

Iron fortification with special reference to the role of iron EDTA

TH Bothwell

Department of Medicine, University of the Witwatersrand Medical School, Johannesburg, South Africa

SUMMARY. Iron fortification has been used for decades in a number of industrialized countries to combat iron deficiency and seems to have played a significant role in reducing its prevalence, especially in infants and women. The overall strategy has been one in which staples such as wheat, flour, have been fortified with iron. While the effects appear to have been positive, there are still problems not yet completely resolved. In this context, the selection of the fortificant always represents a compromise between a choice of chemically reactive compounds of high bioavailability, such as ferrous sulfate, and inert compounds, which are poorly absorbed. Ferrous sulfate is very effective when added during the preparation of bread and bakery products and infant formulas, but cannot be used in stores flour because of organoleptic problems and inert compounds, such as elemental iron powders, have to be used. The search, therefore, continues for compounds of high bioavailability which do not cause organoleptic changes in the vehicles to which they are added. Problems associated with effective iron fortification programmes are compounded in a number of developing countries by a variety of factors. Most potential vehicles are not centrally processed, inhibitory ligands in staple cereal diets depress the absorption of both intrinsic and fortification iron, anemia is often of multifactorial in etiology, financial resources are scanty and governmental support sometimes lacking. Despite such difficulties there are encouraging signs of progress in a number of countries, using a variety of fortificants and vehicles. In the present review particular attention is paid to the potential role of NaFeEDTA as a fortificant in developing countries. It is much less affected by the inhibitors of iron absorption present in diets of low bioavailability, it can be added to a number of vehicles without causing organoleptic problems and its efficacy has been underlined in three intervention studies.

Keywords: Iron deficiency, iron fortification, iron EDTA, NaFe EDTA, iron bioavailability.

RESUMEN. La fortificación de alimentos con hierro: papel del hierro EDTA. La fortificación con hierro ha sido ampliamente utilizada por varias décadas en muchos países industrializados para combatir la deficiencia de hierro y parece haber jugado un papel significativo en su reducción, particularmente en niños y mujeres. La estrategia general ha sido la fortificación con hierro de alimentos básicos como la harina de trigo. Aunque los efectos parecen ser positivos, aún persisten problemas por resolver. En este contexto, la selección del fortificante siempre representa un compromiso entre compuestos químicamente reactivos de alta biodisponibilidad como el sulfato ferroso y los compuestos inertes, de muy poca absorción. El sulfato ferroso es muy efectivo cuando es agregado durante la preparación del pan y productos de panificación y de fórmulas infantiles, pero no puede usarse en harinas de reserva por los problemas organolépticos, por lo que compuestos inertes de hierro elemental en polvo tienen que usarse. La búsqueda por compuestos de alta biodisponibilidad que no causen cambios organolépticos en los vehículos al que son agregados continúa. Los problemas asociados a la efectividad de los programas de fortificación en países en desarrollo están influenciados por una variedad de factores. La mayoría de los vehículos potenciales no son procesados centralmente, existen ligandos inhibidores en las dietas básicas que deprimen la absorción tanto del hierro intrínseco como del hierro agregado, la anemia es de etiología multifactorial, los recursos financieros son limitados y el apoyo gubernamental está a veces ausente. A pesar de todas las dificultades, hay signos de progreso prometedores en varios países, utilizando una variedad de fortificantes y vehículos. En la presente revisión se ha dado particular atención al papel potencial del hierro EDTA como fortificante en países en vías de desarrollo. Este compuesto está menos afectado por los inhibidores de la absorción de hierro presente en las dietas de baja biodisponibilidad, puede agregarse a varios vehículos sin provocar cambios organolépticos y su eficacia ha sido afirmada en tres estudios de intervención.

Palabras clave: Fortificación, hierro EDTA, biodisponibilidad, deficiencia de hierro, anemia.

INTRODUCTION

There has been a steady decline in the prevalence of iron deficiency anemia in industrialized countries over the last several decades, with the drop being particularly striking in two most vulnerable groups, infants (1) and women (2,3). The prevalence in Sweden dropped from between 25 and 30% in the 1960's to about 7% in the mid seventies (2), while an even

lower figure of 2.6% was noted in the USA in the NHANES II survey carried out between 1976 and 1980 (3). The low prevalence in the USA was confirmed in phase 1 of the NHANES III survey (1988-1991), with only 3 to 4% having iron deficiency anemia (4). The improvement in iron nutrition, both in infants and women, has been ascribed, in part at least, to the high consumption of iron-fortified foods. It is not, however, the only factor. Over the same period, there has been

a significant decrease in menstrual blood losses due to increased use of oral contraceptives and 'over the counter' preparations containing iron and vitamins have been widely consumed (2). In addition, there are also other factors which stem from increased affluence, including smaller families and a wider selection of foods (2).

In contrast to affluent industrialized countries, iron deficiency anemia remains a major and pressing problem in most of the developing world (5). This can be ascribed to a number of factors. The major one is the low bioavailability of the iron in staple diets, composed largely of grains and legumes (6). The problem is compounded in many areas by intestinal worm infections and particularly blood loss from hookworm infestation (7). In addition, associated conditions, such as malaria, HIV infection and vitamin A deficiency, might be expected to blunt the effects of any intervention programme (8-11) whether it involves fortification (12), supplementation (13) or dietary modification (14).

It is against this background that the status of iron fortification in the world today must be viewed. On the one hand, there is the example of industrialized countries, such as the USA, in which a number of foods have been successfully fortified, while on the other, there is most of the developing world in which it has proved extremely difficult to develop viable programs to prevent iron deficiency through food fortification. In the present review, factors affecting the availability of fortification iron will first be discussed. The current status of iron fortification in developed countries will then be covered briefly, while major attention will be directed to the constraints that have limited the successful application of similar approaches in most developing countries. In this context, it should be noted that the relative advantages of different iron fortificants and vehicles have been addressed in some detail over the last years in several reviews (12,15-25). In seeking ways of overcoming current challenges, particular attention will be paid to the potential value of NaFeEDTA as a fortificant, since it has been shown to be effective in three pilot trials carried out in developing countries (26-29). It is perhaps appropriate that this compound should be discussed at a meeting in honor of Doctor Miguel Layrisse, since his own group was involved in seminal studies demonstrating its beneficial effects on the absorption of iron from food (30).

FACTORS AFFECTING BIOAVAILABILITY OF IRON FORTIFICANTS

The amounts of fortification iron absorbed from a particular diet are dependent on three factors. These include the composition of the diet, the iron status of the individuals consuming the diet, and the relative bioavailability of the iron fortificant (22). There are two types of iron in the diet, heme iron (derived from hemoglobin and myoglobin) and nonheme iron (derived mainly from cereals, vegetables, and fruit). Most forms of nonheme iron in a meal, whatever their origin,

enter a common pool during digestion and are thus equally susceptible to a number of promoters and inhibitors of iron absorption (31). The major promoters of iron absorption are meat and ascorbic acid (31) while the major inhibitors are phytates (32) and polyphenols (33).

Soluble iron fortificants, such as ferrous sulfate, enter the common pool of nonheme iron completely and are absorbed to the same degree as is the intrinsic nonheme iron in the diet. Such iron is thus well absorbed when the diet contains adequate amounts of ascorbic acid and/or meat, while it is poorly absorbed from diets in which inhibitors of iron absorption predominate (16). Ferrous sulfate and other soluble iron complexes can only be used as fortificants in certain limited situations, since they are chemically reactive and tend to produce undesirable organoleptic changes in the vehicles to which they are added (15,23,25). As a result, several other fortificants, which are less soluble under the conditions prevailing in the upper gastrointestinal tract, are in common use. Although such inert compounds do not cause organoleptic changes when stored in a variety of vehicles, they tend to be less well absorbed.

A list of currently available fortificants, including the vehicles to which they can be added, their relative bioavailabilities in comparison with ferrous sulfate $7H_2O$. (designated as 100), their iron contents and relative costs are shown in Table 1, which is adapted from previous ones by Hurrell (12,19,23,25) and Bothwell and MacPhail (22). Their relative bioavailability has been assessed on the basis of their physical characteristics (particle size, relative surface areas and solubility in acid), their capacity as compared with ferrous sulfate to restore the hemoglobin level in iron deficient rats and the degree to which they exchange with the common pool of nonheme iron in the diet in human absorption studies using radioactive or stable isotopes (23,25). While reasonable agreement between the various methods has been reported (34), there are still doubts about results obtained when testing compounds, such as elemental iron powders, which are poorly soluble in dilute acids. In such circumstances there are always questions as to whether the labelled experimental compound has the same physicochemical characteristics as the commercial compound (12). This may explain the wide range of values for relative bioavailability found in different human experiments (e.g. 13 to 148 with reduced iron).

Included in Table 1 is the interesting compound, ferrous bis-glycine, which is currently being actively studied. This water-soluble complex is not only highly bioavailable but can also be added to a number of foodstuffs without causing organoleptic changes (35). The degree, if any, to which it is protected from inhibitory ligands in the diet, is a topic of active current debate. In one study in adult human subjects the chelated iron was absorbed several fold better than ferrous sulfate when fed in a maize meal (36), while in another, it was no better absorbed than ferrous sulfate when given to infants in a vegetable weaning food (37).

of iron is considerably enhanced by ascorbic acid (41-44). The weight ratio of ascorbic acid to iron should be at least 5 to 1 in such formulas (41,44). Using this approach, the bioavailability of fortified formulas is so high that it has recently been suggested that it may be appropriate to reduce the current level of iron fortification of ± 12 mg iron, since it allows for the absorption of approximately twice the infant's requirements (45).

Infant cereals are more difficult to fortify with iron. Not only do they have a high phytate content but they are also very sensitive to fat oxidation, so that highly bioavailable forms of iron, such as ferrous sulfate, cause organoleptic problems (12), while more stable forms of iron are much less bioavailable (46). Such preparations can, however, still be effective, even when the fortificant is electrolytic iron, provided very large amounts are present (55 mg iron/100 g dry cereal) (47). Alternatively, ferrous fumarate, which is poorly soluble in water but soluble in acid, can be used as the fortificant (48). As with infant formulas, absorption from infant cereals is enhanced by the presence of adequate amounts of ascorbic acid (48).

While iron deficiency is a less critical problem in childhood than it is in late infancy, school programs lend themselves to targeted iron fortification. In this context, milk has proved an effective vehicle (49) and milk-based chocolate drinks fortified with ferrous succinate and ascorbic acid, can be usefully targeted to children and adolescents (50).

Fortification of wheat and other cereal products

Bakery products and wheat flour are currently the most frequently used vehicles for iron fortification that reach the whole population, with wheat flour enrichment mandatory in the USA, United Kingdom and Denmark but not in France, Italy and Spain (25). In those countries in which iron fortification of flour is mandatory, the actual extent of fortification varies widely - up to 44 mg/kg in the USA, 30 mg/kg in Denmark and only 16.5 mg/kg in the United Kingdom (25). In the USA it has been calculated that the iron contribution from foods that are normally fortified, such as whole bread, rolls, crackers, corn flour, corn grits, pasta and breakfast cereals, represents more than 20 percent of the dietary intake (25).

Insofar as cereal products are concerned, iron has been added for the most part in two forms, as ferrous sulfate and as elemental iron powders. Ferrous sulfate is very satisfactory as a fortificant when added during the preparation of bread and bakery products, since it is absorbed as well as the intrinsic iron in the wheat. It is also widely used to fortify infant formulas, pasta and cereal flour, which are only stored for short periods. The more widespread application of ferrous sulfate and other water soluble ferrous salts is limited by their chemical reactivity, which causes organoleptic problems. As a result, several other compounds which are poorly soluble in dilute acids, including gastric juice, have been the most widely used compounds in the fortification of cereals. They include

elemental iron powders and ferric ortho- and pyrophosphate. While hemoglobin regeneration studies in rats suggest a relative bioavailability about half that of ferrous sulfate, studies in humans have given conflicting and variable results (22). As previously discussed, this could be due to the fact that the compounds tested had different physicochemical characteristics from those produced commercially and to the influence of meals on their dissolution in gastric juice (34,51). Such variable results have made it difficult to assess the overall effects of flour fortification on iron nutrition.

The current dilemma is underlined by the Swedish experience. Until 1995 wheat flour was heavily fortified with carbonyl iron (65 ppm), the elemental powder with the smallest particle size and the highest solubility in gastric acid (21). It was calculated that it provided up to 40% of the dietary iron intake in Sweden and there seemed good reason to believe that it had played a significant role in reducing the prevalence of iron deficiency in the country (2). When, however, the relative bioavailability of radioactive carbonyl iron was measured in human subjects it was very low, varying between 5 and 20% depending on the nature of the meal (52).

The doubts raised by such findings may be resolved over the next few years, since the fortification of flour in Sweden was stopped in 1995. Monitoring the iron status of the population, and especially premenopausal females, should therefore provide a unique opportunity of finally deciding what the impact of Sweden's national program of iron fortification actually was. While no firm data are yet available, there is already some indirect evidence that iron fortification may, indeed, have been exerting an effect. It has recently been shown that subjects with the iron-loading disorder, hereditary hemochromatosis, who have had their increased stores removed by repeated venesections, now reaccumulate iron more slowly (53). Calculations based on their need for maintenance phlebotomies before and after the cessation of iron fortification, suggest that an average of 0.65 mg fortification iron was previously absorbed by these subjects daily (53).

Safety of universal iron fortification

While there is still debate on the relative efficacy of universal fortification programmes, this debate is much less vigorous than the one centering on the safety of such programmes. It has been argued that attempts to reduce the prevalence of iron deficiency in women of fertile age expose iron replete subjects to excessive quantities of iron. In this context, it should be noted that iron deficiency anemia was noted in only 0.2% of adult men in the NHANES II study in the USA (3). At particular potential risk are subjects homozygous for the HFE mutation, which is responsible for the iron-loading disorder, hereditary hemochromatosis (54). It occurs with a prevalence of about 0.3% in Caucasoid populations (55). From what is known of iron balance it seems likely that increasing the quantities of bioavailable iron in the diet, would have two effects. Firstly, subjects destined to present clinically

iron overload due to the pathologic effects of excessive iron deposits would do so at younger ages than they would do otherwise. Secondly, iron fortification, would be expected to cause a proportion of asymptomatic homozygotes with only moderate iron overload to accumulate enough iron to develop clinical symptoms. Recently the debate has widened, with disturbing claims, based on epidemiologic data, that normal subjects with only modestly raised iron stores are at greater risk of developing ischaemic heart disease (56). While such claims have not gone unchallenged (57), the debate does raise issues relating to the desirability of fortification programs which supply increased amounts of iron not only to those who need it but also to those who do not.

As a result of these various doubts concerning universal fortification, increasing attention needs to be focussed on programs that are targeted at the most vulnerable sectors of the population. These include infants, young children and pregnant mothers. To do this effectively needs a multiprolonged approach. As mentioned in an earlier section, infant formulas and cereals can be effectively fortified with iron, with a resulting improvement in iron nutrition. In addition, fortified food items such as cookies and beverages, can be administered as part of school feeding programs (12). Insofar, as women of fertile age are concerned, targeted fortification is obviously not feasible and more effective supplementation programs may have to serve as an alternative. Programs are currently being advocated to extend supplementation beyond pregnancy to include adolescent and young adult females, with the emphasis on intermittent supplementation at schools, clinics and in the work place (13).

IRON FORTIFICATION IN DEVELOPING COUNTRIES

While several developing countries, including Chile, Guyana, Kenya, Zambia and Nigeria require that iron be added to flour (21), logistic problems have largely prevented the development of fortification programs in the majority. There are several reasons for this. Firstly, most potential vehicles are not centrally processed in a number of countries and, as a result, the use of alternative ones has been explored. These have included salt (58), sugar (27,29) and condiments (26,28). The second problem relates to the predominantly cereal diets consumed by many of the poorer populations. They are of low bioavailability insofar as iron is concerned. This means that any fortification iron that is added to such diets is equally poorly absorbed. An equally important constraint has been the low priority that the prevention of iron deficiency has had on the health agendas of many countries. Fortunately, there are signs that this is changing, with an increasing commitment to the development of coordinated programs for the prevention of micronutrient deficiencies, including iron deficiency (59).

Two major fortification strategies have been used in attempts

to prevent iron deficiency in developing countries. The first involves the conventional approach of adding iron to staple cereals and the second is to administer the iron in forms that are less susceptible to inhibitory ligands in the diet. In this latter approach the iron has been given together with enhancers of iron absorption, such as ascorbic acid (41) or sodium hydrogen sulfate (58), or in protected forms, such as hemoglobin (60,61) and NaFeEDTA (30,62,63). As discussed previously, ferrous bis-glycine, which has been shown to be effective in treating iron deficiency anemia, may also fall into this category (36).

Fortification of cereals and dairy products with iron

Fortification of cereals has not been widely applied in developing countries but programs are developing in several countries. Rice has been successfully fortified in the Philippines, using ferrous sulfate as the fortificant (64). It has been shown to be effective in a clinical trial and there are current plans for making iron-enriched rice more widely available (64). In addition, instant noodles, triply-fortified with encapsulated reduced iron, iodide and vitamin A, are being marketed in Thailand (65). Furthermore, milk and dairy products fortified with the chelate, ferrous bis-glycine, are currently available in a number of Latin American countries (35,66) as are a variety of Kellogg's cereal products fortified with NaFeEDTA. Of particular interest is a national program started in 1993 by the Venezuelan health authorities to fortify both precooked maize and wheat flour with ferrous fumarate and vitamins (67). The maize and wheat, which are enriched with 20 mg/kg and 50 mg/kg iron respectively, account for 45% of the total energy consumed daily by the lower economic sector. A preliminary survey after 1 year in groups of children suggested a significant drop in the prevalence of anemia and further follow-up studies should provide valuable data on the efficacy of the program (67).

Role of absorption enhancers in iron fortification

While there must be a number of other countries in which iron fortification of staple cereals could be applied, the nature of the diets consumed in such countries suggests that beneficial effects will be limited unless the effects of inhibitory ligands can be overcome. In this context, it is worth recalling pioneering Chilean studies which showed the beneficial effects on iron nutrition of infant formulae fortified with both iron and ascorbic acid (40). More recently the same group has shown that infant cereals fortified with high levels of elemental iron together with ascorbic acid could be beneficial in preventing iron deficiency (47).

Enhancers of iron absorption have also been used in studies in which salt has been the vehicle. In one study, a stable form of iron, namely iron orthophosphate, was used as a fortificant together with ascorbic acid (68). Refined salt tolerated the addition of this combination moderately well, but even more satisfactory results were obtained in India with a combination of iron orthophosphate and sodium hydrogen sulfate (69). When added to salt (1 mg iron/g salt), the color,

taste, storage properties and bioavailability were all reported to be good; furthermore, the fortified salt proved efficacious in a multicenter trial. Community trials of fortified salt are currently underway (58).

Fortification of cereals with dried bovine hemoglobin

An alternative approach to iron fortification is to use compounds for iron fortification that are themselves less affected by inhibitory ligands in the diet. The two compounds which have received most attention are dried hemoglobin and NaFeEDTA.

Hemoglobin iron is well absorbed because it is not released from the porphyrin ring prior to uptake by mucosal cells and is therefore not affected by the dietary inhibitors which reduce the absorption of nonheme iron (70). Stekel and his coworkers, who used hemoglobin to fortify cookies distributed as part of a school lunch program in Chile, found that the absorption of the heme iron was about 20%, which was equivalent to an absorption of 1 mg iron per day (60). In a further study by Stekel's group, a weaning food composed of extruded rice flour was fortified with 5% bovine hemoglobin (61). The geometric mean absorption of the iron in the hemoglobin was 14.2%, which is severalfold greater than figures previously obtained with various forms of inorganic iron (1). While problems in ensuring the sterility of hemoglobin during collection and storage could lead to problems in gaining approval for its use in some countries, its potential as a fortificant merits further investigation.

Potential role of NaFeEDTA as an iron fortificant

The peculiar advantages in food fortification of the iron chelate NaFe EDTA have been demonstrated in a number of physiologic and clinical studies, (26-30,71-74) and its potential use as a food additive has recently been reviewed in a monograph prepared by the International Nutritional Anemia Consultative Group (75). In this context, it should be noted that Na₂EDTA and Ca Na EDTA have been used by the food industry for a considerable time to protect foods from metal-induced organoleptic changes.

Chemistry

The hexadentate chelate EDTA (ethylene diamine tetraacetic acid) can combine with virtually every metal in the periodic table, with the nature of the metal complex formed depending on such factors as stability constants, molar ratios and pH (12). Binding to iron is favored in the acid environment of the stomach, but in the more alkaline surroundings of the duodenum the iron is exchanged in part for other metals, such as calcium, copper and zinc (75). EDTA acts, therefore, as a shuttle, protecting iron from inhibitory dietary ligands in the stomach, such as phytates and polyphenols, and releasing it in the duodenum, where it is absorbed (75). The absorption of iron from meals fortified with NaFeEDTA is controlled by the same physiologic mechanisms that determine food iron

absorption, with the amounts of iron absorbed being inversely related to body iron stores (30,62,63,71). When NaFeEDTA is added to a meal, less than 5% of the EDTA is absorbed intact as metal complexes, with less than 1% being complexed with iron. Absorbed EDTA complexes are rapidly and completely excreted in the urine (63,72).

Effects on iron absorption

Since iron is less affected by inhibitors when given as NaFeEDTA, its relative bioavailability when fed with meals is between 1.05 and 2.8 times that of ferrous sulfate (17) (Table 2). Its relative bioavailability appears to be greater in meals with a high content of inhibitors (17). NaFeEDTA has another advantage as a fortificant. Within the lumen of the gut the iron dissociates from the EDTA and exchanges completely with the common pool of nonheme iron in the diet (63). As a result, when NaFeEDTA is added as a fortificant to the diet the relative bioavailability of the intrinsic iron is increased to the same degree as is the fortificant iron (30,62,63). It should be noted that a similar enhancement in iron absorption can be obtained if other complexes of EDTA, such as, Na₂EDTA, are administered. For example, when Na₂EDTA and ferrous sulfate were added in equimolar quantities to Egyptian Baladi bread, iron absorption was 5.3% as compared with 2.1% when ferrous sulfate was fed alone (73). The optimal ratio of EDTA to iron appears to be 1:2 (74).

Organoleptic considerations

NaFeEDTA causes many fewer organoleptic problems than most other water soluble compounds and is suitable for fortifying wheat flour, other cereal and legume products and many other foods (12,75). It has also been successfully added to condiments, such as fish sauce and curry powder, and to sugar (26-28). It does, however, cause the sugar to turn slightly yellow and when the sugar is added to tea it discolors it (12). It can, also, cause unwanted color changes when added to certain foods, such as chocolate drink powders and infant cereals containing banana and other fruits (12).

Intervention trials

Thus far, three fortification trials have been carried out using NaFeEDTA. The results in these three studies will be reviewed briefly:

NaEDTA-fortified fish sauce (10-15 mg/day) was provided to a Thai village for 1 year (26). Packed cell volume (PCV) values showed a significant rise as compared with a control village supplied with unfortified fish sauce. The largest mean change of + 4.6 was seen in a sub-group of women who were anemic at the start of the study. This change was calculated to be equivalent to an increase in body iron of about 190 mg, which represented an increase in iron absorption of about 0.5 mg daily (26,75).

TABLE 2
Comparison of the mean absorption of iron from meals fortified with FeSO₄ or NaFeEDTA
in several different studies reviewed by Hallberg (17)

Number of subjects	Amount of iron added (mg)	Absorption (%)		B/A	Reference dose absorption ^a (%)	Type of meal
		FeSO ₄ (A)	NaFe EDTA (B)			
7	2.5	3.5	9.8	2.80	76.8	Milk, rice, sugar formula
21	5.0	3.3	7.4	2.20	51.3	Black bean gruel, corn tortillas, wheat bread, coffee
12	3.0	7.7	18.0	2.30	49.3	Wheat dough
11	3.0	12.4	14.4	1.20	35.2	Sweet manioc
12	5.0	3.5	7.2	2.06	35.3	Maize porridge
18	5.0	3.9	6.3	1.62	43.0	Rice, boiled vegetables, curry
8	2.7	6.6	7.9	1.20	47.8	Wheat-oat meal
10	5.0	5.4	5.7	1.05	35.0	Hamburger, string beans, potatoes
11	3.0	1.6	4.1	2.58	30.8	Egyptian flat bread

a) The percentage absorption of a 3-mg dose of ferrous iron.

b) The results in the last study, (73) which were obtained by adding equimolar amounts of ferrous sulfate and Na₂ EDTA, were reported after Hallberg's review.

NaFeEDTA-fortified sugar (± 4.3 mg/day) was administered to 3 out of 4 Guatemalan communities for 32 months (27,29). All pregnant women and subjects with severe anemia received iron therapy or supplements and were excluded from the analysis. Interpretation of the findings was complicated by certain confounding factors, including differences in the initial iron status of the communities, distribution problems and variations in compliance. Despite these drawbacks, iron stores in the fortified communities increased significantly, except for women aged 18 to 48 years, in one community and greater than 49 years in another. In addition, children in two of the communities showed a significant improvement in hemoglobin concentrations when compared with children in the control community.

NaEDTA-fortified masala (± 7.7 mg/day) was administered for two years in an Indian community living in Durban, South Africa (28). While the prevalence of iron deficiency anemia in Indians was known to be high, they lived in an area where the local black population was iron replete, with a proportion suffering from dietary iron overload. In looking for a suitable food vehicle it was therefore important to identify a dietary component that was consumed by the target population but not

by blacks. Curry powder or masala was found to have a number of advantages. It was universally consumed by the Indian population, most of it was obtained from one supplier and it tolerated well the addition of NaFeEDTA. It provided a further and unexpected dividend. Iron absorption from a typical meal was moderately enhanced in the presence of curry powder, an effect which was probably due to enhanced gastric acid secretion (76).

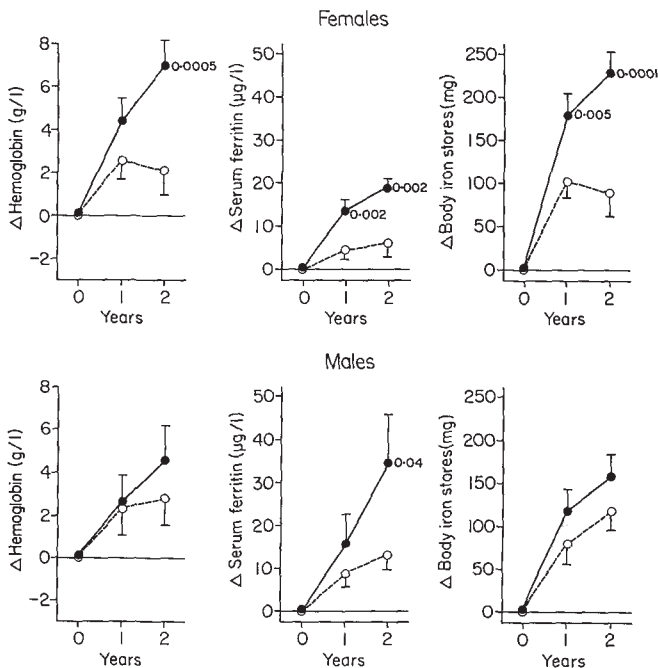
The trial differed from the other two studies in that it was double blinded and was conducted in a single community, with the 263 families randomly assigned to control and test groups, which were matched for iron status. Care was taken to ensure that crossover between groups did not occur and the masala, fortified or unfortified, was distributed directly to each family. In addition to evaluating the usual monitors of improving iron status (increasing hematocrit or hemoglobin and ferritin), an attempt was made to estimate the total body iron in each individual by using a composite of the hemoglobin concentration, percent transferrin saturation, and the serum ferritin concentration (3). This comprehensive index of iron nutrition made it possible to compare subjects with wide variations in iron status and thus to assess both the beneficial and potentially adverse effects of additional iron, i.e.

development of iron overload (28).

A significant improvement in body iron as assessed by the index was detectable in the group of women receiving fortified masala after 1 year of the program (Figure 1). This improvement continued during the second year, when the rise in hemoglobin concentration became significantly greater than that in the control group. The prevalence of iron deficiency dropped dramatically in the women receiving fortified masala. Iron deficiency anemia was detected in 22% of individuals at the start of the study but only in 4.9% after 2 years of fortification. The most significant improvement in iron status was noted in women who entered the trial with iron deficiency and especially in those with anemia. Those with anemia showed an increase in calculated body iron of 505 mg, which is equivalent to the absorption of an additional 0.7 mg iron/day. The latter figure is close to the predicted improvement in iron balance of 0.8 mg/day based on radioisotope absorption studies using NaFeEDTA-fortified masala (76).

FIGURE 1

Changes (Δ) in measurements of iron status after 1 and 2 years of iron fortification in fortified (\ominus) and control (\circ) groups of males and females (mean \pm SE). The probability (one tailed, Student's test) that individual changes were greater in the fortified group than in the control group are also shown (Source: Ballot et al (28); with permission)



In iron replete males the rise in calculated body iron was modest and reached statistical significance only in alcohol abusers receiving fortified masala. This suggests that iron-replete males are unlikely to accumulate excessive amounts of iron under these fortification conditions.

Regulatory issues relating to the use of NaFeEDTA

With so much evidence suggesting that NaFeEDTA is a potentially effective food fortificant, it is important to examine the nature of the constraints that have limited its more widespread use. Based on animal toxicological studies, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) sanctioned in 1974 the use of other salts of EDTA, (CaNa_2EDTA and Na_2EDTA) as food additives up to 2.5 mg/kg body weight/day, with a maximal acceptable daily intake of 150 mg/person/day (77). They are useful as sequestering agents to prevent organoleptic changes in canned products and foods, such as mayonnaise, other sauces and margarine, and are used in many countries throughout the world. In the USA they may be added to 34 different foods and the estimated daily intake is about 15 mg, ten times less than the ADI (75).

There has been some concern that the feeding of EDTA compounds over long periods of time might affect the absorption of other micronutrients, such as zinc and copper. Calcium and magnesium would be unlikely to be affected since the amounts in the diet are, on a molar basis, many fold greater than the amounts of NaFeEDTA that would be used for fortification (75). The actual effects of EDTA compounds on zinc and copper metabolism have been studied in several animal studies and have shown an increase rather than a decrease in absorption and retention when added to diets of low bioavailability (75). Similar conclusions have been reached in a recent study in human subjects in which the effects of NaFeEDTA on the absorption of zinc and calcium were measured (78). It was concluded that the use of NaFeEDTA in populations consuming staple inhibitory diets would not only improve iron but also zinc nutrition. In contrast, calcium metabolism would not be affected. The concern that the administration of NaFeEDTA might increase the absorption of potentially toxic elements, such as lead, mercury, aluminum and manganese has recently been partially addressed. Manganese absorption and excretion were found to be unaffected by NaFeEDTA in human studies (79).

In contrast to the other salts of EDTA, NaFeEDTA was only recently recognised by JECFA as a food additive. Provisional approval was, given by JECFA in 1993 for its use in supervised food fortification programs in populations in which iron-deficiency anemia is endemic (80). At the time, JECFA requested further animal toxicological data and these were subsequently provided by the International Nutritional Anemia Consultative Group. As a result, in 1999 JECFA concluded that NaFeEDTA could be considered safe when used in supervised food fortification programs in response to a need for iron supplementation of the diet of a population as determined by public health officials. Such programs would provide daily iron intakes of approximately 0.2 mg/kg bw (81).

Cost considerations

While NaFeEDTA is currently six times more expensive than ferrous sulfate, it is twice as well absorbed. There is now

a need for food-grade NaFeEDTA to become more widely available and affordable (56). In this context, it seems entirely possible that the cost will drop if there is a large demand for fortification grade NaFeEDTA. Alternative strategies to reduce costs might include the use of ferrous fumarate together with Na₂EDTA in a 2:1 ratio; it is a ratio which has proved effective in radioisotopic absorption studies (73). One final point to be born in mind is the expectation that an effective fortification program would reduce after several years the costs of current supplementation and therapeutic programs for the control of iron deficiency (29).

FINAL COMMENTS

Iron fortification is an important component in any overall strategy to control iron deficiency anemia but before any comprehensive program is developed there should be an understanding of the extent and severity of the problem and its causes. It is a particularly attractive option where the intake of bioavailable iron is low but it should not be seen in isolation but rather as one part of a multipronged approach involving other complementary strategies, such as iron supplementation, dietary modification and the elimination of hookworm infestation. In operational terms, it is essential to identify the particular segments of the population which are to be the major targets of the program and then to choose suitable iron sources and vehicles to reach these targets. For this to be successful, active partnerships must be built up between many sectors, both public and private. In this context, government departments, private industry, the scientific community, the media, non-governmental organisations, consumer groups and donors all have important roles to play (59). In addition, such a coordinated program must be firmly embedded within the primary health care system and must address not only iron deficiency but also other micronutrient deficiencies. The comparative lack of success of fortification programs in many developing countries thus far can be ascribed to a number of factors but a central one has been a lack of commitment on the part of governments and the food industry to deal with the problem of iron deficiency in general and fortification in particular in a coordinated way (59). For success in the future, it would seem necessary for governments in developing countries to mandate fortification and to back this mandate with political will (59).

REFERENCES

- Dallman PR. Progress in the prevention of iron deficiency in infants. *Acta Paediatr Scand* 1990; 365: 28-37.
- Hallberg L. Iron nutrition and food iron fortification. *Semin Hematol* 1982; 19: 41-41.
- Cook JD, Skikne BS, Lynch SR, Reusser ME. Estimates of iron sufficiency in the US population. *Blood* 1986; 68: 726-731.
- Dallman P, Looker AC, Johnson CL, Carroll L. Influence of age on laboratory criteria for the diagnosis of iron deficiency in infants and children. In: L Hallberg, N-G Asp (Eds). *Iron nutrition in health and disease*. 65-74, London, J Libbey, 1996: 65-74.
- De Maeyer E, Adiels-Tegman M. The prevalence of anaemia in the world. *World Health Stat Q* 1985; 38: 302-316.
- MacPhail P, Bothwell TH. The prevalence and causes of nutritional iron deficiency anemia. In: SJ Forman, S Zlotkin (Eds.) *Nutritional anemias*. New York, Raven Press, 1992: 1-12.
- Stoltzfus RJ, Dreyfuss ML, Chwaya HM, Albonico M. Hookworm control as a strategy to prevent iron deficiency. *Nutr Rev* 1997; 55: 223-232.
- Hercberg S, Galan P, Dupin H. Iron deficiency in Africa. *World Rev Nutr Diet* 1987; 54: 201-236.
- Suharno D, West CE, Muhial, et al. Supplementation with vitamin A and iron for nutritional anaemias in pregnant women in West Java. *Lancet* 1993; 342: 1325-1328.
- Van den Broek NR, White SA, Neilson JP. The relationship between asymptomatic human immunodeficiency virus infection and the prevalence and severity of anemia in pregnant Malawian women. *Am J Trop Med Hyg* 1998; 59: 1004-1007.
- Layrisse M, Garcia-Casal MN, Salano L, et al. Vitamin A reduces the inhibition of iron absorption by phytates and polyphenols. *Food Nutr Bull* 1998; 19: 3-5.
- Hurrell RF. Preventing iron deficiency through food fortification. *Nutr Rev* 1997; 55: 210-222.
- Viteri FE. Iron supplementation for the control of iron deficiency in populations at risk. *Nutr Rev* 1997; 195-209.
- Hallberg L, Rossander-Hulten L, Brune M. Prevention of iron deficiency by diet. In: SJ Foman, S Zlotkin (Eds.) *Nutritional anemias*. New York, Raven Press, 1992: 169-181
- Cook JD, Reusser ME. Iron fortification: an update. *Am J Clin Nutr* 1983; 38: 648-659.
- Hurrell RF. Bioavailability of different iron compounds used to fortify formulas and cereals: technological problems. In: A Stekel (Ed.) *Iron nutrition in infancy and childhood*. New York: Raven Press, 1984, 147-148.
- Hallberg L. Factors influencing the efficacy of iron fortification and the selection of fortification vehicles. In: Clydesdale FM, Weiner KL. (Eds.) *Iron fortification of foods*. New York: Academic Press, 1985, 7-28.
- Patrick J Jr. Types of iron fortificants: elemental sources. In: Clydesdale FM, Weiner KL, (Eds.) *Iron fortification of foods*. New York: Academic Press, 1985: 31-38.
- Hurrell RF. Types of iron fortificants: elemental sources. In: Clydesdale FM, Weiner KL, (Eds.) *Iron fortification of foods*. New York: Academic Press, 1985: 39-53.
- MacPhail P, Charlton R, Bothwell TH, Bezwoda WR. Experimental fortificants. In: Clydesdale FM, Weiner KL (Eds.) *Iron fortification of foods*. New York: Academic Press, 1985, 55-71.
- Barrett F, Ranum P. Wheat and blended cereal foods. In: Clydesdale FM, Weiner KL (Eds). *Iron fortification of foods*. New York: Academic Press, 1985: 75-109.
- Bothwell TH, MacPhail P. Prevention of iron deficiency by food fortification. In: Foman S, Zlotkin S (Eds). *Nutritional anemias*. New York, Raven Press, 1992: 183-192.
- Hurrell RF. Prospects of improving the fortification of foods. In: Forman S, Zlotkin S (Eds.) *Nutritional anemias*, New York, Raven Press, 1992: 193-208

24. Bothwell TH. Strategies to prevent iron deficiency in adults. In: Iron nutrition in health and disease. Hallberg L, Asp N-G. (Eds.) London, J Libbey, 1996: 339-348.
25. Hurrell RF, Jacob S. Role of the food industry in iron nutrition: Iron intake from industrial food products. In: Iron nutrition in health and disease. Hallberg L, Asp N-G (Eds.) London, J Libbey, 1996: 339-348.
26. Garby L, Areekul S. Iron supplementation in Thai fish sauce. *Ann Trop Med Parasitol* 1974; 68: 467-476.
27. Viteri FE, Alvares E, Torun B. Prevention of iron deficiency by means of iron fortification of sugar. In: Underwood BA (Ed.) Nutrition intervention strategies in national development. New York, Academic Press, 1983: 287-314.
28. Ballot DE, MacPhail AP, Bothwell TH, Gillooly M, Mayet F. Fortification of curry powder with NaFe(III)EDTA in an iron-deficient population: report of a controlled iron-fortification trial. *Am J Clin Nutr* 1989; 49: 162-169.
29. Viteri FE, Alvarez E, Batres R, Torun B, Pineda A, et al. Fortification of sugar with iron sodium ethylenediamine tetraacetate (FeNaEDTA) improves iron status in semi rural Guatemalan population. *Am J Clin Nutr* 1995; 61: 1153-1163.
30. Layrisse M, Martinez-Torres C. Fe (III)EDTA complex as iron fortification. *Am J Clin Nutr* 1977; 30: 1166-1174.
31. Hallberg L. Bioavailability of dietary iron. 1981; *Annu Rev Nutr* 1: 123-147.
32. Hallberg L, Rossander L, Skanberg A-B. Phytates and the inhibitory effect of iron on iron absorption in man. *Am J Clin Nutr* 1987; 45: 988-996.
33. Gillooly M, Bothwell TH, Torrance JD et al. The effects of organic acids, phytates and polyphenols on the absorption of iron from vegetables. *Br J Nutr* 1983; 49: 331-142.
34. Forbes AL, Adams CE, Amaud MJ et al. Comparison of in-vitro animal and clinical determination of iron bioavailability: International Nutritional Anemia Consultative Group Task Force Report on Iron Bioavailability. *Am J Clin Nutr* 1989; 49: 225-238.
35. Olivarez M, Pizarro F, Pineda O et al. Milk inhibits and ascorbic acid favours ferrous bis-glycine chelate bioavailability in humans. *J Nutr* 1997; 127: 1407-1411.
36. Allen LH. Properties of iron amino acid chelates as iron fortificants for maize. International Conference on Human Nutrition (January 24-25). Abbot Laboratories Inc. 1998; 96-108.
37. Fox TE, Eagles J, Fairweather-Tait SJ. Bioavailability of iron glycine as a fortificant in infant foods. *Am J Clin Nutr* 67: 664-668, 1998.
38. Foman SJ. Reflections on infant feeding in the 1970s and 1980s. *Am J Clin Nutr* 1987; 46: 171-182.
39. Committee on Nutrition Iron-fortified infant formulas. *Pediatrics* 1989; 84: 114-115.
40. Stekel A, Olivares M, Cavazzo M, et al. Prevention of iron deficiency by milk fortification. 11. A field trial with a full-fat acidified milk. *Am J Clin Nutr* 1988; 47: 265-269.
41. Derman DP, Bothwell TH, MacPhail AP, et al. Importance of ascorbic acid in the absorption of iron from infant foods. *Scand J Haematol* 1980; 25: 193-201.
42. Stekel A, Olivares M, Pizarro F, et al. Absorption of fortification iron from milk formulas in infants. *Am J Clin Nutr* 1986; 43: 917-922.
43. Davidsson L, Walczyk T, Morris A, Hurrell RF. Influence of ascorbic acid on iron absorption from an iron-fortified, chocolate-flavored milk drink in Jamaican children. *Am J Clin Nutr* 1998; 67: 873-877.
44. Gillooly M, Torrance JD, Bothwell TH, et al. The relative effect of ascorbic acid on iron absorption from soy-based and milk-based infant formulas. *Am J Clin Nutr* 1984; 40: 522-527.
45. Hertrampf E, Olivares M, Pizarro F, Walter T. High absorption of fortification iron from current infant formulas. *J Pediatr Gastroenterol Nutr* 1998; 4: 425-430.
46. Foman SJ. Bioavailability of supplemental iron in commercially prepared dry infant cereals. *J Pediatr* 1987; 110: 660-661.
47. Walter T, Dallman PR, Pizarro F. Effectiveness of iron-fortified cereal in preventing iron deficiency anemia. *Pediatrics* 1993; 91: 976-982.
48. Hurrell RF, Furniss DE, Burn J et al. Iron fortification of infant cereals: a proposal for the use of ferrous fumarate or ferrous succinate. *Am J Clin Nutr* 1989; 49: 1274-1282.
49. Rivera R, Ruiz R, Hegenauer J et al. Bioavailability of iron- and copper-supplemented milk for Mexican school children. *Am J Clin Nutr* 1982; 32: 1162-1169.
50. Hurrell RF, Reddy MB, Dassenko SA et al. Ferrous fumarate fortification of a chocolate milk powder. *Br J Nutr* 1991; 65: 271-283.
51. Bjorn-Rasmussen E, Hallberg L, Rossander L. Absorption of fortification iron: bioavailability in man of different samples of reduced iron, and prediction of the effects of iron fortification. *Br J Nutr* 1977; 37: 375-388.
52. Hallberg L, Brune M, Rossander L. Low bioavailability of carbonyl iron in man: studies on iron fortification of wheat flour. *Am J Clin Nutr* 1986; 43: 59-67.
53. Olsson KS, Varsanen M, Konar J, Bruce A. The effect of withdrawal of food fortification in Sweden as studied with phlebotomy in subjects with genetic haemochromatosis. *Eur J Clin Nutr* 1997; 51: 782-786.
54. Feder JN, Gnrke, A, Thomas W, et al. A novel MHC class 1-like gene is mutated in patients with hereditary hemochromatosis. *Nature Genet* 1996; 12: 399-408.
55. Bothwell TH, Charlton RW, Motulsky AG. Hemochromatosis. In: CR Scriver, AL Beaudet, WS Sly, D Valle (Eds). The metabolic and molecular bases of inherited disease. New York, McGraw Hill, 2237-2269, 1995.
56. Salonen JT, Nyssnen K, Korpela H et al. High stores iron levels are associated with excess risk of myocardial infarction in Eastern Finnish men. *Circulation* 1992; 86: 803-811.
57. Sempos CT, Looker AC, Gillum RF, Makuc DM. Body iron stores and the risk of coronary heart disease. *N Engl J Med* 1994; 330: 1119-1124.
58. Rao BSN. Fortification of salt with iron and iodine to control anaemia and goitre: Development of a new formula with good stability, and bioavailability of iron and iodine. *Food Nutr Bull* 1994; 15: 32-39.
59. Gillespie S. Major Issues in the Control of Iron Deficiency. The Micronutrient Initiative, UNICEF, 1998; 1-104.
60. Stekel A. Prevention of iron deficiency. In Stekel AM (Ed.) Iron Nutrition in Infancy and Childhood. New York: Raven Press, 1984: 179-194.
61. Calvo E, Hertrampf E, de Pablo S, Stekel AM. Haemoglobin - fortified cereal: an alternative weaning food with high iron bioavailability. *Eur J Clin Nutr* 1989; 43: 237-243.

62. Viteri, FE, Garcia-Ibanez R, Torun B. Sodium iron NaFeEDTA as an iron fortification compound in Central America. Absorption studies. *Am J Clin Nutr* 1978; 32: 961-971.
63. MacPhail AP, Bothwell TH, Torrance JD et al. Factors affecting the absorption of iron from Fe(III)EDTA. *Br J Nutr* 1981; 45: 215-227.
64. Florentino RF, Pedro MRA. Update on rice fortification in the Phillipines. *Food Nutr Bull* 1998; 19: 149-153.
65. Chavasit V, Tontisirin K. Triple fortification of instant noodles in Thailand. *Food Nutr Bull* 1998; 19: 164-167.
66. Iost C, Name JJ, Jeppsen RB, Ashmead HD. Repleting hemoglobin in iron deficiency anemia in young children through liquid milk fortification with bioavailable iron aminoacid chelate. *J Am Coll Nutr* 1998; 17: 187-194.
67. Layrisse M, Chávez JF, Mendez-Castellano H, et al. Early response to the effect of iron fortification in the Venezuelan population. *Am J Clin Nutr* 1996; 64: 903-907.
68. Sayers MH, Lynch SR, Charlton RW, Bothwell TH, Walker RB. The fortification of common salt with ascorbic acid and iron. *Br J Haematol* 1974; 28: 483-495.
69. Working Group on Fortification of Salt with Iron. Use of common salt fortified with iron in the control and prevention of anemia - a collaborative study. *Am J Clin Nutr* 1982; 35: 1442-1451.
70. Bothwell TH, Charlton RW, Cook JD, Finch Ca. Iron metabolism. Oxford, Blackwell Scientific Publications, 1979: 1-576.
71. Martinez-Torres, C, Romano EL, Layrisse M. Fe(III) EDTA complex as iron fortification. Further studies. *Am J Clin Nutr* 1979; 32: 809-816.
72. Candela E, Camacho MV, Martinez-Torres et al. Iron absorption by humans and swine from Fe(III) EDTA. Further studies. *J Nutr* 1984; 114: 2204-2211.
73. El Guindi M, Lynch SR, Cook JD. Iron absorption from fortified flat breads. *Br J Nutr* 1988; 59: 205-213.
74. MacPhail AP, Patel RC, Bothwell TH, Lamparelli RD. EDTA and the absorption of iron from food. *Am J Clin Nutr* 1994; 59: 644-648.
75. Lynch SR, Hurrell RF, Bothwell TH, MacPhail AP. Iron EDTA for food fortification. A Report of the International Anemia Consultative Group (INACG). Washington DC: ILSI-Nutrition Foundation, 1993.
76. Lamparelli RD, MacPhail AP, Bothwell TH et al. Curry powder as a vehicle for iron fortification; effects on absorption. *Am J Clin Nutr* 1987; 46: 335-340.
77. Joint FAO/WHO Expert Committee on Food Additives. Toxicological evaluation of some food additives including anticaking agents, antimicrobials, antioxidants, emulsifiers and thickening agents. World Health Organisation Tech Rep Set No 539, 1974.
78. Davidsson L, Kastenmayer P, Hurrell RF. Sodium iron EDTA (NaFe(III)EDTA) as a food fortificant: the effect on the absorption of zinc and calcium in women. *Am J Clin Nutr* 1994; 60: 231-237.
79. Davidsson L, Almgren A, Hurrell RF. Sodium iron EDTA (NaFe (III) EDTA) as a food fortificant does not influence absorption and urinary excretion of manganese in healthy adults. *J Nutr* 1998; 128: 1139-1143.
80. Joint FAO/WHO Expert Committee on Food Additives. Summary and Conclusions. Forty-first meeting, Geneva 9-18 February, 1993.
81. Joint FAO/WHO Expert Committee on Food Additives. Rome 2-10 June 1999.