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Bioavailability of iron, vitamin A, zinc, and folic acid when added to condiments and seasonings

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Seasonings and condiments can be candidate vehicles for micronutrient fortification if consumed consistently and if dietary practices ensure bioavailability of the nutrient. In this review, we identify factors that may affect the bioavailability of iron, vitamin A, zinc, and folic acid when added to seasonings and condiments and evaluate their effects on micronutrient status. We take into consideration the chemical and physical properties of different forms of the micronutrients, the influence of the physical and chemical properties of foods and meals to which fortified seasonings and condiments are typically added, and interactions between micronutrients and the physiological and nutritional status of the target population. Bioavailable fortificants of iron have been developed for use in dry or fluid vehicles. For example, sodium iron ethylenediaminetetraacetic acid (NaFeEDTA) and ferrous sulfate with citric acid are options for iron fortification of fish and soy sauce. Furthermore, NaFeEDTA, microencapsulated ferrous fumarate, and micronized elemental iron are potential fortificants in curry powder and salt. Dry forms of retinyl acetate or palmitate are bioavailable fortificants of vitamin A in dry candidate vehicles, but there are no published studies of these fortificants in fluid vehicles. Studies of zinc and folic acid bioavailability in seasonings and condiments are also lacking.

Keywords: seasonings; condiments; bioavailability; iron; zinc; folate; folic acid; vitamin A

Introduction

Food fortification strategies involving the addition of micronutrients are designed to reduce deficiencies within defined populations. Identifying which food to fortify (known as the vehicle) is a multidisciplinary task, as there are many requirements of the candidate vehicle that need to be fulfilled.[1] For example, the vehicle is required to be a food that is regularly consumed by the target population. Spices, herbs, seasonings, and condiments are used with the intention of enhancing the aroma and taste of food and, for this purpose, are widely and regularly consumed. Hence, they are candidate vehicles for micronutrient fortification. There is no overall agreement about the exact definition of a seasoning or condiment, and some candidate vehicles may be regarded as both. We may refer to them as powdered and fluid mixtures of dried aromatic plants or animals, such as bouillon powder and cubes, curry powder, and soy and fish sauce. Salt and monosodium glutamate (MSG) are also used for conserving and flavoring foods. Powdered mixes of vitamins and minerals (sprinkles) added immediately before consumption are generally neutral in taste, but may be used for flavoring if sweetened.

Another crucial requirement is that the fortified micronutrient is made available for use by the body after the vehicle is ingested, a process known as bioavailability. The aim of this review is to assess the bioavailability of iron, vitamin A, zinc, and folic acid when added to condiments and seasonings, taking into consideration the chemical and physical properties of both the foods and the fortificants.

Bioavailability

Despite the given content of a nutrient in a food, the amount finally acquired by the human body depends on a set of variables that come into play
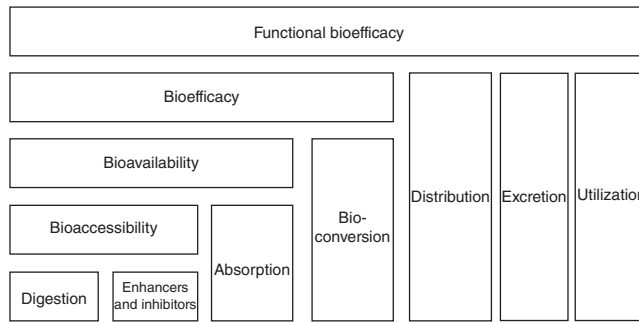
Figure 1. Hierarchical terminology relevant to the concept of bioavailability.

during storage and preparation of the food, digestion and absorption in the gastrointestinal tract, and when the nutrient is inside the host. After ingestion of the meal containing the fortified vehicle, an important determinant of bioavailability is the food matrix, which constitutes the vehicle itself and co-ingested foods. Nutrients can be encapsulated and confined within other components, including plant and human cells and tissues. Before absorption in the small intestine, adequate digestion in the mouth, stomach, and intestines is needed to release the nutrients from encapsulation. Additionally, the food matrix contains other constituents that may bind to or change the chemical structure of the nutrient and thereby influence absorption. As an example, phytate chelates metal ions, such as iron and zinc, forming insoluble complexes in the gastrointestinal tract.[2] The human gastrointestinal tract lacks phytase activity and therefore the phytate-bound minerals will be excreted in the stool. Phytate is primarily found in cereals, legumes, and oil seeds. In most cereals, phytate is concentrated in the bran, resulting in high amounts in whole-grain flour and low amounts in refined products. In legumes, phytate is uniformly distributed throughout the cotyledon, meaning that dehulling actually increases the concentration of phytate.[2] In addition to phytic acid from cereals, polyphenols from chocolate drinks and calcium and casein from dairy also chelate iron and zinc.[3]

Bioavailability can be defined as the proportion or fraction of a nutrient in the diet that is ultimately available for utilization or storage in the body after digestion, absorption, and distribution.[4] In order to address the individual components within this broad encompassing definition, we expanded the terminology with complementary terms (Fig. 1).[5,6] Bioaccessibility is defined as the fraction of an ingested nutrient that is released from the food matrix and made available for absorption in the gut.[6] This term takes into account the effect of digestion, encapsulation, and binding. Bioavailability is defined as the amount of an ingested nutrient that is absorbed, thereby including the steps of bioaccessibility and absorption. When applicable, bioconversion describes the fraction of a bioavailable nutrient that is converted to the active form, and bioefficacy describes the sum of bioavailability and bioconversion.[7] Finally, functional bioefficacy refers to the fraction of an ingested nutrient that performs a certain metabolic function,[8] thereby including factors relevant to bioavailability and bioefficacy, as well as host factors that influence distribution, excretion, and utilization of the nutrient.

Study methodologies

Digestion and encapsulation can be studied *in vitro* by adding commercially available digestive enzymes to the fortified food. A static model can be manipulated to become more dynamic and to mimic physical and mechanical processes that occur during digestion.[6] Absorption of micronutrients occurs by passive diffusion or active transport involving membrane protein interaction. Passive transport can be studied using a synthetic membrane and the principles of dialysis, suitable for minerals. Active transport can be studied using dissected intestines from animals or through the use of cell cultures.[6] The Ussing chamber system measures the absorption of micronutrients across the epithelial layer of dissected intestines and is considered an *ex vivo* method.[9] Absorption can also be assessed *in vitro* using the polarized human colon carcinoma cell line (Caco-2 cells).[6] An advantage of *in vitro* and *ex vivo* models is that they can be readily used to study the effects of chelating agents and enhancers

Table 1. Outcomes used in human studies to assess bioavailability, bioefficacy, and functional bioefficacy of iron, vitamin A, zinc, and folic acid

Fortificant	Bioavailability and bioefficacy	Functional bioefficacy
Iron	Hemoglobin (Hb), serum ferritin (SF), serum transferrin receptor (STfR), zinc protoporhyrin, erythrocyte incorporation from stable isotope studies, total body iron calculated from biomarkers	Prevalence of anemia based on cutoffs from biomarkers Children: growth, height for age, weight for age
Vitamin A	Serum and breast milk retinol, retinol-binding protein (RBP), retinol:RBP ratio, RBP:transthyretin ratio, dose–response test, retinol isotope dilution	Bilot's spots, healing of xerotic lesions, dark adaption, histology of ocular epithelium
Zinc	Plasma, erythrocyte, lymphocyte, neutrophil, hair and urinary zinc, plasma metallothionein, alkaline phosphatase	Children: height for age, linear growth, diarrheal episodes, dermatitis, infections
Folate	Serum folate, red blood cell folate, serum homocysteine, macrocytic red blood cells, mean corpuscular volume	Anemia

in the food matrix on the dialysability or absorption of a micronutrient. Estimates of bioavailability can be assessed by combining studies of digestion and absorption.

In humans, bioavailability can be studied using different methods, such as the balance method, where the ingested amount of a nutrient is compared to the amount excreted during the hours and days after ingestion. This method requires extensive collection and measurement of relevant metabolites in sources of excretion. Another method of studying bioavailability in humans is to measure the responsiveness of a biochemical marker to dietary intake, in which the validity depends on the responsiveness of the marker throughout the continuum of dietary status. A drawback of both of these methods is that they do not allow researchers to distinguish nutrients in the experimental diet from endogenous nutrients present in the body. This issue can be resolved with the use of tracer technology,[5] where atoms in the micronutrient molecule are replaced with radioactive isotopes or stable isotopes with a different molecular mass. The traced micronutrients are then measured separately, resulting in accurate quantification of bioavailability or bioefficacy.

Distribution and storage in humans are often assessed with the use of biochemical markers in blood, while most direct measures of storage tissues, such as liver or adipose tissue biopsies, are mainly performed in animal models because of ethical considerations. Animal studies have shown that two fortificants of the same micronutrient can be distributed and stored differently and that

a single biochemical marker may underestimate bioavailability.[10] Functional bioefficacy can be measured by determining the rate by which deficiency symptoms are cured, changes in appropriate biochemical markers, weight gain and growth, or by using other established measures specific to the nutrient's function.[4] Under normal circumstances the functional measures correspond to bioavailability, but the relationship may be influenced by host factors. Early clinical observations and animal studies showed that symptoms of deficiency occurred more rapidly during infections, independent of dietary intake.[11] Infections or other inflammation may cause sequestration of the bioavailable micronutrient[12] or undesirable excretion from the kidneys,[11] thereby reducing delivery to target cells. A summary of outcomes used in human studies to assess bioavailability, bioefficacy, and functional bioefficacy of iron, vitamin A, zinc, and folic acid is provided (Table 1).

Iron

Fortificants
Fortificants of iron can be categorized on the basis of their water solubility and properties, as these influence how well the fortificants dissolve in gastric juice, which, in most cases, is a prerequisite to absorption.[13] Freely water-soluble fortificants, such as ferrous sulfate, are considered to be the most bioavailable, but also cause the most organoleptic problems, such as precipitation and changes in color and flavor. Ferrous fumarate is poorly water soluble, but still dissolves adequately in gastric juice and is almost as bioavailable as the freely water-

soluble fortificants. A reduction in gastric acid secretion (e.g., from mucosal atrophy from protein energy malnutrition[14,15] or bacterial-induced gastritis[16]) may therefore reduce bioavailability. Ferric pyrophosphate and elemental iron powders are insoluble in water and poorly soluble in gastric juice, and thereby less bioavailable. However, particle-size reduction results in more bioavailable candidates.[17] In order to be absorbed,[3] the iron metal must be present in the ferrous (Fe^{2+}) form. Iron absorption is enhanced by reducing agents, such as ascorbic acid, which facilitates the conversion of ferric (Fe^{3+}) to ferrous iron. Ascorbic acid not only enhances iron absorption per se, but also balances the negative effects of inhibitors, such as phytate, by acting as a competitive chelating agent. Ascorbic acid chelates iron in the stomach, but in contrast to phytate-bound iron, the iron bound to ascorbic acid is made soluble and absorbable in the duodenum. Ethylenediaminetetraacetic acid (EDTA) is also a chelator that binds various metals and thereby reduces the percentage of iron compounds bound to inhibitors.[13] Sodium iron EDTA (NaFeEDTA) has been demonstrated to have superior iron bioavailability when added to foods with a high content of phytic acids, such as cereals and legume grains.[18] However, it is unclear how iron bound to NaFeEDTA enters the intestinal wall.[19] Depending on the pH in the medium, as well as the molar ratio of EDTA and the metal, the affinity to different metals can vary during digestion. Iron can also be encapsulated by a wide range of methods in order to prevent it from reacting with other components in the vehicle during storage. Encapsulation has been widely used in attempts to fortify salt with multiple nutrients.[20–22]

Outcomes

Bioavailability and bioefficacy of iron can be assessed by measuring hemoglobin, serum ferritin, the serum transferrin receptor, erythrocyte zinc protoporphyrin, or a combination, and functional bioefficacy can be assessed by measuring changes in the frequency of iron-deficient anemia and growth rates in children.[1] Absorption of iron is regulated by the iron stores of the host, and hemoglobin is therefore a more responsive marker in deficient than sufficient subjects.[23] Hookworm infections can severely reduce the amount of bioaccessible iron, and deworming should precede studies related to fortification in relevant areas.[1]

Bioavailability in dry vehicles

In vitro bioavailability studies indicate that many spices and herbs contain relatively large amounts of polyphenols and phytic acid that may inhibit iron absorption and dictate which fortificant is more appropriate, while other spices and herbs contain ascorbic acid or tartaric acid, which may counterbalance some of the inhibitory effect.[24] It was shown that ferrous sulfate and NaFeEDTA iron were equally bioavailable in curry powder containing 27 mg (676 mg/100 g) phytate and 13 mg (336 mg/100 g) polyphenols per serving, as assessed by measuring intracellular ferritin concentration in Caco-2 cells after inoculation of samples that underwent *in vitro* digestion with pepsin and pancreatin–bile extract.[18] Despite equal performance in a cell model, only NaFeEDTA has been evaluated in humans. In a double-blind controlled trial, 264 South African families were randomized to curry powder fortified with chelated NaFeEDTA or placebo.[25] In women in the fortified group, mean hemoglobin concentrations increased from 125 ± 14 to 133 ± 11 g/L and serum ferritin from 12 (minimum: 4; maximum: 42) to 27 (minimum: 10; maximum: 73) μg/L. In men in the fortified group, serum ferritin, but not hemoglobin concentrations, increased. Stratified analysis showed that the increase in hemoglobin concentrations was profound in women with low iron status before the intervention ($\Delta\ 19 \pm 2.4$ g/L), but not in those with normal concentrations ($\Delta\ 0 \pm 1.3$ g/L). Mean serum ferritin increased throughout the continuum of initial iron status. On the basis of hemoglobin concentrations, serum ferritin, and a daily intake of 5.5 g masala, bioavailability was approximately 9% in women with low initial iron stores and approximately 3% in women in the fortified group altogether. Fortification did not interfere with zinc concentrations.

In a randomized placebo-controlled trial (RCT) lasting 31 weeks in Thailand, a multiple micronutrient-fortified seasoning powder was investigated in iron-replete children (aged 5–13 years; frequency of serum ferritin <12 μg/L: 4%).[26,27] The powder was administered through school lunches 5 days/week and contained MSG, salt, sugar, hydrolyzed vegetable protein, and dried meat powder. Hydrogen-reduced elemental iron was used as a fortificant and each serving provided 5 mg, equivalent to one-third of the recommended daily intake (RDI). There were no differences in hemoglobin

concentrations (baseline >118 g/L), serum ferritin (baseline >46 μg/L), mean red blood cell volume (baseline >75 fL), or in anthropometric measurements between the fortification and non-fortification groups. The fortification group experienced a lower incidence of respiratory-related illnesses and symptoms of runny nose, cough, and diarrhea, and improved cognitive function as measured by visual recall.

A tracer study investigated the bioavailability of vacuum-dried iron compounds in chocolate drink powder, with the use of radioactive iron tracers.[28] The bioavailability of iron compounds did not differ when added directly to prepared chocolate drinks, but when added before manufacturing and preparation of the drink, ferrous fumarate (5.27%) was twice and 10 times as bioavailable as ferrous sulfate (2.62%) and ferric pyrophosphate (0.55%), respectively.

Bioavailability in fluid vehicles

Bioavailability and functional bioefficacy of NaFeEDTA added to soy and fish sauces have been studied in parallel in different Asian countries, using a similar approach starting with a tracer study, followed by a controlled feeding study in iron-deficient individuals and, finally, by a study in community-dwelling individuals representing the target population. As a fortificant in fish sauce, NaFeEDTA was tested during the 1970s with promising results with respect to bioavailability of the added iron and organoleptic changes.[29,30] However, NaFeEDTA was not approved for use in monitored food fortification programs by the Joint Food and Agriculture Organization (FAO)/World Health Organization (WHO) Expert Committee on Food Additives until 1999.[31] Ferrous sulfate was also shown to be bioavailable when added to soy sauce, but it precipitated and caused organoleptic changes to the extent that it was disqualified as a fortificant.[32]

Soy sauce has been assessed as a potential vehicle for iron fortification in China. An initial stable isotope tracer study aimed to accurately assess the bioavailability of NaFeEDTA added to soy sauce and compare it to ferrous sulfate. According to apparently identical studies (one only available in Chinese), absorption was 10.5% from NaFeEDTA and 4.7% from ferrous sulfate.[33,34] The following studies evaluated the bioavailability and functional bioefficacy in populations with low iron

status and in the general Chinese population. The first study, published in 2002, screened students older than 15 years of age for anemia and randomized the anemic students to receive a school lunch for 3 months with 5 mL soy sauce that was either unfortified or fortified with 1 or 4 mg/mL iron.[32] When compared to the unfortified group, the fortified groups showed significant improvements in all relevant biomarkers of iron bioavailability, including the concentration of hemoglobin, serum ferritin, serum iron, serum transferrin, serum-free erythrocytic porphyrin, and total iron-binding capacity. The results were comparable between subjects who received 5 and 20 mg/day of iron. In the second study,[33] published in 2005, the authors investigated the effect of fortification in a free-living Chinese population of about 14,000 residents randomized to receive either soy sauce fortified with NaFeEDTA or unfortified soy sauce, free of charge for 18 months. Biomarkers of bioavailability and measures of functional bioefficacy were assessed and the results stratified according to age and gender. The average consumption of soy sauce during the trial was 16.4 mL in the intervention group. The fortified sauce contained 29.6 mg/100 mL iron, and the contribution to daily iron intake accumulated to approximately 4.9 mg. The diets of the participants were predominantly composed of cereals, fruits, and vegetables; only 5% animal foods; and consequently high in phytic acid and polyphenols. For most age and gender groups, fortification led to significantly higher and sustained concentrations of hemoglobin and serum ferritin from 6 months and throughout the 18 months of the study. Also, the change in weight for age was higher among children between 3 and 6 years of age in the fortified group than in the control group. On the basis of these studies, the Chinese government approved and implemented the fortification of soy sauce with NaFeEDTA in the concentration range of 175–210 mg/100 mL, equivalent to approximately 4.1 mg iron per serving (16.4 mL) of soy sauce.[35]

In studies from Vietnam, the bioavailability of NaFeEDTA added to soy and fish sauces was assessed using stable isotope tracers, with ferrous sulfate as a reference.[31] The absorption varied between approximately 3% and 6% in fish and soy sauces, but no differences were observed between NaFeEDTA and ferrous sulfate. The authors also assessed whether unfortified soy and fish sauces per se could affect the

absorption of iron in a meal and reported that soy sauce reduced the absorption of iron in a meal significantly. Overall, the bioavailability of NaFeEDTA in fish sauce was considered adequate, and bioavailability and functional bioefficacy were tested in anemic women who were provided controlled servings at the factory where they worked.[36] For 6 days/week for 6 months, these women were served a mid-morning snack with rice or noodles and randomized to 10 mL fish sauce that was either unfortified or fortified with 1 mg/mL iron. Fortification improved hemoglobin concentrations (from 111 to 116 g/L) and serum ferritin (14 to 31 μg/L) and reduced levels of serum transferrin receptors (from 10 to 7 mg/L). Another study tested bioavailability and bioefficacy in free-living individuals from rural villages who were randomized to receive fish sauce that was unfortified or fortified with 0.5 mg iron per milliliter of fish sauce.[37] The study lasted for 18 months, and fortification led to improvement in hemoglobin concentrations (126–131 g/L) and serum ferritin (30–66 μg/L), but there were no effects on anthropometrical measurements.

In studies from Thailand and Cambodia, bioavailability of water-soluble iron fortificants was assessed in fish sauce after discovering that the addition of citric acid improved organoleptic qualities without affecting sensory qualities.[38] In a stable isotope tracer study conducted in Thailand and published in 2005,[39] the absorption of ferrous sulfate was shown to be 12–14% and approximately 50–100% higher than ferric ammonium citrate and ferrous lactate when added to fish sauce with citric acid. The next step was to assess the bioavailability and functional bioefficacy of ferrous sulfate in anemic individuals under controlled feeding conditions. It was also necessary to compare ferrous sulfate to NaFeEDTA, which was now considered to be the established fortificant on the basis of the findings from studies from China and Vietnam. Therefore, a comparative intervention trial screened 6- to 21-year-old Cambodian school children for anemia and randomized those who were anemic to receive 10 mL Khmer fish sauce that was unfortified or fortified with either 1 mg/mL of ferrous sulfate or NaFeEDTA.[40] Fish sauce was given 6 days/week for 6 months together with a mixed meal. Both fortificants were shown to improve hemoglobin and serum ferritin concentrations compared to placebo, but they did not differ from each other.

Fortification did not affect markers of functional bioefficacy, including the prevalence of infectious diseases and diarrhea, or anthropometric markers.

Bioavailability of iron in dual-fortified salt with iodine

A long-sought goal has been to fortify salt with both iodine and iron, but nutrient instability, unacceptable organoleptic changes, and low bioavailability have slowed the process. As a result of innovative and persistent work, different fortificants have been produced from different production strategies. The Indian National Institute of Nutrition developed a dual-fortified salt (DFS) with ferrous sulfate and potassium iodide, and sodium hexametaphosphate to stabilize the two nutrients.[41] Another strategy was to encapsulate either iron or iodine to create a barrier between the nutrients and the impurities, which may increase stability and reduce organoleptic changes during storage.[20] With respect to bioavailability measured by hemoglobin and other biochemical measures, encapsulation of iron resulted in adequate iron bioavailability, but also unacceptable organoleptic changes.[42–45] One of the few candidate fortificants of DFS was ferric pyrophosphate, but its use has been limited by inferior bioavailability. However, parallel work demonstrated that particle-size reduction of ferric pyrophosphate could enhance bioavailability.[46] Specifically, the effect of particle-size reduction of ferric pyrophosphate appeared to be inversely related to bioavailability when studied in rats, with relative bioavailability to ferrous sulfate of 59%, 69%, and 95% for regular (21 μm), reduced (2.5 μm), and micronized, emulsified ferric pyrophosphate (0.5 μm), respectively.[17] Field trials in iron-deficient individuals confirmed that this fortificant was bioavailable.[47,48] Furthermore, in a 2008 study, the bioavailability of a fortificant with reduced particle size was compared to an encapsulated fortificant as well as to plain salt iodization in iron-deficient Indian schoolchildren. They received either iodized salt or DFS with 2 mg Fe/g salt of microencapsulated ferrous fumarate (EFF) developed by the Micronutrient Initiative[20] or micronized ground ferric pyrophosphate (MGFePP) (2.5 μM).[49] With respect to bioavailability, both fortificants performed equally well, with bioavailability estimated to be 0.9% for MGFePP and 1.1% for EFF. However, the loss of

iodine (86%) from DFS with MGFePP was unacceptable in comparison to EFF and iodized salt (∼20%). For some local foods, EFF-fortified salt caused organoleptic changes that prevented consumption. Efforts have been made to continue to reduce the organoleptic changes of EFF by modifying the production method.[50] The bioavailability of EFF as measured by serum ferritin and hemoglobin was also recently confirmed in female workers in India.[51] In addition, a review of the literature concluded that DFS with iodine and iron results in improved iron status as measured by hemoglobin.[52] Salt is also under investigation as a candidate for triple and multiple fortification with iron and iodine together with vitamin A[53,54] and folic acid.[55]

Vitamin A

Fortificants

Vitamin A is acquired in the form of retinol after bioconversion of provitamin A carotenoids from dietary vegetables or preformed vitamin A (i.e., retinyl esters) from animal sources. Retinol itself is unstable and not suitable for fortification.[1] Carotenoids have low bioefficacy, with the exception of β-carotene, which may yield 1 µg of retinol per 2–3 µg dietary β-carotene.[7] Retinol esters of palmitic acid (retinyl palmitate) and acetic acid (retinyl acetate) are stable and highly bioavailable when added as forticants and have been the preferred choice in different vehicles.[56,57] β-Carotene has been used as a fortificant in margarine and oils, but may not be the most optimal alternative in vehicles ingested in small amounts, such as condiments and seasonings.

Two fortificant options of retinyl esters are commercially available.[1] First, a fat-soluble substance, retinyl esters can be added directly to fat-based and oily foods, such as margarines, or emulsified in water-based solutions, such as milk. The second fortification option is as a dried compound coated with gelatin, gum, or starch that can be added to dry vehicles or, if required, designed to dissolve in cold water and used in fluid vehicles. Fortificants of vitamin A also contain added antioxidants to increase stability. The bioavailability of retinyl acetate in oil in healthy individuals is approximately 90%.[57,58] Retinyl palmitate has been the ester most frequently used in fortification programs, both as an oil fortificant in margarine and

oils and as a dry fortificant in cereal products, flour, and sugar.[1]

Outcomes

Functional bioefficacy of vitamin A can be assessed by investigating clinical signs of deficiency, including visual or ocular indicators.[59] Bioefficacy can be assessed using blood retinol concentration; however, circulating retinol is homeostatically controlled by the liver, which is the main storage organ (Fig. 2). Serum retinol is responsive when liver stores are very low (below approximately 0.07 µmol/g (20 µg/g), and very high (above approximately 1.05 µmol/g (200 µg/g)).[60,61] Hence, a valid assessment of vitamin A fortification is limited to populations with a high prevalence of vitamin A deficiency.

Plasma retinol concentrations decrease by as much as 50% during infections[62,63] and systemic inflammation.[12] The reduction is caused by increased urinary excretion, which is proportional to the severity of the disease,[64,65] and it has been estimated that 10 days of illness can reduce liver stores by 18% (6 µmol) in children.[65] However, low retinol concentrations are also caused by a reduction of plasma carrier proteins, including retinol-binding protein and prealbumin, which may be lowered due to either systemic inflammation or macronutrient deficiency. Resolution of the cause of inflammation[66] or re-feeding children suffering from kwashiorkor with a standard skim milk diet low in vitamin A[67] resulted in partial restoration of serum retinol concentrations.

Bioavailability in dry vehicles

Bioavailability and functional bioefficacy of dry retinyl palmitate fortified to MSG was demonstrated in children in the Philippines[68] and Indonesia.[69,70] The Philippine study lasted 2 years and intervened with packets of MSG (2.2 g each) containing 0.1 g dry retinyl palmitate embedded in silicon dioxide.[71] Mean serum retinol concentrations in the children (*n* = 387) increased from 1.01 ± 0.60 to 1.37 ± 0.71 µmol/L, and the prevalence of clinical signs of xerophthalmia was reduced from 4.2% to 1.0%. Improvements were restricted to those with a low vitamin A status at baseline. In the Indonesian study, which lasted 11 months and intervened with MSG containing dry vitamin A palmitate coated with gelatin, the content of vitamin A was intended to provide 50% of the RDI to children in rural villages. Two publications reported on bioavailability[69]
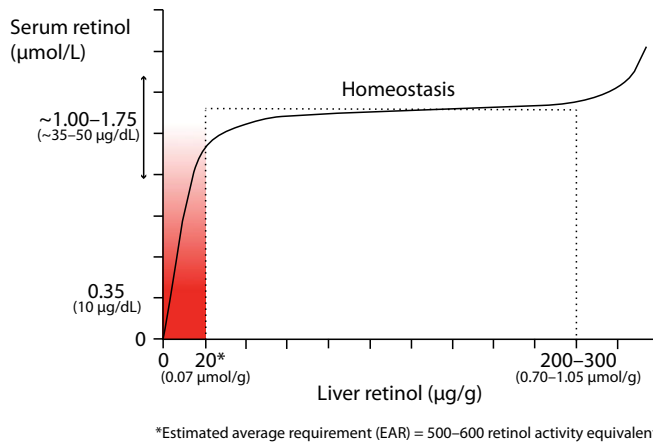
Figure 2. Functional relationship between liver and serum concentrations of retinol.

and functional bioefficacy.[70] Retinol concentrations increased from 0.67 ± 0.33 to 0.92 ± 0.33 µmol/L in serum from children and from 0.60 ± 0.29 to 0.67 ± 0.30 µmol/L in breast milk of mothers from villages receiving fortified food, while no changes occurred in control villages. Mean hemoglobin concentrations increased from 113 ± 16 to 123 ± 16 g/L in the children. Functional markers showed that the prevalence of xerophthalmia, as measured by Bitot's spots, was reduced, and linear growth and survival improved.

The importance of baseline vitamin A status in the ability to evaluate the efficacy of vitamin A fortification was demonstrated by two studies from Thailand and India. A seasoning powder fortified with multiple micronutrients including dry retinyl palmitate was evaluated in a 31-week RCT in Thailand of relatively well-nourished children aged 5.5 to 13.4 years.[26,27] Baseline retinol and hemoglobin concentrations were >1.3 µmol/L and >118 g/L in both groups, and the prevalences of vitamin A deficiency and suboptimal status were approximately 3% and 19%, respectively. The seasoning powder was administrated through school lunches 5 days/week and contained MSG, salt, sugar, hydrolyzed vegetable protein, and dried meat powder, and each serving provided 270 µg of retinol equivalents, aimed to provide one-third of the RDI. It was found that fortification did not affect serum retinol concentrations, prevalence of vitamin A deficiency, or hemoglobin concentrations when compared to the nonfortified group. The Indian study investigated multiple-micronutrient fortification of

salt in children, by intervening in the school kitchen for 1 year.[72] One gram of salt contained 300 IU (100 µg) dry retinyl acetate and other micronutrients, and the average consumption was 10 g/day per child, equivalent to a daily dietary intake of 3000 IU of vitamin A from fortified salt. Baseline and follow-up serum retinol concentrations were 1.27 ± 0.44 and 1.45 ± 0.52 µmol/L in the experimental group and 1.49 ± 0.45 to 1.61 ± 0.65 µmol/L in the control group, with no between-group differences.

In a 6-month intervention study in Chinese preschool children, multiple-micronutrient fortification with three seasoning powders was investigated, in which there were 2.5 g per packet containing either vitamin A acetate (500 µg), vitamin A and ferric sodium edetate (12 mg), or vitamin A and iron with additional folic acid (0.2 mg); zinc oxide (12 mg); thiamine; riboflavin; niacinamide; and calcium. The seasoning was added to foods served at the children's nursery, such as porridge, bean milk, soups, or noodles after cooking;[73] thus, it was used as a micronutrient powder (MNP), which was the focus of recent reviews.[74,75] The study showed that serum retinol concentrations remained constant or increased among the three groups. Anthropometric markers also improved, indicating that vitamin A acetate alone or in combination with other micronutrients was able to improve growth-related functions in preschool children.[76]

Bioavailability in sugar
In one of three studies that investigated fortification of sugar with vitamin A, fortification of sugar in

Guatemala resulted in an increase in dietary intake of vitamin A as well as increased serum concentrations of the combined measurement of retinol and retinol carrier protein after 2 years.[77] Those with the lowest vitamin A status benefited the most from fortification. Another study in Guatemala investigated the effect of iron and vitamin A fortification of sugar and found that double-fortified sugar was homogeneous and stable for several months, but no relevant outcomes for vitamin A were reported.[78] Lastly, in a study from Nicaragua,[79] consumption of fortified sugar for 1 year resulted in an increase in total body and liver vitamin A content and a 20% increase in plasma retinol in the 21 children studied.

Zinc

Fortificants

The zinc compounds listed as safe by the U.S. Department of Agriculture are zinc sulfate, chloride, gluconate, oxide, and stearate, with zinc oxide being the least expensive and most commonly used by the food industry;[80,81] however, concerns have been raised about its bioavailability because of its near insolubility at neutral pH. However, three studies have shown no difference in zinc absorption from foods fortified with zinc oxide compared with the same level of fortification using zinc sulfate either in children or adults.[82–84] Increased solubility in gastric acid can explain this similarity.[85] Zinc oxide used for fortification is a fine-particle, light-grade compound that mixes well with flour without clumping.[81] Particle-size reduction to increase bioavailability is an alternative for zinc fortificants.[86] In addition, the metal-chelating compound EDTA has been shown to enhance absorption of zinc from foods fortified with zinc and EDTA in some, but not all, studies.[84,87,88]

The most important dietary factor determining the bioavailability of zinc is phytate or phytic acid.[89] The effect of dietary phytate on zinc absorption is dose dependent. Therefore, the phytate content of staple foods to which condiments and seasonings are added is the main determinant of the bioavailability of zinc. The International Zinc Nutrition Consultative Group (IZiNCG) estimates zinc absorption to be 26% for men and 34% for women on mixed and refined vegetarian diets with phytate-to-zinc molar ratios of 4:18, but only 18% for men and 25% for women on diets with ratios higher than 18.[90]

Outcomes

At the population level, serum or plasma zinc is the best available biomarker of zinc bioavailability: it reflects dietary zinc intake, the response to zinc supplementation is consistent, and reference data exist for most age and sex groups.[91] At the individual level, serum zinc concentration is not a reliable indicator because of factors that independently affect serum zinc concentrations, such as infection and muscle loss. Zinc is involved in RNA, DNA, and protein synthesis, as well as cellular division, differentiation, and growth.[92] Zinc status is therefore relevant to the immune system, which depends on cells with rapid turnover and proliferation demands, and important during developmental periods of rapid growth. Hence, the incidence of infectious diseases, such as diarrhea, and linear growth are potential markers of functional bioefficacy.[26]

Bioavailability in dry vehicles

Since there is only one published study examining the bioavailability of zinc from zinc-fortified condiments and seasonings, we also draw from the literature on MNPs and zinc-fortified flour and porridge. Several aspects of bioavailability of zinc from zinc-fortified foods have been reviewed by Hess and Brown[93] in more detail.

In a previously mentioned RCT in 569 Thai children aged 5.5–13 years,[26,27] children were randomized to receive school lunches with either an unfortified or fortified seasoning powder containing zinc, iron, iodine, and vitamin A for 7.5 months. Children assigned to the fortified seasoning powder had a significantly lower risk of developing zinc deficiency defined by serum zinc[27] and a lower incidence of individual symptoms of diarrhea and respiratory symptoms compared to children in the unfortified group.[26] Of the nutrients included in the micronutrient formulation, the evidence that zinc has an effect on the incidence of both diarrhea and respiratory infections is much stronger than the evidence available for the other nutrients included in the formulation.[94–96]

We are aware of four trials of home fortification with zinc-containing MNPs versus placebo. Only one study found a significant effect on serum zinc concentration, and none found any effects on growth.[97–100] Among studies of zinc fortification of cereal products, such as bread and porridge,

few studies found a beneficial effect on plasma or serum zinc and growth, and one study reported an effect on the incidence of diarrhea.[93] These inconsistent results may be due to differences in pre-existing zinc nutritional status, growth, and burden of infections.[93]

Folic acid

Fortificants

Folate fortification uses folic acid that differs from naturally occurring folates in the chain length of glutamates and in oxidation status, but is relatively stable as a fortificant. Methylated forms ((6S)-5-methyltetrahydrofolate) have also been used in clinical trials, but because of the high cost and lower stability, this is not a widely used option in folic acid fortification. It has been suggested that oxidized folic acid is absorbed differently from food folates, thus making direct comparisons of folic acid with reduced folates from food difficult.[101,102] Folic acid from supplements and fortified food has, in general, a higher bioavailability than natural folates from foods, a fact that was considered when the dietary folate equivalents were introduced in 1998.[103]

Outcomes

Folic acid bioavailability has been studied in controlled, short-term intervention studies with serum folate concentrations as the primary outcome. These studies have shown that relatively high amounts are required in order to achieve a measurable increase in serum folate.[101] Responsiveness of serum folate is even lower in depleted subjects; this has been attributed to a concept known as first-pass metabolism, in which the nutrient is retained in the tissues through which it passes and therefore does not result in a strong increase in systemic circulation.[102] Thus, short-term bioavailability studies have been replaced by either long-term studies with serum folate, homocysteine, or red blood cell folate as outcomes or tracer studies with labeled folates of different folate forms at lower doses. In long-term studies, it appeared that a consistent dose–response relationship was only seen at daily doses of 50–400 μg, and that a doubling of the dose of folic acid resulted in an increase in serum or plasma folate of approximately 60% and of red blood cell folate of approximately 30%.[104] Although serum homocysteine can also be used as a biomarker for folate status, it has to be noted that homocysteine

concentrations are also dependent on other host factors, such as vitamin B12 status or renal function, and are therefore less reactive to an acute change of folate status. In conclusion, interpretation of folate or folic acid bioavailability depends heavily on study design, host factors, and the biomarker chosen.

Bioavailability in condiments and seasonings

Fortification of staple foods, such as flour, with folic acid is mandatory in the United States, Canada, and many other countries in the Americas, and has been shown to be effective in reducing the occurrence of birth defects.[105] In contrast, there are few studies that investigated the bioavailability of folic acid added to seasonings and condiments. Interestingly, although there are salt products available that are fortified with folic acid, we are not aware of studies on the bioavailability of folic acid from fortified salt. Folic acid has been investigated as a fortificant in multiple-micronutrient powders, but in most studies, the main focus was not on biomarkers of folate nutritional status; it was on other more nonspecific biomarkers or functional markers, which may change due to increased intake of other micronutrients added.[106,107] In general, it is accepted that the bioavailability of folic acid added to foods is slightly lower than that of supplemental folic acid.[108] These estimates have been made mainly on the basis of a well-designed tracer study using fortified grain products,[109] but there are no studies on whether this also applies to other food sources, such as seasonings or condiments. However, folic acid absorption is not limited by inhibitors, and the capacity of the gastrointestinal tract is sufficient to absorb large amounts of folic acid.

Summary and conclusion

Seasonings and condiments offer an effective route for micronutrient fortification, as shown for iron and vitamin A. Iron is bioavailable when added to soy and fish sauces in the form of NaFeEDTA or ferrous sulfate with added citric acid, and such fortification is a strategy implemented in several Asian countries. Iron in the form of ferrous sulfate with citric acid is bioavailable when dual fortified with iodine in fish sauce. In dry vehicles, such as curry powder, NaFeEDTA is adequately bioavailable. Although the least water-soluble fortificants are poorly bioavailable, such as hydrogen-reduced elemental iron, particle-size reduction may produce

better candidates. In salt, MGFePP is bioavailable, but also reduces co-fortified iodine, and EFF is bioavailable in salt and does not reduce co-fortified iodine.

Vitamin A is bioavailable from the dry form of retinyl acetate or palmitate added to salt, MSG, and sugar. Serum retinol is the biomarker most commonly used to evaluate vitamin A fortification but is only responsive in deficient populations. Hence, smaller-sized tracer studies are required to evaluate the bioavailability of fortification in less-deficient populations.

There is a lack of studies examining the bioavailability of zinc and folic acid from condiments or seasonings. In principle, fortification of both micronutrients will increase dietary intake and absorption. Similar to iron, the bioavailability of zinc depends strongly on the content of the food matrix. Hence, some of the technological progress made with iron fortificants over the last few decades may be applied to zinc. The food matrix is not an issue in folic acid bioavailability, and the use of condiments or seasonings as vehicles for fortification with folic acid should be further investigated.

Acknowledgments

Conflicts of interest

The authors declare no conflicts of interest.

References

1. Allen, L. *et al.* 2006. *Guidelines on Food Fortification with Micronutrients.* Geneva, Switzerland: World Health Organization and Food and Agricultural Organization of the United Nations.
2. Gibson, R.S. *et al.* 2010. A review of phytate, iron, zinc, and calcium concentrations in plant-based complementary foods used in low-income countries and implications for bioavailability. *Food Nutr. Bull.* **31**: S134–S146.
3. Hurrell, R. & I. Egli. 2010. Iron bioavailability and dietary reference values. *Am. J. Clin. Nutr.* **91**: 1461S–1467S.
4. Aggett, P.J. 2010. Population reference intakes and micronutrient bioavailability: a European perspective. *Am. J. Clin. Nutr.* **91**: 1433S–1437S.
5. van Lieshout, M., C.E. West & R.B. van Breemen. 2003. Isotopic tracer techniques for studying the bioavailability and bioefficacy of dietary carotenoids, particularly beta-carotene, in humans: a review. *Am. J. Clin. Nutr.* **77**: 12–28.
6. Parada, J. & J.M. Aguilera. 2007. Food microstructure affects the bioavailability of several nutrients. *J. Food Sci.* **72**: R21–32.
7. van Lieshout, M. *et al.* 2001. Bioefficacy of beta-carotene dissolved in oil studied in children in Indonesia. *Am. J. Clin. Nutr.* **73**: 949–958.
8. Brouwer, I.A. *et al.* 2001. Bioavailability and bioefficacy of folate and folic acid in man. *Nutr. Res. Rev.* **14**: 267–294.
9. He, L. *et al.* 2013. Use of the Ussing chamber technique to study nutrient transport by epithelial tissues. *Front. Biosci. (Landmark Ed.)* **18**: 1266–1274.
10. Srikantia, S.G. & V. Reddy. 1970. Effect of a single massive dose of vitamin A on serum and liver levels of the vitamin. *Am. J. Clin. Nutr.* **23**: 114–118.
11. Scrimshaw, N.S., C.E. Taylor & J.E. Gordon. 1968. Interactions of nutrition and infection. *Monogr. Ser. World Health Organ.* **57**: 3–329.
12. Duncan, A. *et al.* 2012. Quantitative data on the magnitude of the systemic inflammatory response and its effect on micronutrient status based on plasma measurements. *Am. J. Clin. Nutr.* **95**: 64–71.

13. Hurrell, R.F. 1997. Preventing iron deficiency through food fortification. *Nutr. Rev.* **55**: 210–222.

14. Adesola, A.O. 1968. The influence of severe protein deficiency (kwashiorkor) on gastric acid secretion in Nigerian children. *Br. J. Surg.* **55**: 866.

15. Gracey, M. *et al.* 1977. The stomach in malnutrition. *Arch. Dis. Child.* **52**: 325–327.

16. Kusters, J.G., A.H. van Vliet & E.J. Kuipers. 2006. Pathogenesis of *Helicobacter pylori* infection. *Clin. Microbiol. Rev.* **19**: 449–490.

17. Wegmuller, R. *et al.* 2004. Particle size reduction and encapsulation affect the bioavailability of ferric pyrophosphate in rats. *J. Nutr.* **134**: 3301–3304.

18. Karn, S.K. *et al.* 2011. Shelf stability, sensory qualities, and bioavailability of iron-fortified Nepalese curry powder. *Food Nutr. Bull.* **32**: 13–22.

19. Garcia-Casal, M.N. & I. Leets. 2014. Carotenoids, but not vitamin A, improve iron uptake and ferritin synthesis by Caco-2 cells from ferrous fumarate and NaFe-EDTA. *J. Food Sci.* **79**: H706–H712.

20. Diosady, L.L., J.O. Alberti & M.G.V. Mannar. 2002. Microencapsulation for iodine stability in salt fortified with ferrous fumarate and potassium iodide. *Food Res. Int.* **35**: 635–642.

21. Li, Y.O., L.L. Diosady & A.S. Wesley. 2011. Folic acid fortification through existing fortified foods: iodized salt and vitamin A-fortified sugar. *Food Nutr. Bull.* **32**: 35–41.

22. Li, Y.O. *et al.* 2011. Feasibility and optimization study of using cold-forming extrusion process for agglomerating and microencapsulating ferrous fumarate for salt double fortification with iodine and iron. *J. Microencapsul.* **28**: 639–649.

23. Zimmermann, M.B. *et al.* 2011. Iron deficiency up-regulates iron absorption from ferrous sulphate but not ferric pyrophosphate and consequently food fortification with ferrous sulphate has relatively greater efficacy in iron-deficient individuals. *Br. J. Nutr.* **105**: 1245–1250.

24. Tuntipopipat, S. *et al.* 2009. Inhibitory effects of spices and herbs on iron availability. *Int. J. Food Sci. Nutr.* **60**(Suppl. 1): 43–55.

25. Ballot, D.E. *et al.* 1989. Fortification of curry powder with NaFe(111)EDTA in an iron-deficient population: report of a controlled iron-fortification trial. *Am. J. Clin. Nutr.* **49**: 162–169.

26. Manger, M.S. *et al.* 2008. A micronutrient-fortified seasoning powder reduces morbidity and improves short-term cognitive function, but has no effect on anthropometric measures in primary school children in northeast Thailand: a randomized controlled trial. *Am. J. Clin. Nutr.* **87**: 1715–1722.

27. Winichagoon, P. *et al.* 2006. A multimicronutrient-fortified seasoning powder enhances the hemoglobin, zinc, and iodine status of primary school children in North East Thailand: a randomized controlled trial of efficacy. *J. Nutr.* **136**: 1617–1623.

28. Hurrell, R.F. *et al.* 1991. Ferrous fumarate fortification of a chocolate drink powder. *Br. J. Nutr.* **65**: 271–283.

29. Garby, L. & S. Areekul. 1974. Iron supplementation in Thai fish-sauce. *Ann. Trop. Med. Parasitol.* **68**: 467–476.

30. Viteri, F.E., R. Garcia-Ibanez & B. Torun. 1978. Sodium iron NaFeEDTA as an iron fortification compound in Central America. Absorption studies. *Am. J. Clin. Nutr.* **31**: 961–971.

31. Fidler, M.C. *et al.* 2003. Iron absorption from fish sauce and soy sauce fortified with sodium iron EDTA. *Am. J. Clin. Nutr.* **78**: 274–278.

32. Huo, J. *et al.* 2002. Therapeutic effects of NaFeEDTA-fortified soy sauce in anaemic children in China. *Asia Pac. J. Clin. Nutr.* **11**: 123–127.

33. Chen, J. *et al.* 2005. Studies on the effectiveness of NaFeEDTA-fortified soy sauce in controlling iron deficiency: a population-based intervention trial. *Food Nutr. Bull.* **26**: 177–186.

34. Huo, J.S. *et al.* 2007. NaFeEDTA fortified soy sauce showed higher iron absorption rate in Chinese females. *Biomed. Environ. Sci.* **20**: 126–130.

35. Wang, B. *et al.* 2009. Social mobilization and social marketing to promote NaFeEDTA-fortified soya sauce in an iron-deficient population through a public–private partnership. *Public Health Nutr.* **12**: 1751–1759.

36. Thuy, P.V. *et al.* 2003. Regular consumption of NaFeEDTA-fortified fish sauce improves iron status and reduces the prevalence of anemia in anemic Vietnamese women. *Am. J. Clin. Nutr.* **78**: 284–290.

37. Van Thuy, P. *et al.* 2005. The use of NaFeEDTA-fortified fish sauce is an effective tool for controlling iron deficiency in women of childbearing age in rural Vietnam. *J. Nutr.* **135**: 2596–2601.

38. Chavasit, V., P. Nopburabutr & R. Kongkachuichai. 2003. Combating iodine and iron deficiencies through the double fortification of fish sauce, mixed fish sauce, and salt brine. *Food Nutr. Bull.* **24**: 200–207.

39. Walczyk, T. *et al.* 2005. Iron absorption by human subjects from different iron fortification compounds added to Thai fish sauce. *Eur. J. Clin. Nutr.* **59**: 668–674.

40. Longfils, P. *et al.* 2008. A comparative intervention trial on fish sauce fortified with NaFe-EDTA and FeSO₄+citrate in iron deficiency anemic school children in Kampot, Cambodia. *Asia Pac. J. Clin. Nutr.* **17**: 250–257.

41. Sivakumar, B. & K.M. Nair. 2002. Double fortified salt at crossroads. *Indian J. Pediatr.* **69**: 617–623.

42. Wegmuller, R., M.B. Zimmermann & R.F. Hurrell. 2003. Dual fortification of salt with iodine and encapsulated iron compounds: stability and acceptability testing in Morocco and Cote d'Ivoire. *J. Food Sci.* **68**: 2129–2135.

43. Zimmermann, M.B. *et al.* 2002. Addition of microencapsulated iron to iodized salt improves the efficacy of iodine in goitrous, iron-deficient children: a randomized, double-blind, controlled trial. *Eur. J. Endocrinol.* **147**: 747–753.

44. Rajagopalan, S. & M. Vinodkumar. 2000. Effects of salt fortified with iron and iodine on the haemoglobin levels and productivity of tea pickers. *Food Nutr. Bull.* **21**: 323–329.

45. Asibey-Berko, E. *et al.* 2007. Dual fortification of salt with iron and iodine in women and children in rural Ghana. *East Afr. Med. J.* **84**: 473–480.

46. Fidler, M.C. *et al.* 2004. A micronised, dispersible ferric pyrophosphate with high relative bioavailability in man. *Br. J. Nutr.* **91**: 107–112.

47. Zimmermann, M.B. *et al.* 2004. Dual fortification of salt with iodine and micronized ferric pyrophosphate: a randomized, double-blind, controlled trial. *Am. J. Clin. Nutr.* **80**: 952–959.

48. Wegmuller, R. *et al.* 2006. Salt dual-fortified with iodine and micronized ground ferric pyrophosphate affects iron status but not hemoglobin in children in Cote d'Ivoire. *J. Nutr.* **136**: 1814–1820.

49. Andersson, M. *et al.* 2008. Dual fortification of salt with iodine and iron: a randomized, double-blind, controlled trial of micronized ferric pyrophosphate and encapsulated ferrous fumarate in southern India. *Am. J. Clin. Nutr.* **88**: 1378–1387.

50. Li, Y.O., L.L. Diosady & A.S. Wesley. 2009. Iron in vitro bioavailability and iodine storage stability in double-fortified salt. *Food Nutr. Bull.* **30**: 327–335.

51. Haas, J.D. *et al.* 2014. Double-fortified salt is efficacious in improving indicators of iron deficiency in female Indian tea pickers. *J. Nutr.* **144**: 957–964.

52. Horton, S., A. Wesley & M.G.V. Mannar. 2011. Double-fortified salt reduces anemia, benefit: cost ratio is modestly favorable. *Food Policy* **36**: 581–587.

53. Zimmermann, M.B. *et al.* 2004. Triple fortification of salt with microcapsules of iodine, iron, and vitamin A. *Am. J. Clin. Nutr.* **80**: 1283–1290.

54. Raileanu, I. & L.L. Diosady. 2006. Vitamin A stability in salt triple fortified with iodine, iron, and vitamin A. *Food Nutr. Bull.* **27**: 252–259.

55. McGee, E.J.T. 2012. The fortification of salt with iodine, iron, and folic acid. Master thesis. Department of Chemical Engineering and Applied Chemistry, University of Toronto.

56. Sivakumar, B. & V. Reddy. 1972. Absorption of labelled vitamin A in children during infection. *Br. J. Nutr.* **27**: 299–304.

57. Reddy, V. *et al.* 1986. Absorption of vitamin A by children with diarrhoea during treatment with oral rehydration salt solution. *Bull. World Health Organ.* **64**: 721–724.

58. Reddy, V. & B. Sivakumar. 1972. Studies on vitamin A absorption in children. *Indian Pediatr.* **9**: 307–310.

59. Singh, R. *et al.* 2005. Impression cytology of the ocular surface. *Br. J. Ophthalmol.* **89**: 1655–1659.

60. Olson, J.A. 1984. Serum levels of vitamin A and carotenoids as reflectors of nutritional status. *J. Natl. Cancer Inst.* **73**: 1439–1444.

61. Tanumihardjo, S.A. 2000. Vitamin A status assessment in rats with (13)C(4)-retinyl acetate and gas chromatography/combustion/isotope ratio mass spectrometry. *J. Nutr.* **130**: 2844–2849.

62. Kagan, B.M. 1955. Observations on infection and certain vitamins. *Ann. N. Y. Acad. Sci.* **63**: 214–219.

63. Arroyave, G. & M. Calcano. 1979. Decrease in serum levels of retinol and its binding protein (RBP) in infection. *Arch. Latinoam. Nutr.* **29**: 233–260.

64. Lawrie, N.R., T. Moore & K.R. Rajagopal. 1941. The excretion of vitamin A in urine. *Biochem. J.* **35**: 825–836.

65. Mitra, A.K., J.O. Alvarez & C.B. Stephensen. 1998. Increased urinary retinol loss in children with severe infections. *Lancet* **351**: 1033–1034.

66. Mitra, A.K. *et al.* 1998. Predictors of serum retinol in children with shigellosis. *Am. J. Clin. Nutr.* **68**: 1088–1094.

67. Arroyave, G. *et al.* 1961. Serum and liver vitamin A and lipids in children with severe protein malnutrition. *Am. J. Clin. Nutr.* **9**: 180–185.

68. Solon, F.S. *et al.* 1979. Planning, implementation, and evaluation of a fortification program. Control of vitamin A deficiency in the Philippines. *J. Am. Diet. Assoc.* **74**: 112–118.

69. Muhilal *et al.* 1988. Vitamin A-fortified monosodium glutamate and vitamin A status: a controlled field trial. *Am. J. Clin. Nutr.* **48**: 1265–1270.

70. Muhilal *et al.* 1988. Vitamin A-fortified monosodium glutamate and health, growth, and survival of children: a controlled field trial. *Am. J. Clin. Nutr.* **48**: 1271–1276.

71. Solon, F. *et al.* 1979. An evaluation of strategies to control vitamin A deficiency in the Philippines. *Am. J. Clin. Nutr.* **32**: 1445–1453.

72. Kumar, M.V. & S. Rajagopalan. 2007. Multiple micronutrient fortification of salt and its effect on cognition in Chennai school children. *Asia Pac. J. Clin. Nutr.* **16**: 505–511.

73. Chen, K. *et al.* 2008. Effects of vitamin A, vitamin A plus iron and multiple micronutrient-fortified seasoning powder on preschool children in a suburb of Chongqing, China. *J. Nutr. Sci. Vitaminol. (Tokyo)* **54**: 440–447.

74. De-Regil, L.M. *et al.* 2011. Home fortification of foods with multiple micronutrient powders for health and nutrition in children under two years of age. *Cochrane Database Syst. Rev.* CD008959.

75. Salam, R.A. *et al.* 2013. Effectiveness of Micronutrient Powders (MNP) in women and children. *BMC Public Health* **13**(Suppl. 3): S22.

76. Chen, K. *et al.* 2011. Effects of vitamin A, vitamin A plus iron and multiple micronutrient-fortified seasoning powder on iron metabolic homeostasis. *Zhonghua Er Ke Za Zhi* **49**: 926–932.

77. Arroyave, G., L.A. Mejia & J.R. Aguilar. 1981. The effect of vitamin A fortification of sugar on the serum vitamin A levels of preschool Guatemalan children: a longitudinal evaluation. *Am. J. Clin. Nutr.* **34**: 41–49.

78. Viteri, F.E. *et al.* 1995. Fortification of sugar with iron sodium ethylenediaminotetraacetate (FeNaEDTA) improves iron status in semirural Guatemalan populations. *Am. J. Clin. Nutr.* **61**: 1153–1163.

79. Ribaya-Mercado, J.D. *et al.* 2004. Use of the deuterated-retinol-dilution technique to monitor the vitamin A status of Nicaraguan schoolchildren 1 y after initiation of the Nicaraguan national program of sugar fortification with vitamin A. *Am. J. Clin. Nutr.* **80**: 1291–1298.

80. Rosado, J.L. 2003. Zinc and copper: proposed fortification levels and recommended zinc compounds. *J. Nutr.* **133**: 2985S–2989S.

81. Brown, K.H. *et al.* 2010. Zinc fortification of cereal flours: current recommendations and research needs. *Food Nutr. Bull.* **31**: S62-74.

82. Herman, S. *et al.* 2002. Cofortification of iron-fortified flour with zinc sulfate, but not zinc oxide, decreases iron absorption in Indonesian children. *Am. J. Clin. Nutr.* **76**: 813–817.

83. Lopez de Romana, D., B. Lonnerdal & K.H. Brown. 2003. Absorption of zinc from wheat products fortified with iron and either zinc sulfate or zinc oxide. *Am. J. Clin. Nutr.* **78**: 279–283.

84. Hotz, C. *et al.* 2005. Zinc absorption from zinc oxide, zinc sulfate, zinc oxide + EDTA, or sodium-zinc EDTA does not differ when added as fortificants to maize tortillas. *J. Nutr.* **135**: 1102–1105.

85. Allen, L.H. 2006. New approaches for designing and evaluating food fortification programs. *J. Nutr.* **136**: 1055–1058.

86. Hilty, F.M. *et al.* 2009. Development and optimization of iron- and zinc-containing nanostructured powders for nutritional applications. *Nanotechnology* **20**: 475101.

87. Tripathi, B. & K. Platel. 2010. Finger millet (*Eleucine coracana*) flour as a vehicle for fortification with zinc. *J. Trace Elem. Med. Biol.* **24**: 46–51.

88. Hettiarachchi, M. *et al.* 2004. Na_2EDTA enhances the absorption of iron and zinc from fortified rice flour in Sri Lankan children. *J. Nutr.* **134**: 3031–3036.

89. Hambidge, K.M. 2010. Micronutrient bioavailability: dietary reference intakes and a future perspective. *Am. J. Clin. Nutr.* **91**: 1430S–1432S.

90. International Zinc Nutrition Consultative Group *et al.* 2004. International Zinc Nutrition Consultative Group (IZiNCG) technical document #1. Assessment of the risk of zinc deficiency in populations and options for its control. *Food Nutr. Bull.* **25**: S99–S203.

91. Hess, S.Y. *et al.* 2007. Use of serum zinc concentration as an indicator of population zinc status. *Food Nutr. Bull.* **28**: S403–S429.

92. MacDonald, R.S. 2000. The role of zinc in growth and cell proliferation. *J. Nutr.* **130**: 1500S–1508S.

93. Hess, S.Y. & K.H. Brown. 2009. Impact of zinc fortification on zinc nutrition. *Food Nutr. Bull.* **30**: S79–S107.

94. Lukacik, M., R.L. Thomas & J.V. Aranda. 2008. A meta-analysis of the effects of oral zinc in the treatment of acute and persistent diarrhea. *Pediatrics* **121**: 326–336.

95. Singh, M. & R.R. Das. 2011. Zinc for the common cold. *Cochrane Database Syst. Rev.* CD001364.

96. Science, M. *et al.* 2012. Zinc for the treatment of the common cold: a systematic review and meta-analysis of randomized controlled trials. *CMAJ* **184**: E551–E561.

97. Smuts, C.M. *et al.* 2005. Efficacy of multiple micronutrient supplementation for improving anemia, micronutrient status, and growth in South African infants. *J. Nutr.* **135**: 653S–659S.

98. Giovannini, M. *et al.* 2006. Double-blind, placebo-controlled trial comparing effects of supplementation with two different combinations of micronutrients delivered as sprinkles on growth, anemia, and iron deficiency in Cambodian infants. *J. Pediatr. Gastroenterol. Nutr.* **42**: 306–312.

99. Sharieff, W. *et al.* 2006. Micronutrients (including zinc) reduce diarrhoea in children: the Pakistan Sprinkles Diarrhoea Study. *Arch. Dis. Child.* **91**: 573–579.

100. Zlotkin, S.H. *et al.* 2004. Controlling iron deficiency anemia through the use of home-fortified complementary foods. *Indian J. Pediatr.* **71**: 1015–1019.

101. Melse-Boonstra, A., P. Verhoef & C. West. 2004. Quantifying folate bioavailability: a critical appraisal of methods. *Curr. Opin. Clin. Nutr. Metab. Care* **7**: 539–545.

102. Ohrvik, V.E. & C.M. Witthoft. 2011. Human folate bioavailability. *Nutrients* **3**: 475–490.

103. Suitor, C.W. & L.B. Bailey. 2000. Dietary folate equivalents: interpretation and application. *J. Am. Diet. Assoc.* **100**: 88–94.

104. Duffy, M.E. *et al.* 2014. Biomarker responses to folic acid intervention in healthy adults: a meta-analysis of randomized controlled trials. *Am. J. Clin. Nutr.* **99**: 96–106.

105. Das, J.K. *et al.* 2013. Micronutrient fortification of food and its impact on woman and child health: a systematic review. *Syst. Rev.* **2**: 67.

106. Chen, K. *et al.* 2011. Effect of vitamin A, vitamin A plus iron and multiple micronutrient-fortified seasoning powder on infectious morbidity of preschool children. *Nutrition* **27**: 428–434.

107. Kumar, M.V. & S. Rajagopalan. 2007. Multiple micronutrient fortification of salt and its effect on cognition in Chennai school children. *Asia Pac. J. Clin. Nutr.* **16**: 505–511.

108. Caudill, M.A. 2010. Folate bioavailability: implications for establishing dietary recommendations and optimizing status. *Am. J. Clin. Nutr.* **91**: 1455S–1460S.

109. Pfeiffer, C.M. *et al.* 1997. Absorption of folate from fortified cereal–grain products and of supplemental folate consumed with or without food determined by using a dual-label stable-isotope protocol. *Am. J. Clin. Nutr.* **66**: 1388–1397.