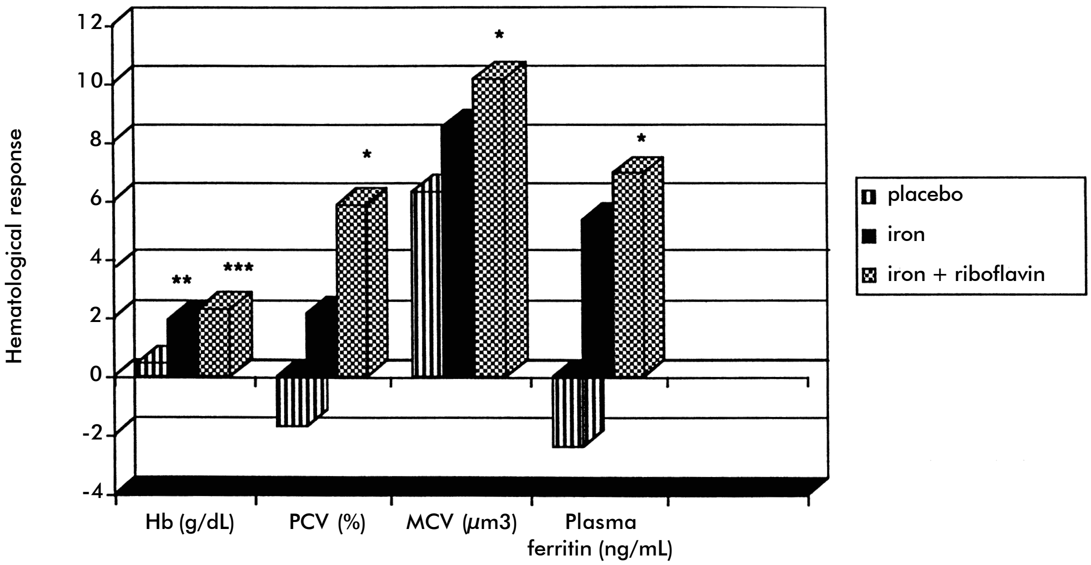

Figure 2. Hematological response to 6 weeks of supplements of iron or iron with riboflavin in Gambian men with an initial hemoglobin value of 115 g/L or less (Powers and Bates 1987). Change significantly different from placebo: *$p<0.05$, **$p<0.01$, ***$p<0.001$.

been confirmed in humans. A single study carried out in adult males in The Gambia reported measurements of iron absorption using the stable isotope ^{58}Fe and neutron activation analysis but, within the limits of sensitivity provided by the method, did not show an effect of correcting riboflavin deficiency (Fairweather-Tait et al. 1992). Improving riboflavin status without increasing iron intake, however, led to an increase in hemoglobin concentration, which could have resulted from increased iron absorption or increased efficiency of using endogenous iron for heme synthesis.

Table 1. Effect on measures of iron absorption of feeding rats a diet deficient in riboflavin

System Studied	Age of Animals (weeks)	Depletion Period (weeks)	Reduction Resulting from Riboflavin Deficiency (%)	Reference
Plasma appearance of gastric ^{59}Fe	3	7	33	Adelekan and Thurnham 1986b
Whole body retention of ^{59}Fe test meal	5	7	10	Powers et al. 1988
Whole body retention of intraperitoneal ^{59}Fe	5	5	10	Powers et al. 1991
Plasma appearance of gastric ^{59}Fe	3	6	56	Butler and Topham 1993
^{59}Fe uptake into duodenal brush border membrane vesicles	3	6	65	Butler and Topham 1993

Riboflavin Deficiency Disturbs Gastrointestinal Structure and Cytokinetics

The observed acceleration of iron loss resulting from riboflavin deficiency is difficult to explain on the basis of impaired iron mobilization from ferritin, and an alternative explanation is needed. Detailed investigations have revealed that gastrointestinal morphology and cytokinetics are profoundly disturbed as a result of feeding young rats a riboflavin-deficient diet. These observations are particularly relevant to weaning infants because the gastrointestinal disruption does not occur if the dietary depletion is initiated in young adults (Williams 1996).

An early effect of riboflavin depletion from weaning is a failure to produce the normal number of villi in the duodenum. In normal weanling rats fed a complete diet, the number of villi in the duodenum increases over the 3 weeks following weaning; such an increase does not occur in rats fed a riboflavin-deficient diet (Williams et al. 1996a). Crypt and villus hypertrophy also occur in response to riboflavin depletion, possibly as an adaptation to a reduction in surface area (Williams et al. 1996a, Powers et al. 1993). The rate of production of new cells in the crypts of the duodenum was also reported to increase in riboflavin deficiency induced in rats (Powers et al. 1993) and mice (Miyali and Hala 1965). Data also suggest that the rate of transit of enterocytes along the villi of the duodenum is accelerated in riboflavin deficiency (Williams et al. 1996a). The precise timing of these changes is under study. Of importance is that some of these morphological and cytokinetic disturbances resulting from riboflavin deficiency appear not to be readily reversible, which has implications for the long-term effects of periods of riboflavin inadequacy around the weaning period (Williams et al. 1996b).

If newly formed enterocytes move more rapidly out of the crypts and along the villi, the duration of their functional maturity may be decreased. This may be a factor determining the effects of riboflavin deficiency on iron absorption. Studies are needed to explore whether the disturbances seen in the duodenum in response to riboflavin depletion are associated with changes in the concentration of or binding capacity of iron-binding proteins in the duodenum thought to play a role in the control of iron absorption. The increased rate at which iron is lost from the body is entirely compatible with the hyperproliferative response to riboflavin depletion.

Implications for the Formulation of Complementary Foods

Riboflavin deficiency, characterized by using conventional biochemical methods, is endemic in many developing countries in which a very high incidence has been reported (Padmaja et al. 1990, Bates et al. 1981, Thurnham et al. 1971). Studies to determine daily requirements for this vitamin have been limited to some extent by the uncertainty surrounding the most appropriate biochemical cutoff point for deficiency. Current reference values set in the United Kingdom are 0.4 mg/day up to 12 months of age, rising thereafter to 0.6 mg between I and 3 years, 0.8 mg between 4 and 6 years, and 1.0 mg up to 10 years of age (Department of Health 1991). The biochemical response to different intakes of riboflavin, however, is not consistent across communities, which may be explained by an increase in requirements in response to such factors as infection and other nutrient deficiencies (Bamji et al. 1987, Bates et al. 1981).

Strategies for improving the micronutrient status of infants and children in developing countries should include steps to normalize riboflavin deficiency. In view of the importance of an adequate riboflavin status to normalize iron economy, strategies to improve iron status in particular should consider riboflavin. As has been pointed out elsewhere, the relatively low cost of this vitamin, coupled with a negligible risk of toxicity, are arguments for its inclusion in fortification programs (Liu et al. 1993).

Conclusions

Riboflavin depletion disturbs the normal handling of iron. This is true whether the deficiency is induced at around weaning or in adulthood. It impairs absorption and increases the rate of gastrointestinal loss of endogenous iron. It may also reduce the efficiency of iron use for heme synthesis. The effects on absorption and gastrointestinal loss appear to result from a hyperproliferation of crypt cells and an increased rate of transit of enterocytes along the villi, probably leading to functionally immature villi. A failure to increase villus number in the small intestine, observed after weaning when there is riboflavin deficiency, reduces the absorptive surface area and contributes to the effect on iron absorption. An increase in villus length may be an adaptation to the observed failure to increase villus number. The effects of changes in the small intestine are unlikely to be specific to iron.

Ethical Considerations

All studies involving human subjects were carried out with the approval of the University of London or University of Cambridge Ethics Committee.

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Effects of Food Processing Techniques on the Content and Bioavailability of Vitamins: A Focus on Carotenoids

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Introduction

Nutrient-rich foods are essential for proper development and growth both in adults and children, but good nutrition is especially crucial during weaning. Infants are not born with nutrient stores adequate to sustain them when faced with insufficient or imbalanced diets. Therefore weaning foods should be processed so that vitamins and minerals remain bioavailable and are not destroyed. The following will summarize the general factors that affect vitamin bioavailability from foods, including processing, food matrix, and chemical effects.

Adequate vitamin A nutriture is particularly essential during weaning. Vitamin A deficiency is a worldwide problem that increases childhood death from infections approximately 20% and results in blindness in an estimated three million children under 5 years old (Fjeld 1995). Vitamin A can be obtained from animal products as retinol or retinyl esters or from fruits and vegetables as provitamin A carotenoids. In developing countries most vitamin A value is derived from fruits and vegetables. It is hypothesized that vitamin A deficiency would be less prevalent if foods containing vitamin A or carotenoids were processed and consumed under more favorable conditions. Therefore, optimization of carotenoid bioavailability from foods will be explored in detail.

Food Processing and Nutrient Retention

Foods are processed for many beneficial reasons: to preserve and extend shelf life, increase digestibility, increase nutrient bioavailability, improve palatability and texture, eliminate microorganisms, destroy toxins, remove inedible parts, destroy antinutritional factors, and create new types of foods. Food processing can also increase consumer convenience and make foods available throughout the year (Erdman and Poneros-Schneier 1994). Unfortunately, an overall decrease in nutrient content can accompany many food processing techniques, with larger losses occurring under more strenuous conditions. In most cases, the benefits of food processing outweigh the nutrient losses. However, an understanding of the factors that affect nutrient retention and bioavailability can be used to maximize nutritional value.

Environmental conditions such as pH, temperature, light, and oxygen can affect nutrient retention during processing. Manipulation of these environmental conditions can minimize spoilage and nutrient damage. For example, riboflavin is very sensitive to light and its rate of destruction increases as temperature and pH increase. To minimize the exposure of riboflavin to light and its subsequent degradation, milk is sold in cardboard or opaque plastic containers.

The most heat-labile vitamins are vitamin C, thiamin, and riboflavin, whereas vitamin C, thiamin, and folate are most sensitive to pH extremes. Therefore, vitamin C and thiamin are considered to be the most processing-sensitive vitamins and are often used to indicate the harshness of a process. It is generally assumed that if these two vitamins are well retained during processing and storage, other nutrients also are well retained. Lysine is the most processing-sensitive amino acid. Unlike water-soluble vitamins that are leached out when excess water is used, amino acids are most sensitive to the dry heat that is commonly used in roasting or toasting of legumes or cereals.

Milling, drying, canning, and home cooking are common food processing procedures where nutrients can be lost. In some cases, the final food products are fortified to compensate for the nutrient depletion. However, the best option may be to optimize the processes to minimize the damage.

Milling removes the bran and germ fractions from cereal grains. When wheat is refined to make white flour, a 40–60% loss of vitamins and minerals occurs. In the United States most wheat flour and breads are enriched with thiamin, niacin, riboflavin, and iron and soon will be enriched with folate to compensate for the nutrients lost (Institute for Food Technology [IFT] 1986). Similarly, brown rice contains about 15.0 nmol thiamin/g whereas highly polished rice contains as little as 2.63 nmol/g. In Asia, rice is often washed before cooking, resulting in additional losses of thiamin. Thiamin deficiency can result in beriberi, which at one time was a major cause of death in many countries. To compensate for the processing losses, white rice is usually enriched with niacin, thiamin, and iron.

Multiple factors influence nutrient retention in dried foods. For example, the preservative sulfur dioxide adversely affects thiamin but protects ascorbic acid. The presence of copper, iron, light, or dissolved oxygen can decrease the ascorbic acid concentration. Dehydrated foods are susceptible to vitamin A and carotenoid losses during storage if oxygen is present. The drying process can also affect the final nutrient retention. Sun drying, a common but time-consuming drying method used in developing countries, is accompanied by substantial nutrient losses. This process is particularly harsh for heat-labile vitamins. In general, low-temperature processing such as freeze-drying will produce products with the least amount of deterioration, but this procedure is very costly.

Canning is widely used for many weaning foods, fruits, vegetables, and juices. In canned vegetables, water-soluble vitamins and minerals equilibrate between the solids and liquids. Therefore the liquid, usually discarded by the consumer, contains significant quantities of nutrients. For example, approximately 30% of the available thiamin has been found in the liquid portion (Borenstein and Lachance 1988). Also, more than 50% of the manganese, cobalt, and zinc may end up in the liquid portion of canned spinach, beans, and tomatoes (IFT 1986). Incorporation of this liquid into soups, sauces, and gravies is a way for the consumer to avoid these nutritional losses. Additionally, unpeeled fruits and vegetables show higher nutrient retention during canning, an important consideration for the home canner.

Fermentation is used to make products like cheese, yogurt, pickles, summer sausage, soy sauce, wine, and beer. Nutrient losses during processing are minimal. In fact, the vitamin content of the final products is often increased. An increase in B vitamins except for B-12 has been observed (IFT 1986).

In general, vitamin retention can be improved by cooking with less water, trimming fruits and vegetables minimally and chopping coarsely, cooking in covered pans to lessen cooking time, cooking vegetables only until tender, and reusing cooking water in soups and gravies. As an example, it was reported that baked or unpeeled boiled potatoes retained almost 100% of the initial nutrients whereas boiled, peeled potatoes retained as little as 63% (Killeit 1994). In general, steaming or stir-frying will result in greater nutrient retention than will boiling or typical pan frying (IFT 1986, Erdman 1979). Boiling with minimal amounts of water is better than boiling with larger amounts of water. Finally, vitamin losses increase with the surface contact area (Selman, 1994). In more practical terms, more nutrients are lost during the cooking of leafy vegetables than root vegetables.

Other factors that affect the bioavailability of nutrients from foods include the chemical form of the nutrient, the presence of absorption inhibitors or promoters, the amount of dietary fat in the meal, and the health and nutritional status of the individual. For instance, a food may be high in calcium, but if the calcium is bound in an oxalate as it is in spinach, the body is unable to absorb it. Heme iron, bound in a porphyrin ring, is absorbed more efficiently than nonheme iron, which must be liberated enzymatically before being absorbed (Groff et al. 1995). Further, nonheme iron absorption is improved if the iron is in the ferrous (Fe^{+2}) or reduced state versus the ferric (Fe^{+3}) state; the chemical form of a nutrient can affect bioavailability. Similarly, certain compounds act as inhibitors or promoters of absorption. For example, thiaminases can inhibit the absorption of thiamin whereas vitamin C enhances the absorption of nonheme iron.

The fat content of a food or a meal affects nutrient bioavailability because fat is required for the absorption of the fat-soluble vitamins A, D, E, and K and the carotenoids that are precursors to vitamin A. Fat stimulates bile flow and acts as a carrier for these hydrophobic nutrients.

Health and nutritional status affect the bioavailability of nutrients. In developing countries, intestinal parasites compete with a host for essential vitamins and minerals in food. Therefore, even if sufficient nutrients are present in the diet, parasites could prevent adequate consumption by the host. Nutrient deficiencies can alter the absorption and metabolism of other nutrients. For instance, the utilization of dietary folate is impaired in individuals with vitamin C or iron deficiencies (Comb 1992). Although cleavage of provitamin A carotenoids to vitamin A appears to be more efficient in individuals who are marginally vitamin A deficient, adequate protein and zinc status is required for the absorption and conversion of carotenoids to vitamin A (Erdman et al. 1988).

Carotenoid Bioavailability

Carotenoid and vitamin A absorption depend on many factors, including the food matrix (especially fiber), the fat in the meal, and the processing conditions used. Absorption has been defined as the movement of carotenoids and vitamin A from the intestine into the lymphatic circulation (Erdman et al. 1993). Uptake into mucosal cells does not guarantee that the carotenoids will be transported into general circulation before the cells are sloughed off. Absorption of β-carotene from a spinach leaf will be used to illustrate how food matrix, fat, and processing are involved.

Within a spinach leaf, carotenoids are bound by proteins in the chloroplast where photosynthesis occurs. Carotenoids can also be trapped in the fibrous regions of the chromoplast (Olson 1996). Mild heating, chewing, and digestive enzymes help to rupture the chloroplasts, denature the binding proteins, and release the entrapped β-carotene into the partially digested meal (chyme). More efficient release of β-carotene occurs when smaller particle sizes are achieved (Parker 1996). Because β-carotene is a fat-soluble hydrocarbon, it only associates with the lipid portion of the chyme. Once the chyme moves into the small intestine, bile and the lipid portion of the meal combine to form mixed micelles. Without bile or sufficient fat to form micelles, β-carotene cannot be solubilized or transported into the intestinal mucosal cells. Carotenoids appear to be absorbed by passive diffusion (Parker 1996).

Once in the intestinal mucosal cells, β-carotene can remain intact or can be cleaved to vitamin A. This absorption and cleavage process is relatively inefficient. It has been estimated that only one molecule of retinol is produced from six molecules of dietary β-carotene. From the mucosal cell, chylomicrons containing β-carotene, vitamin A, or both are transported into the lymphatic circulation. Again, without sufficient fat chylomicrons will not be formed and the β-carotene and vitamin A will be left in the mucosal cells eventually to be sloughed off into the lumen of the small intestine. For vitamin A to be derived from spinach, many conditions must be optimal.

In 1995, dePee et al. reported that a supplement of dark-green vegetables did not improve the vitamin A status of breast-feeding women in Indonesia. Although the vegetables were stir-fried with 7.8 g of fat, the bioavailability of the β-carotene did not compare with a supplemental wafer containing a highly bioavailable form of β-carotene with only 4.4 g of fat: β-carotene locked in the vegetable matrix was less available. It appears that even with mild heating and 7.8 g of fat, carotenoid-rich vegetables are not the best source of vitamin A. However in many countries, carotenoid-rich vegetables are still the only source for the population. Under what conditions can vitamin A bioavailability from vegetables be optimized?

In the spinach example, mild heat helped to release bound carotenoids from the food matrix and binding proteins. Unfortunately, higher temperatures used in both home cooking and food processing can result in isomerization of the carotenoid double bonds. For example, the β-carotene in fresh spinach is about 80% all-*trans*, yet after canning only about 60% is in the all-*trans* conformation. All-*trans* β-carotene has the highest vitamin A value, and isomerization decreases this value. Therefore, after canning the β-carotene utilizable for vitamin A decreased 20% (Chandler and Schwartz 1987). Similarly, Marty and Berset (1988) examined the fate of all-*trans* β-carotene in a model extrusion system of starch and water at 180 °C. Extrusion cooking is a high-temperature, high-shear, short-time processing technology primarily applied in grain processing to produce breakfast cereals and snacks. After processing, only 8% of the original β-carotene was recovered as all-*trans* β-carotene. Accompanying degradation products included epoxides, diepoxides, mono- and poly-*cis* isomers and apocarotenals. This model system may exaggerate the processing effects because carotenoids are more protected in natural systems, but the take-home message remains clear: mild heating increases carotenoid bioavailability whereas excessive heat is detrimental. In addition to enhancing isomerization, heat has been implicated in the oxidative degradation of carotenoids and in the aggregation

Table 1. Food matrix effects on bioavailability of carotenoids

		Excellent Bioavailability
Commercial beadlets		
Carotenoids from oil	Palm oil	↑
Fruits	Papaya, peach, pumpkin, melon	|
Tubers	Squash, yam, sweet potato	|
Mildly cooked yellow/orange vegetables	Carrots, peppers	|
Raw yellow/orange vegetables	Carrots, peppers	↓
Green leafy vegetables	Spinach	
		Poor Bioavailability

of fiber, which can further entrap carotenoids in the food matrix (Olson 1996).

Fat is required at three points in the carotenoid absorption process: stimulation of bile secretion, mixed-micelle formation, and chylomicron formation. Without sufficient fat, absorption is limited. Prince and Frisoli (1993) showed a fourfold higher serum β-carotene response when β-carotene capsules were consumed with 50 g of fat than when consumed without fat. Similarly, in India Reddy and Vijayaraghavan (1995) fed spinach to preschool children (1.2 mg β-carotene daily) for 4 weeks, either without fat or with the addition of 5 or 10 g vegetable oil (Reddy and Vijayaraghavan 1995). Initial serum vitamin A concentrations were similar, but significant increases in serum vitamin A were observed only in the groups receiving the additional fat. It is hypothesized that spinach was an effective source of vitamin A in this study because the children were more vitamin A deficient than were the breast-feeding women in the dePee study.

Roels et al. (1958) fed 22 vitamin A–deficient boys one of five supplements at lunch and dinner: group A received 100 g grated raw carrots; group B, 100 g grated raw carrots + 9 g olive oil; group C, 14 mg crystalline β-carotene + 9 g olive oil; group D, 9 g olive oil; and group E, placebo. Group A absorbed less than 5% of the carotenoid from the supplement whereas groups B and C absorbed 25% and 45%, respectively. This study reinforces the fact that fat is required for carotenoid absorption but also suggests that more complex food matrices inhibit absorption.

Researchers postulate that this food matrix effect is primarily a fiber effect. When subjects were fed a meal supplemented with 25 mg β-carotene from commercial beadlets and 12 g pectin, the mean percentage increase in serum -carotene was more than 50% less than serum values for subjects fed a meal supplemented only with β-carotene (Rock and Swendseid 1992). Pectin is hypothesized to interfere with gastric emptying and with mixed-micelle formation, thereby disrupting β-carotene absorption (Erdman et al. 1986). Drugs that affect normal fat-absorption mechanisms, such as cholesterol-lowering drugs, will also impair carotenoid absorption.

Some sources of β-carotene are more bioavailable than others (Table 1). Commercial β-carotene beadlets contain antioxidants to inhibit oxidation and emulsifiers to enhance -carotene absorption. For example, we found that β-carotene beadlets were six times more bioavailable than an equivalent amount of β-carotene from carrot juice (White et al. 1993). Similarly, β-carotene absorption from oil solutions or aqueous dispersions can be as high as 50% (Erdman et al. 1993).

Carotenoids naturally present in oils, such as palm oil, are very bioavailable. Additionally, fruits and tubers are good sources of β-carotene. However for reasons described above, carotenoid utilizability from carrots and green leafy vegetables is poor, particularly when the vegetables are consumed raw.

Conclusions

A balanced diet begins with the selection of proper foods. However, food processing and food preparation procedures must be optimized to maintain the bioavailability and nutrient content of the foods. Mild heating, minimal water, and controlled storage conditions usually enhance the vitamin value whereas excess heat, water, or harsh storage conditions are destructive. Deriving vitamin A value from some vegetables is a challenge and depends on the matrix of the vegetable, the composition of the meal, food processing conditions, and the health of the gastrointestinal tract. Simple matrices, sufficient fat, and mild heating appear to favor carotenoid absorption for vitamin A usage. Attention to these key items can lead to continuing improvement of the nutritional status of the world population.

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Food Processing Methods for Improving the Zinc Content and Bioavailability of Home-based and Commercially Available Complementary Foods

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Introduction

The nutritional adequacy of dietary zinc depends on its amount in the diet and its bioavailability. Dietary-induced zinc deficiency is likely to occur when diets are predominately plant based and low in flesh foods, a rich source of readily available zinc. In less industrialized countries complementary foods used for feeding infants and children are generally thin porridges prepared from flour mixtures based on cereals, legumes, starchy roots, and tubers. Flesh foods are rarely added to these porridges because of economic, cultural, and religious constraints. Cereal-based porridges have a higher zinc content than do those from starchy roots and tubers but their content of phytic acid (myoinositol hexaphosphate) is also higher (Table 1).

Phytic acid is the major determinant of zinc absorption, especially when the content of animal protein is low. Zinc and phytic acid form insoluble complexes, and the negative effect of such complexes on zinc absorption can be predicted by phytate-to-zinc molar ratios when the dietary zinc intake is close to the requirement (Oberleas and Harland 1981). Ratios above 15–20 appear to compromise zinc status (Bindra et al. 1986, Turnland et al. 1984). Phytate-to-zinc ratios of the cereal-based porridges are all above 15 whereas those for porridges based on starchy roots and tubers are generally below 15. Such cereal-based complementary foods, when consumed with breast milk, may actually reduce the bioavailability of zinc from breast milk. Bell et al. (1987), using an in vivo absorption rat pup model, showed that even commercially processed infant cereals lowered ^{65}Zn uptake from extrinsically radiolabeled milk diets.

High amounts of calcium exacerbate the inhibitory effect of phytate on zinc absorption by forming a calcium-zinc-phytate complex in the intestine that is even less soluble than phytate complexes formed by either ion alone (Fordyce et al. 1987, Wise, 1983). The calcium content of most plant-based porridges is not high enough to potentiate the negative effect of phytate on zinc bioavailability unless the porridges contain, for example, lime-soaked maize.

Insoluble cereal and vegetable fibers may also exacerbate the adverse effect of phytate on zinc absorption, especially when protein intakes are low (Sandstrom et al.

Table 1. Energy and selected nutrient and antinutrient content of 750 mL/day of some porridges used for infant feeding in Ghana and Malawi

	80% S Potato + 20% Amaranth Leaves (Ghana)	80% R-Maize + 20% Soya (Malawi)	80% U-Maize + 20% Soya (Malawi)	70% U-Maize, 20%GN + 10% Sorg (Malawi)
% Dry flour		24%	24%	22%
Energy (kJ)	2604	2747	2866	2628
Protein (g)	8	26	27	27
Ca (mg)	170	80	76	29
Zn (mg)	1.4	2.6	4.6	3.7
NSP (g)	13	7.3	14.8	12.2
Phytate (mg)	102	723	1403	849
Phy/Zn	7	28	30	23

S Potato, sweet potato; R-Maize, refined maize; U-Maize, unrefined maize; GN, ground nuts; Sorg, sorghum; NSP, nonstarch polysaccharide.

1989), but whether dietary fiber alone can inhibit zinc absorption independent of phytic acid is less clear. Porridges based on starchy roots or tubers such as sweet potatoes, sago, and bananas (as in Papua New Guinea) or cassava and plantains (as in the forest regions of Ghana) generally have a relatively low phytate but high dietary fiber content (Table 1).

Some plant-based porridges may also contain appreciable amounts of oxalic acid, known to impair calcium bioavailability in humans. Oxalic acid forms fiber-zinc-oxalate complexes that are less readily degraded in the digestive tract than are mineral-fiber complexes alone (Kelsay and Prather 1983). Whether oxalic acid per se influences zinc absorption in humans has not been established (Kelsay et al. 1988); studies on zinc-deficient rats have shown no effect of oxalic acid (House et al. 1996).

The amount and type of protein also affects zinc bioavailability. For example, inclusion of small amounts of animal and fish protein can increase the apparent absorption of zinc and counteract the negative effect of phytic acid, even when the amount of zinc in the diet is only modestly increased (Sandstrom et al. 1989). The mechanism is unclear: naturally occurring mineral chelates may exist in animal protein (Scott and Zeigler 1963). Alternatively, L-amino acids and cysteine-containing peptides formed during the digestion of proteins may form soluble ligands with zinc and facilitate its absorption (Snedeker and Greger 1983) or form complexes with zinc, thereby preventing the formation of the zinc-phytate complex (Sandstrom et al. 1980). In rats, supplemental methionine or cysteine enhanced the bioavailability of ^{65}Zn provided intrinsically in corn kernels (Welch, personal communication).

Competitive interactions between chemically related minerals in the intestine (e.g., copper and zinc, and nonheme iron and zinc) also decrease zinc bioavailability, but the amounts intrinsic to complementary foods are unlikely to be high enough to compromise zinc status. Care must be taken, however, to avoid antagonistic interactions when complementary foods are fortified with multiple micronutrients.

Food Processing Methods for Improving the Zinc Content and Bioavailability of Complementary Foods

Certain food preparation and processing methods can be used to increase both the content and bioavailability of zinc in complementary foods. Many of these methods are economically feasible, culturally acceptable, and sustainable and can be used at the household and commercial levels. They are described below.

Plant Breeding

New cereal varieties are being bred that tolerate zinc-deficient soils. These varieties—termed zinc-efficient genotypes—have a superior ability to extract zinc from zinc-deficient soils by growing deeper roots. They are also more disease resistant and have improved seedling vigor, enhanced germination, and a higher grain yield (Graham et al. 1992). Hence, they have an economic advantage. Although these cultivars do not necessarily have grains with the highest zinc concentrations, plant breeders are confident that because grain zinc concentrations are under genetic control, they can be increased in these zinc-efficient varieties. Increases in grain zinc concentrations could also be achieved by soil fertilization or foliar application.

Breeding strategies are being developed to improve the bioavailability of trace minerals from cereal staples by increasing their content of certain amino acids (e.g., methionine and cysteine) (House et al. 1996), reducing their phytic acid content, or both.

Germination

During germination of cereals (but not legumes), amylase activity increases, especially -amylase in tropical cereals such as sorghum and millet. Amylases hydrolyze amylose and amylopectin to dextrins and maltose, which do not gelatinize on cooking. Hence, by adding 1–5% of a germinated cereal flour (e.g., sorghum), the viscosity of thick porridges (25% dry flour) can be reduced to that of an easy-to-swallow semi-liquid consistency (i.e., from 50,000 to 3000 mPa s) without diluting with water. As a result, both the energy and nutrient density including zinc will be increased (Ashworth and Draper 1992) and the taste will become sweeter and more appealing to infants.

The bioavailability of zinc in cereal-legume based porridges can also be enhanced by the addition of germinated cereal flours. Germination increases phytase activity in seeds as a result of synthesis and activation of endogenous phytase. Seeds contain 6-phytases (EC 3.1.26), the level of phytase activity depending on the species and variety; rye has the highest phytase activity, followed by wheat and barley; activity in oats and maize is negligible (Cheryan 1980). Phytase enzymes hydrolyze phytic acid to yield inorganic orthophosphate and myo-inositol via intermediate myoinositol phosphates (pentaphosphates to monophosphates); only the higher inositol phosphates (IP-5 and IP-6) inhibit zinc (and iron) absorption (Lonnerdal et al. 1989). Hence, knowledge of factors enhancing phytase activity in plant-based complementary foods is important. Optimal conditions depend on stage of germination, pH, moisture content, temperature, solubility of the phytate, and presence of certain inhibitors. Sodium and certain metal ions form salts with phytate that are resistant to phytase. By contrast, the concentration of minerals (including zinc) does not change during germination; any reported increases may arise from losses in dry matter as the result of respiration during sprouting (Lorenz 1980). Care must be taken, however, to ensure that all porridges prepared from germinated flours are decontaminated by heating before use because germination may increase pathogenic and toxinogenic species of bacteria and fungi (Nout 1993).

Fermentation

Microbial fermentation can also enhance the bioavailability of zinc in complementary foods via hydrolysis induced by microbial phytases (EC 3.1.8). The latter may be derived from the microflora present on the surface of seeds and legumes or from microbial starter cultures (Chavan and Kadam 1989). Alternatively, commercial phytase enzymes prepared from *Aspergillus oxyzae* or *A. niger,* which act over a broader pH range (i.e., pH 1.0–7.5) than cereal phytases (pH 4.5–5) and are stable over a wider temperature range, may be used (Turk and Sandberg 1992). Their use will facilitate phytate hydrolysis over a longer fermentation period and at physiological pH conditions of the stomach (Sandberg et al. 1996).

The nature of the substrate as well as physiological differences in fermenting organisms will also influence the rate of phytate hydrolysis. Reported reductions in the hexainositol phosphate and pentainositol phosphate content of fermented porridges prepared from white sorghum and maize were about 50% (Svanberg et al. 1993) (Figure 1). Note that phytate degradation by microbial phytase is less in high-tannin cereals such as brown sorghum and finger millet, possibly because of the inhibitory effect of polyphenols on phytase activity.

Other investigators reported phytate reductions after fermentation of 35–40% for idli batter in India prepared from rice and black gram (Chavan and Kadam 1989) and 52–98% for a variety of West African fermented products based on cassava, cocoyam, maize, and assorted legumes (Adewusi et al. 1991, Marfo et al. 1990), depending on preparation, storage, and cooking conditions.

In the future, pure culture microorganisms may be available that reduce the fermentation times necessary to decrease or eliminate phytic acid and other antinutrients (e.g., polyphenols), enhance the nutrient content and quality, and avoid toxin production problems (Chavan and Kadam 1989).

The organic acids produced during fermentation (lactic, acetic, butyric, propionic, and formic acids) may also potentiate zinc absorption via formation of soluble ligands with zinc (Desrosiers and Clydesdale 1989), whereas the low pH and possible pro-

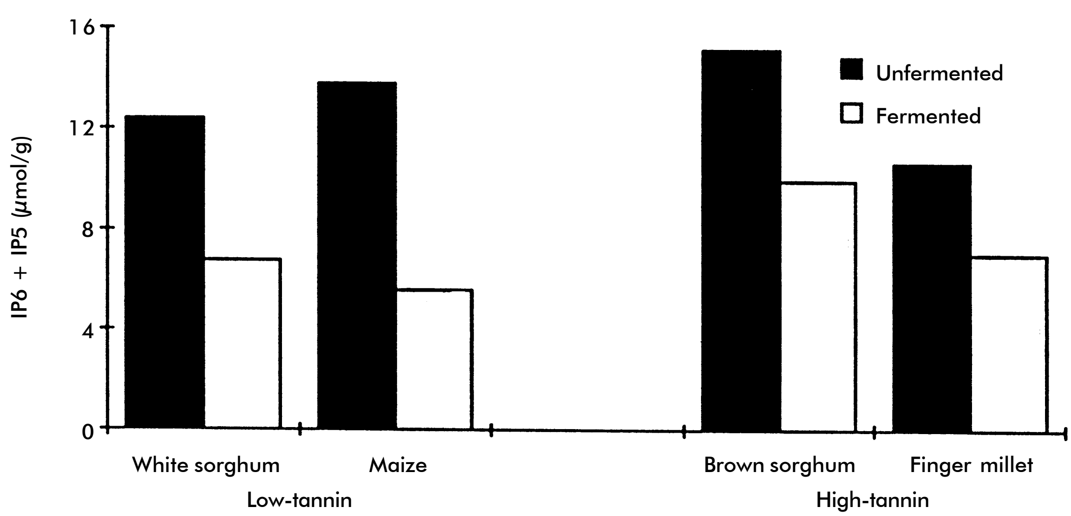

Figure 1. IP6 + IP5 content of porridges (10% solids) made from low- and high-tannin cereal flours (modified from Svanberg et al. 1993).

duction of antimicrobial substances may inhibit the growth of diarrheal pathogens. In general, fermentation, unlike germination, does not increase the energy and zinc density because lactic acid bacteria are not amylolytic and hence do not reduce the viscosity of cereal porridges (Ashworth and Draper 1992).

Soaking

Soaking is a practical household method that can also be used to reduce the phytic acid content of complementary foods prepared from certain cereals (e.g., maize) and most legumes, including soybeans, because their phytic acid is stored in a relatively water-soluble form such as sodium or potassium phytate and hence can be removed by diffusion. Levels of water-soluble phytate range from 10% in defatted sesame meal to 70–97% in California small white beans, red kidney beans, corn germ, and soya flakes (Chang et al. 1977, De Boland et al. 1975). Discrepancies occur in the reported levels of soluble phytic acid in these staples, attributed to variations in the conditions used to extract the phytic acid, pH, and the content of protein, calcium and magnesium ions (Tabekhia and Luh 1980). Magnesium, calcium, and zinc salts of phytic acid tend to be soluble at lower pHs but insoluble at higher pHs (Cheryan 1980). Theoretically, at the commercial level, the pH of the water could be adjusted to yield insoluble salts of phytic acid that could then be removed by filtration or centrifugation. This more sophisticated approach has been used to prepare low-phytate soy protein isolates and rapeseed protein. Soaking may also remove other antinutrients such as saponins and polyphenols (Kataria et al. 1988).

Milling

Milling can also be used to reduce the phytic acid (and dietary fiber) content of cereals and some oilseeds (except soybeans) used for complementary foods if phytic acid is localized within a specific part of the grain or seed. For example, most of the phytic acid is localized in the outer aleurone layers in wheat, triticale, rice, sorghum, and rye, whereas in maize it is in the germ (O'Dell et al. 1972). Because a major proportion of the zinc (as well as iron, calcium, magnesium, manganese, and copper) is also removed by refining, the phytate-to-zinc ratio of porridge prepared from refined cereals is not dramatically reduced (Table 1). By contrast, the cotyledons and not the seed coat contain most of the phytate in peas and beans so that if the testa is removed, the phytate concentration actually increases.

Thermal Processing

Thermal processing at high temperatures may induce some partial nonenzymatic hydrolysis of phytic acid, although in general, use of excessive or harsh heat treatments (e.g., extrusion cooking) is not a practical method for destroying phytic acid. Such treatment also reduces nutrient bioavailability (including that of zinc) by causing chemical changes (Kivisto et al. 1986, Lykken et al. 1986). Mild heat treatment will increase the digestibility of most porridges and may reduce the phytic acid content of tubers but probably not of cereals and legumes (Marfo et al. 1990).

Combination of Soaking, Germination, and Fermentation

The most effective way to enhance the content and bioavailability of zinc in cereal-legume based porridges is to soak flour blends containing some germinated flour as an additive (at 5%) for 24–48 hours followed by the addition of a microbial starter culture to accelerate fermentation. Prior soaking of flour blends allows endogenous cereal phytases to hydrolyze phytic acid for a longer time at their optimal pH (4.5–5.0). When the pH falls below 4.5, phytic acid can only be hydrolyzed by microbial phytases, because they act over a broader pH range

(2.5–5.5) than do cereal phytases. Water is then added to the fermented flour to form a slurry that is cooked to a porridge. These combined strategies can reduce the phytic acid content by as much as 90% (Figure 2) (Svanberg et al. 1993) while enhancing zinc bioavailability, protein quality and digestibility, microbiological safety, and keeping quality.

Caution must be used when evaluating the effect of enzymatic and nonenzymatic hydrolysis of phytate in plant-based staples. Inconsistencies arise because of methodological differences in the phytate and phytase assays (Xu et al. 1992). High-performance liquid chromatography should always be used to separate, identify, and quantify the higher and lower inositol phosphates (Lehrfeld 1989).

Multimicronutrient Fortification of Cereal- and Legume-based Complementary Food Enriched with Germinated Sorghum

Commercial methods for improving the content and bioavailability of complementary foods in less industrialized countries include multimicronutrient fortification of cereal- and legume-based complementary foods containing some germinated cereal (i.e., 5–10%), possibly combined with the addition of commercial microbial phytase enzymes. The fortificants selected must be safe, stable, acceptable, bioavailable, and at amounts that do not induce any adverse nutrient-nutrient interactions or influence the organoleptic qualities and shelf-life of the complementary food. Ideally, protected fortificants that are resistant to common inhibitors of zinc absorption should be used. Our knowledge of the effects of food matrices and processing condition on the bioavailability of zinc fortificants is limited. Currently, zinc oxide is most frequently used despite its low solubility. Solubility could be improved by the concomitant addition of certain organic chelators such as cysteine; ligands such as malate, citrate, and lactate are ineffective (Desrosiers and Clydesdale 1989).

Summary

The content and bioavailability of zinc in plant-based complementary foods used in less industrialized countries are often low and can be improved by using certain food

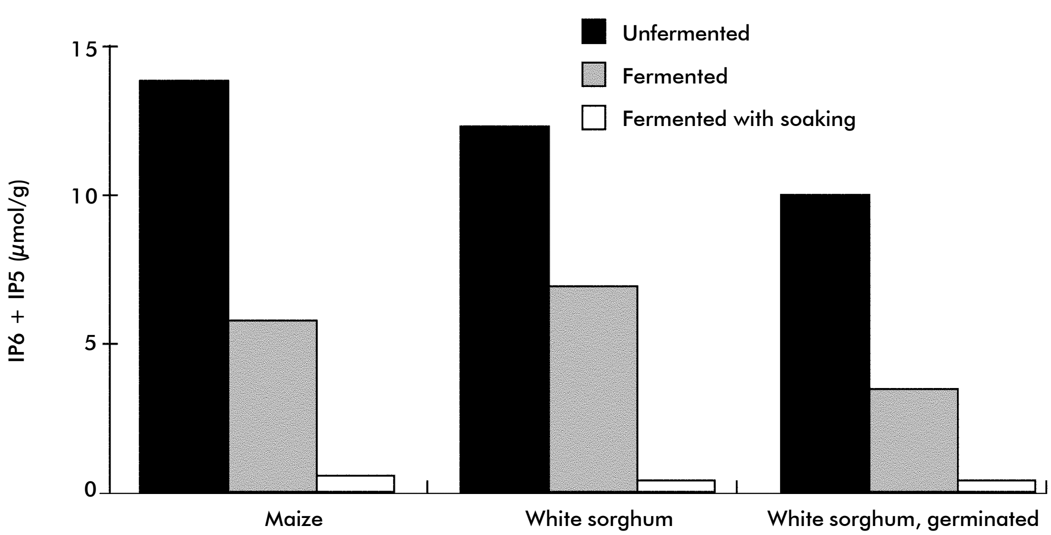

Figure 2. IP6 + IP5 content of porridges (10% solids) made from unfermented, fermented, and fermented + soaked cereal flours (modified from Svanberg et al. 1993).

preparation and processing methods. These methods include reducing the phytic acid content via phytase hydrolysis induced by germination and fermentation as well as via nonenzymatic degradation by soaking or thermal processing. Addition of germinated flours also enhances the energy and nutrient density (including that of zinc) by amylase-induced hydrolysis of starch to dextrins and maltose which do not gelatinize on cooking. Finally, commercially based complementary foods can be fortified with multimicronutrients and enriched with germinated cereals or commercial phytase. To be effective, these strategies must be integrated with ongoing national food, nutrition, and health education programs and implemented at the household level using education and social marketing techniques.

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Effects of Cooking and Food Processing on the Content of Bioavailable Iron in Foods

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Introduction

The capacity of foods and diets to meet iron requirements depends on both iron content and bioavailability. Iron bioavailability is the proportion of iron in a food or diet that is available for intestinal absorption in a form that is physiologically active. Except for elemental iron added as a fortificant, the valence of iron in foods is either +2 (ferrous) or +3 (ferric). Little, if any, of the iron in foods is present as free ions. Rather, it is bound to various ligands (proteins, carbohydrates, organic acids, porphyrin rings, and other species). Thus the number of different forms of iron in foods is very large. However, for purposes of defining iron bioavailability, food iron is often categorized as either heme or nonheme (Hallberg 1981).

Heme iron is tightly complexed at the center of a porphyrin ring and does not exchange with nonheme iron during digestion. Major sources of heme iron in the diet are hemoglobin and myoglobin; only meats and blood products provide significant amounts dietary heme iron. Virtually all of the iron in plant-based foods and about half of the iron in meats is in the form of nonheme iron. These two forms of iron are absorbed by different pathways. The bioavailability of heme iron is relatively high and is not much affected by the composition of the diet. The bioavailability of added and intrinsic nonheme iron is largely determined by the solubility of the iron in the upper gastrointestinal tract (International Nutritional Anemia Consultative Group [INACG] 1993). Iron solubility varies widely and depends on pH and the chemical form of the iron.

As foods move through the gastrointestinal tract, there is an exchange of nonheme iron among ligands that were present in the ingested food or that are products of the digestion of proteins, carbohydrates, and lipids. It is generally accepted that most or all of the nonheme iron in ingested foods enters a common pool as a result of this exchange (Hallberg 1981). Iron added to foods as a fortificant may also enter this pool if it is soluble in the acidic environment of the stomach. Components of the digesta interact with the iron in this pool to either enhance or inhibit iron absorption. Dietary phytates, proteins, polyphenols, and calcium inhibit nonheme iron absorption whereas ascorbic acid and meats enhance it.

Food processing can markedly affect the content of bioavailable iron in foods. Iron content may be increased by fortification and by cooking in iron vessels. The principle route of loss of iron from foods during processing is through physical separation during the milling of cereals. Milling removes about two-thirds of the iron present in the whole grain (Ranum and Loewe 1978). Processing may also alter the bioavailability of food iron. Addition of ascorbic acid enhances bioavailability whereas destruction or leaching of ascorbic acid reduces it. Removal or hydrolysis of phytates may also enhance iron bioavailability.

Iron Content of Complementary Foods

Cereals, pulses, and milk are widely used as complementary foods for infants. Cereals and pulses have the advantage of being relatively inexpensive and locally available throughout the world. Milk is an excellent source of protein, calcium, and riboflavin but is very low in iron.

Iron Content of Selected Foods

The reported iron content of foods varies considerably even within a plant or animal species. Several factors contribute to this variability, including maturity of the plant or animal; growing conditions (soil fertility and rain fall); iron status of the animal; genetics; and contamination from soil, dust, and equipment used for harvesting, processing, and cooking. Also, inaccuracies in analytical methods may be a factor.

The iron content of selected cereals, pulses, and weaning foods formulated from cereal, pulses, and jaggery (crudely refined sugar available in India) is shown in Table 1. It is striking that the iron content of the weaning foods is more than double that commonly reported for cereals and pulses. The authors (Gahlawat and Sehgal 1993) speculated that the high iron content of the weaning foods was from the jaggery.

Table 1. Iron content of selected cereals, pulses, and weaning foods from India

Food	Iron Content
	(mg/100 g fresh weight)
Raw staples[a]	
Brown rice	1.2
White rice	0.6
Wheat	4.2
Green gram	4.4
Weaning foods[b]	
Wheat:green gram:jaggery (70:30:25)	16.0
Barley:green gram:jaggery (70:30:25)	13.9

[a]Data from Srikumar (1993). Values are on a wet weight basis. Moisture content range: 9–13%
[b]Data from Gahlawat and Sehgal (1994). Values are on a dry matter basis.

Effects of Milling on the Iron Content of Cereals

The iron content of cereals is affected by milling. Much of the iron in cereal grains is concentrated in the outer layers of the kernels. When the outer layers are removed during milling, the milled products (white flours and white rice) have markedly lower iron content than does the whole-grain product. The iron content of wheat flours varies with the extraction rate, which is a term used in the flour industry to characterize the degree of refinement of the flour. For example, if the weight of the flour produced by milling 100 kg of wheat kernels is 70 kg, the extraction rate for the flour is 70%.

The extraction rate of whole wheat flour is 100%, so the iron content should be very close to the iron content of the intact wheat kernels, or about 3.5 mg/100 g. Extraction rates for most white flours in the United States are around 76%. These flours contain about 1.12 mg iron/100 g, or slightly less than 40% of the iron in the intact wheat kernel (Barrett and Ranum 1985). Flours with lower extraction rates have lower iron contents than do the intact grains. A large proportion of the wheat flour produced in the world has extraction rates ranging from 70% to 80% (Barrett and Ranum 1985).

Much of the rice available for human consumption has also been milled or polished. As with flour, milling of rice removes the bran and germ portions of the kernel, leaving the endosperm. The iron contents of brown rice and polished rice are about 1.1 and 0.5 mg/100 g, respectively (Hunnell et al. 1985).

Effects of Cooking on the Iron Content of Foods

Cooking in iron pots can markedly increase the iron content of foods (Brittin and Nossaman 1986). The gain in iron varies considerably depending on the pH of the food and the cooking time. Acidic foods cooked for long periods gain the most iron. In one study the correlation between the iron increase in foods cooked in a steel wok and pH was -0.82; the correlation between the increase and cooking time was +0.79, respectively (Zhou and Brittin 1994). A comparison of the iron contents of selected foods cooked in either noniron (glass or aluminum) or iron utensils is shown in Table 2.

Although cooking in iron pots increases the iron content of many foods, the increases are variable because different iron pots may release different amounts of iron to foods (Brittin and Nossaman 1986). The iron from the cooking vessel may discolor foods or impart a metallic taste. Presumably, the iron from the pots enters the nonheme iron pool as it enters the food. Thus, the bioavailability of the iron from the pot will be the same at that for the iron intrinsic to the food and would be low in foods containing inhibitors. Cooking in iron pots as a strategy for improving iron status, therefore, may not be reliable.

Effects of Processing on Iron Bioavailability

Phytates and Iron Bioavailability

Phytic acid (myoinositol hexaphosphate) is the primary storage form of phosphorous in plants (Reddy et al. 1982). The multiple phosphate residues in each molecule of phytic acid make it an excellent chelating

Table 2. Iron content of foods cooked in noniron or iron cooking utensils

Food	Cooking Time (min)	pH	Noniron Utensil (mg Fe/100 g)	Iron Utensil (mg Fe/100 g)
Pureed vegetables[a]	23	5.25	0.67	7.67
Cornmeal mush[a]	18	5.66	0.13	4.30
Milk (boiled)[a]	10	6.32	0.08	1.96
Rice[a]	22	6.41	0.13	3.11
Applesauce[b]	20	3.90	0.35	7.38
Scrambled egg[b]	4.0	7.2	1.79	4.76
Rice[b]	20	6.22	0.67	1.97
Corn tortillas, fried[b]	1.0	9.34	1.14	1.23
Cornbread, baked[b]	15	6.81	0.83	0.86

[a]Data from Borigato and Martinez (1992).
[b]Data from Brittin and Nossaman (1986).

agent for metal ions. Metal-phytate chelates are often insoluble under conditions found in the gastrointestinal tract. There is convincing evidence that phytic acid is a major factor contributing to the poor bioavailability of iron in diets rich in legumes and whole-grain cereals (Zhou and Erdman 1995, Sandberg 1991).

A few examples from recent well-designed studies illustrate the size of the effects of phytate on iron absorption. Graded amounts of sodium phytate (2–250 mg) were added to simple meals composed of wheat buns made from 60%-extraction flour (Hallberg et al. 1989). Iron absorption in human subjects was reduced by 18% and 82% for meals containing 2 and 250 mg of phytate, respectively. The addition of 50 mg of ascorbic acid to the meals partially counteracted the inhibitory effects of phytate. The authors concluded that large amounts of ascorbic acid (80 to several hundred milligrams, depending on the amount of phytate present) would be necessary to fully overcome the inhibitory effects of phytate.

Another study in humans investigated the inhibitory effects of soy protein isolates on iron absorption (Hurrell et al. 1992). Iron absorption from soy protein isolates was only 10–25% of absorption from an egg-white control. Removal of the phytate from the isolates by washing with an acid-salt mixture or by adding phytase (which hydrolyzes the phosphate groups on phytate) increased iron absorption four- to fivefold, but the absorption was still only about 50% of that from the egg-white control. This study shows that removal of phytate can dramatically improve iron absorption but that other factors in soy protein isolates also inhibit iron absorption.

A third study in humans compared iron absorption from breads made from combinations of whole wheat, whole rye, and low-extraction wheat flours fermented for various times (Brune et al. 1992). Iron absorption was inversely related to the phytate content of the breads. Furthermore, fiber content of the breads appeared to have little effect on iron absorption.

Phytic acid in foods is complexed with metal ions, primarily Ca^{+2}, Mg^{+2}, and K^{+1} (Reddy et al. 1982). It is concentrated in the aleurone layer and germ in monocotyledonous seeds (wheat, rice, barley, and rye) but is more uniformly distributed in dicotyledonous seeds (beans). The concentration of phytic acid ranges from 0.5% to 1.9% in cereal grains and from 0.4% to 2.1% in legume seeds (Reddy et al. 1982). The concentrations and distributions of phytic acid in wheat and rice kernels is shown in Table 3.

Table 3. Concentrations of phytic acid in various fractions of wheat and rice kernels and the distribution of total phytic acid in each of the fractions.[a]

Fraction	Phytic Acid Concentration (%)	Distribution of Total Phytic Acid (%)
Soft wheat	1.4%	—
Endosperm	0.004	2.20
Germ	3.91	12.90
Hull	0.00	0.00
Aleurone	4.12	87.10
Brown rice	0.89	—
Endosperm	0.01	1.20
Germ	3.48	7.60
Pericarp	3.37	80.0

[a]After Reddy et al. (1982).

Effects of Milling on Phytates

Because the phytate in cereal grains is concentrated in the aleurone layer and germ, milling to white flour or white rice should dramatically reduce the phytate content because milling of cereals removes most of the bran layer and germ. A comparison of the phytate content of whole and milled products shows that this is the case (Table 4).

Effects of Cooking on Phytates

The phytate content of foods may be reduced during cooking. Presumably the loss is due to leaching of soluble sodium phytate into cooking water as well as to hydrolysis of the phytate. Phytate reductions during cooking vary. Cooking white rice in tap water reduced the phytate content by 70% but cooking in distilled water had little effect (Toma and Tabekhia 1979). Cooking in boiling water for 3 hours reduced the phytate content of black-eyed, red kidney, mung, and pink beans by 8–36% whereas canning (115 °C for 3 hours) reduced phytate by 70–91%.

Effects of Fermentation on Phytates

Plants contain phytases that, when activated, can catalyze the hydrolysis of phytates. As already mentioned, the fermentation involved in bread making can significantly reduce the phytate content. Fermentation of maize, soy beans, and sorghum also reduces phytate content (Sandberg 1991). The extent of phytate reduction depends on the type of fermentation. Sourdough fermentation reduced phytate amounts in rye bread by 86–98% (Sandberg 1991). Conventional baking of whole wheat bread reduced phytate only by about 50% (Sandberg 1991).

Table 4. Phytate contents of whole and milled cereal products[a]

Product	Phytate Content (mg/100 g)
Whole wheat flour	960
White flour, enriched	154
Rice Brown	518
Rice, polished	255
Bread, whole wheat	390
Bread, white, enriched	69

[a]After Harland and Oberleas (1987).

Iron Fortification

To compensate for iron losses from milling, several countries encourage or mandate fortification of refined cereal products with iron. These countries include Canada, Chile, Denmark, Guyana, Kenya, Zambia, Great Britain, Nigeria, and the United States (Barrett and Ranum 1985). In the United States, the Food and Drug Administration regulates the amounts of iron added to grain products, specifying ranges or lower limits. When a range is specified, amounts outside the range would constitute a violation. When a lower limit is specified, reasonable overages within good manufacturing practices are allowed. Current requirements for enriched products in the United States are shown in Table 5.

Sources of iron commonly used in food fortification include ferrous sulfate, ferric orthophosphate, and various forms of elemental iron powders. Three different types of elemental iron powders are available: reduced, electrolytic, and carbonyl iron (Patrick 1985). Reduced iron is produced by reducing iron oxide with hydrogen or carbon monoxide. Electrolytic iron is produced by the electrolytic deposition of iron onto a cathode made of flexible sheets of stainless steel. The deposited iron is removed by flexing the sheets and then is milled to a fine powder. Carbonyl iron is produced by heat-

Table 5. Iron contents of selected cereal products[a]

Product	Iron Content (mg/100 g [mg/lb])
Whole wheat flour, unenriched	3.5 [15.9]
White flour (76% extraction), unenriched	1.12 [5.1]
Enriched flour[b]	4.41 [20]
Enriched bread, rolls, and buns[b]	2.75 [12.5]
Enriched corn grits and corn meals[c]	2.86 [13–26]
Enriched rice[c]	2.86–5.73 [13–26]
Enriched macaroni products[c]	2.86–3.63 [13–16.5]

[a]Values for enriched products are as specified by the U.S. Food and Drug Administration (1995).
[b]Added iron may be supplied by any safe and suitable substances.
[c]Added iron may be added only in forms which are harmless and assimilable.

ing scrap or reduced iron in the presence of carbon monoxide under high pressure to form pentacarbonyl, $Fe(CO)_5$. The pentacarbonyl is then decomposed by further heating to yield a very fine powder of high purity.

The bioavailability of iron from various sources is difficult to predict because components of the food vehicle may markedly affect bioavailability. The physical and chemical forms of the iron source can also affect bioavailability (Hurrell 1984). For example, the bioavailability of iron from iron powders varies inversely with particle size. Fritz (1976) compared the iron bioavailabilities of different forms of electrolytic iron by using a hemoglobin-repletion method in rats and found that bioavailability from a powder with a particle size of 7–10 µm was nearly double that from particles ranging from 20 to 40 µm.

Unfortunately, many iron sources with good bioavailability are not suitable for fortifying foods because they cause adverse organoleptic changes. These changes include off-odors produced by iron-catalyzed oxidation of lipids in the cereals and off-colors that develop when the cereals are mixed with warm water or milk. Hurrell et al. (1989) reported that hydrated ferrous sulfate and ferric pyrophosphate added to wheat or wheat-milk infant cereals generated unacceptable odors when the cereals were stored at 37 °C for 3 months. Encapsulation of the ferrous sulfate in hydrogenated vegetable oil prevented the odor development. Color formation on mixing the iron-fortified cereals with warm water or milk is also a problem. Cereals fortified with ferrous sulfate turned green when mixed with 80 °C water or milk. Encapsulation did not prevent the color formation (Hurrell et al. 1989). Carbonyl iron, ferric pyrophosphate, ferric fumarate, and ferric succinate did not cause organoleptic problems when added to cereals (Hurrell et al. 1989).

Iron-fortified Infant Cereals

Cereals are an attractive vehicle for fortification because they are widely available as complementary foods for infants and young children and they are low in cost (Walter et al. 1993). Iron-fortified infant cereals are widely used in the United States, where they provide nearly 50% of dietary iron during the first year of life (Glinsmann et al. 1996). Electrolytic iron powder is the preferred iron source for fortification in the United States, whereas ferric pyrophosphate is preferred by European manufacturers (Hurrell 1985). The iron content of commercial fortified infant cereals in the United States is 55 mg/100 g dry cereal (Walter et al. 1993). This exceeds the iron content stated on the label (45 mg/100 g) so that inadvertent falling below the label claim, which would violate federal regulations, is avoided. Handbook 8 values for fortified infant cereals are even higher (74 mg/100 g [U.S. Department of

Agriculture 1978]), suggesting that manufacturers have reduced levels in recent years.

The bioavailability of electrolytic iron appears to be low compared with ferrous sulfate when assessed in short-term studies (Howard et al. 1993, Hallberg et al. 1986). However, the effectiveness of fortification could still be significant given the high amounts of iron that are commonly added to infant cereals. The effectiveness of iron-fortified infant cereals in preventing iron-deficiency anemia was addressed directly by Walter et al. (1993). They randomly assigned 515 infants from a low-income population in Santiago, Chile, to five groups: fortified cereal + unfortified infant formula, unfortified cereal + unfortified formula, unfortified cereal + fortified formula, breast-fed + fortified cereal, and breast-fed + unfortified cereal. The iron-fortified rice infant cereal contained 55 mg electrolytic iron per 100 g dry cereal. The iron-fortified infant formula contained 12 mg iron as ferrous sulfate per reconstituted liter. The formula-fed infants consumed approximately 30 g cereal per day, providing more than 16 mg iron as electrolytic iron in the fortified cereals. Cereal consumption in the breast-fed groups was lower, approximately 25 mg per day. Iron status was assessed at 8, 12, and 15 months of age using venous blood. The prevalence of anemia (hemoglobin <105 g/L) at age 15 months was 8%, 24%, 4%, 13%, and 27% for the five groups, respectively. This study shows that fortification of infant cereal with electrolytic iron can be effective at preventing anemia in infants.

In a similar study in the United States, Fuchs et al. (1993) recruited infants 4–6 months of age and assigned them to receive either whole cow milk plus iron-fortified infant cereal or iron-fortified infant formula. Iron intakes in both groups exceeded the recommended dietary allowance. At 12 months of age, only one infant in the group receiving whole cow milk plus infant cereal had a low hemoglobin value and no infant receiving infant formula had low hemoglobin. However, serum ferritin values for infants in the first group were only about half those for infants receiving iron-fortified infant formulas. This study corroborates the study by Walter et al. (1993) in that iron-fortified infant cereal seemed to protect infants against anemia.

In a study designed to evaluate infant formulas fortified with different amounts of iron, Bradley et al. (1993) observed that infants who were fed iron-fortified infant cereals before 4 months of age had higher serum ferritin values at 6 months than did infants who did not get cereal before 4 months.

Thus it appears that although iron-fortified infant cereals reduce the risk of iron-deficiency anemia, they may not provide absorbable iron sufficient for building up iron stores.

Iron-fortified Milk and Infant Formula

The prevalence of anemia in U.S. children from both low-income and middle-class groups has been declining in recent years (Yip et al. 1987a, 1987b). The increased use of iron-fortified infant formulas likely has been a major factor in this trend.

Most powdered and liquid formulas available in the United States are fortified with ferrous sulfate at a level of 12.7 mg iron per reconstituted liter (Bradley et al. 1993). These formulas also contain added ascorbic acid, which may enhance iron absorption. Iron absorption from fortified infant formulas ranges from 3% to 10% (Hurrell 1984) but the high levels of iron present make up for this relatively low bioavailability. Bradley et al. (1993) compared the iron status during the first year of life for 347 breast- and formula-fed infants from the United States. Two milk-based formulas were used, one containing 7.4 and the other 12.7 mg iron per liter. Parents were instructed to introduce iron-fortified cereals when the infants were 4–6 months old. At 12 months there were no significant differences in hemoglobin concentrations in the three groups. The authors concluded that infant formulas containing either 7.4 or 12.7 mg iron per liter are adequate to meet iron requirements of healthy term infants. One implication of this study is that 12.7 mg iron per liter of formula may be unnecessarily high, although no adverse effects were observed.

Infant formulas available in the United States are carefully formulated to mimic breast milk and meet nutrient requirements of infants. The manufacture of these formulas requires highly sophisticated technology and rigorous quality control. Thus, they are expensive and probably out of reach for many low-income families in developing countries. Therefore, powdered milk fortified with a few select nutrients may be more suitable in developing countries because the product would be cheaper and could be manufactured with more widely available technologies.

Stekel and his co-workers (Olvares et al. 1989, Stekel et al. 1986a, Stekel et al. 1986b) carefully tested fortified powdered milk in infants in Chile. In one trial (Stekel et al 1986a), the milk was fortified with 15 mg iron as ferrous sulfate, 100 mg ascorbic acid, 1500 IU vitamin A, and 400 IU vitamin D per 100 g powder. Infants from seven community clinics in Santiago were given either fortified or unfortified milk powder on weaning and followed until they were 15 months old. At age 9 months, the prevalence of anemia was 11.8% and 32.5% in the infants receiving fortified and unfortified milk, respectively. At age 15 months, the prevalence was 5.5% and 29.9%, respectively. The authors concluded that iron-fortified milk could prevent iron deficiency in most Chilean infants.

Stekel's studies clearly demonstrate that iron fortification of milk powder can be highly effective in preventing iron deficiency in young children.

Iron Fortification with EDTA

Two of the major problems associated with iron fortification of foods—reduced bioavailability caused by inhibitors of iron absorption and undesirable organoleptic changes caused by interactions of the added iron with food constituents—may be largely avoided with the use of sodium iron ethylenediaminetetraacetic acid (NaFeEDTA). INACG reviewed the use of iron EDTA as a food fortificant and concluded that sufficient information exists to recommend the use of NaFeEDTA in fortification programs designed to improve the iron status of iron-deficient populations (INACG 1993).

EDTA is a strong chelating agent capable of forming chelates with virtually all metal ions. It is widely used as a food additive in the United States and elsewhere to retard peroxidation of food lipids during prolonged storage and to prevent color changes caused by the interaction of trace metals such as iron and copper with polyphenolic compounds in foods. EDTA functions by sequestering mineral ions, thereby reducing their reactivities toward other food constituents. Moreover, metal-EDTA chelates are usually water soluble over a wide range of pH values.

The bioavailability of iron from iron EDTA has been extensively studied. Relative iron bioavailability (bioavailability from iron EDTA compared with that from ferrous sulfate) varies depending on the amounts of enhancers and inhibitors present in the meal. Relative bioavailability is highest in meals containing inhibitors of iron absorption. Presumably, this is because the EDTA prevents iron from binding to inhibitors that would otherwise reduce absorption. This concept is illustrated by a study where known inhibitors of iron absorption (bran and tea) were added to aqueous solutions of radiolabeled ferrous sulfate or iron EDTA (MacPhail et al. 1981). The solutions were given to human subjects and iron absorption was measured. Bran reduced iron absorption from ferrous sulfate 11-fold but had no effect on iron absorption from iron EDTA. Tea, however, reduced iron absorption from iron EDTA, suggesting that the polyphenols in tea have a higher affinity for iron than does EDTA.

Similarly, the effect of iron absorption enhancers is blunted when the iron source is iron EDTA. MacPhail et al. (1981) reported that 25 or 50 mg of ascorbic acid had no effect on iron absorption from maize meal porridge fortified with iron EDTA.

The efficacy of iron EDTA as a food fortificant is best established through field trails. Four such trials have been conducted. Garby and Areekul (1974) provided fish sauce fortified with NaFeEDTA (1 mg Fe/mL) to residents in a Thai village for 1 year. A second village served as a control. There was a significant improvement in packed erythrocyte volume in the trial village but not in the control village. Viteri et al. (1983) distributed NaFeEDTA-fortified sugar (130 mg Fe/kg) to residents in three villages in Guatemala. A fourth village served as the control. After 20 months hemoglobin values of children significantly improved in the trial villages compared with the control village. Hemoglobin values in adults also increased but not significantly. Ballot et al. (1989) distributed NaFeEDTA-fortified curry powder (1.4 mg Fe/g) to families in one community in a 2-year trial. A group of families from the same community matched for iron status served as the control. Improvement in hemoglobin and serum ferritin values was significantly greater for women in the test group than in the control group. Viteri et al. (1995) distributed NaFeEDTA-fortified sugar (130 mg Fe/kg) through local stores to be purchased by the residents of the communities. Three semirural Guatemalan communities received the fortified sugar and a fourth community served as the control. The trial lasted 32 months. Iron stores in residents in the test communities significantly improved whereas those in the control community were unchanged except in adult males. These four studies show that fortification with NaFeEDTA can effectively improve iron status in populations where iron deficiency is prevalent and diets are high in cereals and legumes.

On the basis of these and other studies, it has been concluded that iron EDTA has promise as an iron fortificant in populations where the diet is rich in iron-absorption inhibitors (INACG 1993). Iron EDTA is not recommended in diets that contain an abundance of iron-absorption enhancers (INACG 1993).

Conclusions

The knowledge and technology required for effectively enhancing the amount of bioavailable iron in complementary foods is now available. Several well-done studies have shown that iron-fortified milk and cereal can dramatically reduce the prevalence of iron deficiency in young children. The task that lies ahead is to put into place sustainable fortification programs that will deliver appropriate, affordable iron-fortified complementary foods to the children of the world who are suffering from iron deficiency.

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Thailand's Experiences with Fortified Weaning Foods

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Introduction

Over the past two decades undernutrition, especially protein-energy malnutrition, among Thai children has dramatically improved. Nationwide, the prevalence of protein-energy malnutrition declined from 45% in 1982, to 25% in 1986, and to only 12% by 1995. Factors important to this improvement include more effective nutrition-education strategies (including the use of social marketing), higher incomes for food purchasing, a more efficient health-monitoring system, better transportation and communication capabilities, and the application of appropriate weaning foods during a child's early critical years of development.

In the past the early introduction of semisolid weaning foods was very common among rural Thai families. Chewed-rice paste, gruel, and banana were often given to infants as a supplement to breast milk beginning at a very early age, not uncommonly within the first few days of life. Rice and banana then remained the sole weaning foods for more than 6 months. The problem of supplementary feeding in rural Thai families was therefore the too early introduction of relatively less nutritious foods. In urban areas bottle feeding also usually replaced breast milk at a rather early age, with weaning foods based on rice and banana being introduced when the child reached the age of about 3-4 months. The problem of weaning foods in urban Thailand was thus similar to that in rural areas. Growth faltering in urban children, and especially those from low socioeconomic families, could occur at an earlier age because of the early cessation of breast feeding.

Appropriate weaning foods should contain enough nutrients to meet infants' requirements at different ages. Moreover, weaning foods should be sensory-acceptable products that are affordable and require preparation by methods suitable for community members. In Thailand weaning foods have been prepared at different levels, including family, community, and industrial levels. To make weaning foods appropriate for the population, food composition is usually based on such factors as parents' nutrition knowledge, available time, and income.

Development of Standards for Weaning Foods in Thailand

Government sectors, and particularly the Ministry of Public Health along with universities, have played important roles in promoting the suitable feeding of weaning foods to infants of different ages by using nutrition-education strategies based on nutrition science and social marketing. The Ministry of Public Health is also responsible for enforcing regulations concerning standards for weaning foods to be sold in the country.

Thailand's first weaning food standard was developed by the Thai Food and Drug Administration (FDA) in 1979 and regulated both nutritional and safety qualities. For example, standards were set for amounts of reference protein, amino acid pattern, amounts of fat and essential fatty acids, as well as kinds and amounts of vitamins and minerals; safety standards were also designated for microbial load, toxic substances, and certain food additives.

In 1985 the Thai FDA filed a new standard—Supplementary Food for Infants and Children—that classified weaning foods into two types: complete and partially supplemented. The standards for each type covered both powdered and liquid forms. The standard for the complete-type weaning food was similar to the previous one in 1979, and some of the nutritional qualities of this type of weaning food are listed in Table 1. The standard for the partially supplemented type, however, emphasized only the safety quality and was similar to the safety standard for the complete type. The only exception was that the amount of sodium was not allowed to be higher than 200 mg/100 g of the cooked product. Vitamins and minerals could be added to the partially supplemented type of weaning foods in amounts that were not harmful and were under the approval of the FDA.

In 1994 Thailand's latest standard for Supplementary Food for Infants and Children was approved. Under this standard, the complete-type weaning food was eliminated and only standards for the partially supplemented type remained. Therefore, the definition of a supplementary food in the Thai standard now is food that is used either for infants aged 6–12 months or children aged 1–3 years to supplement nutrients and allow children to become familiar with different varieties of foods. As mentioned above, product nutritional quality is not regulated. A weaning food or supplementary food, referred to as a complementary food as suggested by the Ministry of Public Health, means a food that is provided to an infant to complement breast milk or a breast-milk substitute after 4–6 months of life.

Table 1. Thai standard, 1985, for nutritional quality of complete-type weaning foods

Protein	
Reference protein	>2.5 g/100 kcal
Amino acid score	>70%
Fat	
Total fat	>2.0 g/100 kcal
Linoleic acid	>300 mg
C20+ fatty acid	<1% of total energy
Vitamins	
Vitamin A	75-150 RE/100 kcal
Vitamin D	40-80 IU/100 kcal
Vitamin E	>0.7 IU/100 kcal and >0.7 IU/1 g linoleic acid
Vitamin B-1	>40 mcg/100 kcal
Vitamin B-2	>60 mcg/100 kcal
Nicotinamide	>250 mcg/100 kcal
Vitamin B-6	>38 mcg/100 kcal or > 15 mcg/g protein (if the food contains protein of more than 2.5 g/100 kcal)
Folic acid	>4 mcg/100 kcal
Vitamin B-12	>0.15 mcg/100 kcal
Vitamin C	>8 mg/100 kcal
Minerals	
Sodium	20-100 mg/100 kcal
Potassium	80-250 mg/100 kcal
Chloride	55-250 mg/100 kcal
Calcium	> 60 mg/100 kcal
Phosphorus	>35 mg/100 kcal
Ca/P	1.2–2.0
Iron	1–2 mg/100 kcal
Iodine	5–20 mcg/100 kcal

Experiences with Food-to-Food Fortification

Food-to-food fortification is a food-based strategy and has been a very appropriate tool for solving Thailand's malnutrition problems. Thailand is one of the main food producers in the world, and many kinds of foods grown in the country are actually sources of multiple nutrients. By providing nutrition information and education, one can encourage families to select suitable foods for use as weaning foods, and nutritious foods can also be used as raw materials for producing weaning foods at community and industrial levels.

Food-to-Food Fortification at the Family Level

The Subcommittee on Maternal and Child Nutrition of the National Food and Nutrition Committee in Thailand suggested that supplementary feeding should be started after the first 3 months of life. To ensure adequate nutrients, a supplementary feeding guideline was issued: at 3 months begin gruel, rice, and banana; at 4 months add egg yolk, liver, or legumes; at 5 months add fish, green leafy vegetables, and pumpkin; at 6 months begin one meal; at 7 months add ground meat and begin whole egg; at 8–9 months give two complete meals; and at 10–12 months give three complete meals.

The above guideline is intended for use in supplementary foods at the family level, where parents can easily obtain the ingredients by growing, gathering, or purchasing. The guideline also considers facets of Thailand's nutritional situation; for example, orange juice is not included on the list because vitamin C deficiency is very rare in Thailand. Furthermore, the preparation of orange juice, if not properly done, can cause diarrhea because of microbial contamination from the orange skin, especially in when children are bottle fed.

Weaning foods have traditionally been prepared by parents who have adequate available time. In the past, mothers in both rural and urban areas played a very direct role in child care and food preparation. However, changes in Thailand's socioeconomic and political climates during the past decade have transformed the country's economic system towards a cash-based market economy. Such changes have resulted in a large increase in rural-to-urban migration, particularly for women. Simultaneously, urban parents have had to spend more time working and commuting to work, hence they have less time for child-care activities. As a result, home-based preparation of weaning food is becoming impractical, especially among low- and medium-income populations. In many cases children are being cared for by their grandparents in the rural countryside, which additionally limits the appropriate preparation of weaning foods because of the grandparents' limited nutrition knowledge.

Food-to-Food Fortification at the Community Level

In the mid to late 1970s, when Thailand's transportation and communication infrastructure was not well established, seven recipes for semicooked food-to-food weaning foods were formulated and processes for their preparation were developed at the Institute of Nutrition at Mahidol University. The formulas and processes were intended for producing preserved weaning foods for community-level distribution and using locally available raw materials and appropriate technologies. The product used rice (ground and broken) as a carbohydrate source, legumes (e.g., soybean and mung bean) or fish meal as a protein source, and oil or oil seeds (e.g., groundnut and sesame) as a fat source.

This food-to-food fortification formulation provides enough good-quality protein, energy, and essential fatty acids to satisfy the nutritional requirements of a 6-month-old infant according to the Thai standard for a complete-type weaning food of 1979. Table 2 shows different combinations of nutritious foods that can provide enough protein, fat, and energy to meet the requirement.

Preparation of the weaning foods is relatively simple. The beans and sesame are roasted for 5–10 minutes to give well-cooked ingredients; roasting also imparts a pleasant aroma to the food mixture. Rice is roasted for a shorter period of 3–5 minutes to destroy contaminating organisms. Roasting rice also reduces the moisture content and increases shelf life. After roasting, each ingredient is weighed, mixed, and ground with an electric or manual grinder. The ground mixture (100 g) is packed and sealed in a small plastic bag, which can be kept for 6–8 weeks in a metal or plastic can. These products are semicooked and need to be boiled in water for 10–15 minutes before being consumed. These recipes were also modified for older children by using raw materials without grinding to provide a coarser texture. For example, three roasted ingredients were packed together in the proper proportions in a cloth bag. The mixture was soaked overnight and then steamed in the same container as the family's rice, which avoids additional cooking effort, and the food could be self-fed by the children.

Such mixtures could be prepared in villages both with and without electricity by using inexpensive machines and locally available raw materials. Hence, this supplementary food formulation was adopted by the Ministry of Public Health for a nationwide campaign in 1980. Thereafter, community-based supplementary food production was carried out in many areas nationwide for another 10 years. This activity slowed and then ceased mainly as a result of changes in the socioeconomic and political climates that caused migration of young laborers into the city and thus a reduction in the commununity manpower needed to produce supplementary foods.

Certain allied research studies were also conducted to improve the quality of the weaning foods, such as producing an amylase-rich food. The Institute of Nutrition at Mahidol University used technology from India to develop amylase-rich food from local staple cereals and legumes. The amylase-rich food could be added into the cooked weaning food to increase sensory acceptability by decreasing viscosity and also to increase the energy density. This research project, however, has not yet been implemented at the community level.

Food-to-Food Fortification at the Industrial Level

As noted above, Thailand's weaning food standard now refers only to the partially supplemented type, although weaning foods that are similar to the complete type are readily available in the market. Most of these industrially produced complete-type weaning foods are cereal based and in a powdered instant form. Milk powder and soybean flour are commonly used as a protein source, and fat is usually from vegetable oils and sometimes from butter oil. These cereal-based products are cooked in a drum drier or a cooker extruder. The cooked product is ground and packed in a metal can or laminated aluminum foil bag. The product must be mixed with warm water before consumption. There are at least three factories in Thailand producing this type of product, but one multinational company is responsible for 90% of all sales. Another part of the market is shared by a government-owned research institute, which now does not exactly follow the old standard for complete-type supplementary food in their production.

The partially supplemented weaning foods sold in Thailand are usually ready to eat and are hermetically packed in a glass jar. Almost all are imported from abroad

Table 2. Ingredients and composition of community-prepared weaning food formulas

Formula/Ingredients	Composition per 100 g Protein (g)	Fat (g)	Energy (kcal)
I. Rice, soybean, groundnut (70:15:15)	16.5	10.6	437
II. Rice, soybean, sesame (70:15:15)	14.8	11.0	448
III. Rice, mung bean, groundnut (60:15:20)	14.5	11.9	443
IV. Rice, mung bean, sesame (60:20:15)	13.2	13.2	451
V. Rice, fish meal, groundnut (70:10:20)	18.5	11.8	454
VI. Rice, fish meal, sesame (70:10:15)	17.2	10.4	444
VII. Rice, fish meal, oil (70:10:8)	14.4	9.2	437

and provide a wide range of product varieties, such as fruit juice, fruit and vegetable purees, mixtures of meat and cereal or noodles, and so forth. Bottled weaning foods are expensive and thus normally affordable only by higher-income families. Locally produced partially supplemented weaning foods are powdered and semicooked, such as a dried ground mixture of precooked rice with carrot or pumpkin.

Experiences with Nutrient-to-Food Fortification

Vitamins and minerals are usually fortified only in industrially produced weaning foods. Most fortification does not follow any standard because Thailand's standard for weaning foods does not specify nutritional qualities. For certain brands, fortification amounts are based on either the old standard for complete-type supplementary foods or the CODEX standard for supplementary food. Such amounts usually provide enough micronutrients to satisfy a 1-day requirement for a child aged 6–8 months. Other brands with the same general appearance as the sufficiently fortified foods may not contain a quantity of nutrients adequate to meet a child's requirements. Only some imported products, especially the bottled ready-to-eat products, have been fortified (e.g., fruit puree and juice are fortified with vitamin C).

Appropriate Weaning Foods for Thai Children

Weaning foods used for Thai children should come from fresh produce, which is possible because of Thailand's rich agricultural supply. Nutrition education should enable parents to choose materials and prepare a suitable, affordable weaning food for their infants. However, in Thailand's present socioeconomic climate, many Thai parents and other child caregivers do not have enough time and knowledge for such activities. Consequently, quickly and easily prepared weaning foods are necessary. The industrially produced complete-type weaning food is one of the best choices, because it can be inexpensive and conveniently stored and prepared, and it can provide enough nutrients to meet an infant's requirements. The cost of weaning foods can be lowered and made more affordable by domestic production using locally available raw materials and packed in suitable packaging materials in practical quantities.

In our opinion, the partially supplemented type is not suitable for the present Thai society because most Thai parents, especially in rural areas, do not have adequate nutrition knowledge to combine suitable food types to fulfill an infant's nutritional requirements. Furthermore, some partially supplemented weaning foods, such as the highly nutritious, bottled ready-to-eat foods, are too expensive and are not affordable by most of the population. The use of industrially produced partially supplemented weaning foods is practical only if parents have adequate nutrition knowledge and a relatively high income. Local production of partially supplemented weaning foods, such as those that are bottled ready-to-eat, can lower cost and thus encourage greater use. However, the formulation should be based on a guideline for preparing food at the family level to avoid confusing parents. Furthermore, restoration of nutrients should also be performed in the finished products.

Conclusion

Thailand's experience with fortified weaning foods has mainly emphasized food-to-food fortification. In line with the nation's development, and responding to changes in food obtainment practices (from gathering or bartering to purchasing), family income, and work-activity patterns as well as migration from rural communities, weaning food production has progressed from a family- and community-based focus to one of industrial preparation. Family-based weaning food preparation has usually followed the guideline for supplementary feeding. Weaning foods prepared at the community level have entailed using preserved forms of readily available raw materials such as rice, legumes or fish meal, and oil seeds and applying appropriate preparation technologies. Two types of industrially produced weaning foods, complete and partially supplement, can be found in Thailand's market. Although the latest Thai standard for supplementary food for infants and children has eliminated the complete type, it still holds a 90% share of the market. Changes in Thailand's way of life are pointing to a growing demand for industrially produced weaning foods. Instant cereal containing complete nutrients should be the best choice because of its quick and easy preparation, low cost, and suitability in a population with low nutrition knowledge.

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High-protein-quality Vegetable Mixtures for Human Feeding

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Introduction

A number of nutrition and food consumption surveys conducted in the Central American countries in the early 1950s showed that large segments of the population, particularly children, suffered from protein-energy malnutrition as well as from deficiencies of iodine, vitamin A, and iron (Scrimshaw et al. 1955). This situation gave rise to various research programs with the objective of providing practical solutions to the nutritional problems indicated.

The iodization of salt was the first solution to be implemented. Time has shown that salt iodization is effective, but problems have occurred occasionally because of the logistics of iodine addition and the quality control of the process. The addition of vitamin A to sugar was also implemented and is a successful approach to eliminating vitamin A deficiency in Central America.

To provide practical solutions to the protein-energy problem, a research program was initiated in 1950 to develop multipurpose high-quality-protein vegetable foods mainly for child feeding but also useful for other population groups with nutritional deficiencies (Béhar et al. 1959). The food to be developed had to be presented as a flour, follow dietary habits and practices of the population, be formulated from locally available ingredients with a corn-like flavor, be similar in protein content and quality to animal proteins, be supplemented with B vitamins as well as with vitamin A and iron, contain no antiphysiological factors, be stable and have an acceptable shelf life, and be cooked before consumption. The final objective was to develop a food that could efficiently supplement the habitual diets of children.

Available High-quality Foods

Four high-quality weaning foods, developed at the Instituto de Nutrición de Centro América y Panama (INCAP), are available in Guatemala. Two of the foods—Incaparina (INCAP mixture no. 9) and Bienestarina (INCAP mixture no. 14)—were licensed by INCAP, and the other two—Vitatol (INCAP mixture no. 15) and Innovarina (INCAP mixture no. 14)—were produced by the manufacturer of Incaparina, using information from the published literature.

Production of Vitatol and Innovarina was begun because cottonseed flour is becoming difficult to obtain in Central America, and mixture no. 15 contains less cottonseed flour than does mixture no. 9 (Bressani and Elías 1966, Bressani et al. 1966, Bressani et al. 1961) (Table 1). All four foods are blended products containing degermed corn flour and variable amounts of oilseed flour. Incaparina, the first food to be produced commercially, contains human-grade cottonseed flour. Smaller amounts of this flour are used in Vitatol, which also contains soybean flour; Innovarina and Bienestarina contain soybean flour as the supplementary protein source. A cookie made from one of the above mixtures and wheat flour is used for school food programs.

The four weaning foods are packaged in polyethylene-lined cellophane bags that hold 454 g. Some products are also sold in 75-g packages. The container labels list ingredients, chemical composition, instructions for cooking, and the date of manufacture. Product shelf life is around 4 months under tropical conditions.

The nutritional information listed on the label of the four weaning foods is shown in Table 2. Protein content varies from 21% to 23% and energy content varies from 335 to 386 kcal/100 g (1.40 to 1.62 MJ/100 g). Vitamin A is given as vitamin A acetate 500 and iron is given as ferrous fumarate. The label also indicates the percentage of the recommended dietary allowance provided by 20 g of the product (Table 3).

Table 1. Basic quantities of main ingredients in high-nutritional-quality multipurpose foods (%)

	Mixture No.		
	9	14	15
Corn flour	60	70	70
Cottonseed flour	40	—	15
Soybean flour	—	30	15

Protein Quality of Mixtures

The main ingredients of the various high-protein-quality weaning foods were determined by protein-complementation studies in rats (Bressani and Elías 1966, Bressani et al. 1966, Bressani et al. 1961) (Table 4). The highest protein quality for cottonseed flour and corn protein resulted from a 60:40 weight ratio (protein-energy ratio [PER] of

Table 2. Nutritional information on the label of the four foods (per 100 g)

Nutrient	Incaparina	Vitatol	Innovarina	Bienestarina
Moisture, g	8.0	10.0	—	—
Calories, kcal	367	335	360	386
Protein, g	23.0	23.0	21.2	21.0
Fat, g	3.0	3.5	3.10	5.6
Crude fiber, g	3.5	4.0	2.4	2.5
Ash, g	4.0	4.5	—	—
Carbohydrate, g	62.0	53.0	62.4	63.0
Vit A, IU	4500	5000	5980	4500
Niacine, mg	13.62	13.62	20.50	14.25
Thiamin, mg	1.70	1.70	2.15	1.12
Riboflavin, mg	1.01	1.01	2.05	1.23
Iron, mg	11.20	11.20	20.00	20.00
Lysine, mg	250	250	—	—

Table 3. Percentage of the daily dietary recommendation from 20 g/cup

Nutrient	Percentage	Amount
Vitamin A	27–37	900–1195 IU
Thiamin	15–30	0.34–0.43 mg
Riboflavin	15–24	0.20–0.41 mg
Niacin	15–22	2.7– 4.1 mg
Calcium	5–10	61–64 mg
Iron	20–36	2.2–4.0 mg

1.93). For soybean and corn protein, a 70:30 weight ratio gave the highest PER (2.24). For mixtures with cottonseed, soybean, and corn, 70:15:15 gave the highest PER (2.20) (Bressani et al. 1972). The protein quality of the cottonseed flour and corn mixture could be improved substantially by adding 0.250% lysine (Bressani and Elías 1962).

The basic blends indicated above were tested extensively in various experimental animals before feeding tests were conducted with children. In some nitrogen balance studies in children, the blends were the sole source of protein (Bressani et al. 1972) (Table 5). The protein quality of the four blends was also found to be high for children. As expected, the blends had a lower true protein digestibility but a higher biological value for the absorbed nitrogen than did animal protein sources.

The Manufacture and Fortification Procedure

The manufacturing process used by at least one manufacturer is shown in Figure 1. Although lime-treated corn flour was used in the past, white corn is now milled and converted into a flour without the seed coat, which reduces the amount of dietary fiber. The germ is eliminated to increase stability of the flour during storage and marketing, which also significantly decreases the amount of phytic acid in corn (O'Dell et al. 1972). Cottonseed flour is produced from cottonseed that is solvent-extracted before or after pressing. This raw material is milled and screened to give at least 100-mesh flour with 50% protein and low amounts of free gossypol (Bressani and Elías 1968). The soybean flour used is a solvent-extracted product.

The ingredients are mixed in a horizontal blender to which the premixed vitamins, minerals, flavors, and antioxidants are added. (Premixing is done to ensure an efficient distribution of the added nutrients.) The particle size of each of the four commercials foods is shown in Table 6. To protect the flours and supplements, 0.005–0.010% of the antioxidants butylated hydrixyanisole, butylated hydroxytoluene, or tertiary butyl hydroquinone are added. However, the relation of shelf life to the stability of vitamin A or the bioavailability of iron has not been studied.

Table 4. Protein quality of quality-protein vegetable mixtures (in rats)

Food	Commercial Name	Protein-efficiency Ratio
Skim milk	Milk	2.55 ± 0.30
Whole egg	Egg	2.90 ± 0.27
Mixture no. 9	Incaparina	1.93 ± 0.13
Mixture no. 14	Innovarina, Bienestarina	2.24 ± 0.23
Mixture no. 15	Vitatol	2.20 ± 0.20
Casein	Casein	2.88 ± 0.20

Source: Bressani et al. (1972).

Table 5. Protein digestibility and protein quality of vegetable mixtures in children

Food	Commercial Name	Nitrogen Intake (mg kg^{-1} day^{-1})	True Protein Digestibility (%)	Biological Value (%)
Milk	Milk	163	92.0	80.6
Egg	Egg	168	95.2	78.1
Mixture no. 9	Incaparina	174	80.4	65.7
Mixture no. 14	Innovarina, Bienestarina	158	91.8	78.6
Mixture no. 15	Vitatol	168	88.7	82.5

Source: Bressani et al. (1972).

Processing Effects on Nutrient Content and Protein Quality

The recommendation in the label of the four weaning foods is that the flours should be cooked for at least 15 minutes. Studies were conducted to determine whether cooking decreases the nutrient content and quality of the foods. Most of these studies were done with the mixtures containing cottonseed flour because of the negative effects that gossypol can induce. One study examined cooking time effects (Table 7); thiamin decreased by 32% after 25 minutes of cooking, riboflavin decreased by 23% after 20 minutes, available lysine decreased by 18% after 10 minutes, and free gossypol decreased by 62%

Figure 1. Flow diagram for manufacture of Incaparina and other high-quality-protein foods.

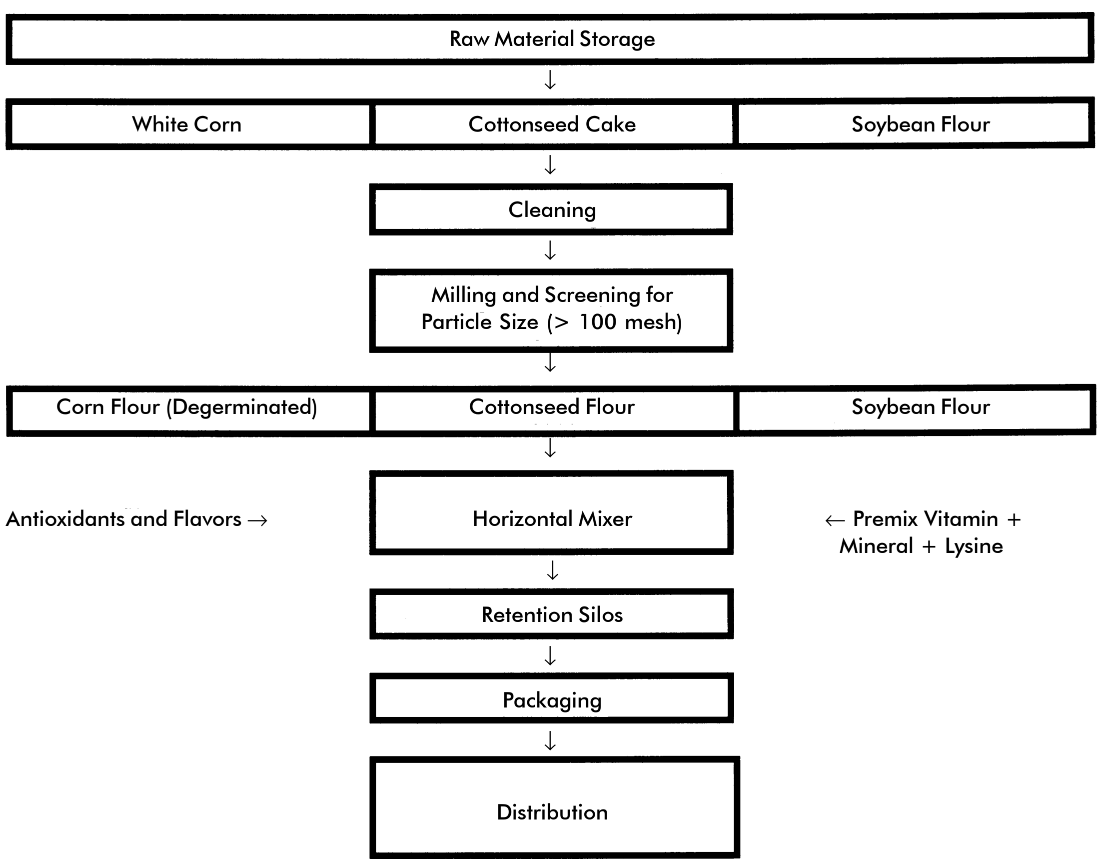

Table 6. Particle size distribution of commercial weaning foods in Guatemala (%)

Mesh	Incaparina	Vitatol	Innovarina	Bienestarina
Above 40	0.2	0.2	1.9	1.5
Above 80	9.1	7.6	16.4	24.4
Above 120	21.5	16.2	12.6	11.2
Passes 120	69.2	76.0	69.1	62.9

Bulk density (g/mL): Incaparina, 0.67; Vitatol, 0.65; Innovarina, 0.62; Bienestarina, 0.62.

after 25 minutes, probably becoming bound to other chemical compounds during the cooking process (Bressani et al. 1964b). Protein quality, expressed as nitrogen retained as percentage of nitrogen intake, did not change after up to 24 minutes of cooking (17.5%, 16.2%, 16.4%, and 16.3% at 0, 8, 16, and 24 minutes, respectively) (Bressani et al. 1964b). Cooking Incaparina for 30 minutes resulted in a vitamin A loss of around 23.3%.

Monitoring and Impact

Quality Control

To protect the consumer by guaranteeing the quality of the mixtures licensed by INCAP to qualified industries, INCAP has established licensing requirements as well as a control system for the packaging, identification, advertising, and analysis of the quality of the product (INCAP 1960). Quality includes chemical composition, microbiological quality, and (less frequently) biological nutritional quality. Samples are collected at the production site or the marketplace, depending on the quantity produced. An example of a quality-control scheme is shown in Table 8.

Thus far, no major discrepancies have been found between the label claims and actual nutrient content. Problems have been associated more with the microbiological quality of the various weaning foods because of inefficient systems for storing grain and other raw materials.

Table 7. Effect of cooking time on changes in several nutrients in experimental Incaparina (mixture no. 9)

Free Gossypol Cooking Time (min)	(mg/100g)	Thiamin (mg/100g)	Free Amino Riboflavin (mg/100g)	Groups of Lysine (mg/100 g)
0	2.25	1.12	1.16	7.7
5	1.96	1.07	1.09	5.3
10	2.01	1.05	0.95	6.4
15	1.99	1.14	1.18	8.4
20	1.75	0.86	1.19	5.3
25	1.54	1.18	1.09	2.7

Source: Bresani et al. (1964b).

Table 8. Quality control scheme[a]

Production (metric ton)	No. of Samples
10	1
11–24	2
25–49	3
Each additional 25	1 additional

[a] Chemical analyses: moisture, protein, fat, fiber, ash, minerals, thiamin, vitamin A, free gossypol (only in foods with cottonseed flour), available lysine, trypsin inhibitors (only in foods with soybean flour). Microbiological analyses: total bacterial and *Escherichia coli* count. Biological assays: protein-efficiency ratio or net protein ratio, occasionally or when requested.

Impact

The impact of weaning foods as sources of vitamin A and iron have not been evaluated in field studies. For the suggested daily consumption of 20 g, these products would provide around 27–37% of the recommended dietary allowance (RDA) for vitamin A and 20–36% of the RDA for iron. The monthly sales of all weaning foods is around 1.0 x 10^6 lb/month. On the basis of an intake of 60 g/day and of the number of children aged 0–4 years in Guatemala (Organización de la Salud [OPS] 1990), these foods may be reaching around 260,000 children. The possible number of children covered is therefore relatively small, about 16% of the existing population aged 0–4 years in Guatemala (OPS 1990). Preparation of these weaning foods includes the addition of sugar in a weight ratio of about 3:7. Therefore, the vitamin A intake is from the multipurpose food and from sugar, which in Guatemala is fortified with vitamin A.

Iron may not be as readily available because of factors in the weaning foods that could affect bioavailability, such as dietary fiber, phytate, gossypol, and other compounds. Oilseed flours, including cottonseed and soybean, contain large amounts of phytic acid (Erdman 1979, Hartman 1979), and although the amounts can be reduced, they remain relatively high. Refining cottonseed flour by air classification reduced phytic acid about 20% (Wozenski and Woodburn 1975). Because these flours are the supplementary protein source in the weaning foods, it is of interest to know the extent of their interference with the bioavailability of iron, which is also being added. Storage conditions could further reduce iron bioavailability. This area needs more study even though tests in rats fed Incaparina showed no blood hemoglobin and erythrocyte counts different from those of rats fed control diets (Bressani et al. 1964a).

Problems Encountered

No problems have been encountered with respect to the availability of raw materials and other ingredients, processing, and distribution. However, the cost of the foods has been increasing since the first product, Incaparina, was introduced. The price of all four mixtures is now around US$0.50 per pound; in the 1960s and 1970s it was around US$0.26 per pound for Incaparina. The cost increase is due to increased in the cost of ingredients, packaging materials, labor, electrical power, and facilities as well as distribution. This higher cost probably limits the accessibility of the products, particularly in rural areas. The vegetable mixtures were intended for small children but they are being consumed by older groups.

The quality-control process is progressing well. However, it could be developed as a means to solve the problems that the foods may have, such as the potential problem of iron bioavailability.

Conclusion

Incaparina was the first product launched by INCAP through a private industry, and its acceptance by the population has been increasing steadily. The fact that three other foods were recently introduced suggests that there is a market for them and that people are purchasing such products. Even though the cost has been increasing, it is much less than the costs of milk, meat, common beans, and baby cereal grains manufactured by international food companies. Although these products have not eliminated protein-energy malnutrition in children, they are contributing towards that goal. Children's lives have been saved. Finally, the manufacture these weaning foods has contributed to industrial and economic development in the region.

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Addendum: A Summary of the Workshop Discussion Sessions

Food Processing Interventions to Improve the Micronutrient Content of Home-based Complementary Foods

Food preparation affects both the amount and bioavailability of certain micronutrients, but the extent of these effects in complementary foods has not received as much attention as is deserved. Most foods consumed in developing countries are prepared in the home, and the nutritional quality of these meals can be improved through changes in home-based preparation methods. The various processing methods that were identified and discussed for improving micronutrient levels in foods are summarized below.

What Food Processing Methods Should Be Used?

Blanching

Blanching involves briefly immersing foods in boiling water and is used to soften foods, which improves their palatability and acceptability. Blanching is commonly used in the home for preparing various types of beans and leaves, including spinach, amaranth, and pumpkin. Often, as is the case in Indonesia, the blanched leaves (e.g., young cassava leaves) are ground, sun dried, and pulverized. The resulting powder can be mixed with staples such as rice flour and crude palm oil and used in complementary foods. The duration of blanching can vary. Because certain vitamins, such as A and C, are sensitive to heat, shorter blanching time results in greater retention of these vitamins. Although blanching reduces the amount of certain heat-sensitive vitamins, it increases the bioavailability of β-carotene, calcium, and zinc in green leafy vegetables.

Steaming

Fruits and vegetables can be steamed by placing them on a rack or in a basket over simmering water in a covered pan, which prevents the leaching out and loss of water-soluble nutrients that occur with boiling.

Frying

There is some evidence that frying may improve the retention of certain micronutrients— including vitamin C and thiamin— compared with boiling in water because frying is a high-temperature, short-time process. Another advantage is that the oil used will increase oil consumption, which can improve the bioavailability of β-carotene and other fat-soluble vitamins. Frying is region specific because oil is not always widely available in developing communities and frying is not always a traditional cooking method.

Solar Drying

Sun drying is a traditional method of preservation for off-season consumption that is practiced in tropical countries where there is 12 hours of free sunshine. Fruits and vegetables are spread on sheets, mats, or the bare ground and exposed to direct sunlight. This practice results in contamination and significant losses of β-carotene. Solar drying is an improved alternative to sun drying in that the food is dried in the shade. Solar drying provides higher air temperature and lower humidity, which increases the drying rate, thus reducing the amount of β-carotene lost, and reduces the final moisture content. Because fruits and vegetables are dehydrated, the micronutrient concentration is increased. Furthermore, dehydrated foods can be stored for longer periods of time. There are many ways to eat fruits and vegetables that have been solar dried. They can be eaten dried or rehydrated by adding water or adding to soups and stews. Rehydration will soften the food and make it easier for young children to eat.

Roasting

Cooking foods by dry heating—roasting—is commonly used for preparing foods such as peanuts, cereals, pulses, and jaggery. Roasted complementary foods comprising a mixture of cereal, rice, and jaggery contain adequate levels of micronutrients and can be stored for up to 60 days. Roasting also increases the bioavailability of iron in complementary foods. Losses of vitamin C and β-carotene are less with roasting than with sun drying.

Nixtamalization

This process involves soaking grains, specifically cornmeal and sorghum, in an alkaline solution usually containing lime. When the grain is more alkaline, niacin becomes unbound and its bioavailability increases. Nixtamalization has been used in Central America for processing corn but may also be used for other cereals and legumes. Nixtamalization of grains typically occurs just before milling for the production of foods such as tortillas. The addition of alkali during extrusion for the preparation of tortillas increases the calcium and ash (total mineral) content.

Germination

Germination involves soaking seeds in water in the dark, usually for up to 3 days, to promote sprouting. During the germination process phytase activity increases, which breaks down phytic acid. Other antinutrients, including polyphenols and tannins, are also reduced with germination. Certain vitamins, including riboflavin, vitamin B-6, and vitamin C, and the bioavailability of calcium, iron, and zinc increase with germination (Sandberg 1991, Camacho 1992). Seeds that are commonly germinated in developing countries include lentils, peas, soy beans, and mung beans.

Fermentation

Fermentation can be used for cereals, legumes, and vegetables. Acid and alcoholic fermentation increase the nutritional value and improve the physical properties of certain foods. Fermentation can be spontaneous (uses the microorganisms that are naturally present in a food) or started with an inoculation. The nutritional advantages of fermentation include improving the bioavailability of minerals, such as iron and zinc, as a result of phytic acid hydrolysis. Some micronutrients, such as riboflavin, are increased by fermentation. Some microorganisms produce vitamin B-12, thus making the vitamin available in plant-derived foods where it is normally not present. There is also some evidence that fermented foods have an antidiarrheal effect in young children (Mensah et al. 1990). Another advantage of fermentation is that it can be a time-saving device for mothers in that fermented foods can be safely used throughout the day without being cooked.

Milling

The micronutrient composition of flours depends on the proportion of the cereal grain removed by the milling process. When the outer layers of grain are removed, most of the thiamin, riboflavin, niacin, and iron are lost and phytic acid is reduced. In some rural households in Latin America, home-based hand milling of grains has been shown to result in higher iron and zinc content compared with motorized milling (Krause et al. 1993). Dry, fine milling of corn bran results in greater bioavailability of the B vitamins than do other milling methods, such as wet milling or fine or coarse grinding (Yu and Kies 1993).

Malting

Malting involves the grinding and softening of grains by soaking them in water until sprouting occurs. Malting is typically followed by drying and milling. Many cereal-based porridges are prepared by malting. This process increases the absorption of indigenous iron and zinc by reducing phytic acid levels (Gahlawat and Sehgal 1994).

What Additional Information Is Needed?

More information is needed in the different food processing areas:

- Drying: Drying green, leafy vegetables, such as cassava, for mixing with rice or other components of complementary foods may decrease β-carotene bioavailability. The effect of this process on the fiber or mineral content of vegetables is unknown. For example, some green leafy vegetables are good sources of iron and calcium: Is the availability of iron and calcium altered by drying?
- Frying: Additional information is needed on vitamin retention in foods that are fried in oil. Because of the short time required for frying, vitamin retention with frying may be superior to that with cooking in water. What is the effect of frying on mineral bioavailability?
- Fermentation: Additional information on the putative antidiarrheal effect of fermented foods is needed. What is the effect of cofermentation on micronutrient content? If cereals and legumes are combined and cofermented, how are micronutrient content and bioavailability affected? Also, should foods be combined before or after they are fermented? Does cofermentation have an antidiarrheal effect?
- Nixtamalization and dehulling: The advantages of nixtamalization followed by dehulling need to be investigated. Because nixtamalization involves elevated pH, which tends to soften and loosen the hulls, this method may improve the dehulling process. What is the effect of nixtamalization on the bioavailability of minerals, such as iron? The addition of lime may enhance the bioavailability of iron in foods that contain phytate because of an exchange of calcium with phytate. The use of nixtamalization in foods other than corn, such as sorghum, millet, and rice, needs to be investigated.

Micronutrients in Fortified Complementary Foods and Supplements

Breast milk is an excellent source of micronutrients for infants during the first 4–6 months of life. However, after this time other foods need to be introduced into the diet to provide required energy and nutrients. In many developing countries, breast milk intake may be compromised by the early introduction of complementary foods, which replace rather than complement breast milk. Some micronutrients (e.g., vitamin A) are low in breast milk as a result of poor maternal status and the concentration of other micronutrients in breast milk are inadequate to meet the relatively high demand for infant growth and development. In developing countries, traditional infant foods are often based on unrefined cereals and legumes that contain a high level of substances that inhibit absorption of some minerals; thus, the bioavailability of micronutrients such as iron, zinc, and calcium may be low. As a result, it may be difficult for traditional complementary foods to meet infants' micronutrient requirements for growth and development. The consumption of appropriately mixed diets made from local foods are encouraged to ensure adequate micronutrient intake. Fortified complementary foods or supplements are another nutritionally-appropriate option to ensure adequate micronutrient intake. However, food fortification may not be realistic for the vast number of poor households .

Which Micronutrients Should Be Added to Complementary Foods and Supplements?

The micronutrients deemed important to add to complementary foods and pharmaceutical supplements can be divided in two categories: priority micronutrients (i.e., those that should be added to any form of complementary food or pharmaceutical supplement) and optimal micronutrients (i.e., those that should be added when financial resources allow).

Nutritionally adequate complementary foods should be the primary source of micronutrients. Pharmaceutical supplements should only be considered as an alternative to unfortified or fortified complementary foods rather than used in addition to these foods.

Priority Trace minerals

Priority micronutrients that should be added to complementary foods and supplements, are divided into trace minerals and vitamins (Table 1). Iron and zinc are the two trace minerals that should always be provided to infants and young children. In many developing countries, traditional complementary foods are based on legumes and unrefined cereals, both containing a form of iron (nonheme iron, which is poorly absorbed) and high levels of phytic acid, which reduces the absorption of both iron and zinc. Iron-deficiency anemia, which is the most widespread nutrition deficiency worldwide, is associated with poor mental and motor development and impaired immune competence in infants and young children. There is evidence that zinc supplemen-

Table 1. Priority and optimal micronutrients that should be added to complementary foods and supplements

Priority Micronutrients		Optimal Micronutrients	
Trace Minerals	Vitamins	Trace Minerals	Vitamins
Iron	Vitamin A	Iron	Vitamin A
Zinc	Riboflavin	Zinc	Riboflavin
Iodine (RS)	Niacin (SS)	Iodine	Niacin (SS)
Selenium (RS)	Vitamin B-12 (RS)	Selenium	Vitamin B-12
	Vitamin C (RS)	Calcium	Vitamin C
	Thiamin (SS)		Thiamin (SS)
	Vitamin D (RS)		Vitamin D
	Vitamin E		Vitamin E
			Folate

SS, staple specific (thiamin: rice, niacin: corn); RS, region specific.

tation can reduce the incidence of diarrheal episodes in children (Rosado et al. 1997). In addition, supplementation of children who are deficient in iron or zinc can improve appetite and growth (Allen 1994, Lawless et al. 1994).

Developing fetuses and infants up to age 2 years are most susceptible to the effects of iodine-deficiency disorders, including cretinism (Maberly 1994). Although iodine-deficiency disorders are easily prevented, most of the characteristics, including hypothyroidism and mental and physical retardation, are irreversible. These disorders are worsened by the consumption of goitrogens, which are present in a few varieties of staple foods consumed in developing countries, including cassava, maize and millet (Delange et al. 1982). Goitrogens, however, can be destroyed by heat during the processing of foods. Although iodized salt is the primary means for providing iodine in the diet, salt is not usually added to complementary foods. The percentage of salt that is iodized varies from country to country and ranges from 0 to 100%, depending on the presence and enforcement of legislation for salt iodization.

The addition of selenium to complementary foods and supplements is recommended in regions where the selenium content in soil is low, including parts of China and Zaire (Jackson 1988, Vanderpas et al. 1992). Region specificity is also necessary to prevent overconsumption in areas where the selenium content in soils is high and, therefore, dietary intake can be high, such as in parts of China and Venezuela (Jackson 1988, Burguera et al. 1990). Children with protein deficiency tend to have low stores of selenium, suggesting that the risk for selenium deficiency is increased. Keshan's disease, known to be associated with selenium deficiency, is characterized by various myocardial abnormalities and often leads to congestive heart failure (Yang et al. 1988).

Priority Vitamins

Priority vitamins recommended to be added to complementary foods and supplements include vitamins A, C, D, B-12, and E; thiamin; riboflavin; and niacin. Like the priority trace elements, some of these vitamins are considered to be region specific and some are also staple-food specific.

Clinical and subclinical vitamin A deficiency are most prevalent in children 6 months through 5 years of age, a period that includes the transition from breast-feeding to dependence on other dietary sources of vitamin A and increased requirements for rapid growth (WHO 1982). The absorption of beta-carotene and vitamin A requires the presence of fat which is often low in the diet of developing countries. The availability of green leafy vegetables, which contain carotenoids (precusors of vitamin A), is affected by seasonality. Because there is a link between vitamin A deficiency and increased mortality and severity of morbidity, various interventions—including fortification, increased consumption of fruits and vegetables rich in vitamin A, and supplementation—have been implemented worldwide.

The vitamin E (α-tocopherol) content in diets varies widely, depending primarily on the type and amount of fat present in the diet and the losses that occur during processing and cooking. Most fruits, vegetables, animal products, and fish contain very little vitamin E whereas green leafy vegetables are a good source. Because green leafy vegetables are seasonal in many developing countries, fortification and supplementation of vitamin E are needed. In many developing countries, fat and oil intakes are negligible, which can impair the absorption of vitamin E. Vitamin E deficiencies have been observed in Mexico and Sri Lanka (Rosado et al. 1995, Christiansen et al. 1988). Some infants born prematurely have low tissue stores of tocopherol, but premature very-low-birth-weight infants respond well to vitamin E supplementation (Phelps 1988). Prolonged inadequate consumption or absorption of vitamin E can result in hemolytic anemia and neurological damage as early as a few months after birth in preterm infants, and impaired immune function is associated with a vitamin E deficiency. Vitamin E helps to prevent the occurrence of damaging oxidation reactions in the body.

Animal proteins (from meat, poultry, and dairy products) as well as some green vegetables are good sources of riboflavin, whereas grain products contain relatively low amounts of riboflavin. A diet poor in riboflavin during infancy and childhood is associated with dermatologic problems and poor growth due, in part, to the inefficient use of dietary energy. Riboflavin deficiency has been observed in Latin America, Africa, and Asia (Rosado et al. 1995, Bates et al. 1982, Lo 1984) and is associated with impaired iron absorption (Fairweather-Tait et al. 1992). Riboflavin deficiency may offer some protection against *Plasmodium falciparum* malaria. Supplementation of riboflavin along with iron has a greater positive effect on iron status than does iron supplementation alone (Powers et al. 1985).

Vitamin C (ascorbic acid) is abundant in many fruits and vegetables whereas low levels are found in animal sources and grains, and vitamin C deficiency is not common in the developing world. Vitamin C fortification or supplementation is recommended in areas where the consumption of fresh fruits and vegetables is limited because of cultural practices and availability. Infectious diseases are more common in people with a vitamin C deficiency than in people with normal vitamin C status (Rose 1981). Vitamin C improves the absorption of iron and therefore enhances the absorption of iron added to complementary foods and supplements (Hallberg and Rossander 1984). The inclusion of appropriate food sources of vitamin C in complementary foods should be encouraged, and infants should be given fruits and vegetables as an integral part of complementary feeding.

Vitamin D is essential for bone development. The main dietary sources of vitamin D are animal and dairy foods, which are often lacking in the diet in developing countries. The ability of human breast milk to provide adequate amounts of vitamin D in the absence of sunlight has been questioned in various studies (Tsang 1983). The absorption of vitamin D requires the coabsorption of dietary fat, which is often consumed at low levels in developing countries. Vitamin D can be made in the body in the presence of sunlight. In certain cultures, exposure to sunlight is minimal and is dependent on the type of clothing worn. In Ethiopia, for example, mothers carry their babies on their backs completely covered up to shelter them from the sun and they discourage their children from playing in the sun. Vitamin D supplementation or fortification is recommended in regions where minimal exposure to sunlight occurs.

Vitamin B-12 is not found in plant-derived foods; thus, deficiency can be common among people who consume a vegetarian diet, such as people in India. Low intakes of vitamin B-12 were reported in other countries, such as Mexico and countries throughout Africa, where the intake of animal products is limited (Allen et al. 1995). A common clinical symptom of a vitamin B-12 deficiency is megaloblastic anemia, which is associated with an increased susceptibility to infections. Therefore, it is recommended that vitamin B-12 be added to foods or supplemented where there is a high prevalence of infection and disease.

The poorest inhabitants in various developing countries often eat a narrow range of foods and usually one staple, which serves as a cheap source of energy and is the dominant food. Examples of such staples include rice and maize. Thiamin should be supplemented in regions where the primary staple is polished rice, because most thiamin is removed during dehulling and milling of rice to make polished rice. The clinical signs of thiamin deficiency (beriberi) include cardiovascular and neurological disorders, which have been observed in populations where the main staple is polished and unenriched rice.

Niacin is widely distributed in plant and animal foods. However, it is found in low levels in cornmeal and sorghum and is not available for absorption in cornmeal unless the corn is soaked in lye or roasted. Clinical signs of niacin deficiency include weakness, anorexia, and diarrhea. These signs are associated with poorer social classes whose chief staple food consists of cereal such as corn or sorghum, which are common for Latin America and parts of Africa and India (Carpenter 1983). Niacin is therefore recommended in areas where such foods are the main staple.

Optimal Micronutrients

The optimal micronutrients, recommended to be added to complementary foods and supplements are the priority micronutrients and calcium and folate (Table 1). Calcium is essential for proper bone development. There is some evidence of low intakes of calcium in young children in developing countries (Belton 1986). Calcium is abundant in breast milk, dairy products, and some green vegetables. Calcium levels are low in grains, fruits, and most vegetables, which are typically the main component in complementary foods.

Folate is widely distributed in foods, but as much as 50% may be destroyed during household preparation and storage of food. In many regions, green leaves that are added to complementary porridges are generally cooked for a long time, and thus the folate levels are low. Folate body stores are small at birth and are rapidly depleted as a result of the increased requirements for growth, especially in premature infants. A deficiency of folate is characterized by poor growth, structural and functional changes in the gastrointestinal tract, and megaloblastic anemia.

What Amounts of Micronutrients Should Be Added?

The amounts of micronutrients recommended to be added to complementary foods or supplements are shown in Table 2. Recommendations from the Food and Agricultural Organization and the World Health Organization of the United Nations (FAO/WHO 1988, 1966) were used when they were available, otherwise the 1991 United Kingdom (UK 1991) requirements were used. Requirements are given for 6–12 months and 12–23 months of age. Requirements for infants between birth and 6 months of age were excluded because it was assumed that all infants are breast-fed up to 6 months of age.

The FAO/WHO recommendations provide categories for iron and zinc requirements based on whether the bioavailability of iron and zinc is low or moderate. Because the diets typically consumed in developing countries are high in phytic acid, the low-bioavailability requirements are used in the table; the other requirements are listed as a footnote. Criteria used to define low- and moderate-bioavailability for iron and zinc are defined by FAO/WHO. Because the FAO/WHO requirements for calcium were not reexamined and included in the FAO/WHO publication, the UK calcium requirements were considered to be appropriate.

Which Micronutrient Interactions Are Important?

On the basis of scientific information on micronutrient interactions and their effect on bioavailability presented in this workshop, the interactions at the amounts recommended in Table 2 that may affect

Table 2. Amounts of micronutrients that should be added to complementary foods and supplements

	6–12 months		12–23 months		
	Per day	Per 100 kcal	Per day	Per 100 kcal	Source
Calcium (mg)	525	70	350	32	UK
Iodine (mg)	50	6.6	90	8.2	FAO/WHO
Vitamin A (mg RE)	350	46	400	37	FAO/WHO
Folate (mg)	32	4.2	50	4.6	FAO/WHO
Vitamin C (mg)	20	2.6	20	1.8	FAO/WHO
Vitamin D (mg)	10	1.3	10	0.9	FAO/WHO
Thiamin (mg)	0.2	0.3	0.5	0.5	UK
Riboflavin (mg)	0.4	0.5	0.6	0.5	UK
Vitamin B-12 (μg)	0.1		0.1		FAO/WHO
Vitamin E (mg/g PUFA)			0.4		UK
Zinc (mg)[a]	10	1.32	10	0.92	FAO/WHO
Iron (mg)[b]	14	1.85	8	0.7	FAO/WHO

RE, retinol equivalent; PUFA, polyunsaturated fatty acid.
Sources: FAO/WHO (Food and Agriculture Organization/World Health Organization 1966,1988) ; UK (Panel on Dietary Reference Values of the Committee on Medical Aspects of Food Policy 1991).
[a]Values in the table are for low bioavailability. For moderate bioavailability: 5 mg/d (0.65 mg/100 kcal) for 6–12 months, 5 mg/d (0.46 mg/100 kcal) for 12–23 months.
[b]Values in the table are for low bioavailability. For moderate bioavailability: 7 mg/d (0.92 mg/100 kcal) for 6–12 months, 4 mg/d (0.4 mg/100 kcal) for 12–23 months.

bioavailability are identified below. Because negative interactions between micronutrients have less of an effect in foods, it is recommended that where there is no option for children, except to take supplements, they should be taken with meals. This practice should be taught to mothers.

Calcium, added in large amounts to a complementary food (e.g., more than 300 mg) may interfere with iron absorption. Research on calcium-iron interactions, conducted in adults but not in infants, suggests that large amounts of calcium may affect iron absorption in the short term but not over the long term (Minihane et al. 1997) . More long-term studies in infants are needed.

Most infants and young children in developing countries consume plant-based complementary foods which are abundant in copper. The copper status for those infants receiving milk-based complementary foods, however, should be of concern because these foods are low in copper. The addition of iron to these foods could exacerbate low copper status. This concern does not extend to plant-based complementary foods, such as cereals and legumes, which contain adequate amounts of copper.

Because vitamin C has a positive effect on nonheme iron absorption, when iron is added to complementary foods and supplements, vitamin C should be added in a 2:1 ratio with iron. If the complementary foods have high levels of iron absorption inhibitors, such as polyphenols and inositol phosphates (e.g., unrefined maize, soya flour, and brown sorghum), the ratio should be increased from 2:1 to 4:1. The 4:1 ratio may not be adequate at much higher phytic acid levels. The effect of vitamin C on iron absorption when complementary foods contain large amounts of polyphenols has not been investigated in infants. These recommended ratios should be at the point of consumption because there are many opportunities for vitamin C to deteriorate; the amount added to the foods should take deterioration during storage into account.

When supplements are consumed with a meal, iron does not have an inhibitory effect on zinc absorption at the amounts recommended in Table 2 (Sandstrom 1985). This inhibitory effect, however, may be significant when a supplement is taken between meals. Iron-fortified foods do not appear to affect the absorption of zinc.

What Further Research Is Needed?

Studies in infants and children are needed to investigate the following:

- long-term effect of iron-calcium interactions on iron bioavailability and status,
- relative absorption of calcium from milk and other calcium sources,
- effect of daily vs. weekly supplementation of priority micronutrients,
- effect of vitamin C on iron absorption when complementary foods contain large amounts of polyphenols, and
- efficacy and effectiveness of providing a supplement vitamin-mineral mix in a sachet that can be added to unfortified commercially prepared foods or home-based complementary foods.

Barriers to Overcome and Knowledge Needed to Implement a Successful Complementary Feeding and Supplementation Program

A number of barriers have impeded the successful implementation of programs to increase micronutrient intake through the use of complementary foods or pharmaceutical supplements. Some of the barriers are technical but many are related to political interests and behavior change. The following summarizes the issues raised.

Consensus

In the 1960s and 1970s, there was a push by organizations such as the United Nations Children's Fund and the U.S. Agency for International Development to implement complementary feeding programs that used fortified foods distributed free of cost to beneficiaries. Although there has been a great deal of controversy as to whether the use of complementary foods is feasible, some countries such as Chile and Thailand have been successful in reducing malnutrition with the help of preprocessed complementary foods for young children. Despite this, there are few examples of success in other countries.

Complementary foods are fortified and supplements are distributed if there is evidence that a particular micronutrient deficiency exists, but few national studies have been done in developing countries to document deficiencies other than iron and vitamin A deficiencies. However, dietary data often suggest that young children also have low intakes of many other micronutrients. A review of experience with private sector marketing of complementary foods would help put the negative attitudes toward these activities in perspective and could highlight current issues that differ from those in the past. For example, production of complementary foods historically has focused on adequate levels of protein and not on micronutrients, but many of the complementary foods that were used were fortified with micronutrients that are currently recommended.

There is a lack of consensus on the need for multivitamin supplements. Although there is consensus on the need for vitamin A supplementation in prophylactic and clinical cases—and even for iron supplementation—there is a lack of understanding about the degree of other micronutrients deficiencies in children. Multiple micronutrient deficiencies often occur, especially in children, for whom lack of adequate energy coincides with lack of micronutrients. However, there has been little program effort to try to address multiple micronutrient deficiencies.

Efficacy

Data are lacking on the efficacy of using processed complementary foods at the local level because few well-designed studies have investigated this issue. There is also a lack of data on the prevalence and location of important micronutrient deficiencies, such as zinc deficiency. This information is essential to the development of a baseline for evaluating the impact of processed complementary foods or vitamin/mineral supplementation programs. Data on both the efficacy and effectiveness of food programs need to be obtained and published so that they are widely available to policy makers.

Legislation

There is a lack of legislation, regulations, guidance, and norms on fortificant levels for complementary foods or vitamin/mineral supplements for young children that could help in defining needs or creating alliances among governments, the public sector, and the private sector. Guidelines should be developed by national committees—including ministries of health, commerce, and agriculture; local university researchers and faculty; and industry representatives—for levels of micronutrient fortificants to help industry to produce fortified complementary foods. There is a need for nutritonists to identify the micronutrient issues of concern and prioritize these issues to aid the policy makers in addressing how to improve child feeding.

Partnerships

Partnerships between the private and public sectors are needed. Especially important is the lack of involvement of the private sector in complementary feeding programs for all sectors of the developing world. There is a need for a structured dialogue, both internationally and nationally. The absence of partnerships is viewed as a barrier because partnerships are necessary for obtaining consensus on levels of fortificants and other technical issues, such as the need for an instant food that requires less cooking, the requirement to cook the food to prevent contamination, or the types of iron that should be added for maximum absorption. Partnerships can be developed by establishing joint committees with regular meetings.

Cost and Sustainability

The major costs for implementing and sustaining complementary feeding programs are production, distribution, packaging, and marketing. Costs implications can be reduced by segmenting markets. For example, complementary feeding programs could be targeted to urban areas whereas supplementation programs could be targeted to the rural areas. Additionally, for complementary feeding programs, the public sector could focus on a small age group (e.g., 6–12 months). The private sector could then focus on older age groups, greatly reducing the costs of programs for the public sector, especially if the critical mass of consumer demand for these products is reached.

Information and Education

Consumers and professionals need information and education on the need for complementary foods and supplements. They also need to understand how to use these products and implement programs. Social marketing and education programs could be developed and sustained through partnerships between public and private sectors.

Distribution

Inadequate distribution results in a lack of available complementary foods and supplements at the local level. Advantage needs to be taken of the distribution systems already in place. Complementary foods and supplements could be distributed through stores, existing public health care networks and through the private sector. Supplements could be delivered through small and private stores, even in remote regions.

Acceptability

Acceptability of complementary foods and supplements can be a barrier to a successful program; the taste, color, and smell of a food can have a large effect on its acceptability by a mother or child. Furthermore, certain cultures are receptive to specific staples, and different complementary foods are needed in different regions. Acceptability of a food or food supplementation program also depends on the amount of time, fuel, and water needed for food preparation.

Safety

To better ensure the safety of fortified feeding and supplementation programs, issues such as toxicity, interaction between micronutrients, and impact of the physiological state (e.g., during infection) on the nutritional status of an individual need to be well understood.

Recommendations

Research on micronutrient interactions has brought about many interesting findings which can influence the bioavailability of a micronutrient, but still a better understanding of the issues involved need to be obtained and much more needs to done to lead to practical solutions benefiting children of all social groups. The complexity of micronutrient interactions should not hinder practitioners to make steps towards increasing the micronutrient content and density in complementary foods. Based on the current findings presented and discussed at this workshop, the following recommendations were made:

- Complementary foods should contain a mixture of foods, preferably animal- and plant-based, to ensure adequate consumption of the micronutrients needed for proper growth and development.
- Mild heating and minimal water is recommended when preparing fruits and vegetables. Extreme heat and excess water should be avoided during the storage of fruits and vegetables.
- For foods containing low levels of ascorbic acid, germination or fermentation should be employed for reducing the level of phytic acid and improving the absorption of iron.
- Copper may need to be considered as part of an iron and/or zinc intervention, particularly when plant-based complementary foods are not being consumed.
- When adding iron to a supplement or a fortified food low in phytic acid, the ascorbic acid:iron ratio should be 2:1. When adding iron to a fortified food, high in phytic acid (maize, brown sorghum, soya flour), the ascorbic acid: iron ratio should be 4:1.
- Vitamin A, along with iron, should be considered as part of an intervention for the prevention and treatment of iron deficiency.

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Appendix 2: Workshop Agenda

July 29, 1996

8:30am	Continental Breakfast
8:45am	Welcome & Purpose of Workshop Mr. David Oot
8:50am	Agenda and Expected Outcomes Dr. Adrianne Bendich

Chair: Dr. Sandra Bartholmey

9:00am	Iron-Zinc-Copper Interactions Dr. Bo Lonnerdal
9:30am	Interactions between Iron, Ascorbic Acid and Calcium Dr. Lindsay Allen
10:00am	Break
10:30am	Iron Bioavailability from Weaning Foods: Effect of Phytic Acid Dr. Lena Davidsson
11:00am	Multi-Micronutrient Studies in Indonesia: Needs for Intervention and Effects of Supplementation Dr. Werner Schultink
11:30am	The Interaction between Riboflavin and Iron: Relevance to Child Health and Nutrition Dr. Hilary Powers
12:00pm	Lunch

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Chair: Dr. David Yeung

1:00pm	Effects of Food Processing Techniques upon the Content and Bioavailability of Vitamins in Weaning Foods Dr. John Erdman
1:30pm	Food Processing Methods for Improving the Zinc Content and Bioavailability of Home-Based and Commercially Available Complementary Foods Dr. Rosalind Gibson
2:00pm	Effects of Cooking and Food Processing on the Content of Bioavailable Iron in Foods Dr. Dennis Miller
2:30pm	Break

Chair: Dr. Frances Davidson

3:00pm	Appropriate Levels and Proportions of Micronutrients to be Added to Complementary Foods and Multi-Micronutrient Supplements Dr. Elizabeth Yetley
3:30pm	Thailand's Experiences with Fortified Weaning Foods Dr. Visith Chavasit
4:00pm	High Protein Quality Vegetable Mixtures for Human Feeding Dr. Ricardo Bressani

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8:30am Continental Breakfast

9:00am Break-out Group Discussion

I. What micronutrient interactions are important in developing fortified complementary foods and supplements for infants and young children?

What micronutrients should be added to complementary foods and supplements and at what level?

II. What food processing methods have been tried to improve the micronutrient content of home-based and commercially available complementary foods?

What additional knowledge is needed for improving the micronutrient content of home-based and commercially available complementary foods?

III. What are the barriers to implementing a successful fortified complementary feeding and supplementation program in developing countries?

What additional knowledge/efforts are needed to promote the consumption of fortified complementary foods and use of supplements in developing countries?

12:00pm Lunch

1:00pm Reporting back of groups

3:30pm Summary and Recommendations
Dr. Suraiya Ismail

This report is the summary of presentations and discussions that took place at a July 29-30, 1996, workshop in the Washington, D.C., offices of the International Life Sciences Institute (ILSI). The information presented herein does not necessarily reflect the scientific recommendations or views of Opportunities for Micronutrient Interventions (OMNI) Research, the U.S. Agency for International Development (USAID), or ILSI.

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