

Special Article

Iron and Calcium Bioavailability of Fortified Foods and Dietary Supplements

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Bioavailability is a key consideration when developing strategies for preventing mineral deficiencies through improved dietary supply. Factors that affect the bioavailability of iron and calcium, forms used for fortification and supplementation, and methods used to assess bioavailability are described. Illustrations of the impact of introducing iron-fortified foods in developing and industrialized countries are given, and the alternative approach of supplementation with iron and calcium is discussed.

Key Words: mineral bioavailability, fortification, supplementation, iron, calcium

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Introduction

There is justification for improving the supply of minerals when deficiency disorders are observed in a population, or when higher levels of intake are associated with a beneficial effect, such as prevention of cancer, cardiovascular disease, or osteoporosis. Minerals can be delivered via supplements or fortified food(s). Principles for fortification strategies aimed at reducing public health problems, such as iron deficiency anemia, are well established.¹ Safety is of paramount importance and is dependent on the chemical form of the element and the dose, which may be subject to labeling or legislative constraints. When examining safety, a distinction should be made between effects in the food, such as undesirable organoleptic changes, and adverse physiologic events. Efficacy encompasses the achievable intake of the mineral by the target population in conjunction with its bioavailability, the latter being influenced by a range of dietary and host-related factors.²

Strategies for improving the supply of a mineral and the problems encountered in developing and industrialized countries are inevitably very different. In industri-

alized countries, the addition of minerals to food is carried out for a number of reasons, such as restoration of nutrient levels lost during processing, ensuring nutritional equivalence of products replacing common foods in the diet, or enriching foods with nutrients they do not usually contain or nutrients present at lower levels. The driving force is usually the food industry, but because safe upper limits and potential toxicity for all prospective consumers of the fortified food must be considered,³ fortification is subject to legislative control. In developing countries, the primary driver for improving the dietary supply is public health (combating deficiency) and responsibility usually lies with government agencies.

Having established the need to increase intake of a mineral, a number of important issues must be addressed (Table 1). Iron will be used to illustrate some of the approaches, with particular emphasis on bioavailability because this is probably the overriding determinant of success or failure of strategies to improve iron nutrition through dietary intervention. Table 2 summarizes the most commonly used techniques for assessing the bioavailability of fortification iron and supplements.⁴ The method selected is dependent on subject characteristics (e.g., age, iron status), the mode of administration (fortification or supplements), resources and skills available, time constraints, and the relevance of the study design to policy issues.

Iron Fortification

Iron deficiency is found in infants, children, and women of childbearing age, but very rarely in men. It is partic-

Table 1. Key Issues for Fortification and Supplementation Strategies

- Identification of target population group
- Use of supplement or fortified food
- Selection of appropriate food vehicle
- Form of nutrient and effect of food matrix and other dietary components on bioavailability
- Dose: how much and when?
- Methods for assessing efficacy

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Table 2. Methods Used to Assess Iron Bioavailability

Isotopic methods that can only be used for forms of iron that can be labeled

- Hemoglobin incorporation
- Fecal monitoring (intake minus excretion)
- Plasma appearance (kinetic modeling)

Methods that can be used for all forms of iron

- Hemoglobin repletion
- Plasma appearance (following >5–10 mg oral dose of iron)
- Fecal monitoring (chemical balance)
- Caco-2 in vitro system

ularly common in pregnancy when the demands of the developing fetus exceed the sum of dietary iron and maternal stores. Although supplemental iron is a feasible route for women with iron deficiency, it is less suitable for infants and children. Fortification is therefore a widely used strategy for countries in which iron deficiency is common. Furthermore, the success of supplements depends on compliance and a suitable infrastructure, and supplement use is mainly restricted to industrialized countries.

In developing countries, where monotonous diets are consumed, staple cereals such as wheat, rice, and maize are usually the vehicle of choice for widespread delivery of controlled amounts of iron to the majority of the population.⁵ In Venezuela, mandatory corn and wheat cereal fortification with ferrous fumarate and electrolytic iron has produced positive results, and in the Philippines, efforts are underway to develop an effective and low-cost method of fortifying rice with iron using coating and extrusion techniques.⁶ Salt,⁷ sugar,⁸ and condiments such as curry powder,⁹ fish sauce, and soy sauce⁶ have also been used as vehicles for iron, with varying success. In all cases, it is crucial that the added iron does not cause adverse organoleptic changes in the food. Equally important, but possibly more challenging, is the addition of a bioavailable form of iron.¹⁰ Unfortunately, most well-absorbed forms of iron are often the most reactive in foods, causing lipid peroxidation and color changes. Exceptions to this rule are iron chelates (e.g., sodium iron EDTA) and microencapsulated iron, in which the iron is protected by chemical or physical means.

Dissolution rate in the acid environment of the stomach and intraluminal conditions (e.g., gastric acid production, gastrointestinal motility, and the presence of iron absorption modulators) are important factors determining the bioavailability of iron fortificants and supplements. Once iron is solubilized in the stomach it is available for absorption by the mucosal cells. However, it is essential to recognize that fortification iron entering the common pool in the gastrointestinal tract will only be

as well absorbed as native iron in the diet. A highly bioavailable form of iron such as ferrous sulfate will be affected by the same dietary enhancers and inhibitors as other sources of iron (Table 3), and if added to a high-phytate food, for example, the fortification iron will be poorly absorbed. Diets that induce iron deficiency because of low bioavailability are therefore notoriously difficult to improve through fortification unless sodium iron EDTA is added, as discussed below, or the general composition of the diet is changed to facilitate iron absorption.¹¹

Legislation on the voluntary addition of nutrients to food differs between countries throughout the world. In the United States, compounds must be “generally recognized as safe” before they can be used commercially. In Europe there is no consensus between countries and in order to promote free movement of food products between Member States, the European Parliament (2002) recently published a directive listing approved forms of vitamins and minerals and maximum levels; this directive takes into account safe upper levels, intakes from other dietary sources, and the contribution of individual food products to the overall diet of the population or population subgroups. Approved forms of iron are listed in Table 4.

Iron fortification of wheat flour is practiced in several countries (Table 5), but the efficacy of this particular public health measure is not clear, mainly owing to differences in the form and level of iron used. The statutory addition of iron to all wheat flours except whole-meal was introduced in the United Kingdom in 1953 to restore the concentration to that of 80% extraction flour; the usefulness of this procedure, however, is subjected to regular debate.¹² In Denmark, flour was fortified with carbonyl iron (30 mg/kg) from 1954 to 1987, after which time the legislation to do so was removed. As a result of the cessation of fortification, iron intake significantly fell in the adult female population, yet there was no change in serum ferritin concentration.¹³ This suggests that the iron had low bioavailability¹⁴ and/or that the Danish population was iron-replete, and therefore had no physiologic response to the additional iron; this example illustrates two key considerations in developing a fortification policy.

There is clear evidence of improved iron status in

Table 3. Dietary Non-heme Iron Absorption

Enhanced By	Inhibited By
Iron deficiency	High iron stores
Pregnancy	Achlorhydria
Altitude, hypoxia	Malabsorption
Animal tissue	Phytate
Ascorbic acid	Polyphenols
Organic acids	Calcium

Table 4. Approved Forms of Iron

U.S. Food and Drug Administration GRAS	European Parliament and Council Food Supplements
Ferric phosphate	Ferrous carbonate
Ferric pyrophosphate	Ferric pyrophosphate
Ferric sodium pyrophosphate	Ferric sodium pyrophosphate
Ferrous gluconate	Ferrous gluconate
Ferrous lactate	Ferrous lactate
Ferrous sulfate	Ferrous sulphate
Sodium iron EDTA (non-GRAS)	Ferrous fumarate
Iron amino acid chelates (non-GRAS)	Ferrous citrate
Carbonyl iron (non-GRAS)	Elemental iron (carbonyl + electrolytic + hydrogen reduced)
Reduced iron	Ferric saccharate

GRAS = generally recognized as safe.

U.S. children (aged 6 to 60 months) from low-income families following public health programs such as the Women, Infants, and Children Supplemental Nutrition Program. This involved replacing cow's milk with iron-fortified infant formulas and widespread use of iron-fortified infant cereals and vitamin C-fortified products. The prevalence of anemia in children has declined steadily from 7.8% in 1975 to 2.9% in 1985,¹⁵ and the latest data from the Third National Health and Nutrition Examination Survey show that iron deficiency anemia affects less than 3%.¹⁶

In developing countries, the diet may be high in phytate and polyphenols and low in meat and ascorbic acid, conditions that render iron poorly absorbed. Several years ago, a report of the International Nutritional Anemia Consultative Group suggested that sodium iron EDTA could be used to combat the adverse effects of high-phytate diets.¹⁷ During the process of digestion, iron remains chelated to EDTA until it is transported into the epithelial cells of the small intestine, thereby preventing it from binding to inhibitory substances in the gut lumen that make it unavailable for absorption; this protection, however, is not 100%.¹⁸ At present, the use of sodium iron EDTA as a fortificant in developing countries is not widespread, mainly because of its relatively high cost, and because it has yet to gain GRAS approval. The addition of di-sodium EDTA to iron-fortified foods appears to counteract the inhibitory effects of phytate

and polyphenols, but only when the iron fortificant is soluble, for example, ferrous sulfate; it has no effect on ferric pyrophosphate.¹⁸ The molar ratio of EDTA to iron is also important: a ratio of 1:3 has no effect¹⁹ whereas ratios of 1:2 and 1:1 improve iron absorption.¹⁸

Another form of chelated iron, iron bisglycinate, has been proposed as an alternative to sodium iron EDTA. Both compounds have the advantage of being stable in food matrices and are therefore not likely to cause adverse organoleptic changes. There is evidence to suggest that iron bisglycinate partially dissociates in the gut, releasing iron into the common non-heme pool, from which it is free to react with dietary enhancers and inhibitors. Polyphenols significantly reduced iron absorption from bisglycinate in adults,²⁰ and a high-phytate weaning food reduced absorption in infants.²¹ Efficiency of absorption of iron from bisglycinate is inversely related to iron status, implying that it is absorbed by the same pathway as other forms of non-heme iron.²² The literature on iron bisglycinate suggests that its behavior is dependent on chemistry. Trisglycinate is less well absorbed than bisglycinate²² and gastrointestinal conditions may be an important determinant of bioavailability.

Iron Supplements

A large number of supplements are available commercially; at least 36 brand names are marketed in the United States. The most common forms are ferrous fumarate, ferrous gluconate, ferrous sulfate, ferrous glycine sulfate, and iron polysaccharide, all of which have varying bioavailability, but are generally more bioavailable than slow-release capsules/tablets or multivitamin/multimineral supplements.²³ Doses to prevent deficiency are usually based on daily intakes from the diet (e.g., 10 mg/day); prenatal supplements are much higher (e.g., up to 200 mg/day). There is no clear evidence that supplements have an impact on the iron status of populations,²⁴ and should really only be used as a short-term measure to address iron deficiency anemia in individuals.

Table 5. Iron Fortification of Wheat Flour

Examples of countries with mandatory enrichment

United Kingdom: 16.5 mg/kg

United States: 44 mg/kg

Chile: 30 mg/kg

Mexico: 24 mg/kg

Nigeria: 35 mg/kg

Guyana, Kenya, Zambia: 29–36 mg/kg

Voluntary enrichment

Germany, Holland, Belgium, Spain, Switzerland

Table 6. Dietary Modulators of Calcium Absorption and Excretion

Constituent	Effect	Evidence		
		Strong	Moderate	Weak
Protein	Calciuria	✓		
Sodium	Calciuria	✓		
Caffeine	Calciuria		✓	
Phosphorus	Increases endogenous Ca secretion Decreases urinary Ca losses		✓	
Fructose	Negative Ca balance			✓
Vitamin D	Enhances Ca absorption	✓		
Oxalate	Inhibits Ca absorption	✓		
Phytate	Inhibits Ca absorption	✓		
CPPs*	Enhances Ca absorption			✓
NDOs ⁺	Enhances Ca absorption			✓

* Caseinophosphopeptides.

⁺ Nondigestible oligosaccharides.

Although fractional absorption is inversely related to dose, the absolute amount of iron absorbed increases with higher doses. Repletion is therefore faster with acute high doses of iron but tolerance is less good; there may also be unpleasant side effects, such as nausea, gastrointestinal disturbances, and metallic taste. In addition, large quantities of iron may have an adverse effect on the absorption or metabolism of other micronutrients. Iron is generally absorbed more efficiently in the fasted state but tolerance may be worse than when it is taken with meals, especially in high doses.

The efficacy of less frequent dosing, which avoids the dose-dependent short-term down-regulation of iron absorption at the mucosal cell,²⁵ has been the subject of investigation in trials throughout developing countries.^{26,27} Results have demonstrated that repletion is faster with acute high doses,^{28,29} but compliance is a greater problem because of adverse gastrointestinal side effects.³⁰

Calcium Bioavailability

Dietary data and indirect measures of bone health indicate that calcium bioavailability is important when habitual intakes of calcium are low, especially during periods of bone growth and loss.³¹ There is wide inter-subject variation in the efficiency of calcium absorption, depending on a number of dietary and host-related factors. Dietary constituents reported to affect calcium absorption and/or excretion to varying degrees are shown in Table 6.³²⁻³⁴ Historic trends suggest that the intake of calcium has decreased since the hunter-gatherer diet of the late Paleolithic era. Other significant changes in modern diets include the introduction of dairy products and decreased intake of plant foods.³⁵ These alterations in dietary composition have resulted in a change in the ratio of various nutrients in relation to calcium, including

phosphate, sodium, and potassium; all of these are associated with calcium absorption, metabolism, and excretion, and hence will affect bone health.

The majority of dietary calcium ($\approx 95\%$) is absorbed in the small intestine by an active (vitamin D-dependent) and a passive (vitamin D-independent) mechanism. The latter is a function of the amount of calcium solubilized and intestinal transit time; there is also a small independent colonic component, which is proportionately more important in individuals with low absorption efficiency.³⁶ Calcium absorption and intake are inversely related, declining from 45% at intakes of 200 mg/day to 15% at intakes above 2000 mg/day. In women absorptive efficiency declines by approximately 20 to 25% between age 40 and age 60.³⁷ There is also evidence that the efficiency of absorption may vary according to vitamin D receptor genotype.^{38,39}

Methods to assess calcium bioavailability can be classified into quantitative, qualitative, and surrogate techniques (Table 7). The choice of method depends on the nutritional question to be answered; the advantages and disadvantages of most of these techniques have been discussed in two recently published reviews.^{34,40} Whereas qualitative techniques may be useful to assess the availability from supplements, quantitative techniques, such as the use of radio and stable isotopes, are needed to assess calcium bioavailability from food sources and fortificants. Surrogate measures of bioavailability that reflect the long-term response to dietary calcium bioavailability examine bone mineral mass or density (BMD), but there are certain limitations with the use of BMD or bone biomarkers as endpoints for bioavailability purposes.³¹

Calcium Fortification

Forms of calcium approved for food supplements in Europe⁴¹ are shown in Table 8. Although calcium ab-

Table 7. Methods to Assess Calcium Bioavailability

Outcome Measures	Methods
Absorption: quantitative	Direct Measures: <ul style="list-style-type: none"> ● Chemical balance ● Intestinal lavage technique ● Radio and stable isotope techniques Indirect Measures: <ul style="list-style-type: none"> ● Postprandial serum calcium ● Urinary calcium increment method
Absorption: qualitative	Postprandial parathyroid hormone Strontium
Bone Health (surrogate measure)	Bone Mineral Mass or Density Bone Biomarkers ⁴¹ Ca technique

sorption is less affected by dietary inhibitors than iron, successful fortification is dependent on employing a calcium compound that is well absorbed but does not cause sensory changes to the food vehicle. In the United Kingdom, mandatory fortification of white flour with calcium carbonate contributes on average approximately 14% to total calcium intake.⁴² A study of the dietary sources of calcium in U.K. adolescents concluded that the removal of fortification calcium would result in a fourfold increase in the proportion of subjects below the lower reference nutrient intake in this age group.⁴³

In countries with a high calcium intake from dairy products, fortification will most likely be targeted toward at-risk subgroups, such as adolescents and postmenopausal women. Examples of this approach include the fortification of fruit juices, soft drinks, and breakfast cereals. One recently published placebo-controlled trial investigating the effect of fortified foods on bone mineral mass in prepubertal girls found a significant increase in bone mass with a greater benefit at habitually lower calcium intakes.⁴⁴ Counteracting inhibitors of calcium absorption such as oxalate and phytate in countries with a high dairy product intake may be regarded as less important than increasing the overall intake of calcium. In developing countries, increased calcium intake may be achieved via fortification of staple foods, such as rice and rice products during processing.⁴⁵ Where there is negligible intake of dairy products, however, reducing inhibitors and/or increasing enhancers of calcium absorption may be the most appropriate strategy for improving the dietary supply of calcium for bone metabolism.

Calcium Supplementation

Supplementation with various calcium preparations⁴⁶ is the most widely used approach to increase calcium intake in postmenopausal women. The dissolution time of commercially available calcium tablets varies and may affect the bioavailability of some calcium preparations, in particular, calcium carbonate and calcium phosphate salts.^{47,48} The bioavailability of a calcium salt is not proportionate to its solubility,⁴⁹ however, and calcium-citrate-malate has been shown to be superior in bioavailability to calcium carbonate.^{50,51} When given with a meal, calcium carbonate and calcium phosphate salts are as well absorbed as milk calcium.⁵² Twice daily administration will enhance efficiency of absorption⁵³ and when given in the evening, calcium supplements suppress the nocturnal increase in bone resorption markers and reverse the nocturnal increase in parathyroid hormone (PTH).⁵⁴ However, the effect of reducing circulating PTH on overall bone turnover does need careful consideration and further research, especially in younger age groups. The administration of smaller doses with main meals (low in inhibitors) rather than one large dose with the main meal may reduce the risk of adverse effects

Table 8. Approved Forms of Calcium

U.S. Food and Drug Administration Generally Recognized as Safe	European Parliament and Council Food Supplements
Calcium carbonate	Calcium carbonate
Calcium chloride	Calcium chloride
Calcium citrate	Calcium salts of citric acid
Calcium gluconate	Calcium gluconate
Calcium glycerophosphate	Calcium glycerophosphate
Calcium lactate	Calcium lactate
Calcium phosphate	Calcium salts of orthophosphoric acid
Calcium hydroxide	Calcium hydroxide
Calcium oxide	
Calcium acetate	
Calcium ascorbate	
Calcium caseinate	
Calcium iodate	
Calcium pantothenate	
Calcium peroxide	
Calcium propionate	
Calcium salts of fatty acids	
Calcium silicate	
Calcium stearate	
Calcium stearyl-2-lactylate	
Calcium sulfate	

on iron absorption⁵⁵ but could magnify compliance problems.

The effectiveness of various calcium supplements in decreasing bone loss and fracture incidence has been demonstrated in longitudinal studies in postmenopausal women in the presence or absence of concomitant hormone replacement therapy; the latter was more effective than calcium and/or vitamin D alone.⁵⁶⁻⁵⁸ There is evidence that a combination of calcium and vitamin D is more effective than either vitamin D or calcium alone. Calcium-citrate-malate (500 mg Ca) and vitamin D supplementation (700 IU) for one year slowed bone loss from the femoral neck, spine, and whole body in the elderly (>68 years old), but the benefits to bone health disappeared within 2 years, emphasizing the need for continuous supplementation in this age group.⁵⁹

Wosje and Specker reviewed a number of randomized trials in children and adolescents that reported incremental increases in bone mass and BMD at various skeletal sites as a result of increased calcium intake in the form of supplements or by increased consumption of dairy products.⁶⁰ However, whether this effect persists into adulthood is difficult to determine and subject to debate.^{60,61}

Advantages and disadvantages of various supplement preparations with respect to side effects and certain medical conditions are discussed elsewhere.⁴⁶ The choice of supplement is a neglected area of research in relation to the possible benefits of the accompanying anion. Alkaline anions may help to maintain acid-base equilibrium, which will reduce the risk of increased bone resorption in response to an acidic environment.⁶² Support for this concept can be derived from both epidemiologic data^{63,64} and trials using potassium bicarbonate supplementation.^{65,66} A higher intake of nonphosphate calcium salts was recently suggested to increase the risk of phosphorus insufficiency, which might have implications in the prevention and/or treatment of osteoporosis.⁶⁷ These observations underline the need for further research on the benefit of various calcium salts for long-term therapy.

Calcium-Iron Interactions

Any extended fortification or supplementation program must take into account possible negative interactions with other nutrients. The most debated and well-documented concern is that of the inhibitory effect of calcium on iron absorption. Although there is evidence that calcium from both supplements and dairy products inhibit iron absorption, this finding is not consistent.⁶⁸⁻⁷⁰ Short-term bioavailability studies suggest that the nutritional significance of an adverse effect of calcium supplementation is only evident in individuals with low habitual calcium intake.⁷⁰ Whereas most prospective cohort stud-

ies suggest no impact on iron stores in various age groups, many cross-sectional studies observe a small but significant inverse relationship between iron stores and calcium intake.⁷⁰ To examine the impact of calcium intake on iron status, however, the interaction of various enhancers and inhibitors in meals must be taken into consideration. This is unlikely to be achieved with cross-sectional studies and illustrates a major limitation in interpreting the data. As yet, it is not possible to draw conclusions about the long-term effects of calcium supplementation or fortification in groups also at risk of low iron status, such as children and premenopausal women.

Conclusion

Bioavailability is a key determinant of success in iron fortification programs; identifying forms of iron that do not cause undesirable organoleptic changes in food, but are bioavailable and subject to normal homeostatic control, is a high priority. Appropriate food vehicle selection is equally important, as is the need for sustainability of the program as long as adverse dietary conditions exist. Bioavailability is less critical with iron supplements, which should be considered a short-term measure because of the fewer side effects and better compliance with less frequently administered therapy. The majority of the calcium compounds used for fortification or supplementation are well absorbed in the absence of inhibitors, and nutritional strategies are therefore primarily targeted at increasing the total intake of calcium rather than dealing with bioavailability issues. Increased prevalence of osteoporosis, however, especially in parts of the world where the consumption of dairy foods is minimal or nonexistent, requires economically sustainable approaches. One of the challenges of fortification is the addition of calcium to nonstaple food products at levels that will result in a nutritionally significant increase in calcium.

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