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International Zinc Nutrition Consultative Group Technical Document #2

SYSTEMATIC REVIEWS OF ZINC INTERVENTION STRATEGIES

Kenneth H. Brown and Sonja Y. Hess, guest editors

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Editorial office:
Food and Nutrition Bulletin
Michelle Badash, Managing Editor
150 Harrison Ave.
Boston, MA, 02111, USA
Tel: 617-636-3778
Fax: 617-636-3727
E-mail: FNB@inffoundation.org

Administrative office:
International Nutrition Foundation
150 Harrison Ave.
Boston, MA, 02111, USA
Tel: 617-636-3771
Fax: 617-636-3727
E-mail: infoperations@inffoundation.org

Subscriptions:
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Contents

International Zinc Nutrition Consultative Group Technical Document No. 2 Systematic reviews of zinc intervention strategies

Kenneth H. Brown and Sonja Y. Hess, guest editors

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The initial drafts of each paper were reviewed by the IZiNCG Steering Committee:

Kenneth H. Brown, M.D. (Chair)
Juan A. Rivera, Ph.D. (Co-chair)
Shawn K. Baker, M.P.H.
Zulfiqar A. Bhutta, M.D., Ph.D.
Omar Dary, Ph.D.
Rosalind S. Gibson, Ph.D.
Christine Hotz, Ph.D.
Janet C. King, Ph.D.
Bo Lönnerdal, Ph.D.
Marie T. Ruel, Ph.D.
Emorn Wasantwisut, Ph.D.
Sonja Y. Hess, Ph.D. (Executive officer)

The comments and suggestions by the IZiNCG Steering Committee were used to revise each of the papers, which were then submitted to the International Union of Nutritional Scientists (IUNS). IUNS and the *Food and Nutrition Bulletin* jointly coordinated the external review by representatives of the United Nations Children's Fund (UNICEF) and the World Health Organization. Responses to the reviewers' comments and suggestions were incorporated into the final published version.

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Recent advances in knowledge of zinc nutrition and human health

Sonja Y. Hess, Bo Lönnerdal, Christine Hotz, Juan A. Rivera, and Kenneth H. Brown

Abstract

Zinc deficiency increases the risk and severity of a variety of infections, restricts physical growth, and affects specific outcomes of pregnancy. Global recognition of the importance of zinc nutrition in public health has expanded dramatically in recent years, and more experience has accumulated on the design and implementation of zinc intervention programs. Therefore, the Steering Committee of the International Zinc Nutrition Consultative Group (IZiNCG) completed a second IZiNCG technical document that reexamines the latest information on the intervention strategies that have been developed to enhance zinc nutrition and control zinc deficiency. In particular, the document reviews the current evidence regarding preventive zinc supplementation and the role of zinc as adjunctive therapy for selected infections, zinc fortification, and dietary diversification or modification strategies, including the promotion and protection of breastfeeding and biofortification.

The purposes of this introductory paper are to summarize new guidelines on the assessment of population zinc status, as recommended by the World Health Organization (WHO), the United Nations Children's Fund (UNICEF), the International Atomic Energy Agency (IAEA), and IZiNCG, and to provide an overview on several new advances in zinc metabolism. The following papers will then review the intervention strategies individually.

Sonja Y. Hess, Bo Lönnerdal, and Kenneth H. Brown are affiliated with the Department of Nutrition and the Program in International and Community Nutrition, University of California, Davis, California, USA; Christine Hotz is affiliated with HarvestPlus and the International Food Policy Research Institute, Washington, DC, USA; Juan A. Rivera is affiliated with the National Institute of Public Health, Cuernavaca, Mexico; Kenneth H. Brown is also affiliated with Helen Keller International, Dakar, Senegal.

Please direct queries to the corresponding author: Kenneth H. Brown, Department of Nutrition, University of California, One Shields Ave., Davis, CA 95616, USA; e-mail: khbrown@ucdavis.edu.

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Background

In 2004, the International Zinc Nutrition Consultative Group (IZiNCG) published a technical review [1] that was designed to provide an overview of current knowledge regarding zinc nutrition in relation to human health, to summarize the available information on assessing population zinc status, and to describe the range of programmatic options for controlling zinc deficiency. Since the publication of that document, recognition of the importance of zinc nutrition for human health worldwide has expanded dramatically, and more experience has been accumulated on the design and implementation of zinc intervention programs. Moreover, during the workshop on zinc supplementation and child mortality and morbidity held by the World Health Organization (WHO) in September 2006, it was concluded that "in view of the results of all the trials examining the impact of zinc supplementation on mortality, morbidity and growth, a consensus was reached on the need to develop new feasible approaches to improve the intake of zinc and its bioavailability in young children, in order to achieve adequate population coverage" [2]. Hence, the IZiNCG Steering Committee concluded that this would be an opportune time to reexamine the latest information on strategies to control zinc deficiency and to reassess the state of knowledge concerning interventions to enhance zinc nutrition.

Adequate zinc nutrition is essential for human health because of zinc's critical structural and functional roles in multiple enzyme systems that are involved in gene expression, cell division and growth, and immunologic and reproductive functions. As a consequence, zinc deficiency affects children's physical growth and the risk and severity of a variety of infections [1]. The results of multiple community-based intervention trials indicate

that zinc supplementation decreases the incidence of diarrhea and pneumonia among young children [3], and clinical treatment studies have shown that zinc supplementation during diarrhea reduces the severity and duration of such illnesses [4]. WHO and the United Nations Children's Fund (UNICEF) now recommend that zinc supplementation should be included as a component in diarrhea treatment regimens [5], and efforts are under way in a number of countries to scale up zinc supplementation during diarrhea.

In the above-mentioned report on the WHO workshop on zinc and mortality, the results of a meta-analysis of available trials of preventive zinc supplementation indicated that there was a statistically significant 9% reduction in overall mortality among young children who received zinc supplementation [2]. The recent *Lancet* series on maternal and child undernutrition concluded that zinc deficiency is responsible for ~4% of child mortality and disability-adjusted life-years [6]. Although the specific number of deaths that might be averted by zinc-related interventions can still be debated because of the numerous assumptions involved in such estimates, both of these foregoing analyses confirm that zinc deficiency is an important risk factor for child morbidity and mortality [7, 8].

In addition to the effects of zinc on morbidity and mortality, a number of studies indicate that preventive zinc supplementation increases linear growth and weight gain in previously stunted or underweight children [9]. Thus, interventions to prevent zinc deficiency also can reduce the overall rates of childhood malnutrition, as defined by anthropometric criteria. For all of these reasons, global commitment is urgently needed to implement policies and programs designed to control zinc deficiency.

Assessment of the risk of zinc deficiency

Because of the serious consequences of zinc deficiency, it is essential to quantify the risk of deficiency in those populations that are most likely to be affected by this problem. Regrettably, there are as yet very limited national-level data on the prevalence of zinc deficiency. To promote the inclusion of zinc status assessment in the context of national health and nutrition surveys, guidelines on the assessment of population zinc status were recently published following a consensus conference convened by WHO, UNICEF, the International Atomic Energy Agency (IAEA), and IZiNCG [10]. The three main types of zinc status assessment that were considered included biochemical, dietary, and functional methods.

Serum or plasma zinc concentration is considered the best available biomarker of the risk of zinc deficiency in populations [11]. Methods for collecting, processing, and analyzing samples for determining serum zinc concentration have been comprehensively reviewed [1, 12]. The prevalence of zinc deficiency should be expressed as the percentage of the population with serum zinc concentration below the specific lower cutoffs in relation to reference data for age, sex, time of day, and fasting status of the individuals examined [13, 14]. When the prevalence of low serum zinc concentration is greater than 20%, the risk of zinc deficiency is considered to be elevated and should be addressed through public health nutrition interventions to improve zinc status. This same indicator also can be used to assess the impact of an intervention program, by comparing the percentage of individuals with low serum zinc concentrations before and after initiation of the intervention. Because serum zinc concentration falls during the acute-phase response to infections, it is advisable to include biochemical indicators of infection, such as C-reactive protein or α_1-glycoprotein, to avoid the possibility of overestimating the prevalence of low serum zinc concentration due to concurrent infections [15, 16].

Inadequate dietary intake of absorbable zinc is one of the major causes of zinc deficiency. Therefore, assessment of the adequacy of zinc intakes through the use of 24-hour recalls or weighed dietary records is an important component in evaluating the risk of zinc deficiency in a population [11]. Dietary assessment can be used to identify subpopulations that have an elevated risk of zinc deficiency and to characterize dietary patterns that contribute to inadequate zinc intakes, thus informing on the appropriate design of food-based interventions. The prevalence of the population with zinc intakes less than the Estimated Average Requirement (EAR) [1, 17] can be used as the specific indicator of the risk of zinc deficiency in the population. Assessment of the adequacy of zinc intakes should take into account dietary zinc bioavailability, preferably through quantification of the phytate:zinc molar ratio of the diet [18] or by using available equations to predict zinc absorption based on dietary zinc and phytate contents [19]. The risk of zinc deficiency is considered to be elevated and of public health concern when the prevalence of inadequate intakes is greater than 25%, in which case an intervention to increase dietary zinc intakes is recommended [11]. The change in prevalence of inadequate zinc intakes can be used to assess the impact and effective targeting of food-based interventions.

Although there are several adverse functional consequences of inadequate zinc intake, these outcomes are not specific to zinc deficiency. For example, the incidence of some types of infections can be reduced by providing supplemental zinc [3, 20], but the disease rates are more closely linked to the level of exposure to specific pathogens. Thus, a high incidence or prevalence of particular infections, such as diarrhea, may suggest that the population could benefit from interventions including zinc, but the illness rates would

not be very useful in quantifying the extent of zinc deficiency in the population. Similarly, low height-for-age is not specific to zinc deficiency and could be attributable in part to maternal short stature, frequent infections, and other nutritional deficiencies. Thus, providing zinc alone should not be expected to fully reverse childhood stunting. Nevertheless, a previous meta-analysis of randomized, controlled trials among prepubertal children found that the severity of stunting in the study populations predicted the response to zinc supplementation [9]. Thus, the percentage of children under 5 years of age with height-for-age z-score (HAZ) less than −2.0 SD with respect to the reference population [21] has been recommended as the best functional indicator to assess the likely risk of zinc deficiency in a population [11]. This risk is considered to be elevated and of public health concern when the prevalence of low height-for-age is greater than 20%, in which case nutrition intervention strategies should include a means to improve zinc status.

The validity of these indicators and proposed cutoffs is still provisional, so they should be evaluated further when opportunities become available during national assessment surveys. As more experience is gained, these recommendations will need to be reviewed and revised as necessary.

Quantifying the risk of zinc deficiency

As with other micronutrient deficiencies, three main factors are responsible for the development of zinc deficiency in lower-income countries: inadequate dietary zinc intake or absorption from predominantly plant-based diets, as discussed above, or suboptimal breastfeeding practices; disease states that either induce excessive losses or impair utilization of zinc; and physiological states that increase zinc requirements, such as periods of rapid growth during childhood and pregnancy. These issues are reviewed in more detail elsewhere [1, 10, 22].

Because so little information is available from nationally representative surveys on the prevalence of low serum zinc concentration or inadequate dietary zinc intake, current estimates of the extent of zinc deficiency must rely on the prevalence of stunting among children under 5 years of age [11]. Fortunately, relevant information is available at the national level for most countries **(fig. 1)** [23]. Approximately 30% of children under 5 years of age worldwide are stunted (HAZ < −2 SD with respect to the distribution of the reference population data). WHO recommends a prevalence of stunting greater than 20% of the population to indicate a public health concern [24]. The highest prevalence rates of stunting (> 30%) are observed in countries in sub-Saharan Africa, South Asia, Southeast Asia, and Central America. Intermediate prevalence rates (20% to 30%) are found in the Andean countries, some Central American countries, Southern Africa, and some countries in North Asia. As zinc deficiency is not the only factor affecting children's growth, assessment of dietary zinc intake and serum zinc levels can be used to confirm the risk of zinc deficiency in these countries [11]. These assessments should be incorporated into existing public health and child nutrition monitoring programs whenever possible.

New advances in zinc metabolism

Although this document focuses primarily on the recent progress that has been achieved with regard to the role of zinc nutrition in public health, some of the advances that have occurred in our understanding of zinc metabolism and the factors that govern zinc homeostasis are also worth noting. A comprehensive review of new research on zinc metabolism is beyond the scope of this paper, but several new discoveries concerning zinc transport proteins will be described briefly, because they provide some insight into the complexities of zinc homeostasis, and this knowledge eventually may yield useful information for estimating dietary zinc requirements and for developing new methods to assess zinc status.

The efficiency of zinc absorption from the diet usually ranges from about 15% to 35% in adults, depending on the amount consumed and the presence of other dietary factors, such as phytate, that may inhibit absorption [25]. Active transport dominates at low or normal intake, whereas passive diffusion contributes more significantly at high intake [26]. The extent of the homeostatic regulation of zinc metabolism in humans is not well known, but both absorption and excretion appear to be involved. Studies in experimental animals suggest that zinc homeostasis is closely regulated, although not to the same extent as for iron. The mechanisms underlying the regulation of zinc absorption have long remained elusive.

Understanding of the mechanisms regulating zinc absorption and homeostasis has progressed considerably because of the discovery of two families of zinc transporters: the so-called ZIP proteins and the ZnT proteins. Members of the ZIP family of proteins (which are also referred to in the literature as Zrt-like proteins and Irt-like proteins, with systemic designation "SLC39") transport zinc from the extracellular space and intracellular organelles into the cytoplasm [27]. Thus, the net effect of these transporters (or "zinc importer proteins") is to increase cytoplasmic zinc. There are 14 known members of the ZIP family encoded by the human genome [28], but only a few of them have been characterized or evaluated with regard to physiological significance. ZIP-1 is expressed ubiquitously in human tissues but is only localized to the

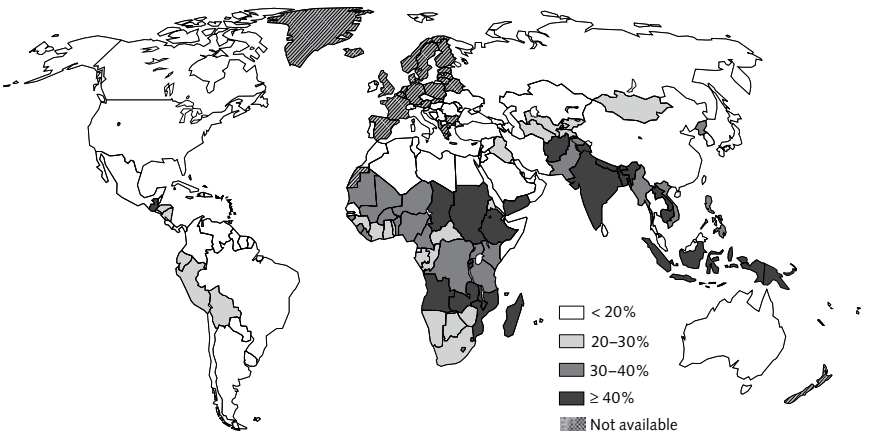

FIG. 1. Prevalence of nutritional stunting in children under 5 years of age. Source: IZiNCG [23]

plasma membrane in some cell types, possibly because of zinc-responsive regulation of its subcellular localization [29, 30]. In zinc-deficient cells, ZIP-1 migrates to the plasma membrane, whereas in zinc-replete cells, it is associated with intracellular compartments [31]. To date, ZIP-1 appears to be mostly involved in zinc uptake by erythroleukemia (K562) cells and prostate cells [29, 32]. ZIP-2 and ZIP-3 have also been shown to be involved in zinc uptake by some cell types, the latter in mammary epithelial cells [33].

The ZIP-4 transporter has been shown to be a key zinc importer in the intestinal cell. This transporter was discovered when mutations in the ZIP-4 gene were linked to the human genetic disorder acrodermatitis enteropathica [34]. Acrodermatitis enteropathica is due to an autosomal recessive mutation of the gene that codes for ZIP-4, causing disrupted transport function and impaired zinc absorption. Patients with acrodermatitis enteropathica need daily zinc supplements throughout life [35]. Because such supplements alleviate the problems of patients with acrodermatitis enteropathica, it is obvious that there are other, but less efficient, zinc transport mechanisms in the enterocyte. The ZIP-5 protein is expressed on the basolateral membrane of the enterocyte [36], where it may be responsible for zinc transport from the systemic circulation into the enterocyte, possibly as a means of drawing zinc into the intestinal cells when dietary zinc is low [27].

Recently, ZIP-14 has been shown to be involved in the uptake of zinc by the liver in response to acute inflammation and infection [37]. Serum zinc concentration falls during these conditions, whereas liver zinc concentration increases, possibly in an attempt to withhold zinc from pathogens. Liver ZIP-14 expression rises in response to the cytokine interleukin-6 (IL-6) during the acute-phase response [37], suggesting that induction of ZIP-14 may be responsible for the hypozincemia associated with infection.

The ZnT ("SLC30") family of zinc transporters has nine members in the human genome. These zinc transporters are primarily involved in cellular efflux of zinc and in uptake of zinc by intracellular organelles. Thus, the net effect of these transporters is to decrease cytoplasmic zinc concentration. ZnT-1 expression in the intestine is regulated by dietary zinc [38] and has been implicated in the regulation of whole-body zinc homeostasis by controlling zinc efflux from the enterocyte. ZnT-2 and ZnT-4 are involved in the flux of zinc in the endosomes, possibly regulating intracellular trafficking of zinc. These membrane transporters all have six transmembrane-spanning domains and a conserved histidine-rich region predicted to have a cytoplasmic loop that is likely to bind zinc [39, 40]. Experiments showing zinc sequestration by endosomal vesicles during overexpression of ZnT-2 suggest that this transporter may be important for controlling intracellular transport of zinc by the enterocyte [41]. All three transporters are found primarily in intestinal villus cells, and much less frequently in crypt cells.

As described for the ZIP proteins, individual members of the ZnT family of transporters are located in specific cell types. For example, in rats ZnT-1 is found mostly in the ileum, ZnT-2 is located primarily in the duodenum and jejunum, and ZnT-4 is found throughout the small intestine [42]. ZnT-5, ZnT-6, and ZnT-7 have been found to be involved in zinc homeostasis in the pancreas, brain, and prostate, respectively [43–45]; these transporters seem to be involved in zinc uptake in the Golgi apparatus [45, 46]. ZnT-3 is localized to synaptic vesicles in some types of neurons [47], and ZnT-8 is associated with the secretory granules of pancreatic beta-cells [48]. Thus, it is evident that the ZnT family is involved in multiple aspects of zinc homeostasis.

Several zinc transporters are involved in zinc secretion. ZnT-4, for example, has been shown to be involved in the secretion of zinc by the mammary gland, and mutations of the gene cause the defect *lethal milk* in mice [49]. The milk of these animals has very low

zinc concentration, resulting in severe zinc deficiency and high mortality among their pups. Several studies have also shown that healthy, well-nourished lactating women can have abnormally low concentrations of zinc in their breastmilk [50]. Supplementation of these women did not increase milk zinc concentrations, suggesting a defect similar to that observed in mice with the *lethal milk* defect, who are unable to secrete zinc into milk. A recent study of a family of women with low milk zinc contents, causing transient neonatal zinc deficiency in their infants, showed that milk zinc secretion was impaired because of a mutation in ZnT-2 [51]. It is not yet known how common this type of mutation is in lactating women or how often it causes zinc deficiency in breastfed infants.

The zinc transporters respond to conditions of low or excessive zinc exposure and changes in zinc status, presumably in an attempt to modulate their effects on particular tissues and biological functions. For example, during zinc deficiency, the abundance of ZnT-1 protein in the small intestine is reduced, decreasing endogenous zinc losses, and the localization of ZIP-4 is changed to the entire villus, maximizing zinc uptake. In the liver, ZnT-1 protein abundance is increased during zinc deficiency, most likely in an attempt to increase zinc in the systemic circulation; however, liver zinc decreases, as has been shown in animal studies [52]. Other tissues, such as the pancreas, muscle, and mammary gland, also respond to alterations in zinc status, but our knowledge regarding homeostasis in these tissues is more limited. Furthermore, little is still known about the regulation of zinc homeostasis and how different tissues contribute to this regulation in humans. It may be possible in the future, however, to combine results from animal models with data from compartmental modeling obtained in humans to unravel relevant information for understanding human zinc requirements and developing new methods to assess zinc status.

As discussed in the first IZiNCG technical document [1], the recommended strategies to control zinc deficiency include supplementation, fortification, and dietary diversification and modification. The present document reviews the current state of knowledge and information gaps regarding each of these intervention strategies. In particular, two papers discuss the available evidence regarding preventive zinc supplementation among infants, preschoolers, and prepubertal children [53] and among pregnant and lactating women [54]. A third paper examines zinc supplementation as adjunctive therapy in the treatment of diarrhea and other diseases [55]. Another paper reviews the current state of knowledge concerning zinc fortification [56], and three separate papers describe the information available on dietary diversification and modification strategies. One of these latter papers focuses on general principles and approaches to dietary diversification and modification [57], another describes the specific contribution of breastfeeding to maintaining adequate zinc intakes among infants and young children [58], and the third covers the potential of recently developed biofortification approaches to improve zinc status [59]. The general conclusions of these reviews, their related programmatic implications, and the most critical remaining research needs are summarized in the last paper [60].

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Preventive zinc supplementation among infants, preschoolers, and older prepubertal children

Kenneth H. Brown, Janet M. Peerson, Shawn K. Baker, and Sonja Y. Hess

Abstract

Zinc supplementation trials carried out among children have produced variable results, depending on the specific outcomes considered and the initial characteristics of the children who were enrolled. We completed a series of meta-analyses to examine the impact of preventive zinc supplementation on morbidity; mortality; physical growth; biochemical indicators of zinc, iron, and copper status; and indicators of behavioral development, along with possible modifying effects of the intervention results. Zinc supplementation reduced the incidence of diarrhea by ~20%, but the impact was limited to studies that enrolled children with a mean initial age greater than 12 months. Among the subset of studies that enrolled children with mean initial age greater than 12 months, the relative risk of diarrhea was reduced by 27%. Zinc supplementation reduced the incidence of acute lower respiratory tract infections by ~15%. Zinc supplementation yielded inconsistent impacts on malaria incidence, and too few trials are currently available to allow definitive conclusions to be drawn. Zinc supplementation had a marginal 6% impact on overall child mortality, but there was an 18% reduction in deaths among zinc-supplemented children older than 12 months of age. Zinc supplementation increased linear growth and weight gain by a small, but highly significant, amount. The interventions yielded a consistent, moderately large increase in mean serum zinc concentrations, and they had no significant adverse effects on indicators of iron and copper status. There were no significant effects on children's behavioral development, although the number of available studies is relatively small. The available evidence supports the need for intervention programs to enhance zinc status to reduce child morbidity and mortality and to enhance child growth. Possible strategies for delivering preventive zinc supplements are discussed.

Key words: Children, growth, infants, iron status indicators, morbidity, mortality, prevention, zinc supplementation

Background

A considerable number of intervention trials have been conducted in a variety of settings to assess the impact of preventive zinc supplementation on children's health and development. The results of these studies are inconsistent, possibly because of differences in the underlying zinc status or other characteristics of the study populations or discrepancies in the research methods. In this paper, we examine the results of controlled supplementation trials to address the following questions:

Section 1: Does preventive zinc supplementation of infants and young children affect their risk of selected illnesses, survival, physical growth, behavioral development, and serum zinc concentration? Are these effects modified by child- or dose-related factors?

Section 2: Are there adverse effects of preventive zinc supplementation?

Section 3: What are the opportunities to link preventive zinc supplementation programs to existing health and nutrition programs, and what technical, social, behavioral, and programmatic challenges must be confronted?

Kenneth H. Brown, Janet M. Peerson, and Sonja Y. Hess are affiliated with the Department of Nutrition and the Program in International and Community Nutrition, University of California, Davis, California, USA; Kenneth H. Brown and Shawn K. Baker are affiliated with Helen Keller International, Dakar, Senegal.

Please direct queries to the corresponding author: Kenneth H. Brown, Department of Nutrition, University of California, One Shields Ave., Davis, CA 95616, USA; e-mail: khbrown@ucdavis.edu.

Section 1

Does preventive zinc supplementation of infants and young children affect their risk of selected illnesses, survival, physical growth, behavioral development, and serum zinc concentration? Are these effects modified by child- or dose-related factors?

Conclusions

Preventive zinc supplementation reduces the incidence of diarrhea by ~20% among children in lower-income countries, although current evidence indicates that this beneficial effect of zinc is limited to children greater than ~12 months of age. Zinc supplementation also lowers the incidence of acute lower respiratory tract infections (ALRI), reducing pneumonia and ALRI by ~15%. Fewer studies have been completed to assess the effects of zinc supplementation on the incidence and severity of malaria, but the limited available information suggests that zinc supplementation may reduce the number of malaria episodes that result in clinic visits. Overall, zinc supplementation produces a 6% reduction in child mortality, although this benefit may be restricted to children 12 months of age or older, in whom the mortality reduction is approximately 18%. There is some information to suggest that zinc supplementation also may reduce mortality among small-for-gestational-age (SGA) infants, but the number of available studies and the numbers of children enrolled in each are too small to allow definite conclusions to be drawn.

Zinc supplementation produces a small, but significant, increase in linear growth and weight gain. Zinc supplementation consistently increases serum zinc concentration, with a moderately large effect size. We did not find evidence of any overall impact of zinc supplementation on mental or psychomotor development. However, the number of available studies is still relatively small, and the duration of these studies may be too short to permit detection of such outcomes.

Zinc supplementation programs should be considered for children in countries with an elevated risk of zinc deficiency to reduce their incidence of diarrhea, pneumonia, and possibly other infections; reduce mortality among children 12 months of age or older and possibly among SGA infants; and increase growth velocity and thereby reduce their risk of nutritional stunting and underweight.

Detailed review of evidence

Overview

To address the aforementioned set of questions, we conducted a systematic review of relevant supplementation trials of infants and prepubertal children. The following sections describe the procedures used to identify individual studies and select those for inclusion in the meta-analyses, the analytic methods that were used, and the specific outcomes of interest.

Identification of references. We sought information on controlled zinc supplementation trials conducted among prepubertal children by completing a computerized bibliographic search in May 2007, using the PubMed bibliographic database with the key word "zinc" and limiting for human studies, English language, clinical trial, and randomized, controlled trials. The results of the search were further expanded by contacting experts in the field and examining subsequent PubMed notifications and one conference report. The search strategy yielded a total of 1,625 individual references for consideration (**fig. 1**).

Selection of studies. The title or abstract of each article was scanned by a research assistant and two of the authors. Full articles were retrieved for further assessment if the available information suggested that zinc was provided as a supplement (exclusive of infant formula), the presence or absence of zinc in the supplement was the only factor that differed between any two intervention groups, and zinc supplementation was provided for prevention of deficiency rather than for treatment of a current disease. Zinc supplementation was considered to be therapeutic when it was provided as a component of the treatment regimen for diarrhea, pneumonia, malaria, or inpatient nutritional rehabilitation of children with severe malnutrition (marasmus or kwashiorkor), and therefore studies of these conditions were excluded from the present analysis. All other zinc supplementation studies were considered preventive zinc supplementation trials.

A total of 95 references were identified from controlled trials of preventive zinc supplementation in children; 8 of these publications were excluded because the article described a prior meta-analysis [1, 2] or pooled analysis [3], insufficient data were presented to address the questions of interest [4, 5], subjects were selected because of sickle-cell disease [6], or some subjects were no longer prepubertal [7, 8]. For two studies that included both prepubertal and postpubertal individuals, we were able to include the results just for the prepubertal children, either as presented in the paper [9] or as provided subsequently by the authors [10].

The 87 acceptable articles were then screened to combine results from those that presented data on the same intervention trial by using key trial characteristics, such as the country site, supplementation scheme, and study population. In some cases, several articles were published from the same study under the names of different first authors, so we refer to individual studies by using the country site and year of first publication. A total of 55 individual trials were identified, which enrolled a total of 202,692 children. If a trial included more than two sets of treatment groups that differed

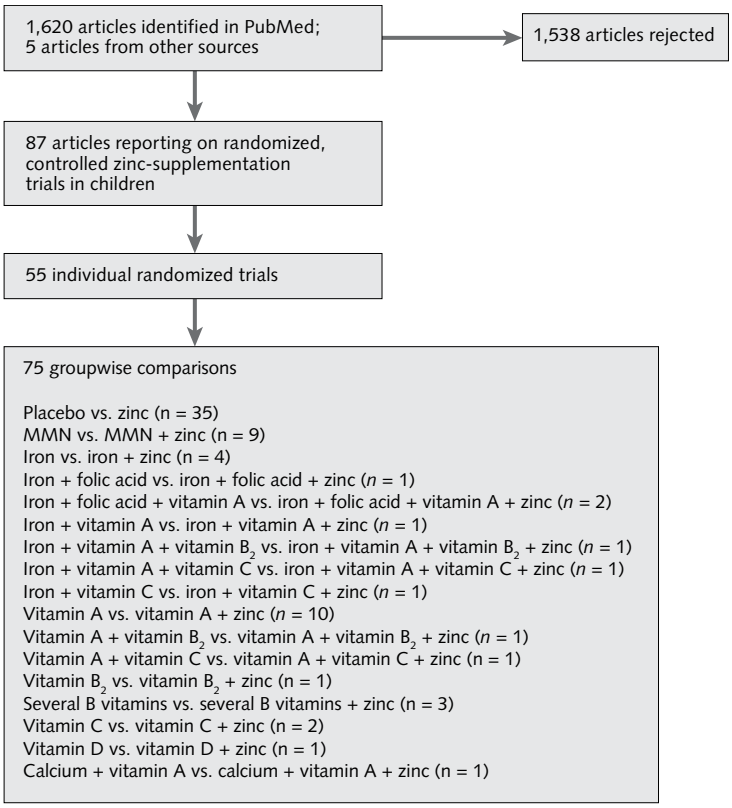

FIG. 1. Number of articles and individual studies included in the meta-analysis on preventive zinc supplementation in children. For groupwise comparisons, the two treatment groups differed only by the presence or absence of zinc in the supplement provided. MMN (multiple micronutrients) indicates at least four micronutrients. For more information on characteristics of the studies and participants, see **table 1.**

only by the presence of zinc (e.g., placebo vs. zinc alone, iron vs. zinc plus iron, or multiple micronutrients (MMN) with or without zinc), each set of groups that differed by zinc only was considered a separate groupwise comparison (**fig. 1**). A total of 75 separate controlled comparisons were identified. Of these comparisons, 73 were derived from studies considered to be well designed because the treatments were randomly assigned to individuals and the research protocol used a double-blind, controlled design. Only one study did not specifically indicate whether treatments were randomly assigned [11]. In the study Brazil 1998 [12, 13], one of the three treatment groups (5 mg of zinc daily) was excluded from consideration because that group was not enrolled concurrently with the placebo group.

Data extraction and management. For studies that fulfilled the inclusion criteria, three research assistants summarized relevant information regarding the study population and intervention design, using a standard data-extraction template. Any relevant information concerning the trial that was missing from the published report was obtained from the original author(s) of the article, if possible. One of the reviewers verified all extracted information by comparing the data with the original publication. When there were differences of opinion among reviewers, these were discussed and resolved by consensus.

Data analyses. The outcomes examined were incidence of diarrhea; incidence of ALRI; incidence of malaria; mortality; change in height (length or stature), expressed in centimeters or height-for-age z-score (HAZ); change in body weight, expressed in kilograms or weight-for-age z-score (WAZ); change in weight-for-height z-score; change in mid-upper-arm circumference; change in serum or plasma zinc concentration; final mental development index score; final physical development index score; change in blood hemoglobin concentration; change in serum ferritin concentration; and change in serum copper concentration. For all outcomes, except morbidity variables and final developmental scores, studies were included in the analyses only if information was available for both the

children's initial status and change during the course of the intervention. Morbidity and mortality variables were converted to rate ratios, and anthropometric, biochemical, and development variables were converted to effect size, which was calculated as the difference between the mean of the values for the zinc and the corresponding control group divided by their pooled standard deviation. In general, effect sizes of ~0.2 are considered of small magnitude, effect sizes of ~0.5 are considered moderately large, and those of ~0.8 or greater are considered large [14].

The overall mean effect size for each outcome variable was estimated from a random-effects model [15]. This model assumes that the observed effect size or log(relative risk) from a particular study is the sum of the true effect for that study plus a normally distributed random error term, which is related to the sample size and effect size or relative risk for that study and, in turn, that the true effects are themselves normally distributed. Because the total variance for the study effect size is different from one study to the next, the best estimate of the overall mean is a weighted mean effect size, in which the weights are equal to the inverse of the total variance. The SAS for WINDOWS (release 9) MIXED procedure was used to estimate the weighted mean effect size and its standard error.

Additionally, the heterogeneity of responses was assessed by using the chi-square test, as described by Hedges [16]. We explored possible sources of heterogeneity with random-effects meta-regression analyses, in which study characteristics were used to explain effect sizes [17, 18]. As with any regression, the number of possible explanatory variables was strictly limited by the number of observations, which in this case is the number of comparisons available. Explanatory variables were examined separately in a series of bivariate models; then a subset of explanatory variables was entered into a regression model and nonsignificant predictors were removed in a stepwise fashion. When appropriate, nonlinearity was initially assessed with polynomial models and, in one case (relation between diarrheal incidence and age), was followed up with the use of a two-phase regression model. The SAS for WINDOWS MIXED procedure was used for all of these procedures except the two-phase regression model, for which the SAS NLMIXED procedure was used.

Description of intervention trials

The general characteristics of the studies included in the meta-analyses are shown in **table 1**. Of the 55 studies included in the analyses, 7 were from Africa, 23 were from Asia, 12 were from South America, 11 were from North America, 1 was from Australia, and 1 was from Europe. The supplementation periods ranged from 2 weeks [19] to 15 months [11], and the number of subjects ranged from 18 to 94,359. The periodic zinc supplementation doses ranged from 1 to 70 mg per dose (median, 10 mg, with one dose unknown). These doses were provided daily [9, 20–68], several times per week [10–13, 69–94], or once per week [94–99], resulting in a daily dose equivalents ranging from 0.9 to 21.4 mg of zinc/day. Most studies provided zinc as zinc sulfate (n = 36), although a few distributed other compounds, including zinc acetate (n = 5), zinc gluconate (n = 5), zinc amino acid chelates (n = 3), and zinc oxide (n = 1). In four studies, the zinc compound was not stated [22, 48, 71, 73], and in another study zinc acetate was provided during the first phase of the study and zinc gluconate was given later [11]. We attempted to evaluate the possible modifying effect of current breastfeeding on the response to zinc supplementation, but this was not possible because of the lack of relevant information in the available reports.

Selected initial characteristics of the subjects enrolled in the trials are presented in **table 1**. The mean initial age at enrollment varied greatly among studies. Some studies enrolled infants within a few days after birth [12, 27, 30, 78], whereas one study enrolled children with a mean initial age of 11.1 years [10].

Results

Diarrhea morbidity. Information on diarrhea incidence was available from 24 studies, which enrolled a total of 16,339 children. These studies provided 33 distinct comparisons of zinc supplements, with or without other nutrients, versus the same preparations without zinc. The treatment groups received just zinc or placebo in 16 comparisons. In five comparisons, both groups also received iron, with or without vitamin A; and in four comparisons, the treatment groups received vitamin A plus a micronutrient other than iron. Three comparisons provided vitamin C or B vitamins, with or without zinc. Five additional comparisons investigated MMN, with and without zinc. The mean age of the study participants ranged from newborns to approximately 4 years.

There was a significant 20% lower incidence of diarrhea among children who received zinc supplementation (relative risk, 0.80; 95% CI, 0.71 to 0.90; p = .0004, random-effects model) (**fig. 2**). Because of significant heterogeneity among studies (p < .0001), a meta-regression analysis was completed. The mean initial age of the study subjects was highly significantly associated with the magnitude of the effect of zinc supplementation (p < .001), and the groupwise comparisons are displayed by mean initial age in **figure 3**. Inspection of the figure indicates that the beneficial effect of zinc supplements on diarrhea incidence was limited to studies of children with a mean initial age greater than 12 months. Among studies of children with mean age initial age greater than 12 months, the relative risk of diarrhea incidence was 0.73 (95% CI, 0.61 to 0.87; p = .0014).

TABLE 1. Selected characteristics of double-blind, randomized, controlled trials in prepubertal children and study subjects for each group comparison

Country, year [reference] author	Selection criteria for study population[a]	Group comparison[b]	Sample size[c]	% male	Supplementation scheme				Mean initial characteristics[c]			
					Duration (mo)	Frequency	Zinc dose (mg)	Other micronutrients[d]	Age (mo)	Serum zinc concentration (μg/dL)	HAZ	WAZ
Bangladesh, 2001 [19–21] Rahman	Unselected infants	Placebo Zinc	325	50.5	0.46	Daily	20	None	23.7	73.5	−2.41	−2.35
		Vitamin A Vitamin A + zinc	328	55.5	0.46	Daily	20	200,000 IU vitamin A[e]	23.7	70.9	−2.41	−2.42
Bangladesh, 2002 [22] Osendarp [23] Hamadani	Unselected children	Placebo Zinc	301	44.5	4.6	Daily	5	None	0.9	NA	NA	NA
Bangladesh, 2003a [24] Albert	Unselected infants	Placebo Zinc	126	57.0	1.38	Daily	20	None	39.0	62.0	NA	NA
		Vitamin A Vitamin A + zinc	123	55.0	1.38	Daily	20	200,000 IU vitamin A[e]	41.0	62.0	NA	NA
Bangladesh, 2003b [95, 97] Baqui [96] Black	Unselected infants[f]	Vitamin A + vitamin B_2 Vitamin A + vitamin B_2 + zinc	318	44.4	6	Weekly	20	100,000 IU vitamin A[e]; 1 mg vitamin B_2	6.3	67.9	−1.20	−1.00
		Vitamin A + vitamin B_2 + iron Vitamin A + vitamin B_2 + iron + zinc	327	44.9	6	Weekly	20	100,000 IU vitamin A[e]; 1 mg vitamin B_2, 20 mg iron	6.3	66.7	−1.20	−1.05
Bangladesh, 2005 [98] Brooks	Unselected infants	Placebo Zinc	1,621	52.0	12	Weekly	70	None	5.3	64.5	−1.30	−1.62
Brazil, 1998 [12] Lira [13] Ashworth	LBW infants	Placebo Zinc	134	44.5	1.84	6/wk	1	None	0.0	NA	NA	NA
Brazil, 2000 [25] Sayeg Porto	Children with HAZ < −2	Placebo Zinc	18	50.0	6	Daily	42	None	118.2	100.5	−2.67	NA
Burkina Faso, 2001 [69, 70] Müller	Unselected preschoolchildren	Placebo Zinc	685	49.5	6	6/wk	12.5	None	18.1	76.5	−1.55	−2.00
Canada, 1989 [26] Gibson	Boys with HAZ < 15%	Placebo Zinc	60	100.0	12	Daily	10	None	75.8	104.9	−1.39	−0.18

Chile, 1994 [9] Castillo-Duran	Children with HAZ < 5% percentile	Placebo Zinc	42	52.4	12	Daily	10	None	104.3	NA	−2.42	NA
Chile, 1995 [27] Castillo-Duran	SGA infants	MMN MMN + zinc	68	47.1	6	Daily	3	1,500 IU vitamin A, 50 mg vitamin C, 400 IU vitamin D; 1–2 mg/kg iron[g]	0.1	NA	NA	NA
Chile, 1997 [28] Ruz	Unselected preschoolchildren	Placebo Zinc	98	50.0	14	Daily	10	None	39.8	114.1	−0.52	0.13
Chile, 2001 [29] Castillo-Duran	Infants with birthweight > 2,300 g	Placebo Zinc	112	50.9	12	Daily	5	1–2 mg/kg iron[g]	0.3	NA	NA	NA
China, 1992 [30] Hong	Infants of high-risk pregnancy	B vitamins B vitamins + zinc	65	50.0	6	Daily	7.6	B vitamins[h]	0.1	86.0	NA	NA
China, 1998 [71] Sandstead [72] Penland	Unselected children	MMN MMN + zinc	230	50.0	2.3	6/wk	20	2,500 IU vitamin A, 0.9 mg vitamin B_1, 1.1 mg vitamin B_2, 12 mg vitamin B_3, 1.1 mg vitamin B_6, 35 µg folic acid, 400 IU vitamin D, 7 mg vitamin E, 20 µg vitamin K, 1 mg copper, 20 µg selenium, 90 µg iodine, 1 mg fluoride, 1.5 mg manganese, 30 µg molybdenum, 30 µg chromium	90.0	86.3	NA	NA
China, 2002 [73] Yang	Preschoolchildren with HAZ < −1	Placebo Zinc	61	49.2	12	5/wk	3.5	None	49.1	NA	NA	NA
		Vitamin A + calcium Vitamin A + calcium + zinc	55	50.9	12	5/wk	3.5	200 µg vitamin A, 150 mg calcium	47.1	NA	NA	NA
Ecuador, 1994 [74] Dirren	Unselected preschoolchildren	Placebo Zinc	96	60.0	15	6/wk	10	None	31.5	74.3	−2.90	−1.76
Ecuador, 1996 [31] Sempertegui	Preschoolchildren with WAZ < 10th percentile or HAZ < 10th percentile	Placebo Zinc	48	56.0	2	Daily	10	None	42.3	86.5	−2.00	−1.40

continued

TABLE 1. Selected characteristics of double-blind, randomized, controlled trials in prepubertal children and study subjects for each group comparison *(continued)*

Country, year [reference] author	Selection criteria for study population[a]	Group comparison[b]	Sample size[c]	% male	Supplementation scheme				Mean initial characteristics[c]			
					Duration (mo)	Frequency	Zinc dose (mg)	Other micronutrients[d]	Age (mo)	Serum zinc concentration (μg/dL)	HAZ	WAZ
Ecuador, 2008[i] [32] Wuehler	Nonanemic preschool-children with HAZ < −1.3 for children 12–23 mo of age, < −1.5 for children 24–30	Placebo	251	53.1	6	Daily	3	None	21.1	71.6	−2.3	−1.3
		Zinc (3 mg)										
		Placebo	253	53.1	6	Daily	7	None	21.0	71.7	−2.3	−1.3
		Zinc (7 mg)										
		Placebo	253	53.1	6	Daily	10	None	20.9	72.0	−2.3	−1.3
		Zinc (10 mg)										
Ethiopia, 2000 [75] Umeta	Stunted infants with HAZ < −2	Placebo	90	53.3	6	6/wk	10	None	9.6	NA	−2.81	−2.58
		Zinc (stunted)										
	Nonstunted infants matched by age and sex	Placebo	94	46.8	6	6/wk	10	None	9.3	NA	−0.64	−1.40
		Zinc (nonstunted)										
France, 1992 [33] Walravens	Breastfed infants	Vitamin D	57	52.7	3	Daily	5	Vitamin D[h]	5.5	NA	0.12	0.76
		Vitamin D + zinc										
Gambia, 1993 [11] Bates	Unselected preschoolchildren	Placebo	109	50.0	15	2/wk	70	None	17.7	NA	NA	NA
		Zinc										
Guatemala, 1993 [76] Cavan [77] Grazioso	Unselected children	MMN	162	50.0	5.75	6/wk	10	1.5 mg vitamin B_1, 1.2 mg vitamin B_2, 20 mg vitamin B_3, 10 mg vitamin B_5, 1 mg vitamin B_6, 6 μg vitamin B_{12}, 100 μg folic acid, 100 mg vitamin C, 10 μg vitamin D, 3.3 mg vitamin E, 50 μg copper, 2 mg chromium, 110 μg iodine, 50 μg selenium, 110 mg magnesium	81.8	93.5	−1.38	−0.85
		MMN + zinc										
Guatemala, 1997 [34] Ruel [35] Bentley [36] Rivera	Unselected infants	Placebo	89	57.1	7	Daily	10	None	7.6	NA	−2.16	−1.18
		Zinc										

India, 1996 [37–42] Sazawal	Preschoolchildren with diarrhea in past 24 h	MMN MMN + zinc	609	52.3	6	Daily	10	240 RE vitamin A, 0.6 mg vitamin B_1, 0.5 mg vitamin B_2, 10 mg vitamin B_3, 0.5 mg vitamin B_6, 100 IU vitamin D, 3 mg vitamin E	16.0	64.8	NA	NA
India, 2001 [43] Sazawal [44] Black	SGA infants	Vitamin B_2 Vitamin B_2 + zinc	584	50.0	9	Daily	5	0.5 mg vitamin B_2	0.5	NA	NA	NA
		MMN MMN + zinc	570	50.0	9	Daily	5	0.5 mg vitamin B_2, 60 μmol folic acid, 180 mg calcium, 90 mg phosphorus, 10 mg iron	0.5	NA	−1.80	NA
India, 2002 [45, 47] Bhandari [46] Taneja	Unselected preschoolchildren	Vitamin A Vitamin A + zinc	2,482	52.4	4	Daily	20[j]	200,000 IU vitamin A[e,j]	15.3	62.0	NA	NA
India, 2003a[i] [94] Gupta	Unselected preschoolchildren	Placebo Daily zinc	189	46.1	3.68	5/wk	10	None	23.5	NA	NA	NA
		Placebo Weekly zinc	185	46.0	3.68	Weekly	50	None	23.5	NA	NA	NA
India, 2003b [78] Sur	LBW infants	B vitamins B vitamins + zinc	100	50.0	12	5/wk	4.5	B vitamins[h]	0.1	114.1	NA	−2.14
India, 2007a [48] Bhandari	Unselected infants and preschoolchildren	Folic acid + iron Folic acid + iron + zinc	94,359	52.7	12	Daily	10[k]	12.5 mg iron,[k] 50 μg folic acid[k]	11.7	64.1	NA	NA
India, 2007b [99] Gupta	Unselected preschoolchildren	B vitamins B vitamins + zinc	1,712	49.0	6	Weekly	49.3	B vitamins[h]	NA	NA	NA	NA
Indonesia, 2001 [79] Dijkhuizen [80, 81] Wieringa	Unselected infants	Placebo Zinc	238	50.0	6	5/wk	10	None	4.2	NA	−0.79	−0.05
		Iron Iron + zinc	240	50.0	6	5/wk	10	10 mg iron	4.2	NA	−0.90	−0.06
		Vitamin A Vitamin A + zinc	129	50.0	6	5/wk	10	2.4 mg β-carotene	4.2	NA	NA	NA
Indonesia, 2003 [49, 50] Lind	Unselected infants[f]	Vitamin C Vitamin C + zinc	336	53.0	6	Daily	10	30 mg vitamin C	6.2	59.3	−0.37	−0.39
		Vitamin C + iron Vitamin C + iron + zinc	330	50.5	6	Daily	10	30 mg vitamin C, 10 mg iron	6.2	58.6	−0.32	−0.39

continued

TABLE 1. Selected characteristics of double-blind, randomized, controlled trials in prepubertal children and study subjects for each group comparison *(continued)*

Country, year [reference] author	Selection criteria for study population[a]	Group comparison[b]	Sample size[c]	% male	Supplementation scheme				Mean initial characteristics[c]			
					Duration (mo)	Frequency	Zinc dose (mg)	Other micronutrients[d]	Age (mo)	Serum zinc concentration (μg/dL)	HAZ	WAZ
Indonesia, 2007 [51] Fahmida	Unselected infants[f]	Vitamin A Vitamin A + zinc	391	49.9	6	Daily	10	100,000 IU vitamin A[e]	5.1	100.0	−0.99	−0.54
Jamaica, 1998 [52] Meeks Gardner	Preschoolchildren with HAZ < −2 and WAZ < median	MMN MMN + zinc	61	42.6	2.76	Daily	5	1,500 IU vitamin A, 0.5 mg vitamin B_1, 0.8 mg vitamin B_2, 7 mg vitamin B_3, 1 mg vitamin B_6, 30 mg vitamin C, 400 IU vitamin D	14.1	NA	−2.85	NA
Jamaica, 2005 [53] Meeks Gardner	Preschoolchildren with WAZ < −1.5	MMN MMN + zinc	114	33.2	6	Daily	10	1,500 IU vitamin A, 0.5 mg vitamin B_1, 0.8 mg vitamin B_2, 7 mg vitamin B_3, 1 mg vitamin B_6, 2 μg vitamin B_{12}, 1 mg folic acid, 30 mg vitamin C, 400 IU vitamin D, 8 mg iron	18.8	NA	−1.42	−2.16
Mexico, 1997 [82] Rosado [83] Allen [84] Munoz	Unselected preschoolchildren	Placebo Zinc	109	48.2	12	6/wk	20	None	28.7	89.8	−1.71	−1.40
		Iron Iron + zinc	108	45.0	12	6/wk	20	20 mg iron	28.2	103.7	−1.55	−1.40
Mexico, 2005 [85] Kordas [86] Rosado [87] Rico	Unselected children[l]	Placebo Zinc	252	56.8	6	5/wk	30	None	84.0	81.6	NA	NA
		Iron Iron + zinc	265	56.2	6	5/wk	30	30 mg iron	84.0	77.6	NA	NA
Mexico, 2006 [54] Long	Unselected infants	Placebo Zinc	364	49.5	12	Daily	20	None	9.9	NA	0.08	0.09
		Vitamin A Vitamin A + zinc	372	54.0	12	Daily	20	20,000 IU vitamin A for ≤ 1 yr of age; 45,000 IU for > 1 yr of age every 2 mo	9.7	NA	0.13	0.10

Nepal, 2006 [55, 56] Tielsch	Unselected infants and preschoolchildren	Vitamin A Vitamin A + zinc	13,385	50.0	13.7	Daily	10[j]	200,000 IU vitamin A[j] every 6 mo	12.4	NA	NA	NA
		Vitamin A + folic acid + iron Vitamin A + folic acid + iron + zinc	17,079	50.0	13.7	Daily	10[j]	50 µg folic acid[j], 12.5 mg iron[j]; 200,000 IU vitamin A[j] every 6 mo	12.4	NA	NA	NA
Papua New Guinea, 2000 [88] Shankar	Unselected preschoolchildren	Placebo Zinc	274	47.0	10.58	6/wk	10	None	31.4	70.5	−1.90	NA
Peru, 2004a [89] Alarcon	Anemic preschoolchildren (hemoglobin 70–99.9 g/L)	Iron Iron + zinc	223	50.0	4.14	6/wk	7.3	3 mg/kg/day iron	17.4	NA	−1.04	−0.25
Peru, 2004b [57] Penny	Children with persistent diarrhea (> 14 days)	Vitamin C Vitamin C + zinc	159	50.3	6	Daily	10	50 mg vitamin C	18.9	70.3	−1.56	−1.13
Peru, 2007 [58] Brown	Infants with LAZ < −0.5 and WLZ > −3	MMN MMN + zinc	200	48.5	6	Daily	3	225 µg RE vitamin A, 0.5 mg vitamin B_1, 0.38 mg vitamin B_2, 3.8 mg vitamin B_3, 2.5 mg vitamin B_5, 0.5 mg vitamin B_6, 50 µg biotin, 20 mg vitamin C, 225 IU vitamin D, 3.8 mg vitamin E, 0.7 mg iron[m]	7.5	77.6	−1.19	−0.75
South Africa, 2005 [59] Bobat	HIV-positive preschoolchildren	MMN[n] MMN + zinc[n]	96	48.9	6	Daily	10	1,000 IU vitamin A, 1.5 mg vitamin B_1, 1.2 mg vitamin B_2, 10 mg vitamin B_3, 1 mg vitamin B_6, 50 mg vitamin C, 400 IU vitamin D	38.3	NA	−1.60	NA
Tanzania, 2006 [60, 61] Sazawal [62] Olney	Unselected infants and preschoolchildren	Vitamin A Vitamin A + zinc	42,546	50.3	12.7	Daily	10[j]	200,000 IU vitamin A[j] every 6 mo	18.1	NA	−1.48	−1.28
		Vitamin A + folic acid + iron Vitamin A + folic acid + iron + zinc	16,070	50.5	12.7	Daily	10[j]	200,000 IU vitamin A[j] every 6 mo; 50 µg folic acid[j], 12.5 mg iron[j]	18.1	NA	−1.45	−1.15

continued

TABLE 1. Selected characteristics of double-blind, randomized, controlled trials in prepubertal children and study subjects for each group comparison *(continued)*

Country, year [reference] author	Selection criteria for study population[a]	Group comparison[b]	Sample size[c]	% male	Supplementation scheme				Mean initial characteristics[c]			
					Duration (mo)	Frequency	Zinc dose (mg)	Other micronutrients[d]	Age (mo)	Serum zinc concentration (μg/dL)	HAZ	WAZ
Thailand, 1992 [90] Udomkesmalee [91] Kramer	Infants with serum retinol concentration < 1.05 μmol/L and serum zinc concentration < 12.2 μmol/L	Placebo Zinc Vitamin A Vitamin A + zinc	68 65	47.4 69.5	6 6	5/wk 5/wk	25 25	None 1,500 RE vitamin A	110.5 113.0	86.3 85.3	NA NA	NA NA
Thailand, 2006 [63] Wasantwisut	Unselected infants[f]	Vitamin A + vitamin C Vitamin A + vitamin C + zinc	304	51.5	6	Daily	10	1,500 RE vitamin A[e], 30 mg vitamin C	4.5	73.8	−0.69	−0.18
		Vitamin A + vitamin C + iron Vitamin A + vitamin C + iron + zinc	305	50.0	6	Daily	10	1,500 RE vitamin A[e], 30 mg vitamin C, 10 mg iron	4.5	71.0	−0.66	−0.12
Uganda, 1998 [92] Kikafunda	Unselected preschool and school-aged children	Placebo Zinc	153	54.1	8	3.75/wk	10	None	55.8	NA	−0.70	−0.41
USA, 1983 [64] Walravens	Preschoolchildren with HAZ < 10th percentile	Placebo Zinc	40	65.0	12	Daily	10	None	50.0	72.0	−2.07	−1.76
USA, 1989 [65] Walravens	Preschoolchildren with documented decline of ≥ 20 percentile in WAZ	Placebo Zinc	50	52.0	6	Daily	5.7	None	15.2	70.0	−1.35	−2.04
USA, 2006 [66] Heinig	Breastfed infants with birthweight > 2,500 g	Placebo Zinc	82	50.0	6	Daily	5	None	4.0	73.3	0.37	0.61

Vietnam, 1996 [67] Ninh	Preschoolchildren with WAZ < −2 and HAZ < −2	Placebo Zinc	146	50.0	5	Daily	10	None	17.6	NA	−2.91	−2.61
Vietnam, 2006 [68] Berger	Unselected infants[f]	Vitamin A Vitamin A + zinc	393	50.4	6	Daily	10	100,000 IU vitamin A[e]	5.8	94.8	−1.01	−0.56
		Vitamin A + iron Vitamin A + iron + zinc	391	47.7	6	Daily	10	100,000 IU vitamin A[e]; 10 mg iron	5.9	92.7	−1.06	−0.58
Zimbabwe, 1997 [10, 93] Friis	Unselected children	Placebo Zinc	313	46.0	12.2	3.5/wk	40	None	133.8	77.8	−1.18	−1.27

HAZ, height-for-age z-score; HIV, human immunodeficiency virus; LAZ, length-for-age z-score; LBW, low-birthweight; MMN, multiple micronutrients with at least four micronutrients; RE, retinol equivalent; SGA, small-for-gestational age; WAZ, weight-for-age z-score; WLZ, weight-for-length z-score; NA, not available

a. Only major selection criteria are listed here. A study population is considered "unselected" if the inclusion and exclusion criteria were such that almost all screened participants were eligible to participate in the study.

b. The study treatment groups included into a group comparison analysis differed only by the presence or absence of zinc in the supplement.

c. Total sample size and mean initial characteristics of both study groups combined, which were included into a group comparison.

d. All nutrients listed were provided to both study groups within a group comparison

e. Single-dose vitamin A was provided in all studies at baseline, except for Bangladesh 2001 [19, 20], in which it was provided at day 14.

f. Selected for breastfeeding, but more than 90% of the population was eligible for the study. The study population is therefore considered representative and defined as "unselected."

g. Iron supplementation after 4 months [27] and 5 months [29] of age.

h. No information on amount of micronutrients provided.

i. If a study included several zinc groups (different dosage or frequency), each groupwise comparison includes the placebo group and the respective zinc group.

j. Half the dose for children less than 12 months of age.

k. Half the dose for children less than 6 months of age.

l. Children with blood lead levels greater than 45 μg/dL and hemoglobin less than 90 g/L were excluded. However, 99.8% of the children screened were eligible and are therefore considered representative and defined as "unselected."

m. Children were also provided iron in iron-fortified cereal porridge separate from an aqueous multivitamin dose (containing zinc in the zinc group).

n. Most children received multivitamin supplement and cotrimoxazole.

Besides mean age, the magnitude of the effect of zinc supplementation on the incidence of diarrhea was negatively associated with the baseline anthropometric status of the study population (initial height, $p = .033$; initial weight, $p = .032$) and positively associated with the mean initial serum ferritin concentration ($p = .036$). However, there were no significant correlations between the daily zinc dose, inclusion of other micronutrients in the supplement preparation, or the duration of supplementation and the impact of supplementation on diarrhea incidence. We also examined whether methodologic issues, such as the duration of recall for illness reporting and whether the reports were based on specific signs of diarrhea or parental perceptions of illness, affected the conclusions. We found that there was no relation between the specific study methods and the effect size of the zinc response.

Data were available on the duration of diarrhea from just nine studies, which provided 13 groupwise comparisons among a total of 1,692 children. The mean age of the study participants ranged from 6 to 29 months. Unlike what has been reported previously from diarrhea treatment studies [100], there was no significant effect of preventive zinc supplementation on the duration of diarrhea in these community-based trials (effect size, 0.041; 95% CI, −0.216 to 0.299; $p = .73$, random-effects model).

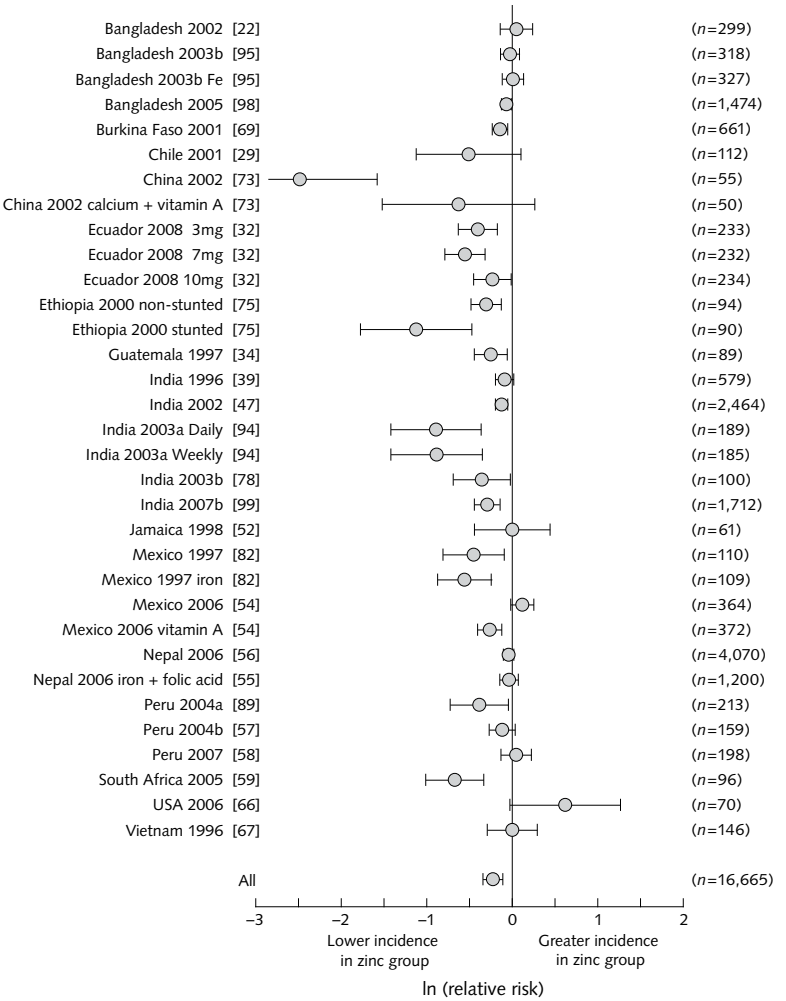

FIG. 2. Effect of zinc supplementation on diarrhea incidence from 24 intervention trials with 33 groupwise comparisons in which the supplements differed only by the presence or absence of zinc.

Respiratory disease morbidity. Analyses related to respiratory disease were restricted to studies that provided information on the incidence of ALRI, using either the World Health Organization (WHO) definition of ALRI, based on age-specific elevated respiratory rates [101], or clinical (auscultory or radiologic) evidence of pneumonia, as defined by the authors. When data were reported for elevated respiratory rates, both with and without associated severity signs, such as cough, difficulty breathing, fever, or lethargy, the illness rates based on the more severe degree of ALRI were used preferentially. Likewise, when information was available from both fieldworkers and physicians, illness rates based on the physicians' examinations were the ones included in the analysis. We also considered a second tier of studies that reported ALRI based on rapid breathing or difficulty breathing, as reported by the caregiver. Information was available from seven studies based on the former, objective criteria [32, 41, 45, 57–59, 98] and from five studies based on reported symptoms only [22, 54–56, 95].

The combined set of studies yielded a total of 16 treatment comparisons from 12 studies with a total of 12,144 subjects. The children's mean initial age ranged from 0.9 to 49 months. Zinc supplements were compared with placebo in six treatment group comparisons. Three comparisons were of MMN, with and without zinc, one of vitamin C, with or without zinc, and six of vitamin A with other micronutrients, such as iron, iron and folic acid, or vitamin B_2, with and without zinc. Overall, there was a significant 15% reduction in ALRI (relative risk, 0.85; 95% CI, 0.75 to 0.97; $p = .017$,

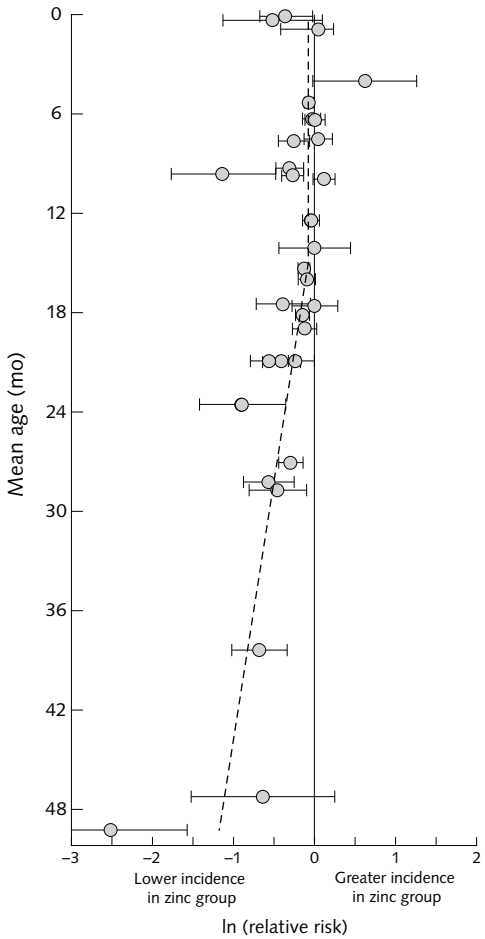

FIG. 3. Effect of zinc supplementation on diarrhea incidence, according to mean initial age of study subjects in each trial. The curve represents ln(risk ratio) = − 0.081 for age less than 15 months, − .081 − .032*(age − 15) for age greater than 15 months

random-effects model) (**fig. 4**). There was significant heterogeneity among studies (p = .008). The two factors that were significantly associated with the magnitude of reduction of relative risk of ALRI following zinc supplementation were the initial height-for-age z-score (HAZ) (p = .010) and the quality of ALRI diagnosis (p = .024). Specifically, studies that enrolled children who initially were more stunted found a greater impact of zinc supplements on ALRI reduction, as did those studies that relied on more rigorous diagnostic criteria. The relative risk for those studies that diagnosed ALRI based on counting respiratory rate or a physician's examination was 21% less in the zinc group than in the comparison group (relative risk, 0.79; 95% CI, 0.67 to 0.94; p = .013, random-effects model). In contrast, the studies that based the diagnosis only on reported rapid breathing or difficulty breathing (without a physician's examination) found no significant difference between the group that received zinc and the comparison group (relative risk, 0.99; 95% CI, 0.91 to 1.08; p = .78, random-effects model). When both factors (initial HAZ and diagnostic rigor) were included in the explanatory models, only HAZ remained statistically significant.

Malaria morbidity. The effects of zinc supplementation on the risk of malaria were examined in the first technical document prepared by the International Zinc Nutrition Consultative Group (IZiNCG) [102]. At that time, the results of just three intervention trials were available, two of which found 32% (Gambia 1993 [11]) and 38% (Papua New Guinea 2000 [88]) reductions in clinic visits for malaria, and one of which found no impact on the incidence of cases detected by daily home visits (Burkina Faso 2001 [69]). The former IZiNCG review concluded that zinc supplementation may ameliorate the severity of malaria infections, hence reducing the number of clinic visits, possibly without affecting the overall incidence of infections. However, the number of available trials was too small to allow definitive conclusions to be drawn.

Since then, only two new relevant studies have become available, neither of which fulfilled the inclusion criteria for the present review. A study in the Peruvian Amazon enrolled children from 0.5 to 15 years of age, some of whom exceeded the age range established for the present review [8]. Children who received either zinc or zinc plus iron had ~15% fewer episodes of *Plasmodium vivax* infections, as assessed by twice-weekly home visits, compared with the placebo group, although the results were not statistically significant (p > .36). However, there was a significant interaction between age group and treatment group, such that, among children less than 5 years of age, those who received zinc without iron had an incidence rate ratio (IRR) of 0.43 (95% CI, 0.17 to 1.10; p = .079), and those who received zinc with iron had an IRR of 0.30 (95% CI, 0.12 to 0.80; p = .016), compared with the placebo group. However, among children aged 5 years or older, there was no significant effect of zinc alone or zinc plus iron. In another study recently completed in Burkina Faso, children were randomly assigned to receive either daily zinc supplements plus a single large dose of vitamin A or placebo supplements [103]. There was a 22% lower rate of fever in the supplemented group, as diagnosed during daily home visits, and a 30% reduction in malaria incidence, as determined during clinic visits. However, because of the intervention design, which included both zinc and vitamin A, it was not possible to determine whether the results were uniquely attributable to the zinc supplements.

In summary, there is still insufficient evidence to allow definitive conclusions to be drawn regarding the effect of zinc supplementation on the risk of malaria, although the weight of currently available information suggests that zinc may reduce the incidence of malaria,

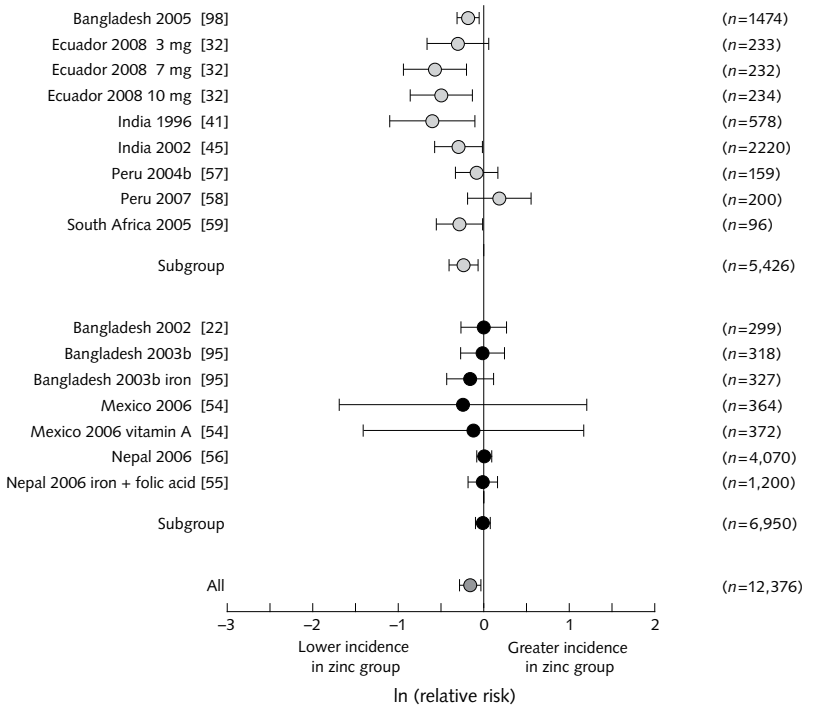

FIG. 4. Effect of zinc supplementation on the incidence of acute lower respiratory tract infection (ALRI)[a] from 12 intervention trials with 16 groupwise comparisons in which the supplements differed only by the presence or absence of zinc.

a. Gray circles indicate studies in which ALRI was diagnosed by fieldworkers or physicians by using objective clinical signs; black circles indicate studies in which the diagnosis was based on caregiver reports of elevated respiratory rate or difficulty breathing

especially that of more severe cases that result in clinic attendance.

Mortality. Thirteen pertinent groupwise comparisons of mortality outcomes were available from 10 studies. Seven of these studies were carried out in unselected study populations [45, 48, 55, 56, 60, 61, 69, 88, 98], one included only low-birthweight infants [12], one included only SGA infants [43], and one enrolled only children with human immunodeficiency virus (HIV) infection [59]. Three of the group comparisons completed among unselected children were from large-scale studies carried out in Tanzania 2006 (n = 16,070 [60]), Nepal 2006 (n = 17,079 [55]), and India 2007a (n = 78,346 [48]), in which zinc plus iron and folic acid was compared with iron and folic acid only, and two were from the same studies in Tanzania 2006 (n = 42,546 [61]) and Nepal 2006 (n = 25,018 [56]), in which zinc was compared with placebo. Four smaller studies also compared mortality outcomes following supplementation with zinc or placebo (Bangladesh 2005, n = 1,474 [98]; India 2002, n = 2,482 [45]; Papua New Guinea 2000, n = 274 [88]; Burkina Faso 2001, n = 685 [69]) in unselected children. These latter studies were not originally designed with sufficient statistical power to detect small differences in mortality outcomes, so the results may be susceptible to publication or reporting bias. Some of the studies also provided a single high-dose vitamin A supplement at baseline [45] or every 6 months during the study period [55, 56, 60, 61]. Although the distribution of high-dose vitamin A supplements was not reported in the other studies [48, 69, 88, 98], children may have received such supplements as part of ongoing national programs.

Overall, there were 1,407 deaths among the 100,081 children in the control groups (1.41%) and 1,328 deaths among the 101,535 children in the zinc-supplemented groups (1.31%). The estimated relative risk of mortality was 0.94 (95% CI, 0.86 to 1.02; p = .11, random-effects model) (**fig. 5**). There was significant heterogeneity in the results (p = .005), but the number of studies was too small to explore systematically the specific sources of heterogeneity.

Because of the heterogeneity among studies and the fact that the results are dominated by the larger trials in Nepal 2006 [55, 56], Tanzania 2006 [60, 61], and India 2007a [48] (with five groupwise comparisons), we reexamined the outcomes for specific age subgroups presented within these three larger trials and whether or not iron and folic acid were provided

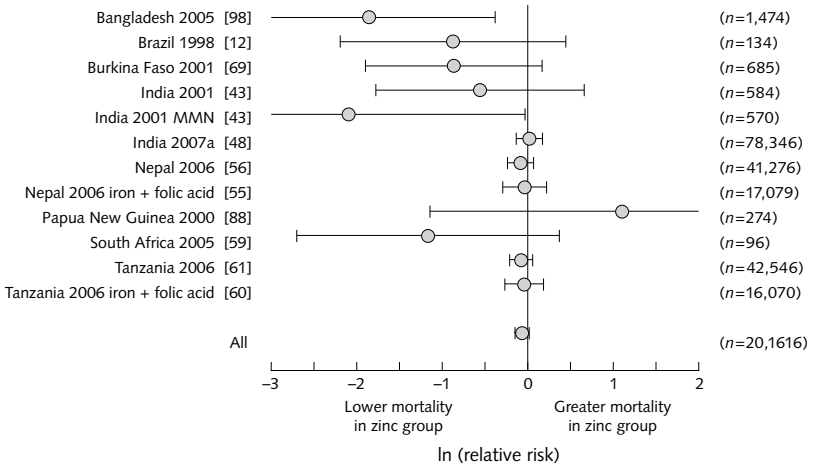

FIG. 5. Effect of zinc supplementation on childhood mortality from 10 intervention trials[a] with 13 groupwise comparisons in which the supplements differed only by the presence or absence of zinc.

a. The figure does not include the study by Bhandari et al. [45] (India, 2002; n = 2,482) because this study had no deaths in the zinc-supplemented group

along with zinc. The authors of these trials graciously provided the results of their respective studies disaggregated by age group (< 12 months or ≥ 12 months) for the comparisons of zinc versus placebo and zinc plus iron and folic acid versus iron and folic acid. We modeled the mortality data using mixed models with log relative risk as the outcome variable, with possible explanatory variables age group (as defined above), iron and folic acid treatment, study (a random effect), and their interactions. Notably, there was a significant (p = .04) interaction between age and supplementation with iron and folic acid, such that when zinc supplements were compared with placebo, there was a significantly lower mortality rate among the older children who did not receive iron and folic acid as compared with the other three groups. When iron and folic acid were provided along with zinc, there was no significant effect of zinc on mortality in either age group. The combined results are summarized in **table 2** by age group and treatment group.

In summary, when the results of these studies are combined, zinc supplementation reduced mortality of children 12 months of age or older by ~18% but had no effect on younger children. However, when iron and folic acid were provided in addition to zinc, the impact of zinc among older children was no longer evident. The remaining studies either enrolled only younger children or did not present the results disaggregated by age, so it is not possible to explore this issue further with the available information.

Among the studies of selected study populations, two enrolled low-birthweight [12] or SGA infants [43]. In the study Brazil 1998, low-birthweight infants received zinc or placebo [12], whereas the study India 2001

TABLE 2. Effect of supplementation with zinc only or zinc plus iron and folic acid on risk of death among children < 12 months or ≥ 12 months of age: Combined analyses of results from three large-scale trials (with five groupwise comparisons) [48, 55, 56, 60, 61]

Age group (mo)	Group comparison[a]	Sample size	Child-yr	Deaths	Mortality rate per 1,000 child-yr	Relative risk	95% CI	p
< 12	Zinc	27,440	15,328	385	25.1	1.05	0.91–1.21	.52
	Placebo	26,974	14,951	360	24.1			
≥ 12	Zinc	14,802	43,595	332	7.6	0.82	0.70–0.96	.013
	Placebo	14,606	43,343	406	9.4			
< 12	Folic acid + iron + zinc	32,859	23,410	349	14.9	0.97	0.82–1.15	.72
	Folic acid + iron	32,456	23,205	352	15.2			
≥ 12	Folic acid + iron + zinc	38,441	34,681	242	7.0	1.05	0.90–1.24	.52
	Folic acid + iron	37,721	33,977	230	6.8			

a. Refers to comparisons of treatment groups that differed only by the presence or absence of zinc. (i.e., zinc versus placebo or zinc plus iron and folic acid versus iron plus folic acid).

included two group comparisons in which SGA infants received vitamin B_2 with and without zinc or MMN with or without zinc [43]. Although the sample sizes were relatively small, both studies found 52% to 68% lower mortality rates among children who received zinc (Brazil 1998, $p = .33$; India 2001, $p = .04$). These results are consistent with the analyses by birthweight in Nepal 2006, where infants with birthweight less than 2,000 g who received zinc had a relative risk of mortality that was nearly half that of their counterparts who did not receive zinc (relative risk, 0.56; 95% CI, 0.30 to 1.04; $p = .06$). These combined sets of results indicate that providing preventive zinc supplementation in settings where there is an elevated risk of zinc deficiency would reduce mortality among children greater than 1 year of age and possibly among low-birthweight infants. However, additional studies are needed to confirm these two sets of results.

Physical growth. Information on change in height was available from 37 studies, which contained 47 groupwise comparisons. The mean initial HAZ ranged from −2.9 [67] to 0.36 [66], and the mean initial age ranged from less than 1 month [12] to 134 months [10]. There was a significantly greater change in height among children who received zinc supplements, with an overall effect size of 0.170 (95% CI, 0.075 to 0.264; $p = .001$, random-effects model) (**fig. 6**). There was significant heterogeneity among studies ($p < .0001$). The effect size for change in height was negatively correlated with concurrent administration of iron ($p = .04$) and vitamin A supplements ($p = .04$). Unlike the results of a previous meta-analysis of the effect of zinc supplementation on children's growth [1], which found a positive response to zinc only among those studies that enrolled children whose initial mean HAZ was less than approximately −1.5 z, there was no correlation between mean initial HAZ and effect size in the present analysis, even when the analysis was restricted to the subset of studies that

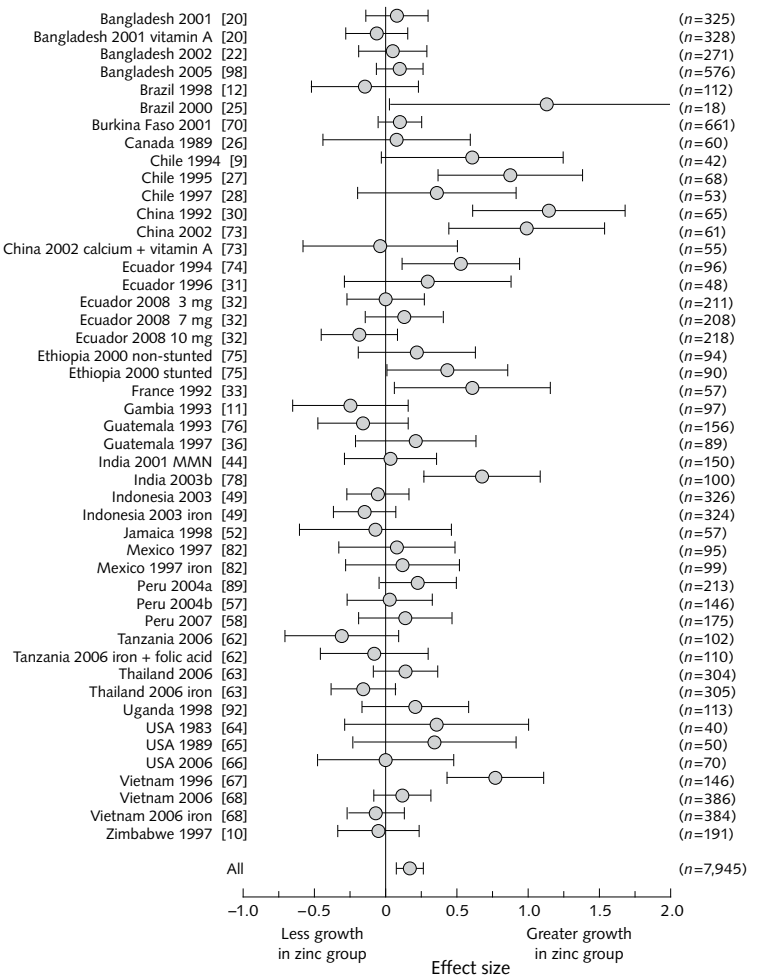

FIG. 6. Effect of zinc supplementation on change in height in prepubertal children from 37 controlled supplementation trials with 47 groupwise comparisons in which the supplements differed only by the presence or absence of zinc.

lasted at least 6 months or to those studies that enrolled children with a mean initial age less than 3 years. This difference from the earlier meta-analysis may be due to the exclusion of studies of hospitalized, severely malnourished children from the present analysis.

Thirty-five studies presented sufficient information to permit assessment of the effect of zinc supplementation on the change in weight from baseline to the end of the intervention. These studies provided 45 groupwise comparisons, and the mean initial weight-for-age z-score (WAZ) ranged from −2.61 [67] to 0.76 [33]. Zinc supplementation had a significant positive overall impact on change in weight, with a mean effect size of 0.119 (95% CI, 0.048 to 0.190; $p = .002$, random-effects model) (**fig. 7**). There was significant heterogeneity among studies ($p < .001$). The effect size for change in weight was negatively correlated with concurrent administration of iron supplements ($p = .002$), but not with any other characteristics of the studies or study subjects.

Twenty-two studies, with 30 groupwise comparisons, provided information on the effect of zinc supplementation on change in weight-for-height z-score (WHZ). There was a small, marginally significant, positive effect of zinc on change in WHZ (**fig. 8**). The estimated effect size was 0.062 (95% CI, 0.000 to 0.123; $p = .049$, random-effects model), and there was no significant heterogeneity among studies ($p = .28$).

There were 11 studies and 14 groupwise comparisons of the effect of zinc supplementation on change in mid-upper-arm circumference. Zinc supplementation did not have a significant effect on change in mid-upper-arm circumference (data not presented here).

In summary, zinc supplementation produced a small, but highly statistically significant, positive impact on children's linear growth and weight gain and a marginal effect on weight-for-height. There was significant heterogeneity in the results of studies of growth velocity,

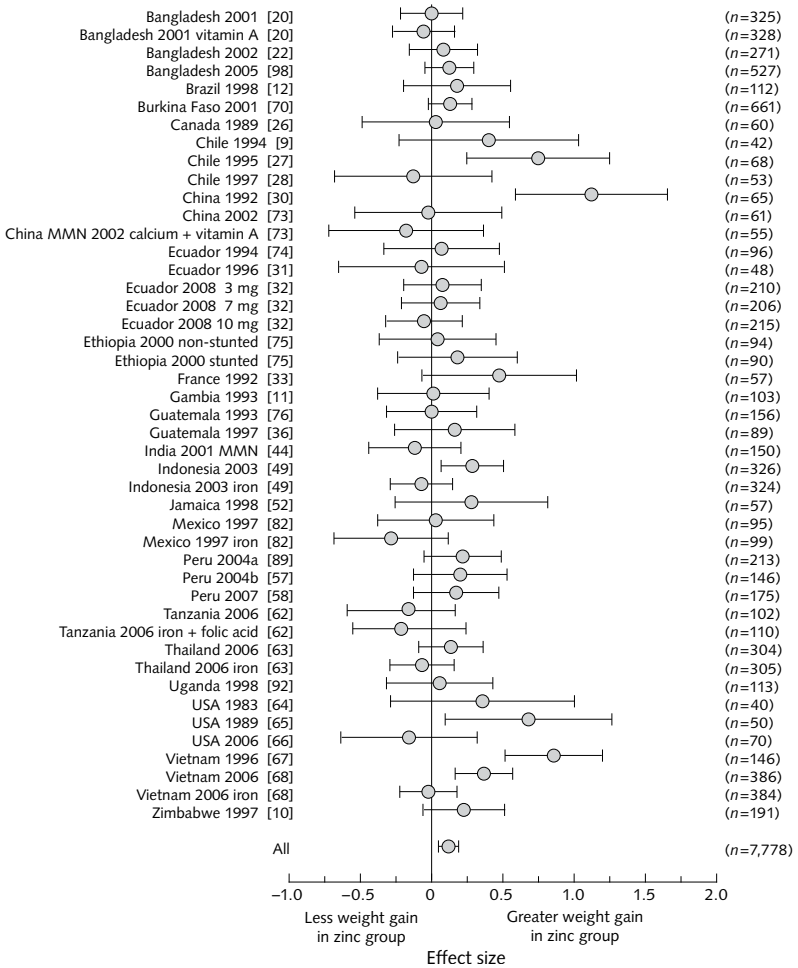

FIG. 7. Effect of zinc supplementation on change in weight in prepubertal children from 35 supplementation trials with 45 groupwise comparisons in which the supplements differed only by the presence or absence of zinc.

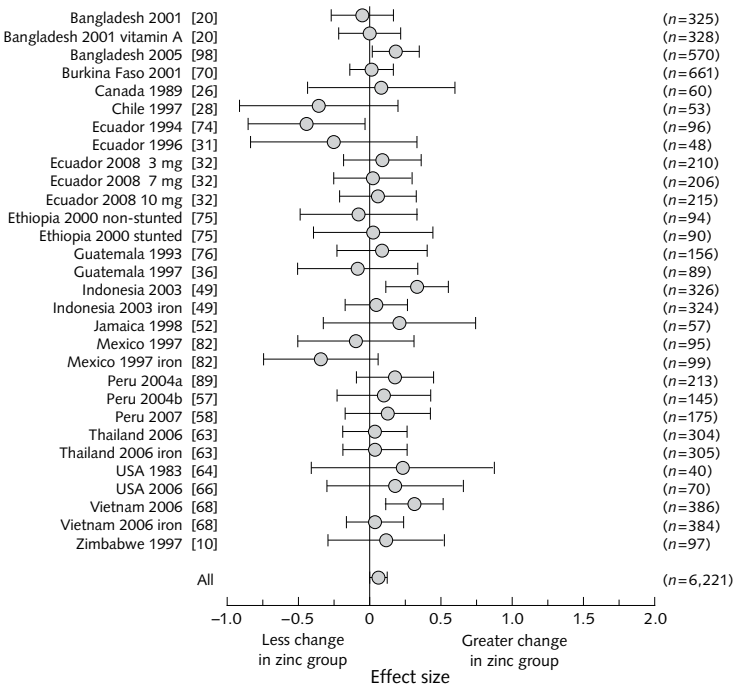

FIG. 8. Effect of zinc supplementation on change in weight-for-height z-score (WHZ) in prepubertal children from 22 controlled supplementation trials with 30 groupwise comparisons in which the supplements differed only by the presence or absence of zinc.

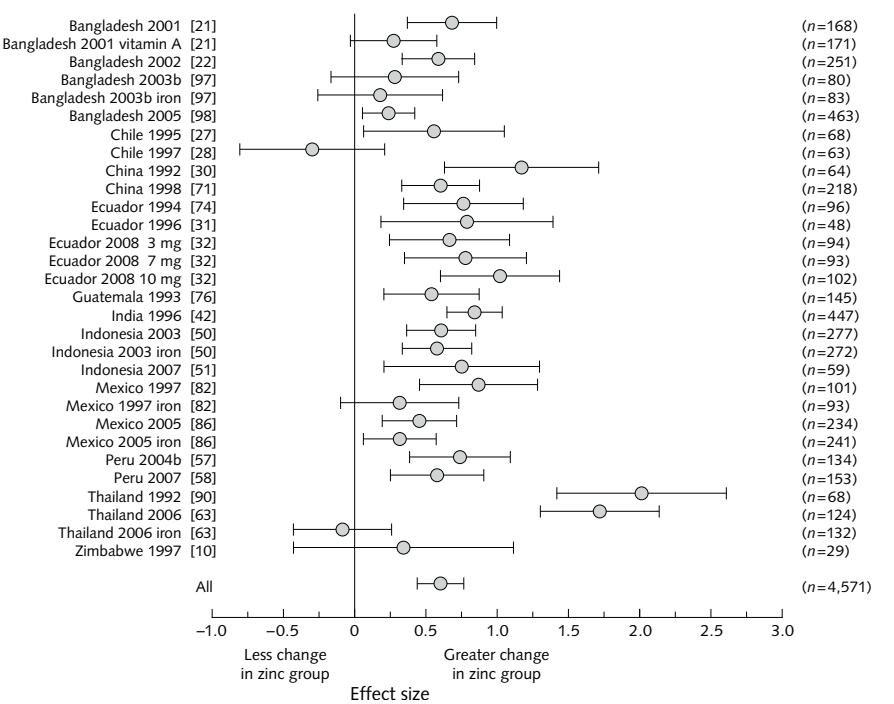

FIG. 9. Effect of zinc supplementation on change in serum or plasma zinc concentration in children from 22 controlled supplementation trials with 30 groupwise comparisons in which the supplements differed only by the presence or absence of zinc.

but the source of heterogeneity generally could not be identified, except for a negative association with concurrent supplementation of either iron or vitamin A for change in height and a negative association with concurrent iron supplementation for change in weight. There was no overall effect of zinc supplementation on mid-upper-arm circumference measurements.

Serum or plasma zinc concentration. Information on the change in serum or plasma zinc concentration was available from 22 intervention trials consisting of 30 groupwise comparisons (**fig. 9**). As in previous meta-analyses [1, 104], there was a consistent, moderately large, statistically significant positive effect of zinc supplementation on the change in serum zinc concentration, with an overall effect size of 0.602 (95% CI, 0.439 to 0.766; $p < .0001$, random-effects model). The daily zinc dose equivalents ranged from 2.9 to 21.4 mg of zinc/day, and the studies lasted from 2 weeks to 14 months. There was significant heterogeneity of results ($p < .001$), but the source of heterogeneity could not be identified.

Mental and motor development. The available studies that reported on children's developmental outcomes in relation to zinc supplementation varied greatly with regard to their developmental assessment methods. For the present analyses, we only considered studies that reported information on the mental development index (MDI) or psychomotor development index (PDI), using the Bayley Scales. Most studies did not present intraindividual changes in developmental scores during the course of the intervention, so only final values could be compared. Final MDI and PDI values were reported from seven studies that provided nine groupwise comparisons. The study duration ranged from 1.9 to 12 months, and just two studies lasted more than 6 months. Two comparisons evaluated the impact of zinc supplementation versus placebo [13, 23], and the others provided additional micronutrients, such as iron [29, 49, 96] or vitamin A [46, 96], to both groups. None of the studies found a significant positive effect of zinc on final MDI (**fig. 10**). The overall estimated effect size was 0.021 (95% CI, −0.133 to 0.175; $p = .76$, random-effects model). There was marginally significant heterogeneity among studies ($p = .065$), and there was a significant association between effect size for final MDI and the percentage of males enrolled in the individual studies ($p = .024$).

As with MDI, there was no significant overall impact of zinc supplementation on final PDI (**fig. 11**). The estimated effect size was 0.025 (95% CI, −0.149 to 0.198,

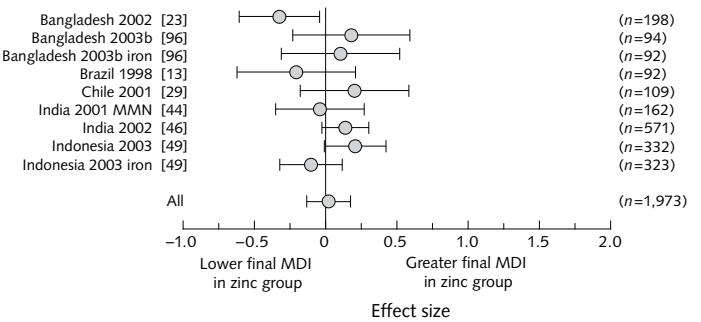

FIG. 10. Effect of zinc supplementation on final mental development index (MDI) among infants and young children from seven intervention trials with nine groupwise comparisons in which the supplements differed only by the presence or absence of zinc.

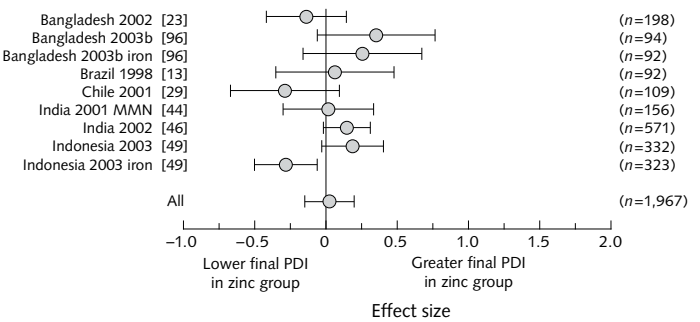

FIG. 11. Effect of zinc supplementation on final psychomotor development index (PDI) in infants and young children from seven zinc supplementation trials with nine groupwise comparisons in which the supplements only by the presence or absence of zinc.

$p = 0.75$, random-effects model). There was significant heterogeneity among studies ($p = .013$), but there were no significant correlations between study or subject characteristics and effect size.

Section 2

Are there adverse effects of preventive zinc supplementation?

Conclusions

According to the previous studies that have been used to define the safe upper level of zinc intake [105], the first signs of excessive intake are perturbations of copper and iron metabolism, resulting in impaired status of these nutrients. Thus, we have reviewed available studies that examined the impact of zinc supplementation on indicators of iron and copper status. There are no overall adverse effects of zinc supplementation on concentrations of hemoglobin, serum ferritin, and serum copper.

Detailed review of evidence

A number of studies have examined the effects of zinc supplementation on iron absorption and vice versa, either by using isotopic tracers during short-term studies to assess mineral absorption or by assessing biochemical and functional responses following longer-term supplementation. The tracer studies indicate that each mineral may interfere to some extent with absorption of the other, but only when they are provided simultaneously in aqueous solutions and in disproportionate molar doses [106]. However, there is no evidence of interference when they are delivered in near isomolar amounts or with food [107]. Some longer-term studies also suggest that when given together each mineral may reduce the magnitude of the response observed with single-nutrient supplementation [50, 68, 79], although nutritional status is still enhanced to a considerable extent despite the nutrient–nutrient interactions [108]. Less information is available with regard to interactions between zinc and copper, but some studies have found a negative effect of large-dose zinc supplementation on indicators of copper status in adults [109, 110].

Because some studies have noted negative effects of zinc supplementation on the absorption or status of other minerals, we completed a systematic analysis of the overall impact of preventive zinc supplementation trials on indicators of children's iron status (namely, hemoglobin and serum ferritin concentrations) and copper status (serum copper concentration). Studies were identified by using the same strategy described above in Section 1.

Hemoglobin and iron status. A total of 11 studies, which included 19 groupwise comparisons, provided information on the change in hemoglobin concentration following zinc supplementation. The daily dose equivalents for those 19 sets of observations ranged from 2.9 to 21.4 mg of zinc/day. Iron supplements were also provided in eight of these groupwise comparisons [50, 58, 62, 63, 83, 86, 89, 97]. Considering all of the available information, there is no overall effect of zinc supplementation on change in hemoglobin concentration (**fig. 12**). The estimated mean effect size was

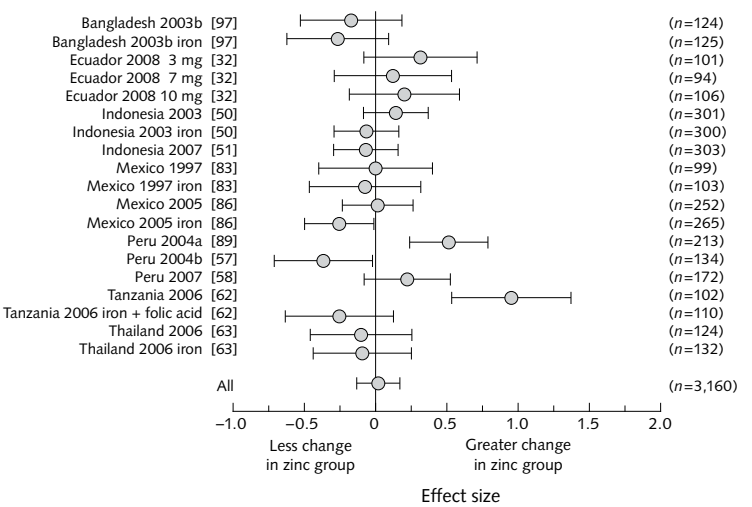

FIG. 12. Effect of zinc supplementation on change in hemoglobin concentration among children from 11 controlled zinc supplementation trials with 19 groupwise comparisons in which the supplements differed only by the presence or absence of zinc.

0.019 (95% CI, −0.132 to 0.170; $p = .80$, random-effects model). There was significant heterogeneity among studies ($p < .0001$), but no particular characteristics of the studies or the study subjects were associated with the magnitude of hemoglobin response. In particular, neither the daily zinc dose nor the presence of iron in the supplement was correlated with effect size of the change in hemoglobin concentration due to zinc.

Similarly, there was no overall effect of zinc supplementation on the change in serum or plasma ferritin concentration among the 17 available groupwise comparisons derived from 10 studies (**fig. 13**), 7 of which also provided iron [50, 58, 63, 83, 86, 89, 97]. The estimated effect size was 0.051 (95% CI, −0.150 to 0.252; $p = 0.60$, random-effects model). There was significant heterogeneity among comparisons ($p < .0001$); the magnitude of the change in serum ferritin concentration in relation to zinc supplementation was negatively correlated with the presence of iron in the supplement ($p = .024$), the mean initial hemoglobin concentration ($p = .018$), and the mean initial ferritin concentration ($p = .019$).

Copper status. Four studies involving eight groupwise comparisons supplied results on the change in serum copper concentration following zinc supplementation (**fig. 14**). There was no overall effect of zinc supplementation on the change in serum copper concentration. The estimated effect size was −0.041 (95% CI, −0.213 to 0.131; $p = .59$, random-effects model), and the daily zinc dose was not correlated with the change in serum copper concentration. However, it should be recognized that serum copper concentration is a relatively insensitive biomarker of copper status [111]. It is possible that more subtle changes in copper metabolism may have occurred, although such changes, if they did occur, would be unlikely to have any functional significance.

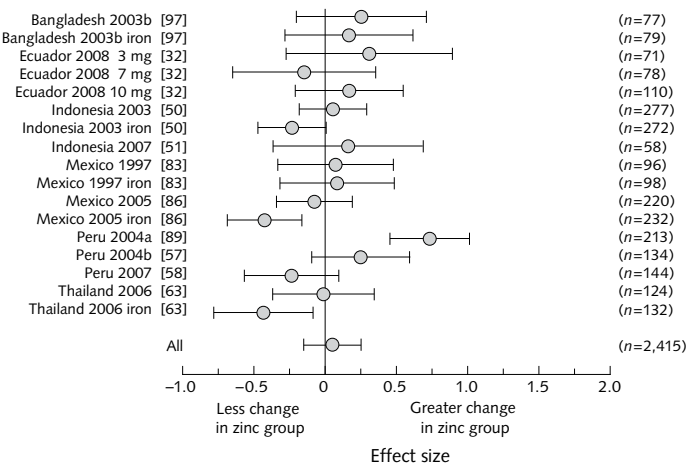

FIG. 13. Effect of zinc supplementation on change in serum or plasma ferritin concentration in children from 10 controlled zinc supplementation trials including 17 groupwise comparisons in which the supplements differed only by the presence or absence of zinc.

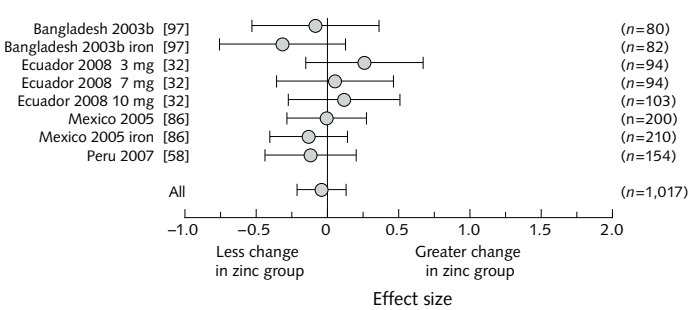

FIG. 14. Effect of zinc supplementation on change in serum copper concentration in children from four controlled zinc supplementation trials with eight groupwise comparisons in which the supplements differed only by the presence or absence of zinc.

Section 3

What are the opportunities to link preventive zinc supplementation programs to existing health and nutrition programs, and what technical, social, behavioral, and programmatic challenges must be confronted?

Available evidence regarding the impact of preventive zinc supplementation of infants and children on morbidity (diarrhea, ALRI, and perhaps malaria), mortality in children greater than 12 months of age and possibly SGA infants, and physical growth argues for the need to develop programs to prevent zinc deficiency in those countries where an elevated risk of zinc deficiency has been identified. There is no evidence of adverse effects of preventive zinc supplementation on markers of iron and copper status, indicating that zinc supplements can be delivered safely, either alone or with other micronutrients. The challenges for scaling up zinc supplementation programs are similar to those faced by other programs that attempt to procure and distribute nutritional supplements or medicines, as discussed below.

It has been stated previously that zinc needs to be provided on a daily basis for an extended period of time [102], although one study found equivalent beneficial effects when supplemental zinc was provided weekly [94]. In either case, the likely need for frequent administration of zinc supplements presents a number of programmatic challenges related to product delivery over an extended period of time and ensuring compliance. The most common, currently existing supplementation program requiring daily dosing and high compliance is iron and folic acid supplementation for pregnant and lactating women. The main operational constraints to successful delivery of such supplements have been described elsewhere [112] and include procurement and distribution of supplements, limited access to and poor utilization of health services by the target population, inadequate training and motivation of frontline health workers, inadequate counseling of target recipients or their caregivers, and low compliance of the intended beneficiaries. These are common obstacles that will need to be addressed by any supplementation program, including programs that distribute potential products such as tablets, powders, and pastes, as discussed below. In addition, there are generic issues of introduction of any new product, which include the regulatory environment, quality assurance and control, costs, supply chain and storage, product acceptability and packaging [113, 114].

The following section examines existing delivery platforms that can be tapped for distribution of zinc supplements and discusses issues that need to be addressed to deliver preventive zinc supplements successfully, either alone or in multiple-micronutrient products.

Twice-yearly vitamin A supplementation (VAS). Globally, the most successful micronutrient supplementation program for children less than 5 years of age is VAS, which is increasingly integrated into twice-yearly events for child survival (combining such interventions as deworming, vaccinations, distribution of insecticide-treated bednets, etc.) [115]. It is estimated that 79% of children 6 to 59 months of age in sub-Saharan Africa and 71% of children 6 to 59 months of age in South Asia received at least one dose of vitamin A in 2005 [116]. A recent publication that describes the progress and future directions of twice-yearly VAS in West and Central Africa [117] documents the success of such programs and calls for institutionalizing the child health day approach to deliver VAS and other low-cost, high-impact services for child survival and development. VAS programs have been very effective in reaching children 12 to 59 months of age, although they have been somewhat less successful in reaching infants 6 to 11 months of age [118]. This platform probably offers the most promising avenue for rapid scale-up of delivery of preventive zinc products, but a number of issues must be addressed:

» What duration of dosing will caregivers be able to administer correctly if the supplement supply is delivered only once every 6 months?
» What combination of zinc dose and duration of supplementation will result in optimal improvement in zinc status when delivered at 6-month intervals?
» What is the optimal presentation of the product (supplement, powder, paste) to maximize compliance and minimize costs and logistical burden?
» Can existing twice-yearly VAS programs support the additional input and logistical costs of adding preventive zinc supplementation?
» What communication strategies are required during twice-yearly events and as follow-up to these events to support optimal compliance by caregivers?
» Twice-yearly VAS programs only need to address coverage, since doses are consumed at delivery. Compliance will be essential for effective preventive zinc programs. How will programs be able to monitor and evaluate compliance?
» What is the effectiveness of these programs?

Growth monitoring and promotion (GMP). GMP programs could be ideal platforms for delivering preventive zinc supplements, because such programs provide frequent contacts with young children, thereby allowing for delivery of zinc-containing products, counseling on their use, and monitoring of compliance. A recent review of GMP programs concluded that these programs should "maximize their potential, strengthen the nutrition counseling elements, [and] combine growth monitoring with other health interventions" [119]. Preventive zinc supplementation is certainly one such health intervention that could easily meld with GMP activities. A particular advantage of such programs is

that they provide routine contacts that can be exploited to ensure delivery of supplements over an extended period and to promote compliance.

Community-based or community-directed distribution programs. Various community-based distribution systems exist in which the supply system is an extension of the health services. In these systems, either health workers visit communities to renew and supervise distribution of supplies or community distributors report to the health center to renew stocks. For example, community-directed treatment with ivermectin (CDTI) is active in 26 countries in sub-Saharan Africa to control onchocerciasis. Community-directed distributors are chosen by the community to provide once-yearly treatment. The scope of CDTI is being expanded to include elimination of lymphatic filariasis and delivery of other services [120, 121]. Another example is provided by traditional birth attendants, who have been trained to deliver a variety of services, including distribution of iron and folic acid tablets to pregnant and lactating women [122]. The issues of integrating preventive zinc products are similar to those described for GMP programs, particularly if the program has ongoing contact with the intended beneficiaries. For programs such as CDTI that only have intermittent contact operations, research is needed to see whether these systems can be expanded to deliver products on an ongoing basis.

Social marketing. This strategy is increasingly used to deliver products through commercial channels or messages to intended beneficiaries. A 1992 review defines social marketing as "a broader, systematic approach to developing strategies to define acceptable concepts, behaviors, or products, to promote them, and in the case of products, to distribute and price them for the market. A complete social marketing strategy not only develops and promotes a good 'product,' but also achieves and maintains political support and trains and motivates program implementers" [123]. Prices are often subsidized or programs have cross-subsidies to enhance reach to low-socioeconomic groups. In addition to issues cited for other delivery strategies, a specific issue is the extent to which such approaches reach the poorest and most remote beneficiaries and how well relevant messages on dosing issues can be communicated.

Point-of-use fortificants. There has been rapid development of point-of-use fortificants, including powders (often called "Sprinkles"), dispersible or crushable tablets, and lipid-based nutrient supplements, which are designed to address deficiencies in MMN and sometimes essential fatty acids and proteins [124]. The programmatic issues of their delivery are not substantially different from delivery of a supplement, and it is assumed that the above-mentioned platforms could be used. These products have the added advantage of favoring a delivery strategy that copromotes the product and optimal infant and young child feeding practices. An issue specific to zinc is determining the level of zinc necessary in the context of specific diets to result in adequate improvement in zinc status [125]. If such products are to include iron, there are several other issues that need to be addressed to ensure safety [126], although a full review of these issues is beyond the scope of this paper

Reaching low-birthweight infants. Low-birthweight infants have multiple special nutritional needs. They have a greater risk of breastfeeding difficulties and have an elevated risk of iron deficiency [127, 128]. Thus, programs to address the special health and nutritional needs of low-birthweight infants would have a scope far broader than just preventive zinc supplementation, although preventive zinc supplementation should be included as a key component. Although birthweight is often measured in clinical settings, this information is seldom used to provide a special package of interventions to meet the needs of low-birthweight infants. Issues that would have to be addressed in designing a strategy to target these infants would include:

- Systematic identification of low-birthweight infants (ensuring accurate weighing at delivery for births attended by trained personnel; integrating weighing at the first contact for other infants, for example, through the expanded program on immunization, etc.);
- Definition of the minimum package of low-birthweight infant care;
- Identifying community and health service contacts that can be mobilized to deliver the package;
- Monitoring and evaluation of delivery of and compliance with the package.

In summary, the available evidence on the impact of preventive zinc supplementation supports the need for intervention programs to enhance zinc status. There are a number of available opportunities to deliver preventive zinc supplementation as one component of programs to prevent MMN deficiencies and to address other nutrition and health needs of infants and children. Efforts are needed to test these delivery mechanisms and evaluate their potential for providing cost-effective preventive zinc supplementation to high-risk target groups on a large scale.

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The effect of therapeutic zinc supplementation among young children with selected infections: A review of the evidence

Batool A. Haider and Zulfiqar A. Bhutta

Abstract

Background*. Zinc deficiency is now widely recognized as a leading risk factor for morbidity and mortality and is estimated to be responsible for approximately 800,000 excess deaths annually among children under 5 years of age.*

Objective. *To evaluate the impact of zinc supplementation as an adjunct in the treatment of diarrhea, pneumonia, malaria, and tuberculosis in children under 5 years of age.*

Methods. *A comprehensive literature search of electronic databases to identify randomized, controlled trials on the topic was undertaken in January 2008. Eligible studies identified on search were reviewed by the authors and data extraction was done. Statistical analyses were performed with the use of Review Manager software.*

Results. *Current analysis of the adjunctive therapeutic benefit of zinc in acute diarrhea corroborates existing reviews and provides evidence of reduction in the duration of acute diarrhea by 0.5 day ($p = .002$) in children under 5 years of age. However, zinc supplementation is found to have no beneficial impact in infants under 6 months of age. A beneficial effect of zinc as an adjunctive treatment is also found in persistent diarrhea, the duration of which is reduced by 0.68 day ($p < .0001$). Evidence of the benefit of zinc supplementation in pneumonia and malaria is insufficient, whereas no studies are available in children with tuberculosis.*

Conclusions. *The existing literature provides evidence of a beneficial effect of therapeutic zinc supplementation in the reduction of the duration of acute and persistent diarrhea. However, evidence for its impact on pneumonia, malaria. and tuberculosis in children under 5 years of age is insufficient and needs further evaluation.*

The authors are affiliated with the Department of Pediatrics and Child Health, the Aga Khan University, Karachi, Pakistan.

Please direct queries to the corresponding author: Zulfiqar A. Bhutta, Department of Paediatrics and Child Health, the Aga Khan University, Karachi, Pakistan; email: zulfiqar.bhutta@aku.edu.

Key words: Diarrhea treatment, infections, malaria, therapeutic, tuberculosis, zinc supplement

Background

Zinc is vital for several body functions, including protein synthesis and cell growth and differentiation. Severe zinc deficiency is characterized by stunted growth, hypogonadism, impaired immune function, skin disorders, cognitive dysfunction, and anorexia [1]. Zinc deficiency is now widely recognized as a leading risk factor for morbidity and mortality [2, 3]. It is estimated that zinc deficiency is responsible for approximately 800,000 excess deaths annually among children under 5 years of age. These deaths are related to diarrhea (176,000), pneumonia (406,000), and malaria (207,000) brought on by inadequate zinc intake [4]. Further, a loss of nearly 16 million global disability-adjusted life years (DALYs) is attributed to zinc deficiency [2]. The aim of this review is to evaluate the impact of zinc supplementation as an adjunct in the treatment of infectious diseases (diarrhea, pneumonia, malaria, and tuberculosis) in children under 5 years of age.

This paper is divided into five sections, which address the following questions in relation to therapeutic zinc supplementation:

Section 1: What is the effect of therapeutic zinc supplementation as an adjunct in the treatment of children with acute diarrhea on the duration of the disease?

Section 2: What is the effect of therapeutic zinc supplementation as an adjunct in the treatment of children with persistent diarrhea on the duration and severity of the disease?

Section 3: What is the effect of therapeutic zinc supplementation as an adjunct in the treatment of children with pneumonia on the duration and severity of the disease?

Section 4: What is the effect of therapeutic zinc

supplementation as an adjunct in the treatment of children with malaria?

Section 5: What is the effect of therapeutic zinc supplementation as an adjunct in the treatment of children with tuberculosis?

Methods

To identify randomized, controlled trials evaluating the effect of zinc supplementation as an adjunctive treatment for diarrhea, pneumonia, malaria, and tuberculosis in children under 5 years of age, we undertook a comprehensive search of the electronic published literature up to January 2008, including PUBMED, POPLINE, EMRO, and African Index Medicus. The Cochrane Databases of Systematic Reviews and Clinical Trials were also searched. The search was conducted by the authors, who reviewed the titles and abstracts, and, if required, full texts were retrieved to assess the eligibility of the studies. All identified eligible studies were then reviewed by the authors, and data were extracted.

Statistical analyses and meta-analyses were performed by using Review Manager software, version 4.2.8 (Cochrane Collaboration, 2003). Weighted mean differences (WMD) for continuous outcomes and relative risks (RR) for dichotomous outcomes were calculated to present impact estimates. Where necessary, data were pooled using a generic inverse variance method. Logarithms of relative risk or hazards and their standard errors were calculated by standard statistical methods. All impacts were presented as summary estimates along with 95% confidence intervals. For each parameter, heterogeneity among the studies was tested by chi-square statistics, I^2 statistics, and visual inspection of forest plots. Heterogeneity was found to be substantial if the chi-square statistic was significant ($p < .01$), I^2 was more than 50%, and the visual inspection of forest plots was suggestive. In case of absence of significant heterogeneity, a fixed-effects model was used; however, in the presence of significant heterogeneity, a random-effects model was used for pooling of data. This was followed by subgroup analysis to explore the possible causes of heterogeneity.

Section 1

What is the effect of therapeutic zinc supplementation as an adjunct in the treatment of children with acute diarrhea on the duration of the disease?

Conclusions

Existing evidence indicates that the inclusion of zinc supplementation in the treatment of acute diarrhea in children reduces the duration of diarrhea by 0.5 day (WMD, −0.50; 95% confidence interval [CI], −0.82 to −0.18). These results support existing recommendations that zinc supplementation should be included as a component of treatment of children with acute diarrhea.

Detailed review of the evidence

Several clinical trials have studied the effect of administration of zinc to children with diarrheal illness to determine whether this intervention can reduce the severity and duration of diarrhea. In a systematic review and pooled analysis of randomized, controlled trials of children under 5 years of age with acute diarrhea in the year 2000, the Zinc Investigators' Collaborative Group [5] found four studies that evaluated the effect of adjunctive therapy with zinc supplements containing at least 50% of the US recommended dietary allowance (RDA) per day. Pooled analysis of data from three of these studies showed that children who received zinc supplementation had a 15% lower probability of continuing diarrhea on a particular day than those not receiving zinc.

To update this previous analysis, a literature search was undertaken in early January 2008. The studies eligible for inclusion in the review were randomized, controlled trials that evaluated the adjunctive therapeutic effect of zinc supplementation in children under 5 years of age with acute diarrhea. The search identified 21 trials completed since 1988, of which 2 were multicenter studies. The main characteristics of the included studies [5–26] are summarized in **table 1**. Most of the studies were conducted in lower-income countries where zinc deficiency is common, and most included children who presented with acute watery diarrhea of unspecified etiology. The data from six studies could not be included in the analysis, because they did not include our outcomes of interest [23], they presented data in a form that precluded inclusion [15], they included cases of shigellosis [18] or cholera [26], or the data were not disaggregated by age (< 5 years vs. ≥ 5 years) or intervention group to allow for a specific focus on the independent effect of zinc in our age range of interest [12, 21]. Zinc was supplemented in doses varying from 5 to 45 mg/day.

The results of the meta-analysis of the 14 included studies showed that the mean duration of acute diarrhea was significantly less in the zinc-supplemented group than in the placebo group (WMD, −0.50; 95% CI, −0.82 to −0.18, random-effects model) (**fig. 1**). Because of the presence of significant heterogeneity among the included trials, subgroup analysis by age group was undertaken using 6 months of age or greater as the cutoff. Zinc supplementation was found to increase the mean duration of acute diarrhea in children under 6

TABLE 1. Randomized, controlled trials of the use of zinc in the treatment of acute diarrhea

Country, year [reference] author	Age group	Study participants	Zinc	Placebo	Interventions	Results
India, 1988 [8] Sachdev	6–18 mo	Children with acute dehydrating diarrhea < 4 days	*n* = 25	*n* = 25	Intervention: zinc sulfate 40 mg/day Control: placebo (glucose)	Zinc supplementation led to a nonsignificant 9% reduction in the duration of diarrhea and an 18% reduction in stool frequency. Children with low rectal mucosal zinc concentrations at baseline had more marked reductions in duration of diarrhea (33%) and in stool frequency (33%) than those with higher tissue zinc
India, 1995 [9] Sazawal	6–35 mo	Children with diarrhea < 7 days	*n* = 456	*n* = 481	Intervention: zinc gluconate 20 mg/day, vitamins A, B, D, and E, and ORS Control: vitamins A, B, D, and E and ORS	Zinc-supplemented group had 23% (95% CI, 12% to 32%) reduction in the risk of continued diarrhea on a given day. There was a 39% (95% CI, 6% to 70%) reduction in the mean number of watery stools per day and a 21% reduction (95% CI, 10% to 31%) in the number of days with watery stools. Reductions in duration and severity were greater in children with stunting than in those without stunting
Bangladesh, 1997 [10] Roy	3–24 mo	Children with acute diarrhea < 3 days and weight-for-age < 76th percentile of NCHS medians	*n* = 57	*n* = 54	Intervention: zinc acetate 20 mg/day and vitamins A, B, D, and C Control: vitamins A, B, D, and C	The median total diarrheal stool output was 28% less in the zinc-supplemented group ($p = .06$). There was a 14% reduction in the duration of diarrhea in the zinc-supplemented group. The subgroup with lower plasma zinc (< 14 μmol/L) at baseline had a significant (22%) reduction in duration of diarrhea
Indonesia, 1998 [11] Hidayat	3–35 mo	Children with acute diarrhea	*n* = 739	*n* = 659	Intervention: zinc acetate 4–5 mg/day Control: placebo	Mean duration of diarrhea reduced by 10% (from 3.8 ± 2.6 days to 3.5 ± 2.4 days) (not significant)
Bangladesh, 1999 [12] Faruque	6–24 mo	Children with acute diarrhea ≥ 3 days	(a) Vitamin A (*n* = 172) (b) Zinc (*n* = 170) (c) Zinc + vitamin A (*n* = 171)	*n* = 171	(a) Vitamin A, 4,500 μg RE daily; (b) 14.2 mg elemental zinc as acetate for zinc or zinc and vitamin A group for the first 417 patients enrolled in the study and 40 mg for the remaining 273 patients randomized to these groups; (c) both vitamin A and zinc at the above doses daily; (d) placebo	Zinc supplementation was associated with a reduced duration of diarrhea (13%, $p = .03$) and markedly reduced rate of prolonged diarrhea (> 7 days) (43%, $p = .017$)

continued

TABLE 1. Randomized, controlled trials of the use of zinc in the treatment of acute diarrhea *(continued)*

Country, year [reference] author	Age group	Study participants	Zinc	Placebo	Interventions	Results
Meta-analysis of 3 trials, 2000 (5) Zinc Investigators group (Sazawal 1995 [9], Roy 1997 [10], and Hidayat 1998 [11])						Zinc supplementation was associated with a 15% (95% CI, 5% to 24%) lower probability of continuing diarrhea on a given day
India, 2000 [6] Dutta	3–24 mo	Male children with acute watery diarrhea ≤ 3 days with some dehydration and < 80% Harvard standard weight-for-age	$n = 44$	$n = 36$	Intervention: zinc sulfate 40 mg/day and ORS Control: ORS	Significantly shorter duration of diarrhea (70.4 ± 10.0 vs. 103.4 ± 17.1 h, $p = .0001$), less liquid stools (1.5 ± 0.7 vs. 2.4 ± 0.7 kg, $p = .0001$), less need for ORS (2.5 ± 1.0 vs. 3.6 ± 0.8 L, $p = .0001$) and other liquids (867 ± 466 vs. 1,355 ± 676 mL, $p = .0001$)
India, 2002 [13] Bahl	6–35 mo	Children with acute diarrhea ≤ 3 days	(a) Zinc ($n = 404$) (b) Zinc + ORS ($n = 402$)	$n = 401$	Intervention: (a) 15 mg (6–11 mo) or 30 mg (12–35 mo) elemental zinc per day as a syrup; zinc gluconate, (b) ORS premixed with zinc (40 mg/L), zinc gluconate Control: ORS only.	Children receiving zinc syrup had lower duration of diarrhea (relative hazard, 0.89; 95% CI, 0.80–0.99) and fewer total stools (rate ratio, 0.73; 95% CI, 0.70 to 0.77) than controls. Children receiving zinc-ORS had fewer total stools (rate ratio, 0.83; 95% CI, 0.71 to 0.96), and had watery stools less often (odds ratio, 0.61; 95% CI, 0.39 to 0.95) than the control children. There was no significant effect on the duration of diarrhea
Nepal, 2002 [14] Strand	6–35 mo	Acute watery diarrhea	Zinc ($n = 442$) Zinc + vitamin A ($n = 447$) Zinc-caretaker ($n = 448$) In the zinc-caretaker group, zinc was administered by the caretaker, who knew that the child was receiving zinc	$n = 449$	Intervention: 15 mg/day (infants) or 30 mg/day (older infants) elemental zinc and massive vitamin A dose at enrollment in the vitamin A group Control: placebo	The relative hazards for termination of diarrhea were 26% (95% CI, 8% to 46%), 21% (95% CI, 4% to 38%), and 19% (95% CI, 2% to 40%) higher in the zinc, zinc + vitamin A, and zinc-caretaker groups, respectively, than in the placebo group. The relative risks of prolonged diarrhea (duration > 7 days) in these groups were 0.57 (95% CI, 0.38% to 0.86), 0.53 (95% CI, 0.35% to 0.81), and 0.55 (95% CI, 0.37% to 0.84), respectively. The effect of zinc was not enhanced by concomitant vitamin A administration

Bangladesh, 2002 [15] Baqui	3–59 mo	Children with acute diarrhea	*n* = 2,483	*n* = 2,502	Intervention: zinc 20 mg/day and ORS Control: ORS	About 40% (399/1,007) of diarrheal episodes were treated with zinc in the first 4 mo of the trial; the rate rose to 67% (350/526) in month 5 and to > 80% (364/434) in mo 7 and was sustained at that level. Children from the intervention cluster had a shorter duration (hazard ratio, 0.76; 95% CI, 0.65 to 0.90) and lower incidence (rate ratio, 0.85; 95% CI, 0.76 to 0.96) of diarrhea than children in the comparison group. Admission to hospital of children with diarrhea was lower in the intervention group than in the comparison group (rate ratio 0.76;95% CI, 0.59 to 0.98)
Brazil, 2003 [7] Al-Sonboli	3–60 mo	Children with acute diarrhea < 7 days and mild dehydration	*n* = 37	*n* = 37	Intervention: elemental zinc 22.5 mg/day (age 3–6 mo) and 45 mg/day (age 7–60 mo) Control: placebo group received vitamin C	Reduction in duration of diarrhea (1.2 ± 0.8 vs. 2.5 ± 1.8 days, $p < .001$) and duration of watery stools (0.4 ± 0.6 vs. 1.3 ± 1.5 days, $p < .001$) in the zinc-supplemented group. Effect was more marked in children with low serum zinc levels
Turkey, 2003 [16] Polat	2–29 mo	Malnourished (weight-for-age < 76th percentile of NCHS) children with normal zinc levels suffering from acute watery diarrhea	*n* = 52	*n* = 54	Intervention: 20 mg zinc per day Control: Placebo	The mean duration of diarrhea was shorter and the percentage of children with consistent diarrhea for more than 3–7 days was lower in the study group than in the control group. Prolonged diarrhea was present in 12% of children in the study group and in 37% of children in the control group. Stool frequency during the first 4 days after enrollment was lower in children in the study group
India, 2004 [17] Bhatnagar	3–36 mo	Male children with acute diarrhea ≤ 3 days with mild or severe dehydration	*n* = 143	*n* = 144	Intervention: zinc sulfate (15 mg/day for those aged up to 12 mo, 30 mg/day for those older) and vitamin B complex (vitamins B, C, D; and niacinamide) Control: vitamin B complex (vitamins B, C, D; and niacinamide)	Zinc treatment reduced total stool output (ratio of geometric means, 0.69; 95% CI, 0.48 to 0.99) and stool output per day of diarrhea (ratio of geometric means, 0.76; 95% CI, 0.59 to 0.98). The risk of continued diarrhea was lower (relative hazard, 0.76; 95% CI, 0.59 to 0.97). Zinc supplementation also reduced the proportion of diarrheal episodes lasting ≥ 5 days (odds ratio, 0.49; 95% CI, 0.25 to 0.97) or ≥ 7 days (odds ratio, 0.09; 95% CI, 0.01 to 0.73)
India, 2005 [19] Patel	6–59 mo	Children with acute diarrhea < 7 days	*n* = 102	*n* = 98	Intervention: 40 mg/day zinc sulfate and 5 mg/day copper sulfate and ORS Control: ORS	The mean survival time with diarrhea was not significantly different in the treatment group (4.34 ± 0.2 [SE] days) and the placebo group (4.48 ± 0.2 days), nor was there any difference in the median time to cure. Cure was less likely if the duration of diarrhea prior to enrollment was greater ($p < .001$), if the time taken for rehydration was greater ($p = .001$), and if intravenous fluids were used ($p = .03$), regardless of the micronutrient supplementation. The proportion of children with diarrhea > 4 days was 46% in the placebo group, with an adjusted odds ratio of 1.19 (95% CI, 1.58 to 0.9; $p = 0.2$), as compared with 39% in the supplemented group

continued

TABLE 1. Randomized, controlled trials of the use of zinc in the treatment of acute diarrhea *(continued)*

Country, year [reference] author	Age group	Study participants	Zinc	Placebo	Interventions	Results
Bangladesh, 2005 [20] Brooks	1–6 mo	Hospitalized male infants with acute diarrhea ≤ 3 days and some dehydration	*n* = 86	*n* = 89	Intervention: (a) 20 mg zinc acetate/day (b) 5 mg zinc acetate/day Control: Placebo	Neither duration of diarrhea nor mean stool volume differed between groups. There were no significant differences between the groups in fluid intake, the need for unscheduled intravenous fluid, weight gain, or vomiting rates
Australia, 2005 [21] Valery	< 11 yr	Children hospitalized with acute diarrhea	(a) Zinc (*n* = 107) (b) Vitamin A (*n* = 109) (c) Zinc + vitamin A (*n* = 112)	108	Intervention: (a) zinc, (b) vitamin A, (c) zinc + vitamin A Control: placebo For children < 12 mo, vitamin A 50,000 IU (days 1 and 5) and zinc sulfate 20 mg daily for 5 days; for children 1–10 yr, vitamin A 100,000 IU (days 1 and 5) and zinc sulfate 40 mg daily for 5 days	Supplementation with zinc, vitamin A, or combined zinc and vitamin A had no significant effect on duration of diarrhea or rate of readmission compared with placebo. The median duration of diarrhea after starting supplementation was 3.0 days for the vitamin A group, the zinc group, and the placebo group ($p = 0.25$ and 0.69, respectively, for the comparison of the vitamin A group and the zinc group with the placebo group). The number of readmissions did not differ significantly between those receiving vitamin A or zinc and the relevant placebo groups (relative risk for vitamin A, 1.2; 95% CI, 0.7 to 2.1; relative risk for zinc, 1.3; 95% CI, 0.8 to 2.1)
Turkey, 2006 [22] Boran	6–60 mo	Acute diarrhea < 14 days (a randomized, open-label non-placebo-controlled trial)	*n* = 150	*n* = 130	Intervention: ORS as required plus 15 mg zinc (for children 6–12 mo) and 30 mg (for children 12–60 mo) as zinc sulfate Control: ORS alone	Mean duration of diarrhea was 3.02 ± 2 days in the zinc group and 3.67 ± 3.2 days in the control group. There was no significant difference between treatment groups in the duration of diarrhea ($p > .05$). The number of stools after starting supplementation was 5.8 ± 3.7 and 5.1 ± 3.9 on day 1, 2.9 ± 1.6 and 3.0 ± 2.2 on day 2, and 1.8 ± 1.1 and 1.6 ± 0.9 on day 3 in the zinc and control groups, respectively. There was no significant difference between treatment groups in the severity of diarrhea ($p > .05$)
India, Pakistan, Ethiopia, 2006 [25] Fischer Walker	1–5 mo	Children with acute watery diarrhea < 3 days	*n* = 554	*n* = 556	Intervention: zinc (10 mg/day for 14 days) with ORS Control: placebo and ORS	The geometric mean duration of the diarrhea episode was 0.21 days longer among infants receiving zinc than among those receiving placebo, but this difference was not statistically significant, and no difference was observed after controlling for sex, exclusive breastfeeding, and LAZ. There were no differences in reported stool frequency or the proportion of episodes lasting > 7 days

Multiple countries, 2006 [23] Awasthi (Effectiveness trials in Brazil, Ethiopia, Egypt, India, and Philippines)	2–59 mo	Children with acute watery diarrhea < 7 days (Outcomes reported included ORS use on days 3–5, adherence to zinc, and any use of an antibacterial or antidiarrheal up to day 14)	*n* = 1,010	*n* = 992	Intervention: zinc (20 mg/day for 14 days) with ORS Control: ORS alone	In 5 of 6 sites, ORS use in cases with continued diarrhea on days 3–5 was the same in the 2 groups or higher in the zinc group. Overall adherence to zinc supplementation was 83.8% (95% CI, 81% to 86%). Overall, less antibiotic or antidiarrheal use occurred in the zinc group (absolute difference, 3.8%; 95% CI, 1.7 to 5.9)
Philippines, 2007 [24] Gregorio	2–59 mo	Children with acute watery diarrhea < 7 days and no evidence of dehydration	*n* = 60	*n* = 57	Intervention: zinc (20 mg/day for 14 days) with ORS Control: ORS alone	There was significantly shorter duration of diarrhea from the time of consultation in the zinc than in the ORS group (mean ± SD, 2.98 ± 0.92 vs. 3.67 ± 1.63 days, $p = .009$) with a difference of 0.69 days (16.6 h). More patients in the zinc group than in the control group had diarrhea lasting < 4 days from admission (54/59 [92%] vs. 43/57 [75%]), but the difference was not significant ($p = .780$)
Bangladesh, 2008 [18] Roy	12–59 mo	Moderately malnourished children with culture-confirmed shigellosis	*n* = 28	*n* = 28	Intervention: 20 mg/day elemental zinc and a multivitamin containing vitamins A and D, thiamine, riboflavin, nicotinamide, and calcium at twice the RDA Control: Same multivitamins alone Standard antibiotic therapy was given to both groups	Children receiving zinc recovered significantly faster than the control children ($p < .05$). The median numbers of days to recovery and to disappearance of blood and mucus were 50% shorter in the zinc group than in the control group, and these differences were significant. The mean body weight of zinc-supplemented children increased significantly ($p < .01$) from 8.8 kg on admission to 9.2 kg at recovery; the body weight of the control children increased from 9.3 to 9.6 kg, a nonsignificant change ($p = .12$).
Bangladesh, 2008 (26) Roy	3–14 yr	Children with cholera	*n* = 82	*n* = 82	Intervention: zinc (30 mg/day) Control: placebo	More patients in the zinc group than in the control group recovered by 2 days (49% vs. 32%, $p = .032$) and by 3 days (81% vs. 68%, $p = .03$). Zinc-supplemented patients had a 12% shorter duration of diarrhea than control patients (64.1 vs 72.8 h, $p = .028$) and 11% less stool output (1.6 vs. 1.8 kg/day, $p = .039$)

LAZ, length-for-age z-score; NCHS, National Center for Health Statistics; ORS, oral rehydration solution; RDA, recommended dietary allowance; RE, retinol equivalent

Review: Therapeutic zinc supplementation
Comparison: 01 Zinc supplementation vs. placebo in the treatment of acute diarrhea
Outcome: 01 Mean duration of acute diarrhea in days (all age groups)

Study or subcategory	Treatment N	Treatment Mean ± SD	Control N	Control Mean ± SD	WMD (random) 95% CI	Weight %	WMD (random) 95% CI
01 All age groups							
Al-Sonboli 2003 [7]	37	1.20 ± 0.80	37	2.50 ± 1.80		6.94	−1.30 (−1.93 to −0.67)
Bahl 2002 [13]	402	1.60 ± 2.20	401	1.70 ± 2.50		8.66	−0.10 (−0.43 to 0.23)
Bhatnagar 2004 [17]	132	2.32 ± 1.54	134	2.69 ± 1.90		8.21	−0.37 (−0.79 to 0.05)
Boran 2006 [22]	150	3.02 ± 2.00	130	3.67 ± 3.20		6.93	−0.65 (−1.29 to −0.01)
Brooks 2005 [20]	86	5.00 ± 4.73	89	5.00 ± 4.81		3.35	0.00 (−1.41 to 1.41)
Dutta 2000 [6]	44	2.93 ± 0.42	36	4.31 ± 0.71		8.94	−1.38 (−1.64 to −1.12)
Gregorio 2007 [24]	60	2.98 ± 0.92	57	3.67 ± 1.63		7.83	−0.69 (−1.17 to −0.21)
Hidayat 1998 [11]	739	3.50 ± 2.40	659	3.80 ± 2.60		8.94	−0.30 (−0.56 to −0.04)
Patel 2005 [19]	102	4.34 ± 2.02	98	4.48 ± 1.98		7.42	−0.14 (−0.69 to 0.41)
Polat 2003 [16]	52	4.70 ± 1.40	54	5.20 ± 1.60		7.31	−0.50 (−1.07 to 0.07)
Roy 1997 [10]	37	5.10 ± 2.50	37	5.50 ± 2.70		4.15	−0.40 (−1.59 to 0.79)
Sachdev 1988 [8]	25	3.40 ± 1.80	25	3.80 ± 1.70		5.10	−0.40 (−1.37 to 0.57)
Sazawal 1995 [9]	456	4.50 ± 3.60	481	5.40 ± 3.40		8.02	−0.90 (−1.35 to −0.45)
Fischer Walker 2006 [25]	554	4.93 ± 3.90	556	4.49 ± 3.17		8.19	0.44 (0.02 to 0.86)
Subtotal (95% CI)	2,876		2,794			100.00	−0.50 (−0.82 to −0.18)

Test for heterogeneity: $\chi^2 = 82.27$, df = 13 ($p < .00001$), $I^2 = 84.2\%$
Test for overall effect: $Z = 3.04$ ($p = .002$)

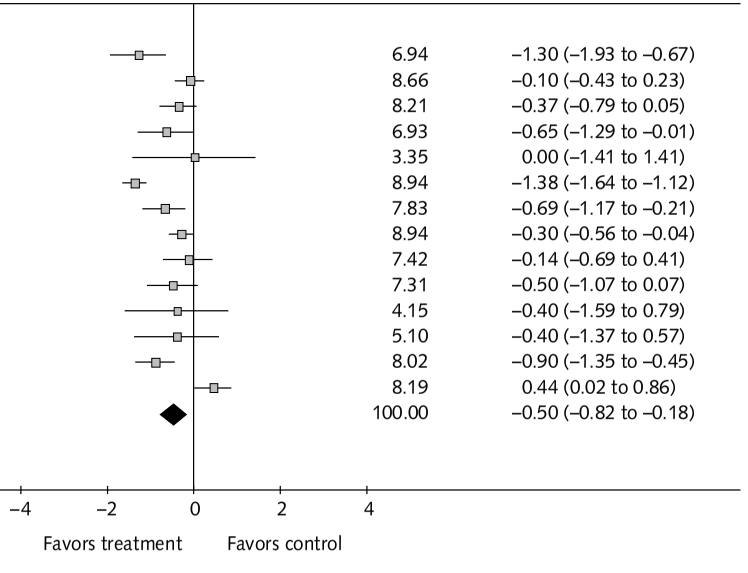

FIG. 1. Impact of zinc supplementation on mean duration of acute diarrhea, all age groups. WMD, weighted mean difference

months of age in two studies (WMD, 0.40; 95% CI, 0.00 to 0.81; $p = .05$) (**fig. 2**). However, given that several previous studies found a beneficial impact of zinc supplementation in infancy also included children under 6 months of age but did not clearly specify outcomes by age group, the currently reported lack of benefit of zinc among children under 6 months of age needs to be further verified by studies evaluating alternative doses and formulations in this age group.

The impact of zinc supplementation on the severity of acute diarrhea episodes was also evaluated, using several different indicators of disease severity. The study by Dutta et al. [6] reported lower total stool output (1.5 ± 0.7 vs. 2.4 ± 0.7 kg, $p = .0001$) and lower intakes of oral rehydration solution (2.5 ± 1.0 vs. 3.6 ± 0.8 L, $p = .0001$) in the zinc-supplemented group than in the control group. The study by Al-Sonboli et al. [7] demonstrated a shorter duration of watery stools in the zinc-supplemented group (0.4 ± 0.6 vs. 1.3 ± 1.5 days, $p < .001$). Pooled analysis for the outcome of episodes lasting for 7 days or more was conducted, including data from six studies. Zinc supplementation was associated with a marginally significant reduction in acute diarrhea episodes that lasted for 7 days or more (six studies; relative risk, 0.68; 95% CI, 0.46 to 1.01, random-effects model) (**fig. 3**).

In recent years, three large-scale effectiveness trials using zinc for the treatment of diarrhea have been completed in India, Pakistan, and Mali through both the public and the private sector health systems. Preliminary evidence suggests that in all instances there was increased zinc usage by child-care providers, with substantial impact on diarrhea outcomes (Bhutta ZA, Soofi S, Huusain A, Black RE, personal communication, 2007). In rural Haryana, India, Bhandari et al. [27] demonstrated utilization of zinc supplements in 36.5% ($n = 1,571$) and 59.8% ($n = 1,649$) of diarrheal episodes occurring in the 4 weeks preceding interviews in the intervention areas. The prevalence rates of diarrhea and pneumonia during the preceding 14 days were lower in the intervention communities during the third quarterly survey (odds ratio for diarrhea, 0.56; 95% CI, 0.41 to 0.75; odds ratio for pneumonia, 0.55; 95% CI, 0.25 to 1.25). The numbers of hospitalizations for any cause, diarrhea, and pneumonia in the preceding 3 months were reduced in the intervention compared with the control areas (survey 3) (odds ratio for diarrhea hospitalizations, 0.69; 95% CI, 0.50 to 0.95; odds ratio for pneumonia hospitalizations, 0.29; 95% CI, 0.15 to 0.54) [27]). In a similar large-scale project in Matiari district in Pakistan, zinc supplements delivered by primary care government health workers (Lady Health Workers) to children with acute diarrhea produced a reduction in the subsequent incidence rates of diarrhea and mortality*. These results have led to the incorporation of oral zinc sulfate for the treatment of diarrhea by the national primary care health program's Lady Health Workers. Given the observed benefits on duration of diarrhea in repeated episodes in these large-scale trials, a potential preventive benefit of repeated therapeutic courses of zinc for diarrhea cannot be excluded. Given the average of three or four episodes of diarrhea per child per year, treatment of all episodes of diarrhea with zinc may be an efficient way of providing a reasonable amount of zinc to deficient populations and may be of some preventive benefit.

Section 2

What is the effect of therapeutic zinc supplementation as an adjunct in the treatment of children with persistent diarrhea on the duration and severity of the disease?

Conclusions

Five studies have been conducted to evaluate the adjunctive therapeutic effect of zinc supplementation in children with persistent diarrhea. Pooled analysis of the available data has identified the beneficial effect of zinc supplementation in reducing the duration of disease by 0.68 day (WMD, −0.68; 95% CI, −1.01 to −0.36).

Detailed review of the evidence

Persistent diarrhea, which is defined as diarrhea lasting for more than 14 days, is associated with significant morbidity and mortality [28]. Thus, rapid institution of effective treatment is needed for these children to prevent complications and death.

A published systematic review and meta-analysis [5] of randomized, controlled trials of zinc supplementation as an adjunct in the treatment of persistent diarrhea in children under 5 years of age included data from four studies. The meta-analysis showed a significant mean reduction of 42% (range, 10% to 63%) in the rate of treatment failure or death. The benefit appeared to be larger in younger children (< 12 months of age) than in older children, in boys than in girls, and in children who were wasted or had lower plasma zinc levels.

We repeated a literature search using the PubMed bibliographic search tool in early January 2008 to identify all trials of therapeutic zinc supplementation. Studies eligible for inclusion were randomized, controlled trials that assessed the adjunctive therapeutic benefits of zinc supplementation in children under 5 years of age with persistent diarrhea. The search identified five studies [29–33], the main characteristics of which are summarized in **table 2**. One trial was conducted in Peru and four in south Asia. Outcomes that were assessed

*Bhutta ZA, Soofi S, Huusain A, Black RE, WHO Zinc in Health Systems Workshop Delhi, February 2008.

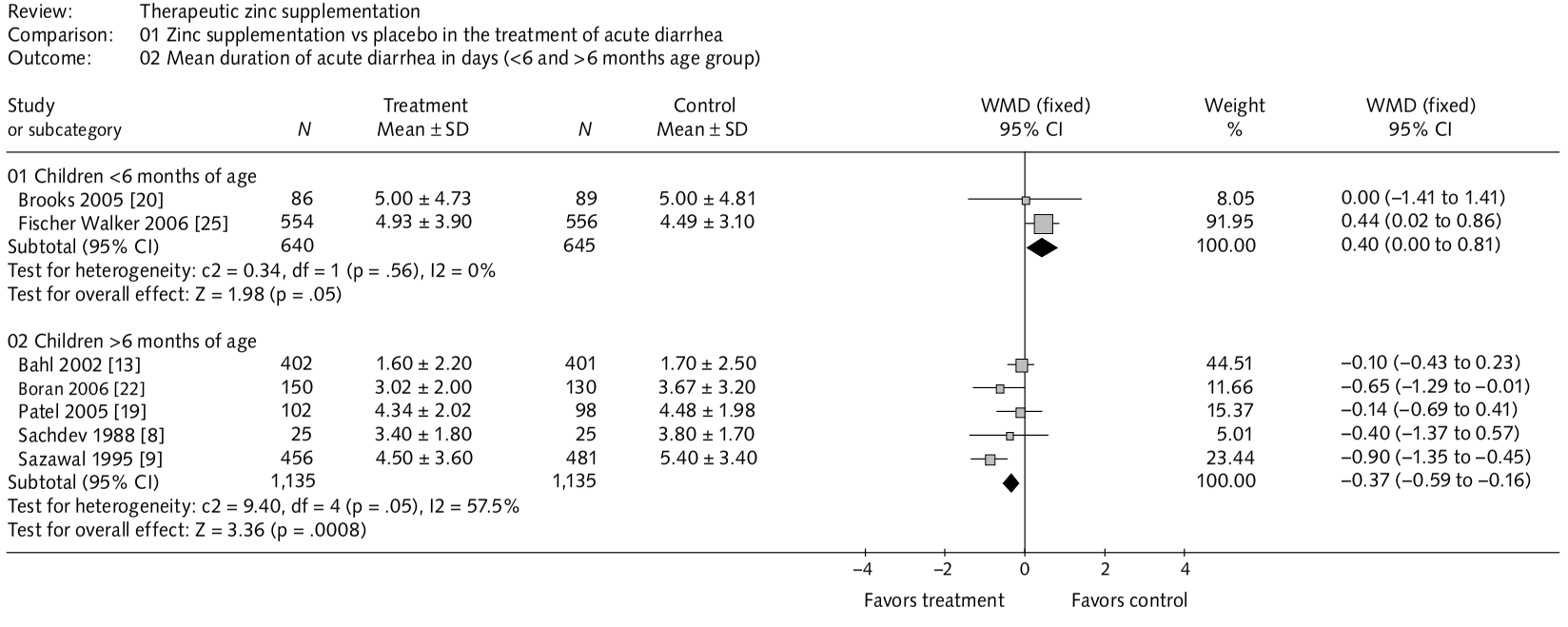

FIG. 2. Impact of zinc supplementation on mean duration of acute diarrhea, according to age group. WMD, weighted mean difference

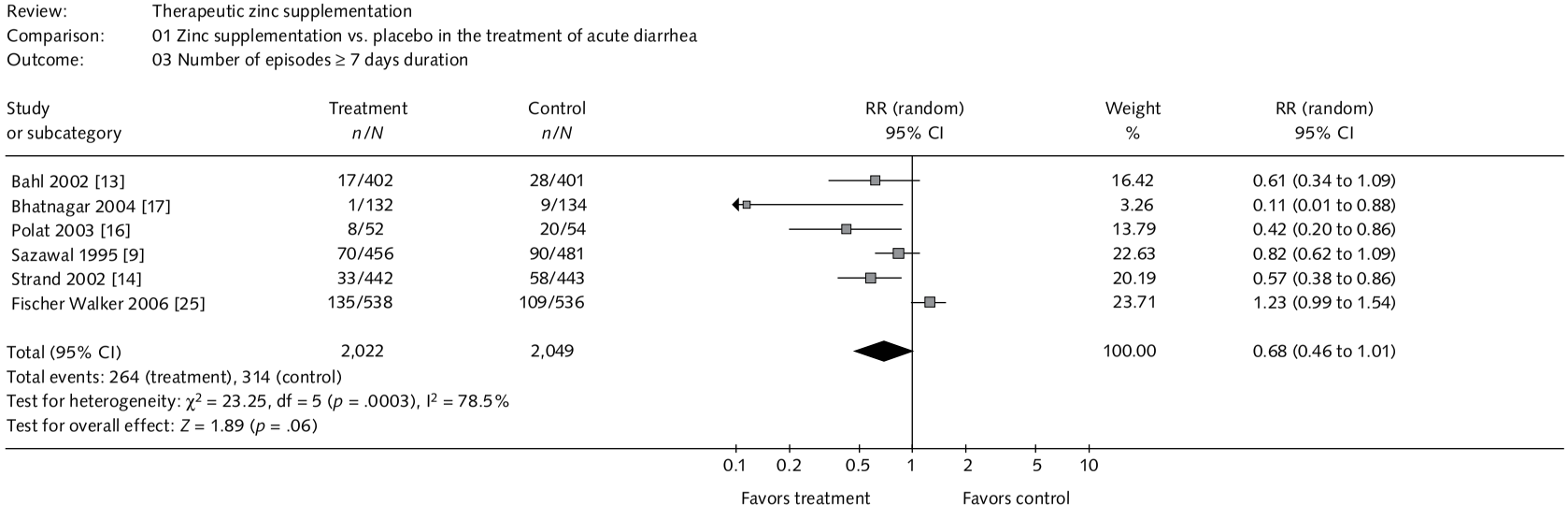

FIG. 3. Impact of zinc supplementation on proportion of acute diarrhea episodes lasting for 7 days or more. WMD, weighted mean difference; RR, relative risk

TABLE 2. Randomized, controlled trials of zinc in the treatment of persistent diarrhea

Country, year [reference] author	Age group	Study participants	Zinc	Placebo	Intervention	Results
India, 1990 [29] Sachdev	6–18 mo	Diarrhea > 2 wk	$n = 20$	$n = 20$	Intervention: 40 mg/day zinc sulfate Control: placebo Both groups received oral nalidixic acid and similar milk-free feeding schedule	A nonsignificant (19%) reduction in the duration of diarrhea was observed
Bangladesh, 1998 [30] Roy	3–24 mo	Persistent diarrhea and weight-for-age < 76th percentile of NCHS median	$n = 95$	$n = 95$	Intervention: 20 mg/day in 3 daily divided doses; zinc acetate and vitamins A, B, D, and C Control: vitamins A, B, D, and C	Overall, there was a nonsignificant reduction in duration of diarrhea. Duration of illness was significantly reduced (33%) with zinc supplementation among underweight children (≤ 70% weight-for-age, $p = .03$). Supplemented children maintained their body weight (5.72 vs. 5.70 kg, $p = 0.62$) during hospitalization, unlike control group, which lost weight (5.75 vs. 5.67 kg, $p = .05$). Deaths: 1/95 in zinc group and 5/95 in control group ($p = .06$)
Pakistan, 1999 [31] Bhutta	6–36 mo	Persistent diarrhea > 14 days and weight-for-age ≤ −2 SD	$n = 43$	$n = 44$	Intervention: 3 mg/kg/day (approx. 20 mg/day) zinc sulfate and vitamins A, B, D, and C Control: vitamins A, B, D, and C	There was no significant difference in the duration of diarrheal episodes. A trend toward shorter episodes in children with lower plasma zinc concentrations at baseline was seen
Peru, 1999 [32] Penny	6–36 mo	Persistent diarrhea > 14 days	(a) $n = 137$ (b) $n = 139$	$n = 136$	Intervention: (a) 20 mg/day zinc gluconate, (b) 20 mg/day zinc gluconate and oral multivitamins Control: placebo	Duration of illness was significantly reduced by 28% in children in the zinc group ($p = .01$) and by 33% in girls in the zinc + multivitamins group ($p = .04$)
Meta-analysis of 4 trials, 2000 (5) Zinc Investigators Group (Roy 1998 [30], Bhutta 1999 [31], Penny 1999 [32] and another unpublished trial from Bangladesh)						There was a 42% (95% CI, 10% to 63%) reduction in treatment failure or death. Effect appeared to be more marked in children who were aged < 12 mo, were male, or had wasting or lower baseline plasma zinc levels
[33] Bangladesh, 2001 Khatun	6 mo –2 yr	Diarrhea > 14 days and moderately malnourished children (61%–75% of NCHS median weight-for-age)	$n = 24$ in each group	$n = 24$	Intervention: (a) zinc (20 mg elemental), (b) vitamin A 100,000 IU for children < 1 yr and 200,000 IU for children > 1 yr , (c) both zinc and vitamin A Control: placebo in 2 doses daily All groups received a multivitamin syrup	The mean daily stool outputs from days 2 to 7 of therapy were significantly less in the zinc and zinc plus vitamin A groups, but not in the vitamin A group, in comparison with the control group. The rate of clinical recovery of children within 7 days was significantly greater in the zinc group (88%) than in the control group (46%, $p = .002$) and the vitamin A group (50%, $p = .005$) but was not significantly different from that in the zinc plus vitamin A group (67%, $p = .086$)

NCHS, National Center for Health Statistics

included duration of diarrhea, stool frequency, stool volume, and body weight. All zinc-supplemented children received zinc dosages to provide at least two times the US RDA daily during the treatment period. Zinc-supplemented children had better clinical outcomes in four of the five trials. A subsequent meta-analysis of the included studies showed that the mean duration of persistent diarrhea was significantly less in the zinc-supplemented group than in the placebo group (WMD, –0.68 days; 95% CI, –1.01 to –0.36) (**fig. 4**). Serum zinc was measured in all studies at baseline, but there were no reported differences in the effect of the treatment associated with baseline serum zinc status.

The impact of zinc supplementation on recovery from persistent diarrhea was also evaluated. Summary estimates, which were calculated from data from four studies, showed that children in the zinc-supplemented group had a 21% lower probability of continuation of diarrhea on a given day than the control group (relative hazard, 0.79; 95% CI, 0.65 to 0.96) (**fig. 5**). Summary impact estimates on the severity indicators of the disease, such as stool frequency and stool output, could not be calculated because of the inconsistent approaches used to analyze these outcomes. The effect of supplementation on the body weight of children at the end of 2 weeks of treatment was reported in only two studies, the analysis of which demonstrated a non-significant impact on this outcome (WMD, –0.09; 95% CI, –0.40 to 0.22) (**fig. 6**).

Section 3

What is the effect of therapeutic zinc supplementation as an adjunct in the treatment of children with pneumonia on the duration and severity of the disease?

Conclusions

Few clinical trials have addressed the therapeutic role of zinc supplementation as an adjunct in the treatment of pneumonia; there is insufficient information available to assess the effect of zinc on pneumonia outcomes.

Detailed review of the evidence

The exact mechanisms by which zinc may affect respiratory infections are not well understood. These effects may be modulated by impacts on the immune system as well as cell membranes. Zinc is known to induce the production of interferon and modulate inflammatory cytokines, which in turn may have beneficial effects on symptoms of respiratory infection [34].

We identified all trials of therapeutic zinc supplementation in children with pneumonia. Studies eligible for inclusion were randomized, controlled trials that assessed the adjunctive therapeutic benefits of zinc

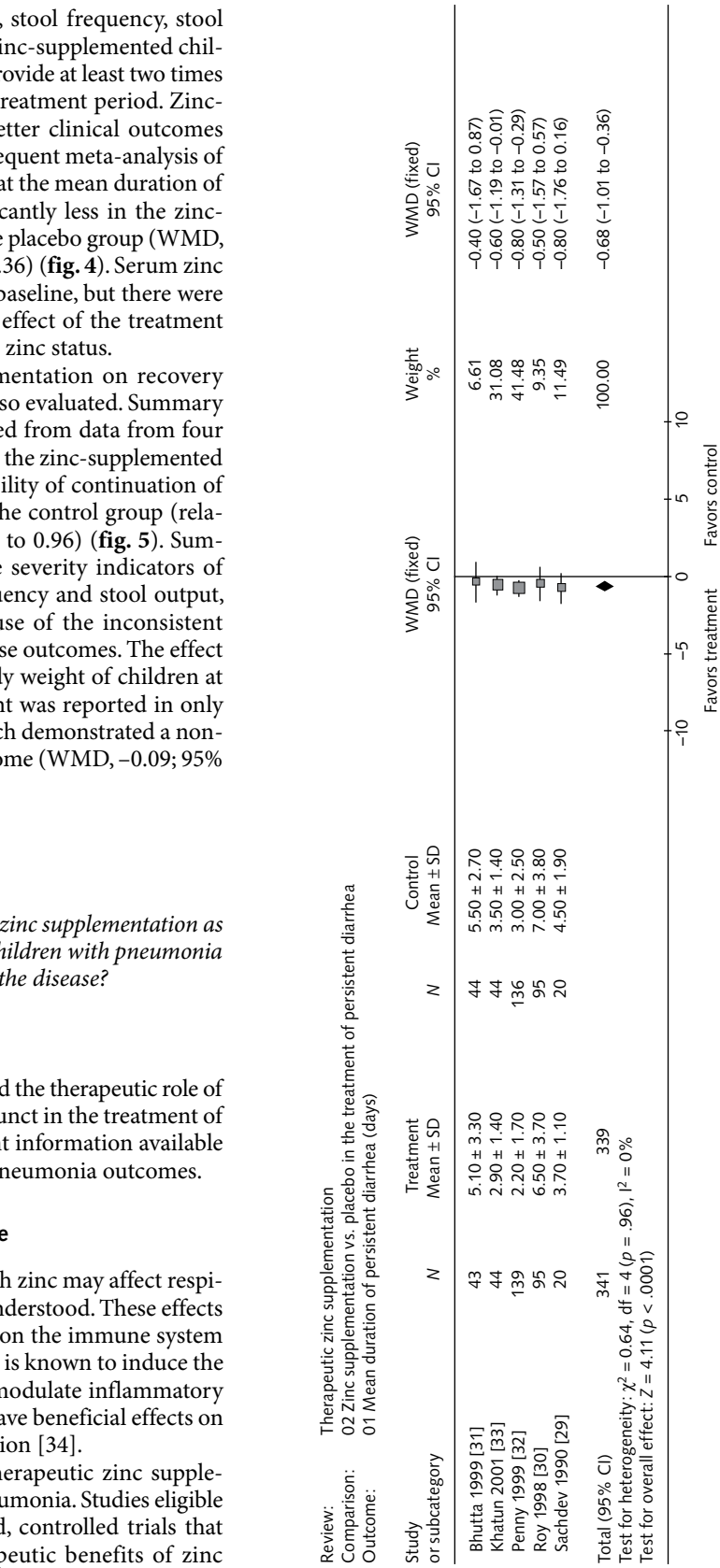

FIG. 4. Impact of zinc supplementation on the mean duration of persistent diarrhea (days). WMD, weighted mean difference

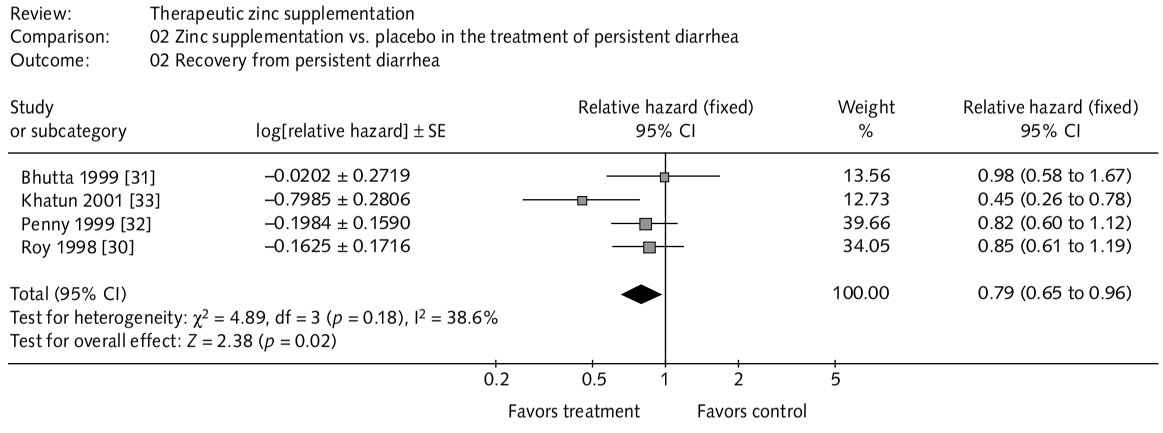

FIG. 5. Impact of zinc supplementation on rates of recovery from persistent diarrhea

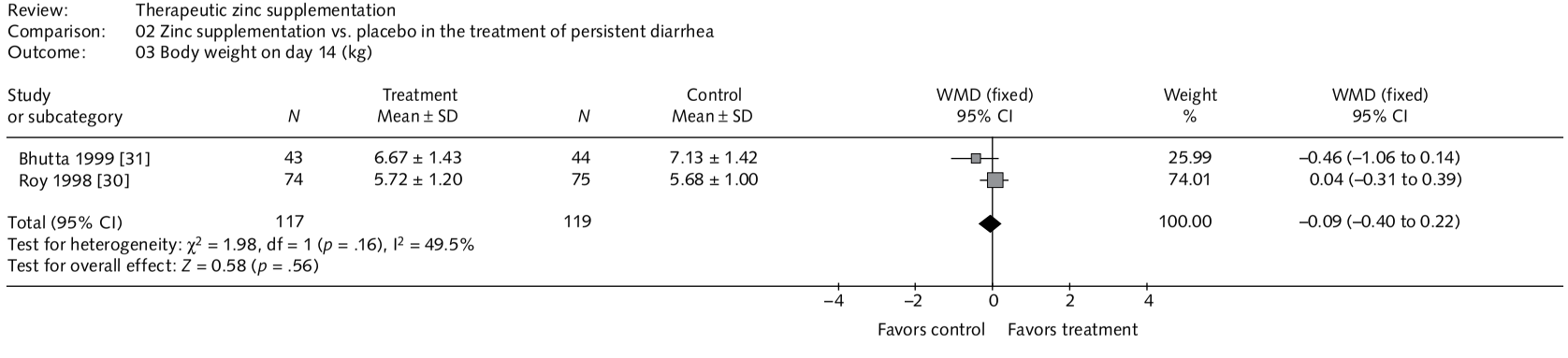

FIG. 6. Impact of zinc supplementation in children with persistent diarrhea on body weight at the end of 2 weeks. WMD, weighted mean difference

supplementation in children under 5 years of age suffering from severe acute lower respiratory infection (ALRI) or pneumonia. We identified five studies that evaluated whether zinc administered for a few days, along with antibiotics, affected the outcome of the disease. All trials except one were conducted in South Asia. The characteristics of these studies [35–39] are presented in **table 3**. The outcomes assessed were time to recovery from pneumonia symptoms, time to complete recovery, time to recovery from respiratory rate > 50/minute, duration of hospital stay, hypoxia, and inability to eat. All treatment doses were at least 2 US RDA daily given for 5 or 6 days or until the child recovered from the current episode of pneumonia. Children receiving zinc showed significantly faster recovery from pneumonia than those receiving placebo in two of the five trials. Serum zinc was measured at baseline in all studies, and no differences in the effect of treatment associated with baseline zinc status were reported. Summary estimates were calculated by the generic inverse variance method utilizing data from two studies [36, 38]. The data from three studies could not be used in calculating summary estimates because two of these studies [35, 39] included children up to 15 years of age and one study reported only sex-based estimates [37]. Analysis showed nonsignificant effects of zinc supplementation on the duration of hospitalization (relative risk, 0.85; 95% CI, 0.71 to 1.02) (**fig. 7**), duration of respiratory rate > 50/minute (relative risk, 0.87; 95% CI, 0.73 to 1.03) (**fig. 8**), and chest indrawing (relative risk, 0.86; 95% CI, 0.70 to 1.04) (**fig. 9**).

Section 4

What is the effect of therapeutic zinc supplementation as an adjunct in the treatment of children with malaria?

Conclusions

On the basis of the available data, there is no evidence that including zinc supplementation in the treatment of malaria affects the course of the illness.

Detailed review of the evidence

A literature search for randomized, controlled trials assessing the impact of zinc supplementation in

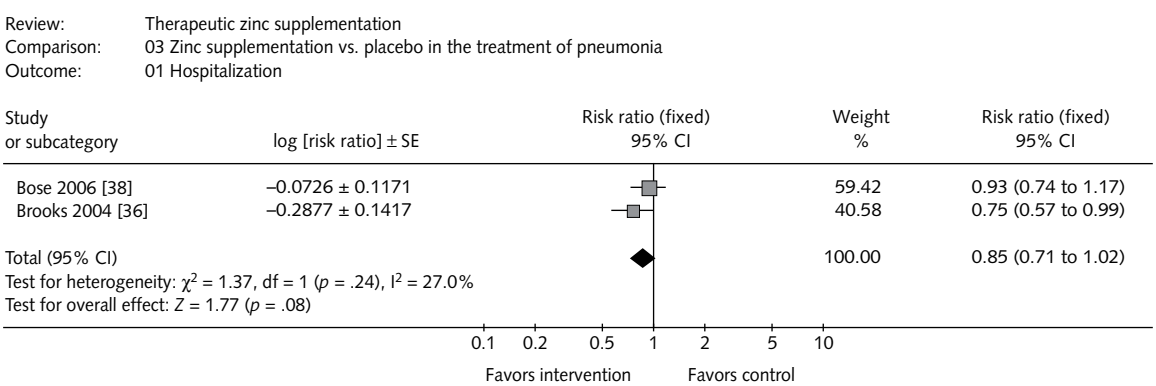

FIG. 7. Impact of zinc supplementation on the duration of hospitalization for pneumonia

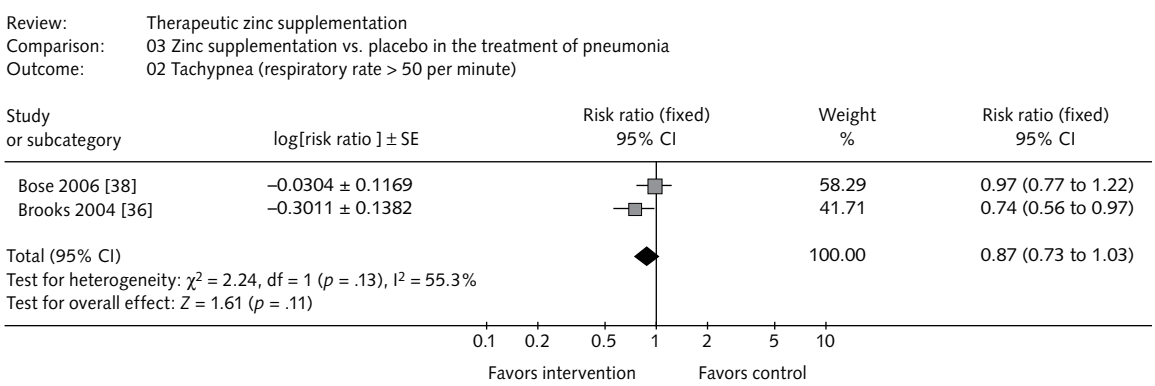

FIG. 8. Impact of zinc supplementation on duration of tachypnea (respiratory rate > 50 per minute) in pneumonia

TABLE 3. Randomized, controlled trials of zinc in the treatment of pneumonia

Country, year [reference] author	Age group	Study participants	Zinc	Control	Interventions	Results
India, 2002 [35] Mahalanabis	9 mo–15 yr	Hospitalized children with clinically severe measles accompanied by pneumonia who had been ill for ≤ 7 days	*n* = 42	*n* = 43	Intervention: zinc acetate 20 mg twice daily Control: Placebo All patients received standard treatment with antibiotics and an initial 100,000 IU dose of vitamin A orally	Time-to-event analysis using the Cox proportional-hazards model showed that the times needed for the resolution of fever and tachypnea, the return of appetite, and the achievement of a "much improved" or "cured" status were not different between the 2 groups
Bangladesh, 2004 [36] Brooks	2–23 mo	Hospitalized children with severe pneumonia	*n* = 132	*n* = 131	Intervention: elemental zinc 20 mg/day Control: placebo All patients received the hospital's standard antimicrobial treatment	The group receiving zinc had reduced duration of severe pneumonia (relative hazard, 0.70; 95% CI, 0.51 to 0.98), including duration of chest indrawing (relative hazard, 0.80; 95% CI, 0.61 to 1.05), respiratory rate > 50/min (relative hazard, 0.74; 95% CI, 0.57 to 0.98), and hypoxia (relative hazard, 0.79; 95% CI, 0.61 to 1.04), and overall hospital duration (relative hazard, 0.75; 95% CI, 0.57 to 0.99). The mean reduction is equivalent to 1 hospital day for both severe pneumonia and time in hospital
India, 2004 [37] Mahalanabis	2–24 mo	Hospitalized children with severe ALRI	*n* = 39	*n* = 38	Intervention: (a) 10 mg zinc as acetate (twice daily for 5 days) plus vitamin A placebo; (b) vitamin A 10,000 μg RE (twice daily for 4 days) plus zinc placebo, (c) zinc plus vitamin A, or Control: zinc and vitamin A placebos	Recovery rates in zinc-treated boys from very ill status and from fever were 2.6 times (p = .004) and 3 times (p = .003) greater than those in non-zinc-treated children; feeding difficulty and tachypnea were not significantly different between groups after an adjusted analysis
India, 2006 [38] Bose	2–23 mo	Hospitalized children with severe pneumonia	*n* = 150	*n* = 149	Intervention: 20 mg zinc sulfate at the time of enrollment. From day 2, 10-mg tablets of zinc sulfate Control: placebo All received standard therapy for severe pneumonia	There were no clinically or statistically significant differences in the duration of tachypnea, hypoxia, chest indrawing, inability to feed, lethargy, severe illness, or hospitalization. Zinc supplementation was associated with a significantly longer duration of pneumonia in the hot season (p = .015)
Australia, 2006 [39] Chang	Children aged < 11 yr	Hospitalized children with ALRI episodes	*n* = 111	*n* = 104	Intervention: (a) zinc plus vitamin A, (b) zinc plus vitamin A placebo, (c) zinc placebo plus vitamin A supplement. Zinc sulfate (20 mg at age < 12 mo, 40 mg at age ≥12 mo) was administered daily for 5 days and vitamin A was administered on days 1 and 5 after admission (50,000 IU at age < 12 mo, 100,000 IU at age ≥12 mo) Control: zinc placebo plus vitamin A placebo	There was no clinical benefit of supplementation with vitamin A, zinc, or the 2 combined, with no significant difference between zinc and no zinc, vitamin A and no vitamin A, or zinc + vitamin A and placebo groups in time to resolution of fever or tachypnea, or duration of hospitalization. Children given zinc had increased risk of readmission for ALRI within 120 days (relative risk, 2.4; 95% CI, 1.003 to 6.1)

ALRI, acute lower respiratory infection; RE, retinol equivalent

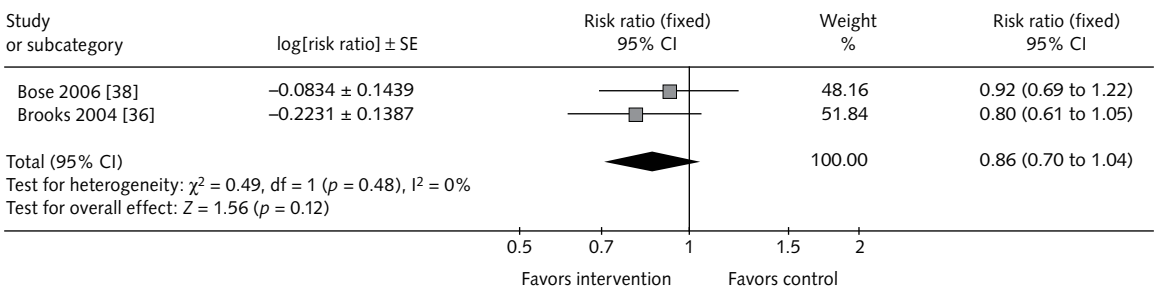

FIG. 9. Impact of zinc supplementation on duration of chest indrawing in pneumonia

children suffering from malaria identified only one multicenter study, which was conducted by the Zinc Against Plasmodium Study Group [40] in five country sites (Ecuador, Ghana, Tanzania, Uganda, and Zambia). Children aged 6 months to 5 years with fever and 2,000 or more asexual forms of *Plasmodium falciparum* per microliter in a thick blood smear were eligible for inclusion. All children were treated with chloroquine and were randomly assigned to receive either a relatively high dose of zinc (20 mg/day for infants, 40 mg/day for older children) or placebo for 4 days. The results showed no effect of zinc on the median time to reduction of fever (zinc group, 24.2 hours; placebo group, 24.0 hours; $p = .37$), the percentage of patients with a reduction of at least 75% in parasitemia from baseline in the first 72 hours (73.4% of the zinc group and 77.6% of the placebo group, $p = .11$), or change in hemoglobin concentration during the 3-day period of hospitalization and the 4 weeks of follow-up. The mean plasma zinc concentrations were low in all children at baseline (zinc group, 55.9 ± 25.7 μg/dL; placebo group, 54.5 ± 21.3 μg/dL), but children who received zinc supplementation had higher plasma zinc concentrations at 72 hours than did those who received placebo (71.6 ± 23.7 vs. 66.4 ± 21.3 μg/dL, $p < .001$).

Low serum zinc levels are common in patients with acute malaria. However, these levels revert to normal within a few days after clinical recovery from malaria. Duggan et al. assessed the relation between plasma zinc concentration and the acute-phase response in an observational cohort study of 689 children with acute falciparum malaria [41]. Plasma zinc was measured by atomic absorption spectrophotometry. On admission, 70% of subjects had low plasma zinc (< 60 μg/dL). On multivariate analysis, the predictors of admission plasma zinc included admission levels of C-reactive protein (a marker of the acute-phase response), parasite density, and study site. The children were randomly assigned to receive either zinc supplements or a placebo. The proportion of children with low plasma zinc at 72 hours decreased from 73% to 30% in the zinc group and from 66% to 41% in the placebo group. The predictors of changes in plasma zinc from admission to 72 hours included baseline C-reactive protein concentration, change in C-reactive protein concentration, treatment group, study site, and baseline zinc concentration.

Section 5

What is the effect of therapeutic zinc supplementation as an adjunct in the treatment of children with tuberculosis?

Conclusions

No studies have been completed to evaluate the role of zinc in the treatment of tuberculosis in children.

Detailed review of the evidence

Our literature search did not identify any randomized, controlled trials evaluating the impact of zinc supplementation among children with tuberculosis. However, trials have been conducted in adults with tuberculosis and have shown beneficial effects when zinc is supplemented along with other micronutrients. In a study from India, 15 patients with pulmonary tuberculosis who received a zinc supplement were compared with 24 controls. The patients who received zinc had an earlier sputum clearance than the control patients; however, the difference was not statistically significant [42]. In a double-blind, placebo-controlled trial in adults aged 15 to 55 years in Indonesia [43], 40 patients newly diagnosed with tuberculosis received either 5,000 IU of vitamin A (as retinyl acetate) and 15 mg zinc (as zinc sulfate) daily for 6 months (micronutrient group) or a placebo, in addition to antituberculosis treatment. The micronutrient-supplemented group had significantly higher rates of sputum conversion and radiologic resolution of lesions, although the results could not be attributed to zinc alone.

Summary

These studies and updated analyses of the effect of including zinc in the treatment of diarrhea corroborate existing reviews and indicate that the evidence is both consistent and robust. The two effectiveness trials in Asia and the one in Africa indicate that scaling up the use of zinc in health systems is feasible and has demonstrable benefits. Thus, for the treatment of diarrhea, the need is to implement the revised diarrhea treatment strategy, including low-osmolality oral rehydration solution and zinc in all cases. It is possible that these benefits may also accrue in developed countries, but there are few studies of zinc supplementation in developed countries, and the benefit of zinc in such circumstances needs further evaluation. Current evidence indicates that zinc treatment of infants with diarrhea has no beneficial impact, although this needs further evaluation.

Comparable evidence of benefits of zinc in the treatment of pneumonia is not available. However, the marked reductions in the prevalence of pneumonia and the rate of hospitalization for pneumonia in one effectiveness trial of zinc treatment for diarrhea suggest that repeated courses of zinc for the treatment of diarrheal episodes may also reduce the incidence and severity of lower respiratory infections. It can therefore be argued that the distinction between preventive and therapeutic uses of zinc for a range of disorders (especially acute diarrhea and respiratory infections in high-burden communities) may become blurred with repeated use.

Given that our current knowledge of the mechanisms of benefit of zinc in these diverse disorders is limited, there is an urgent need for better studies of the mechanisms of action of zinc in such disorders to enable better understanding of the range of disorders and populations for which zinc may be beneficial.

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Effects of maternal zinc supplementation on pregnancy and lactation outcomes

Sonja Y. Hess and Janet C. King

Abstract

Observational studies in human populations suggest that maternal zinc deficiency during pregnancy may cause adverse pregnancy outcomes for the mother and fetus. Therefore, we reviewed the current evidence from studies of zinc supplementation, with or without other micronutrients, during pregnancy and lactation to assess its impact on maternal, fetal, and infant health.

A meta-analysis of supplementation trials indicates a 14% reduction in premature delivery among zinc-supplemented women. Most studies found no significant impact of maternal zinc supplementation on infant birthweight, but a subset of studies conducted in underweight or zinc-deficient women suggests that there may be a positive effect of zinc supplementation in such women. However, the number of relevant studies is limited, and more information is needed to confirm these observations. The results for other pregnancy outcomes are inconsistent, and the number of available studies is small. Likewise, the impact of maternal zinc supplementation during pregnancy on infant postnatal growth and risk of infection is variable, and few studies are available. Thus, more research will be needed to allow definitive conclusions to be drawn, especially for the second half of infancy and later childhood.

Studies found no adverse effects of maternal zinc supplementation on iron status during pregnancy. More information is required on other potential adverse effects, particularly with regard to a possible modifying effect of preexisting maternal zinc status.

In view of the possible benefits of zinc supplementation for reducing the risk of premature delivery, the possible positive impact of zinc supplementation on infant birthweight among undernourished women, and the lack of reported adverse effects, zinc should be included in maternal supplements given during pregnancy in populations at risk for zinc deficiency.

Key words: Lactation, maternal health, neonatal health, pregnancy, pregnancy outcome, prevention, zinc deficiency, zinc supplementation

Background

The results of experimental studies conducted in animals and observational studies in human populations show that maternal zinc deficiency can have adverse effects on reproduction, including infertility, congenital anomalies, fetal growth retardation, prolonged labor, embryonic or fetal death, and early postnatal infant immune dysfunction. The possible mechanisms and pathways of maternal zinc deficiency and adverse health effects on the mother and fetus were previously reviewed [1]. On the basis of the new recommendations by the World Health Organization (WHO), the United Nations Children's Fund (UNICEF), the International Atomic Energy Agency (IAEA), and the International Zinc Nutrition Consultative Group (IZiNCG) to use stunting rates of children under five as an indicator for the risk of zinc deficiency, it is estimated that approximately one-third of the world's population live in countries where the risk of zinc deficiency is high [2]. The prevalence of zinc deficiency among pregnant and lactating women worldwide is unknown.

Zinc requirements during pregnancy and lactation have been estimated from the zinc content of tissues accrued during pregnancy and the zinc content of milk secreted during lactation [3]. The estimated total additional zinc needed for pregnancy is ~100 mg [4]. In addition to the zinc accrued by the fetus, zinc is deposited in the placenta, amniotic fluid, and uterine

Sonja Y. Hess is affiliated with the Department of Nutrition and the Program in International and Community Nutrition, University of California, Davis, California, USA; Janet C. King is affiliated with the Children's Hospital Oakland Research Institute, Oakland, California, USA.

Please direct queries to the corresponding author: Janet C. King, Children's Hospital Oakland Research Institute, 5700 Martin Luther King Jr. Way, Oakland, CA 94609, USA; e-mail: jking@chori.org.

and mammary tissue. Approximately 60% of the total zinc is accumulated in the conceptus and 40% in the maternal tissue. The additional daily need increases during gestation to meet the demands for fetal growth, rising from ~0.1 mg/day additional zinc in the first quarter of pregnancy to ~0.7 mg/day additional zinc in the fourth quarter. As reviewed by Brown et al. [5], the mean amount of zinc transferred in the breastmilk to exclusively breastfed infants declines rapidly from ~4 mg/day during the first few days of life to ~1.75 mg/day by 1 month. Zinc transfer declines more slowly thereafter to ~1 mg/day at 6 months. Studies suggest that maternal zinc absorption is increased and/or exogenous zinc excretion is decreased during pregnancy and lactation, thereby enhancing maternal zinc availability for fetal growth and milk zinc excretion [6, 7]. However, the ability of these homeostatic mechanisms to compensate for diets that are low in total zinc or in bioavailable zinc appears to be limited, so reproductive function may be compromised under these circumstances. In these cases, supplementation of women with low zinc intakes may be necessary to ensure optimal reproductive outcomes.

WHO currently recommends that all pregnant women in areas of high prevalence of malnutrition should routinely receive iron and folic acid supplements, together with appropriate dietary advice, to prevent anemia [8]. In view of the above-mentioned importance of zinc for human health and reproduction, we reviewed the impact of zinc supplementation on various reproductive outcomes in women from developed and developing countries to evaluate whether the addition of zinc to the iron and folic acid supplement should be considered. We compared the results of controlled intervention trials in which zinc was provided, with or without other micronutrients, to pregnant women. We further examined the impact of zinc supplementation during lactation on maternal and infant zinc status and zinc-related outcomes.

This paper is divided into four sections, which address the following questions in relation to zinc supplementation during pregnancy and lactation:

Section 1: What is the effect of preventive zinc supplementation during pregnancy on maternal and neonatal health (i.e., maternal mortality, maternal morbidity, fetal growth, and postnatal growth and morbidity)?

Section 2: What is the effect of preventive zinc supplementation during lactation on maternal and neonatal health?

Section 3: Are there any adverse effects of zinc supplementation during pregnancy or lactation?

Section 4: What are the implications of these outcomes for zinc supplementation programs during pregnancy and lactation and what are the remaining research needs?

Section 1

What is the effect of preventive zinc supplementation during pregnancy on maternal and neonatal health (i.e., maternal mortality, maternal morbidity, fetal growth, and postnatal growth and morbidity)?

Conclusions

The results of experimental studies in laboratory animals and observational studies in human populations both suggest that maternal zinc deficiency during pregnancy can cause adverse pregnancy outcomes for the mother, fetus, and/or newborn infant postnatally. A number of controlled intervention trials have now been completed in humans, but the results are difficult to interpret because of the relatively small number of studies reporting on each of these specific outcomes, the failure to characterize or stratify according to the women's preexisting zinc status and general nutritional condition, and the variable times of initiation, duration, and amount of zinc supplementation. With recognition of these limitations in the existing evidence base, the following conclusions can be drawn from the available studies.

A meta-analysis of supplementation trials indicates a small but significant positive impact of maternal zinc supplementation on the duration of pregnancy and a 14% reduction in premature delivery among zinc-supplemented women. Most available studies found no significant impact of maternal zinc supplementation on infant birthweight, but a subset of studies conducted in underweight or zinc-deficient women suggests that there may be a positive effect of zinc supplementation in such women. However, these results are quite limited, and more studies are needed to confirm these observations. There are inconsistent results with regard to other pregnancy outcomes, and the number of available studies is small, so no definitive conclusions are possible. Likewise, the impacts of maternal zinc supplementation during pregnancy on infant postnatal growth and the risk of infection are inconsistent and the number of studies is quite limited, so more research will be needed to allow definitive conclusions to be drawn, especially for the second half of infancy and later childhood.

Detailed review of evidence

Bibliographic search

Data sets were identified for this section by using a computerized bibliographic search (PubMed) with the key words zinc; limiting for human, English, clinical trial, meta-analysis, randomized, controlled trial. A total of 1,618 articles were identified during the PubMed search using these key words. Three additional manuscripts identified in other reviews of zinc

supplementation during pregnancy [9, 10] were also included, resulting in a total of 1,621 individual references. All titles and abstracts were reviewed. Of these, 44 articles evaluated the impact of zinc supplementation on pregnancy-related outcomes in the mother or infant. We excluded 2 of these 44 studies, 1 because the pregnant women chose the supplement themselves and 1 because the anemia status differed between treatment groups. The remaining 42 articles were then screened to combine those that presented data from the same intervention trial by using key characteristics, such as country, study population, and supplementation scheme. A total of 22 different trials were identified.

Maternal morbidity and mortality

The effect of supplemental zinc, with and without other micronutrients, on maternal morbidity and mortality was evaluated in 10 studies done over the past 30 years [11–22] (**table 1**). With the exception of one study in Nepal [22, 23], the supplement was taken daily from midpregnancy to term. In the study in Nepal [22, 23], the supplement was started early in the first trimester. An additional study in Nepalese pregnant women investigated the impact of short-term zinc supplementation (3 weeks) in women who reported night-blindness [18]. The first zinc supplementation study by Jameson and Ursing [11] was not randomized, and the investigators were not blinded to treatment. In that study of Swedish women with anemia, 90 mg of supplemental zinc per day reduced complications at delivery, such as prolonged labor or excessive bleeding. Mahomed et al. [13] and Jonsson et al. [14] followed up on this observation by conducting two double-blind, randomized, controlled trials of zinc supplementation during pregnancy in England and Denmark, respectively. The women were given either 20 or 44 mg of zinc/day from booking to term. The outcomes evaluated included maternal bleeding, hypertension, and complications of labor and delivery. There was no evidence in either of these randomized, controlled trials that supplemental zinc affected maternal morbidity during pregnancy. Simmer et al. [16], however, found a lower incidence of induced labor (13% vs. 50%) among zinc-supplemented (22.5 mg/day) UK women in a small, double-blind study with a total sample size of 56 women. In contrast, Dijkhuizen and Wieringa [17] found significantly more deliveries with complications in the group receiving zinc (30 mg/day) plus iron and folic acid than in the groups receiving iron and folic acid alone, iron and folic acid plus β-carotene, or iron and folic acid plus β-carotene and zinc. The differences among these studies may be related to the amount of supplemental zinc (90 vs. 20 or 44 mg/day), the initial zinc status of the mothers, the presence of other micronutrient supplements, obstetric practices, or investigator bias in the case of the nonblinded trial.

Hunt and coworkers [19] evaluated the effects of

TABLE 1. Effect of zinc supplementation during pregnancy on maternal morbidity and mortality

Country, year [reference] author	Study design	Population	Amount of zinc supplement	Duration of supplementation	Zinc group	Comparison group	Effect of supplement
Supplementation with zinc alone					Zinc	Placebo	
Chile, 2001 [15] Castillo-Durán	Randomized, double-blind, controlled	Chilean adolescent women	20 mg/day	From before wk 20 to term	*n* = 249	*n* = 258	No effect on preeclampsia
Denmark, 1996 [14] Jonsson	Randomized, double-blind, controlled	Danish women	44 mg/day	From before wk 20 to term	*n* = 415	*n* = 379	No effect
South Africa, 1979 [12] Appelbaum	Randomized, double-blind, controlled	Black South African women	30 or 90 mg/day	From midpregnancy to term	*n* = 32	*n* = 24	No effect on growth-supporting property of amniotic fluid
Sweden, 1976 [11] Jameson	Not randomized, not controlled, not blinded	Swedish women with anemia	90 mg/day	From booking to term; length varied	*n* = 64	*n* = 248	Reduced no. of deliveries with complications
UK, 1989 [13] Mahomed	Randomized, double-blind, controlled	UK women	20 mg/day	From before wk 20 to term	*n* = 246	*n* = 248	No effect

Supplementation with folic acid, iron, and zinc, with or without β-carotene					Folic acid + iron + zinc	Folic acid + iron	
Indonesia, 2001 [17] Dijkhuizen	Randomized, double-blind, controlled	Indonesian rural women	30 mg/day	From before wk 20 to term	Folic acid + iron + zinc (n = 48)	Folic acid + iron (n = 42)	Increased no. of deliveries with complications
					β-Carotene + folic acid + iron + zinc (n = 44)	β-Carotene + folic acid + iron (n = 45)	No effect
Nepal, 2003 [22] Christian	Cluster-randomized, double-blind, controlled	Nepalese rural women	30 mg/day	From wk 5–10 gestation to 3 mo postpartum	Folic acid + iron + zinc (n = 173)	Placebo, folic acid, folic acid + iron, or MMN (n = 606)	AGP concentration decreased most in groups receiving folic acid, folic acid + iron, and folic acid + iron + zinc ($p < .05$). Subclinical infection (CRP concentration) reduced in group receiving folic acid + iron + zinc ($p < .05$).
UK, 1991a[a] [16] Simmer	Randomized, double-blind, controlled	Lower-social-class Englishwomen	22.5 mg/day	From 15–25 wk prior to delivery	n = 30	n = 26	Reduced induction of labor
Supplementation with vitamin A/carotenoid and zinc					Vitamin A/ carotene + zinc	Vitamin A/ carotene	
Nepal, 2001 [18] Christian	Randomized, double-blind, controlled	Nepalese women with night-blindness	25 mg/day	From wk 27 gestation for 3 wk	n = 102	n = 100	No effect on night-blindness
Supplementation with zinc and MMN					MMN + zinc	MMN	
Tanzania, 2005 [21] Villamor	Randomized, double-blind, controlled	HIV-infected pregnant Tanzanian women	25 mg/day	From wk 12–27 gestation to term	n = 198	n = 199	No effect on parasitemia in maternal and cord blood.
USA, 1984 [19] Hunt	Randomized, double-blind, controlled	Low-income Hispanic California women	20 mg/day	From about wk 19 gestation to term	n = 107	n = 106	Reduced incidence of pregnancy-induced hypertension
USA, 1995 [20] Hogg	Randomized, double-blind, controlled	Medically indigent African-American women	25 mg/day	From wk 19 gestation to term	n = 231	n = 206	No effect on serum homocysteine or pregnancy-induced hypertension

AGP, α_1-acid glycoprotein; CRP, C-reactive protein; MMN, multiple micronutrients

a. Iron and folic acid supplement was prescribed if clinically indicated.

20 mg of supplemental zinc/day during the last half of gestation on the incidence of pregnancy-induced hypertension. The incidence was significantly lower in the zinc-supplemented women (2% vs. 12%). Hogg et al. [20] also evaluated the effect of supplemental zinc (25 mg/day) in a larger population of poor African-American women in Alabama and found no effect on the incidence of pregnancy-induced hypertension between the two groups or on serum levels of homocysteine, an amino acid associated with occlusive vascular disease. Similarly, Castillo-Durán et al. [15] found no effect of zinc supplementation on the incidence of preeclampsia in pregnant Chilean adolescents who received 20 mg of supplemental zinc/day. A recent meta-analysis combining the results of seven randomized, controlled trials on pregnancy-induced hypertension and preeclampsia found no significant differences (relative risk, 0.83; 95% CI, 0.64 to 1.08) between the women receiving zinc and the women in the control group [10]. These limited data do not support a role of zinc in reducing hypertension or preeclampsia during gestation.

Zinc plays an important role in maintaining normal immune function [24]. Therefore, maternal infection may be related to zinc status during pregnancy, and three studies have examined maternal infectious morbidity in relation to zinc supplementation.

Supplemental zinc did not alter the microbial growth-promoting properties of amniotic fluid [12] or the risk of malaria parasitemia among HIV-infected women [21]. Christian et al. [22] investigated the impact of micronutrient supplementation during pregnancy on the concentrations of acute-phase response proteins studied before supplementation and at 32 weeks of gestation in a large controlled trial in rural Nepal (779 women in five groups). Serum α_1-acid glycoprotein concentration decreased in all groups but decreased more in the groups receiving folic acid, or folic acid and iron with or without zinc. In contrast, C-reactive protein increased in all groups from baseline to 32 weeks of gestation, but it was significantly lower in the group receiving folic acid and iron with zinc only [22]. This suggests that zinc given with folic acid and iron may ameliorate the inflammatory process in pregnancy, which has positive implications for reproductive health outcomes.

Serum zinc concentration declines progressively during the course of pregnancy in relation to blood-volume expansion [25]. Thus, serum zinc concentration values must be interpreted in relation to the stage of pregnancy or serum albumin concentrations. Unlike studies in young children, in whom serum zinc concentration nearly always increases in response to zinc supplementation [26], only 5 of the 12 zinc supplementation studies that reported serum zinc concentrations during pregnancy found a significant increase in the supplemented group [18, 27–30]. The lack of response may have been due to failure to control for the stage of gestation or serum albumin concentration, in some cases, or to their relatively small sample sizes. Despite these limitations, serum zinc concentration is still the recommended biochemical indicator of zinc status during pregnancy at the population level [2], and this indicator can be used to assess the impact of zinc supplementation in populations. A recent meta-analysis of nine studies of zinc supplementation in pregnant women found a significantly positive overall effect of supplementation on mean serum zinc concentration, with an effect size of 0.20 SD (95% CI, 0.051 to 0.348) [26].

There are no studies of zinc supplementation and maternal mortality. The few studies of maternal morbidity reviewed here do not provide evidence that zinc supplements alone consistently reduce complications of labor and delivery, maternal hypertension, or infection. Since these pregnancy complications may be associated with placentation problems in early gestation, zinc supplementation prior to conception also should be evaluated in relation to maternal morbidity outcomes. However, no studies of zinc supplementation prior to and during gestation have been done to date.

Fetal mortality and growth

The effect of supplemental zinc on stillbirth or neonatal death has been reported in 7 studies, and 17 studies evaluated its effect on fetal growth [13, 15–17, 19, 23, 29–43] (**table 2**). A recent meta-analysis found no overall impact on the rate of stillbirth or neonatal death [10].

Most studies of supplemental zinc and fetal growth have used birthweight as the endpoint. Only three studies found that supplemental zinc significantly increased birthweight as compared with the control group [29, 30, 41]. All three studies were done in populations where maternal zinc depletion was likely. In the study comparing a zinc supplementation group with an untreated control group in India [30], infants born to women in the control group weighed only about 2.6 kg; those born to zinc-supplemented mothers were about 0.3 to 0.8 kg heavier ($p < .001$), depending on the length of time supplemental zinc was provided. Xie et al. [29] compared three different levels of supplemental zinc (5, 10, and 30 mg/day) with placebo in rural Chinese women and found that the infants of mothers receiving the highest amount of zinc supplement (30 mg/day) were on average 283 g heavier ($p = .016$) and had larger head circumferences (0.6 cm, $p = .035$) than the infants of mothers in the placebo group. This difference was not found in mothers receiving 5 or 10 mg of zinc/day. Goldenberg et al. [41] studied the effect of zinc supplementation on birthweight in a group of African-American women who were medically indigent (without health insurance or other health-care coverage). Only women with plasma zinc concentrations below the median for their population at 20 weeks of gestation

TABLE 2. Effect of zinc supplementation during pregnancy on fetal mortality and growth

Country, year [reference] author	Study design	Population	Amount of zinc supplement	Duration of supplementation	Zinc group	Comparison group	Effect of supplement
Supplementation with zinc alone					Zinc	Placebo	
Bangladesh, 2000 [32] Osendarp	Randomized, double-blind, controlled	Poor, urban Bangladeshi women	30 mg/day	From wk 12–16 gestation to term	*n* = 269	*n* = 290	No effect
Chile, 2001 [15] Castillo-Duran	Randomized, double-blind, controlled	Chilean adolescent women	20 mg/day	From before wk 20 gestation to term	*n* = 249	*n* = 258	Significantly lower proportion of LBW and premature infants
China, 2001 [29] Xie	Randomized, double-blind, controlled	Rural Chinese women	5 mg/day 10 mg/day 30 mg/day	From before wk 12 gestation to term	5 mg zinc/day (*n* = 37) 10 mg zinc/day (*n* = 40) 30 mg zinc/day (*n* = 39)	*n* = 40	Increased birthweight and head circumference in high-zinc group compared with placebo group
South Africa, 1979 [31] Ross	Randomized, double-blind, controlled	Black South African women	30 or 90 mg/day	From midpregnancy to term	*n* = 32	*n* = 33	No effect
UK, 1989 [13] Mahomed	Randomized, double-blind, controlled	UK women	20 mg/day	From before wk 20 gestation to term	*n* = 246	*n* = 248	No effect
Supplementation with folic acid, iron, and zinc					Folic acid + iron + zinc	Folic acid + iron	
India, 1993 [30] Garg	Randomized, not blinded No placebo	Urban Indian women	45 mg/day	From booking to term	*n* = 106	*n* = 62	Increased birthweight and gestational age; fewer preterm infants
Indonesia, 2001 [17] Dijkhuizen	Randomized, double-blind, controlled	Indonesian rural women	30 mg/day	From before wk 20 gestation to term	Folic acid + iron + zinc (*n* = 48)	Folic acid + iron (*n* = 42)	No effect on birthweight
					β-Carotene + folic acid + iron + zinc (*n* = 44)	β-Carotene + folic acid + iron (*n* = 45)	No effect on birthweight
Nepal, 2003 [42] Christian	Cluster-randomized, double-blind, controlled	Nepalese rural women	30 mg/day	From wk 5–10 gestation to 3 mo postpartum	Folic acid + iron + zinc (*n* = 827)	Placebo, folic acid, folic acid + iron, or MMN (*n* = 3,295)	No beneficial effect of zinc on fetal loss compared with other groups

continued

TABLE 2. Effect of zinc supplementation during pregnancy on fetal mortality and growth *(continued)*

Country, year [reference] author	Study design	Population	Amount of zinc supplement	Duration of supplementation	Zinc group	Comparison group	Effect of supplement
Supplementation with zinc alone					Zinc	Placebo	
[43] Katz					Folic acid + iron + zinc (n = 827)	Placebo, folic acid, folic acid + iron, or MMN (n = 3,295)	Zinc with folic acid + iron increased birthweight of infants in the 2,400–2,900 g range
[23] Christian					Folic acid + iron + zinc (n = 827)	Placebo, folic acid, folic acid + iron, or MMN (n = 3,295)	Zinc with folic acid + iron had no effect on birthweight
Pakistan, 2005 [38] Hafeez	Randomized, double-blind controlled	Pakistani women	20 mg/day	From wk 10–16 gestation to term	n = 121	n = 121	No effect on birthweight or on length or duration of pregnancy
Peru, 1999 [34] Caulfield	Randomized, double-blind controlled	Poor Peruvian women	15 mg/day	From wk 10–24 gestation to term	n = 521	n = 495	No effect on birthweight or on length or duration of pregnancy
[35] Merialdi					n = 31	n = 24	Zinc-containing supplement increased fetal heart rate range and in utero movement
Peru, 2004 [36] Merialdi	Randomized, double-blind controlled	Poor Peruvian women	25 mg/day	From wk 10–16 gestation to term	n = 94	n = 101	Zinc-containing supplement increased fetal femur diaphysis length
[37] Merialdi					n = 94	n = 101	Zinc-containing supplement increased fetal heart rate variability
UK, 1991a[a] [16] Simmer	Randomized, double-blind controlled	Lower-social-class English women	22.5 mg/day	From 15–25 wk prior to delivery	n = 30	n = 22	Reduced incidence of IUGR
UK, 1991b [33] Robertson	Randomized, double-blind controlled	High-risk English women	62 mg/day	Before wk 18 gestation to term	n = 72	n = 62	No effect on birthweight
Supplementation with zinc and MMN					MMN + zinc	MMN	
Tanzania, 2005 [44] Fawzi	Randomized, double-blind, controlled	HIV-infected pregnant Tanzanian women	25 mg/day	From wk 12–27 gestation to term	n = 198	n = 199	No effect on birthweight, duration of pregnancy, or fetal mortality

USA, 1983 [39] Hambidge	Controlled No statement on randomization and blinding	Middle-income Colorado women	15 mg/day	From mo 1–3 gestation to term	$n = 10$	$n = 36$	No effect on birthweight or other measures of pregnancy outcome
USA, 1984 [19] Hunt	Randomized, double-blind, controlled	Low-income, Hispanic California women	20 mg/day	From about wk 19 gestation to term	$n = 107$	$n = 106$	Zinc supplementation associated with lower incidence of pregnancy-induced hypertension
USA, 1989 [40] Cherry	Randomized, double-blind controlled	US adolescents, primarily black	30 mg/day	From before wk 25 gestation to term	$n = 268$	$n = 288$	Reduced incidence of preterm delivery in normal-weight women
USA, 1995 [41] Goldenberg	Randomized, double-blind controlled	Medically indigent African-American women	25 mg/day	From wk 19 gestation to term	$n = 294$	$n = 286$	Increased birthweight and head circumference; effect greater in women with BMI < 26

BMI, body mass index; IUGR, intrauterine growth retardation; LBW, low-birthweight; MMN, multiple micronutrients

a. Iron and folic acid supplement was prescribed if clinically indicated.

were included in the study. Thus, the intervention was targeted toward women at risk for being zinc deficient. In all women, supplemental zinc increased the infant's birthweight by 128 g ($p = .03$) and head circumference by 0.4 cm ($p = .02$). The effect was greater in nonobese women, among whom zinc supplementation increased birthweight by 248 g ($p = .005$) and head circumference by 0.7 cm ($p = .007$). Eleven other studies failed to find a relationship between zinc supplementation and birthweight [13, 15, 17, 19, 31–34, 38, 39, 44]. However, none of those 11 studies stratified the effects of supplemental zinc on birthweight by maternal weight or zinc status. There was no evidence that whether zinc was given along with other micronutrients or alone influenced the outcome. The recently published meta-analysis of 14 studies found that zinc supplementation had no significant impact on birthweight (WMD, −10.59; 95% CI, −36.71 to 15.54) [10]. Future studies are needed to determine if maternal pregravid or gravid weight modifies the effect of supplemental zinc on fetal growth and birthweight.

Simmer et al. [16] conducted a double-blind trial in the United Kingdom of mothers at risk for delivering infants with intrauterine growth retardation because they had had a small-for-gestational age infant previously, were underweight, or were smoking. Zinc supplementation significantly reduced the incidence of intrauterine growth retardation (7% vs. 27%, $p = .04$). There was no significant effect on overall birthweight. Since this is a small study with insufficient power to allow definite conclusions to be drawn, additional research is needed to evaluate whether zinc supplementation is more likely to improve fetal growth in high-risk, underweight mothers.

Merialdi et al. [36] used ultrasonography to examine the effect of administration of supplemental zinc to pregnant women on fetal bone growth in utero. Although there were no effects of zinc supplementation (given along with iron and folic acid) on birthweight or head circumference, the femur diaphysis length was significantly longer in the fetuses of mothers receiving zinc along with iron and folic acid than in fetuses of mothers receiving iron and folic acid only at all time points measured between 20 and 38 weeks of gestation. No differences in birth length due to zinc supplementation were observed, but the small effects on femur length probably would not be detected in measurements of birth length. There were no other effects of supplemental zinc on growth at specific anatomical sites. These findings are consistent with studies in experimental animals suggesting that zinc has a very specific effect on the growth of long bones [45, 46].

Preterm birth can contribute to a reduction in birthweight. Zinc supplementation significantly reduced the prematurity rate compared with placebo among Chilean adolescents (6% vs. 12%, $p = .016$) [15]. Among Indian women, there were significantly fewer preterm

infants in the zinc-supplemented group than in a nonintervention control group who did not receive a placebo (2% vs. 11%, $p < .05$) [30]. Although most other studies found no significant overall impact on preterm delivery, some studies found a beneficial impact in selected subgroups of women. In a meta-analysis of 13 studies, Mahomed et al. [10] found a small, but significant, reduction in preterm birth (relative risk, 0.86; 95% CI, 0.76 to 0.98). This 14% reduction in preterm birth among zinc-supplemented women was found primarily in the subset of studies involving lower-income women, suggesting that zinc supplementation may be beneficial in areas where poor nutrition and maternal infection are more common.

Animal studies show that zinc is essential for neurologic development in utero. In two studies in Peru, Merialdi et al. [35, 37] evaluated the effect of supplemental zinc on fetal heart rate and fetal movement, using novel methods for measuring fetal cardiac and somatic activity, both of which are influenced by the development of the autonomic nervous system. Fetuses of mothers who received supplemental zinc plus iron and folic acid showed an increased range and variability of fetal heart rate and an increased amount of time spent moving compared with those whose mothers received iron and folic acid supplements without zinc. The differences became significant at 36 weeks of gestation ($p < .05$). Although follow-up studies are needed to verify these observations and assess their longer-term implications, the data suggest that adding zinc to prenatal iron and folic acid supplements may have a beneficial effect on fetal neurobehavioral development.

In summary, the effects of supplemental zinc on maternal labor and delivery complications and on fetal growth and neurobehavioral development are limited and conflicting. There is some evidence that supplemental zinc increases birthweight and duration of gestation in underweight women living in areas where zinc intake is low or zinc is poorly absorbed. However, a number of other studies failed to find a relationship between supplemental zinc, with or without other micronutrient supplements, and birthweight. The results of those studies were not stratified by maternal body weight.

Neonatal and early infant morbidity and mortality, growth, and development

The effects of zinc supplementation on postnatal growth, development, and rates of infections are summarized in **table 3** [9, 42, 47–52]. Osendarp et al. [47] evaluated the effects of zinc supplementation on infant growth and morbidity at 6 months of age in Bangladesh. Maternal supplementation during pregnancy reduced the risk of infant acute diarrhea (risk ratio, 0.84; 95% CI, 0.72 to 0.98), dysentery (risk ratio, 0.36; 95% CI, 0.25 to 084), and impetigo (risk ratio, 0.53; 95% CI, 0.34 to 0.82) in low-birthweight infants but not in infants with normal birthweight. The results from the Peruvian maternal zinc supplementation trial, which were published in a review article [9], also show that maternal zinc supplementation reduced acute diarrhea and dysentery, but the results were only significant for the period from 8 to 12 months of age. In a study in rural Nepal, the women received supplements from early pregnancy to 3 months postpartum that contained vitamin A alone; vitamin A and folic acid; vitamin A, folic acid, and iron; vitamin A, folic acid, iron, and zinc; or a multiple micronutrients (MMN) supplement [48]. None of the combinations of antenatal micronutrient supplements affected symptoms of neonatal morbidity in the first 10 days of life or at 6 weeks of age [48], and there was no evidence that zinc had any effect on infant mortality throughout the first year [42]. These findings suggest that maternal zinc supplementation during pregnancy may influence the infant's risk of selected infections postnatally, but that these benefits may be restricted to older infants. More research is needed to address these issues.

In two studies, no differences in infant growth were observed in relation to maternal zinc supplementation among Indonesian infants at 6 months postpartum [51] and among Bangladeshi children at 13 months postpartum [47]. In contrast, infants born to Peruvian mothers supplemented with zinc during pregnancy had significantly greater anthropometric measures from months 4 to 12 [50]. On average, the infants from the zinc group were 0.58 ± 0.12 kg/month heavier, with weight accrued during the first year of life, than those from the control group. The longitudinal effects of zinc treatment remained significant for weight, calf and chest circumferences, and calf muscle area after control for a range of covariates, including infant-feeding practices and diarrhea morbidity. The reason for these differences among studies is not known, and additional research will be needed to understand the effects.

Neurobehavioral development was evaluated in infants born to zinc-supplemented Bangladeshi [49] and Peruvian women [9]. In Peru, some improvements in infant neurobehavioral development (novelty preference) were observed in the zinc-supplemented group at 6 months of age, whereas no benefit was observed in Bangladesh. In fact, Bangladeshi infants in the placebo group had higher scores on mental development and psychomotor indexes than those in the zinc-supplemented group. Tamura et al. [52] evaluated the effect of prenatal zinc supplementation on the mental and psychomotor development of 355 children of African-American mothers who participated in a double-blind study of zinc supplementation in which the infants of zinc-supplemented mothers had increased head circumference at birth. There was no effect on mental or psychomotor development of the children at 5 years of age, before or after stratification of the sample by maternal body mass index. Identification

TABLE 3. Effect of zinc supplementation during pregnancy on infant and young child postnatal growth, morbidity, and neurobehavioral development

Study	Study design	Population	Amount of zinc supplement	Duration of supplementation	Zinc group	Comparison group	Effect of supplement
Supplementation with zinc alone					Zinc	Placebo	
Bangladesh, 2000 [47] Osendarp	Randomized, double-blind, controlled	Infants of poor, urban Bangladeshi women	30 mg/day	From wk 12–16 gestation to term	*n* = 184	*n* = 199	No effect on growth at 6 mo postpartum. Reduced incidence of acute diarrhea and dysentery
[49] Hamadani					*n* = 83	*n* = 85	Placebo improved mental and psychomotor development. No effect of zinc on behavior or growth
Supplementation with folic acid, iron, and zinc, with or without β-carotene					Folic acid + iron + zinc	Folic acid + iron	
Indonesia, 2001 [51] Dijkhuizen	Randomized, double-blind, controlled	Infants of Indonesian rural women	30 mg/day	From before wk 20 gestation to term	Folic acid + iron + zinc (*n* = 48)	Folic acid + iron (*n* = 42)	No effect on growth at 6 mo postpartum
					β-Carotene + folic acid + iron + zinc (*n* = 44)	β-Carotene + folic acid + iron (*n* = 45)	No effect on growth at 6 mo postpartum
Nepal, 2003 [42, 48] Christian	Cluster-randomized, double-blind, controlled	Nepalese rural women	30 mg/day	From wk 5–10 gestation to 3 mo postpartum	Folic acid + iron + zinc (*n* = 827)	Placebo, folic acid, folic acid + iron, or MMN (*n* = 3,295)	No beneficial effect of zinc on neonatal and infant morbidity and infant mortality
Peru, 1999 [50] Iannotti	Randomized, double-blind, controlled	Infants of poor Peruvian women	15 mg/day	From wk 10–24 gestation to term	*n* = 273	*n* = 273	Larger growth measures beginning at 4 mo through 12 mo
[9] Osendarp					*n* = 521	*n* = 495	Reduced risk of any diarrhea at 8–12 mo and of dysentery at 0–12 mo
[9] Osendarp					NA	NA	Some improvements in neurobehavioral development (novelty preference) at 6 mo
Zinc and MMN supplementation					MMN + zinc	MMN	
USA, 1995 [52] Tamura	Randomized, double-blind, controlled	Infants of medically indigent African-American women	25 mg/day	From wk 19 gestation to term	*n* = 173	*n* = 182	No effect on mental and psychomotor development of children at 5 yr of age

MMN, multiple micronutrients; NA, not available

of long-term effects of prenatal supplemental zinc on neurologic development may be undermined by poor environmental postnatal influences on this aspect of development.

Section 2

What is the effect of preventive zinc supplementation during lactation on maternal and neonatal health?

Conclusions

The limited data available on zinc supplementation and lactation performance and infant growth and zinc status fail to show any consistent benefit to the mother or child. However, studies are available only from relatively healthy women in developed countries. The effect of maternal zinc supplementation during pregnancy and lactation or during lactation only on maternal and neonatal health needs to be studied in undernourished women.

Detailed review of evidence

Bibliographic search

Data sets were identified as described above. Of the 1,618 articles, there were 3 articles that evaluated the impact of zinc supplementation during lactation. Two additional articles not identified during the PubMed search were added. The findings of the five articles are summarized in the following section (**table 4**).

All five studies of the effect of maternal zinc supplementation on milk zinc concentration and infant growth [53–57] were done in women living in developed countries (Finland and the United States). Zinc supplementation was initiated at birth and continued daily throughout the duration of the studies, which varied from 6 to 12 months. It is not clear whether the mothers were exclusively breastfeeding for the entire study period or whether breastfeeding was supplemented with formula and complementary foods during the later stages of lactation.

Milk volume and zinc concentration

Krebs et al. [53] found that the rate of decline in milk zinc concentrations was significantly less in mothers receiving supplemental zinc without any other micronutrients than in non-zinc-supplemented mothers (p = .02). Log-transformed monthly milk zinc concentrations were compared between the two groups to determine the mean differences in rate of decline between birth and 9 months of age. Milk zinc concentration decreased by 0.69 ± 0.27 μg/mL in the non-zinc-supplemented group, whereas it declined by 0.54 ± 0.14 μg/mL in the supplemented group. These changes in milk zinc concentration with zinc supplementation occurred without any increase in maternal plasma zinc levels. The other four studies gave supplemental zinc to lactating women along with other micronutrients. Karra et al. [54] provided 25 mg of supplemental zinc per day to lactating women for 6 months and found a significantly lower decline in breastmilk zinc concentration in the supplemented group than in the placebo group, accompanied by a significant increase in plasma zinc concentration in the zinc-supplemented mothers. Salmenperä et al. [56] found no difference in breastmilk zinc concentrations between the control group and the supplemented group receiving 20 mg of zinc per day. However, breastmilk zinc concentration in the group receiving 40 mg per day declined significantly more slowly—by 6 months—than in the other two groups. There was no correlation between maternal serum zinc concentration and the total zinc transfer into milk in any of the three groups (40, 20, and 0 mg/day) [56]. Krebs et al. [57] and Moser-Veillon and Reynolds [55] did not find an effect of supplemental zinc on milk zinc concentration. These inconsistent findings suggest that neither maternal zinc intake nor plasma zinc levels are major determinants of milk zinc concentration.

Infant growth and zinc status

Only one study is available regarding the effect of maternal zinc supplementation during lactation on infant growth. Salmenperä et al. [56] found no effect of maternal zinc supplementation (20 or 40 mg/day vs. placebo) during lactation on serum zinc concentrations or growth of Finnish infants. Infant serum zinc concentrations throughout the first year of life (mean ± SD, 67 ± 4 μg/dL) tended to decline during periods of rapid growth, especially in boys. There were no associations between serum zinc concentrations and growth rates.

Section 3

Are there any adverse effects of zinc supplementation during pregnancy or lactation?

Conclusions

There is very little information available on adverse effects of zinc supplementation during pregnancy. We reviewed the effects of providing zinc in a prenatal supplement on iron status in nine randomized, double-blind, controlled trials and on copper status in four trials. None of these studies found a difference in final hemoglobin, serum ferritin, transferrin receptor, or serum copper concentration between the zinc-supplemented group and the control group. The four studies reporting on copper status provided information on serum copper concentration only, which is a relatively insensitive biomarker of copper status. Further studies

TABLE 4. Effect of maternal zinc supplementation during lactation on breastmilk zinc concentration

Country, year [reference] author	Study design	Population	Age of infants	Amount of zinc supplement	Duration of supplementation	Zinc group	Placebo group	Effect of supplement
Supplementation with zinc alone						Zinc	Placebo	
USA, 1985 [53] Krebs	Untreated control group. No statement on randomization and blinding	Mothers in Colorado, USA	1–12 mo	15 mg/day	From 1 to 12 mo	$n = 14$[a]	$n = 25$[a]	Rate of decline in milk zinc significantly less than in nonsupplemented group
Supplementation with zinc and MMN						MMN + zinc	MMN	
Finland, 1994 [56] Salmenperä	Controlled. No statement on randomization and blinding	Finnish mothers exclusively breastfeeding	0–12 mo	20 mg/day	From days 4–5 after delivery to 12 mo	$n = 73$[b]	$n = 94$[b]	No effect on infant serum zinc concentration or growth No difference in milk zinc between controls and group receiving 20 mg zinc/day
				40 mg/day		$n = 27$[b]		Smaller decline in milk zinc in group receiving 40 mg zinc/day by 6 mo
USA, 1988 [54] Karra	Controlled. No statement on randomization and blinding	Mothers in Indiana, USA[c]	0–6 mo	25 mg/day	From day 1 after delivery to 6 mo	$n = 24$[d]	$n = 25$[d]	Rate of decline in milk zinc significantly less than in nonsupplemented group
USA, 1990 [55] Moser-Veillon	Randomized, double-blind, controlled	Mothers in Maryland, USA	0–9 mo	25 mg/day	From day 1 after delivery to 9 mo	$n = 20$[e]	$n = 20$[e]	No effect on infant plasma zinc, infant erythrocyte zinc, or milk zinc concentration
USA 1995, [57] Krebs	Randomized, double-blind, controlled	Mothers in Colorado, USA	1–7 mo	15 mg/day	From wk 2 postpartum to 7 mo	$n = 40$[f]	$n = 31$[f]	No effect on milk zinc concentration

MMN, multiple micronutrients

a. Sample size from enrollment to 12 months decreased from 14 to 2 in the zinc group and from 25 to 2 in the placebo group.

b. Sample size from enrollment to 12 months decreased from 73 to 2 in the group receiving 20 mg of zinc and from 94 to 5 in the placebo group. By 9 months, the group receiving 40 mg of zinc was reduced to 4.

c. Study included a comparison group of lactating women in Egypt. Data not presented here.

d. Sample size from enrollment to 12 months decreased from 24 to 19 in the zinc group and from 25 to 22 in the placebo group.

e. Sample size from enrollment to 9 months decreased from 20 to 9 in the zinc group and from 20 to 11 in the placebo group.

f. The number of patients who completed the study is given.

using more sensitive indicators of copper status are required. On the basis of available results, it can be concluded that the addition of zinc to a prenatal iron and folic acid supplement does not adversely affect iron status.

Detailed review of evidence

Any adverse effects related to the addition of zinc to the prenatal iron and folic acid supplement would be of concern. It is important, therefore, to consider the risks, as well as the benefits, of zinc supplementation during pregnancy and lactation. However, manifestations of acute toxicity symptoms, such as nausea and vomiting, occur only at very high zinc intake levels of ~225 to 450 mg/day or more [58]. An adverse effect of zinc on copper metabolism has been shown only at zinc intakes above 50 mg/day in adults, as measured by a decrease in erythrocyte superoxide dismutase activity [59]. There is some evidence from intervention trials in young children that iron indicators do not improve as much when supplemental iron is given with zinc as when iron is given alone [60], although no overall effect was found in a recent meta-analysis [61]. A recent animal study showed that supplementation of zinc-adequate dams with additional zinc induced a immunosuppressive response in the offspring, a finding that needs further investigation but implies a need for caution [62].

The impact of adding zinc to prenatal supplements on iron and copper nutriture was reviewed. A total of nine randomized, double-blind controlled trials in pregnant women [13, 17, 19, 32, 44, 63–67] reporting results on either hemoglobin concentration or other iron status indicators are summarized in **table 5**. One study was not considered in this analysis because the supplementation period was only 3 weeks [18] and, therefore, was not likely to have an impact on hemoglobin concentration. For all other studies, we present the data of the two treatment groups differing by zinc only, if the studies included more than two groups. Two studies [13, 32] compared zinc versus placebo, four studies compared the addition of zinc to an iron [63] or an iron and folic acid supplement [17, 65] with vitamin A or β-carotene [17, 66], and four studies provided a MMN supplement containing iron and folic acid, with or without zinc [19, 44, 64, 67]. One study found that the addition of zinc to an iron supplement had a beneficial impact on hemoglobin concentration in anemic pregnant Iranian women after 12 weeks of supplementation [63]. None of the other studies found a difference in final hemoglobin, serum ferritin, or transferrin receptor concentration between the treatment group receiving zinc and those receiving no zinc (with or without other micronutrients). Dijkhuizen et al. [51] also evaluated maternal and infant micronutrient status 6 months postpartum and found that the addition of zinc to the prenatal supplement had no impact on hemoglobin or plasma ferritin concentration in mothers and infants. On the basis of these results, it can be concluded that the addition of zinc to a prenatal iron and folic acid or a MMN supplement in the range of 15 to 30 mg of zinc/day does not adversely affect iron status.

The studies reporting on copper status provide information on serum copper concentration only, which is the least sensitive biomarker of copper status [68]. Of the three studies reporting on copper status (**table 5**), none of the studies found a significant difference in final serum copper concentration between the two treatment groups receiving MMN with or without zinc. Similarly, Hambidge et al. [39] stated that there was not a difference between groups at 10 months of gestation, but they did not provide the data disaggregated by treatment group. This limited information, as judged by serum copper levels, indicates that the provision of supplemental zinc at a level of 20 to 30 mg/day during pregnancy does not have a negative effect on copper status. Further studies using more sensitive indicators of copper status are needed.

None of the zinc supplementation trials in lactating women reported results on iron or copper status in the mothers. However, on the basis of the lack of any adverse effects on iron and copper status in pregnant women and in children [61], it can be assumed that providing a zinc supplement during lactation at the recommended dosage does not have an adverse effect on maternal iron or copper status.

Section 4

What are the implications of these outcomes for zinc supplementation programs during pregnancy and lactation and what are the remaining research needs?

Adding zinc to prenatal supplements

As mentioned above, WHO recommends that all pregnant women living in areas of high prevalence of malnutrition should routinely receive iron and folic acid supplements to prevent anemia as part of the integrated management of pregnancy and childbirth [8]. The purpose of this review was to evaluate the potential benefits and adverse effects of adding zinc to the prenatal supplement containing iron and folic acid and to draw conclusions for programmatic implications.

There is currently no evidence of adverse effects of supplemental zinc on iron status or the response to iron supplementation during pregnancy. A meta-analysis showed that zinc supplementation during pregnancy reduced the risk of preterm birth by 14% [10]. However, the effects of supplemental zinc on labor and delivery complications, birthweight, and postnatal

TABLE 5. Possible adverse effects of zinc supplementation during pregnancy on hemoglobin and iron and copper status indicators

Country, year [reference] author	Time of gestation at enrollment	Time of gestation at final blood collection	Micronutrient content of supplement	Study group	Sample size [a]	Initial hemoglobin concentration (g/L)[b]	Final hemoglobin concentration (g/L)[b]	Initial serum ferritin concentration (μg/L)[c]	Final serum ferritin concentration (μg/L)[c]	Initial serum copper concentration (μg/dL)[b]	Final serum copper concentration (μg/dL)[b,d]
Supplementation with zinc alone											
UK, 1989 [13] Mahomed	Before wk 20	Wk 28–32	—	Control	232	—	115 (NS)[e]	—	—	—	—
			20 mg zinc	Zinc	238	—	117	—	—	—	—
Bangladesh, 2000 [32] Osendarp	Wk 12–16	Wk 28–32	—	Control	232	115 ± 11	108 ± 11 (NS)[e]	—	—	—	—
			30 mg zinc	Zinc	214	114 ± 14	108 ± 13	—	—	—	—
Supplementation with folic acid, iron, and zinc											
Iran, 2005 [63] Mahmoudian	Wk 16–20	Wk 28–32	100 mg iron	Iron	58	104 ± 2	119 ± 9 ($p < .05$)	—	—	—	—
			100 mg iron, 15 mg zinc	Iron + zinc	60	105 ± 2	128 ± 10	—	—	—	—
Peru, 1999 [65] Zavaleta	Wk 10–24	Wk 37–38	250 μg folic acid, 60 mg iron	Folic acid + iron	320	115 ± 14	115 ± 13 (NS)	19.7 (7.7, 50)	17.8 (8.2, 38.9) (NS)	—	—
			250 μg folic acid, 60 mg iron, 15 mg zinc	Folic acid + iron + zinc	325	116 ± 12	114 ± 13	21.8 (8.9, 53.3)	17.6 (8.1, 38.5)	—	—
Indonesia, 2001 [17] Dijkhuizen	Wk 10–20	Wk 32	400 μg folic acid, 30 mg iron	Folic acid + iron	34	118 ± 14	109 ± 12 (NS)[f]	24 (8.7, 39.6)	14 (7.8, 22.8) (NS)[f]	—	—
			400 μg folic acid, 30 mg iron, 30 mg zinc	Folic acid + iron + zinc	45	114 ± 11	106 ± 14	28 (14.2, 46.7)	14 (7.0, 22.3)	—	—

continued

TABLE 5. Possible adverse effects of zinc supplementation during pregnancy on hemoglobin and iron and copper status indicators *(continued)*

Country, year [reference] author	Time of gestation at enrollment	Time of gestation at final blood collection	Micronutrient content of supplement	Study group	Sample size [a]	Initial hemoglobin concentration (g/L)[b]	Final hemoglobin concentration (g/L)[b]	Initial serum ferritin concentration (μg/L)[c]	Final serum ferritin concentration (μg/L)[c]	Initial serum copper concentration (μg/dL)[b]	Final serum copper concentration (μg/dL)[b,d]
			4.5 mg β-carotene, 400 μg folic acid, 30 mg iron	β-Carotene + folic acid + iron	42	118 ± 12	107 ± 12	25 (16.4, 39.6)	16.2 (8.5, 24.4)	—	—
			4.5 mg β-carotene, 400 μg folic acid, 30 mg iron, 30 mg zinc	β-Carotene + folic acid + iron + zinc	43	116 ± 12	109 ± 11	21 (10.1, 43.2)	16 (8.8, 25.3)	—	—
Nepal, 2003[g] [22, 66] Christian	Wk 10	Wk 32	Vitamin A (1,000 μg RE), 400 μg folic acid, 60 mg iron	Vitamin A + folic acid + iron	202	115 ± 18	116 ± 15 (NS)	11.5 (18.2)	14.4 (12.9) (NS)	148 ± 44	217 ± 48 (NS)[h]
			Vitamin A (1,000 μg RE), 400 μg folic acid, 60 mg iron, 30 mg zinc	Vitamin A + folic acid + iron + zinc	253	116 ± 22	116 ± 15	13.7 (16.7)	13.7 (15.3)	151 ± 44	217 ± 43
Supplementation with zinc and MMN											
USA, 1984[i] [19] Hunt	Average wk 19	Average wk 36	1 mg folic acid, 20 mg iron, other MMN	MMN	37	119 ± 9	123 ± 9 (NS)	—	—	225 ± 40	236 ± 41 (NS)
			1 mg folic acid, 20 mg iron, other MMN, 20 mg zinc	MMN + zinc	27	122 ± 10	123 ± 13	—	—	225 ± 32	238 ± 34
USA, 1985 [67] Hunt	About wk 17	About wk 36	1 mg folic acid, 20 mg iron, other MMN	MMN	26	123 ± 13	124 ± 11 (NS)	29 ± 24.4	10.6 ± 5.7 (NS)	—	—

			1 mg folic acid, 20 mg iron, other MMN, 20 mg zinc	MMN + zinc	36	119 ± 11	120 ± 11	32.9 ± 27.7	10.5 ± 7.5	—	—
USA, 1995 [64] Tamura	Average wk 20	Average wk 40 (delivery)	Folic acid and other MMN[j]	MMN	32	—	—	—	—	222 ± 38	230 ± 46 (NS)
			Folic acid and other MMN,[j] 25 mg zinc	MMN + zinc	31	—	—	—	—	215 ± 34	225 ± 42
Tanzania, 2005 [44] Fawzi	Average wk 23	Wk 6 postpartum	5 mg folic acid, 120 mg iron, other MMN	MMN	193	98 ± 14	114 ± 17 (NS)	—	—	—	—
			5 mg folic acid, 120 mg iron, other MMN, 25 mg zinc	MMN + zinc	192	100 ± 12	112 ± 16	—	—	—	—

MMN, multiple micronutrients; NS, not significant; RE, retinol equivalents

a. Sample size for measurement of initial hemoglobin concentration. Sample size for other indicators may vary.

b. Results for hemoglobin and serum copper concentrations are reported as mean ± SD, except for UK 1989 [13], where SD was not reported.

c. Results for serum ferritin are reported as geometric mean (–1 SD, +1 SD) for Peru 1999 [65], as median (interquartile range) for Indonesia 2001 [17] and Nepal 2003 [22, 66], and as mean ± SD for USA 1985 [67].

d. The study by Hambidge et al. [39] is not shown in this table because the data on copper concentration are not disaggregated between the two treatment groups. However, the authors state that there was no significant difference between final concentrations at 10 months of gestation.

e. No significant difference in final concentration between two treatment groups

f. No significant difference in final concentration among all four treatment groups.

g. Additional information provided by author (Christian P, personal communication, 2007).

h. P-value calculated on the basis of changes in copper concentration.

i. Results reported only for women who were studied long enough to take supplements for more than 60 days.

j. MMN content of supplement not defined.

growth are inconsistent.

In view of the lack of a clear benefit of supplemental zinc during gestation, a recommendation to add zinc to the usual prenatal supplement of iron and folic acid cannot be made. Nevertheless, since toxic effects of supplemental zinc have not been identified, it may be prudent to include zinc in the prenatal supplement in areas at high risk for zinc deficiency, as indicated by a stunting rate of more than 20% among children under five [2] or a maternal body mass index under 18.5.

Zinc supplementation during lactation

There is currently insufficient evidence for any benefit in providing zinc supplements to lactating women. Further research is needed to assess the impact of zinc supplementation on the lactating mothers and their infants, in particular among undernourished women living in lower-income countries.

Research needs

» There is a need for further research on the addition of zinc to prenatal supplements of iron and folic acid for undernourished or low-weight women in lower-income countries.
» Future studies are needed to determine if maternal pregravid or gravid weight is a determinant of the effect of supplemental zinc on fetal growth and birthweight. The association between maternal stature, zinc status, and birthweight also needs further investigation.
» Since pregnancy complications may be associated with placentation problems in early gestation, zinc supplementation prior to conception also should be evaluated in relation to maternal morbidity outcomes.
» Future studies on zinc supplementation during pregnancy should include a follow-up during early childhood to further evaluate the impact on growth, development, and morbidity in infants of mothers who have received zinc supplementation during pregnancy.
» It is further important to assess the risks as well as the benefits of zinc supplementation during pregnancy and lactation.
» The effect of maternal zinc supplementation during lactation on maternal and neonatal health needs to be studied in undernourished women.

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Impact of zinc fortification on zinc nutrition

Sonja Y. Hess and Kenneth H. Brown

Abstract

Food fortification is increasingly recognized as an effective approach to improve a population's micronutrient status. The present report provides a critical review of the scientific evidence currently available on the impact of zinc fortification on zinc nutrition.

The available studies clearly show that zinc fortification can increase dietary zinc intake and total daily zinc absorption. Most absorption studies also indicate that adding zinc to food does not adversely affect the absorption of other minerals, such as iron. Despite the positive effect of zinc fortification on total zinc absorption, only a few studies have found positive impacts of zinc fortification on serum zinc concentrations or functional indicators of zinc status. The reasons for these inconsistent results are uncertain but may relate to the choice of food vehicles, the age group and zinc status of the study populations, or particular aspects of the study design. Thus, additional research is needed to determine the impact of zinc fortification, with or without other micronutrients, in populations at risk for zinc deficiency.

Because of the benefits of increasing intake in populations at high risk for zinc deficiency, the documented increase in total zinc absorption that occurs following zinc fortification, the absence of any adverse effects, and the relatively low cost of adding zinc, public health planners should consider including zinc in mass and targeted fortification programs in such populations. Because of the limited available information on program impact, it will be important to evaluate the outcomes of such programs.

Key words: Bioavailability, household fortification, zinc absorption, zinc fortification, zinc status

The authors are affiliated with the Department of Nutrition and the Program in International and Community Nutrition, University of California, Davis, California, USA; Kenneth H. Brown is also affiliated with Helen Keller International, Dakar, Senegal.

Please direct queries to the corresponding author: Kenneth H. Brown, Department of Nutrition, University of California, One Shields Ave., Davis, CA 95616, USA; e-mail: khbrown@ucdavis.edu.

Background

Food fortification can be defined as the deliberate addition of one or more nutrients to particular foods so as to increase the intake of these nutrients and correct or prevent a demonstrated deficiency, thereby providing some health benefit [1]. Food fortification is often considered the most economical approach to reduce nutritional deficiencies in settings where suitable food vehicles are available, the food industry is sufficiently developed to be able to produce and distribute these foods, and higher-risk subgroups of vulnerable populations have access to adequate amounts of these foods. The recent World Health Organization (WHO) publication on food fortification distinguishes among three possible approaches: mass, targeted, and market-driven fortification [1]. Mass fortification refers to the addition of micronutrients to edible products that are consumed regularly by a large proportion of the general public, such as cereal flours, vegetable oils and fats, milk, and condiments. Targeted fortification is defined as the fortification of foods designed for specific population subgroups, such as complementary foods for young children, foods for institutional programs aimed at schoolchildren or preschoolers, and foods used for emergency situations. Fortification is considered market-driven when a food manufacturer takes the initiative to add one or more micronutrients to processed and branded foods.

Food fortification has become an increasingly attractive strategy in lower-income countries, and a growing number of programs are being implemented. However,

surprisingly few of these fortification programs have been formally evaluated for their impact on the target population's nutrient intake and subsequent nutritional status [1]. The present report provides a critical review of the scientific evidence currently available on the impact of zinc fortification of foods on zinc nutrition.

Although food fortification is most commonly understood to refer to the addition of nutrients to food at the industrial site of food processing or production, fortification can also occur at the community or household level. In some cases, it can be very challenging to decide whether to categorize a particular intervention trial as an example of food fortification or nutrient supplementation, particularly if the nutrient is added to food at the point of consumption rather than at a central site. For the purpose of this review, we included data from intervention trials in which the added micronutrients were consumed together with foods, regardless of the site where the nutrients were added. For example, studies in which nutrients were added to infant formula in the home just before each serving were considered as food fortification trials along with other studies in which the nutrients were added to the formula at the industrial production site. We considered the case of home fortification with products like multiple micronutrient (MMN) powders as a special category of intervention, because the additional nutrients (typically provided in the form of a dry powder to be mixed with foods at the time of serving the meal) are usually administered just once each day, and the food vehicle is generally not stipulated, making these aspects of the implementation strategy different from typical fortification interventions. Other aspects of the household fortification strategy, such as the potential for interactions between the micronutrients and the food components, are more akin to food fortification carried out during the course of the food production [2].

This paper is divided into five sections, which address the following sets of questions in relation to zinc-fortified foods:

Section 1: Does zinc fortification affect total zinc intake and fractional and total absorption of zinc? Are these effects modified by consumer-, fortificant-, or diet-related factors or the presence of other nutrients in the fortified food?

Section 2: Does zinc fortification affect biochemical indicators of zinc status and zinc-related functions?

Section 3: Does household-level fortification with multiple micronutrients (MMN) (including zinc) affect indicators of zinc status and zinc-related functions?

Section 4: Are there any adverse clinical effects of zinc fortification or negative effects of zinc fortification on the utilization of other nutrients due to zinc fortification?

Section 5: What are the steps in implementing zinc fortification programs and what additional research is needed?

Section 1

Does zinc fortification affect total zinc intake and fractional and total absorption of zinc? Are these effects modified by consumer-, fortificant-, or diet-related factors or the presence of other nutrients in the fortified food?

Conclusions

The available studies clearly show that zinc fortification can increase dietary zinc intake and total daily zinc absorption. Although fractional absorption of zinc (i.e., the percentage of dietary zinc intake that is absorbed) decreases with increasing zinc intake, the total amount of absorbed zinc (TAZ) increases in relation to the amount of zinc consumed until it approaches a plateau at higher levels of zinc intake. Zinc seems to be absorbed equally well from foods fortified with zinc oxide or zinc sulfate, the two cheapest sources of zinc that are generally recognized as safe for human consumption, although extension of this conclusion to infants and young preschool children remains to be confirmed. The presence of phytic acid in food reduces zinc absorption from zinc-fortified foods, but a greater amount of zinc is absorbed when these foods are fortified than would have occurred if they were not fortified. There is little evidence of any benefit of putative zinc absorption enhancers on zinc absorption from zinc-fortified foods, but the number of available studies is small. There is insufficient information available to assess the effects of cofortification with other micronutrients on zinc absorption from zinc-fortified foods.

Detailed review of evidence

Overview

Background information on tracer studies to assess zinc bioavailability. Nutrient absorption from foods can be measured by using metabolic tracers. Such tracers are uniquely detectable substances (usually isotope-labeled forms of the nutrient of interest) that presumably are metabolized in the same way as the nutrient that is naturally present in the food or added to it as a fortificant. To assess the bioavailability of a fortificant, in particular, it is important that the tracer be present in food in the same chemical form and phase as the fortificant. In this section, we review the available studies of zinc absorption from zinc-fortified foods, based on studies that used radioisotopes or stable isotope tracers of zinc. Some of this information has been reviewed previously [3].

Bibliographic search. Data sets for inclusion in this analysis were identified by using a computerized bibliographic search (PubMed), with the following key words: 1) zinc; limiting for human, English, clinical trial, meta-analysis, randomized controlled trial; 2) zinc fortification; limiting for human, English; and 3) zinc fortif*; and limiting for human, English. A total of 1,673 articles were identified. All titles or abstracts of every record identified were reviewed. Of these, only nine articles specifically examined zinc bioavailability from zinc-fortified foods. These articles will be summarized in the following section.

Results of tracer studies of zinc absorption from zinc-fortified foods

Zinc absorption from foods fortified with different levels of zinc. Three studies are available in which different levels of zinc fortification (or the same foods with and without fortification) were compared. One study in Danish adults was designed to examine the effect of folic acid on zinc absorption from bread prepared with or without folic acid and either low or high zinc content [4]. The final bread meal contained a total of either 1.2 mg of zinc/meal or 2.9 to 3.0 mg of zinc/meal as added zinc chloride solution (prepared from zinc oxide and dilute hydrochloric acid). Folic acid did not affect zinc absorption from breads containing either amount of zinc. As indicated in **figure 1**, the fractional absorption of zinc (FAZ) was significantly greater from the low-zinc meals (38.8% to 40.6%) than from the high-zinc meals (22.7% to 26.7%), but the total absorbed zinc (TAZ) was greater from the high-zinc meals (0.73 mg of zinc/meal) than from the low-zinc meals (0.48 mg of zinc/meal), as presented in **figure 2**.

In a second study, Swedish adults consumed meals containing bread prepared from either whole-wheat flour (1.3 mg of zinc/meal) or refined-wheat flour (72% extraction rate; 0.4 mg of zinc/meal), with or without additional zinc, as zinc chloride, at a final zinc content of 3.5 to 3.6 mg/meal when the fortified breads were provided [5]. With both types of bread, FAZ from the fortified breads was lower than that of the intrinsic zinc contained in the unfortified food (**fig. 1**). Nevertheless, TAZ was greater when the bread was fortified, although the increment was relatively small with the whole-wheat product (**fig. 2**).

A third study was conducted in Peruvian preschool children who received two meals (breakfast and lunch) that contained a total of 100 g of wheat products (biscuits and noodles) fortified with 3 mg of iron and 0, 3, or 9 mg of zinc, as zinc sulfate, per 100 g of wheat [6]. As with the findings of the above-mentioned studies, the mean TAZ was positively related to zinc intake (**fig. 2**), despite the inverse relation between zinc intake and FAZ (**fig. 1**). The findings of these three studies indicate that increasing zinc intakes by adding greater amounts of zinc to food results in greater net absorption of zinc, although the increments in TAZ

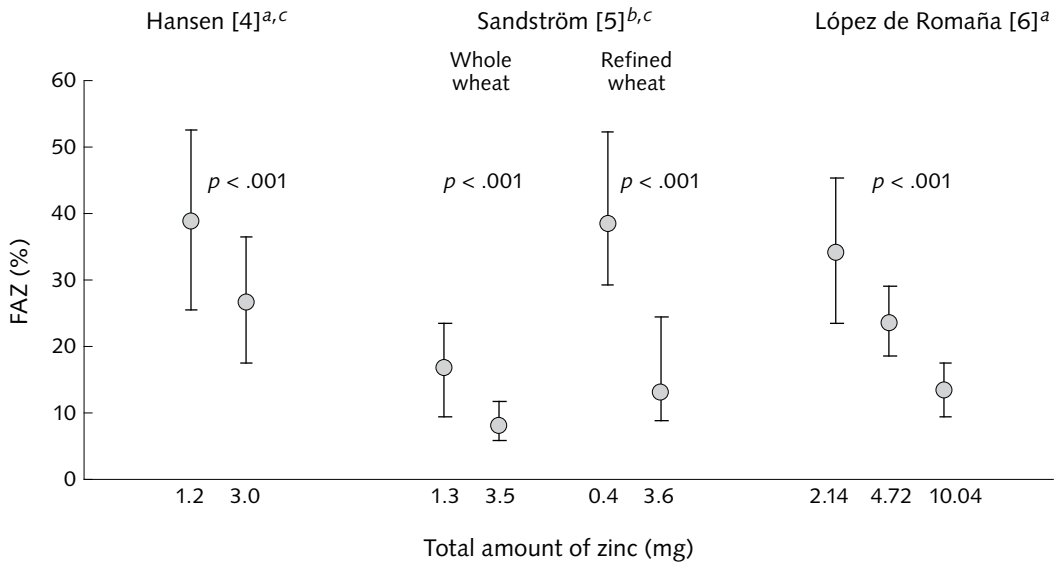

FIG. 1. Effect of the level of zinc fortification of wheat products on fractional absorption of zinc (FAZ) in studies of adults [4, 5] and preschool children [6]

a. Results are shown as mean ± SD.

b. Results are shown as mean (range).

c. For the purpose of this review, we calculated *p* values from the published results, using two-way ANOVA. For Hansen et al. [4], the *p* value was calculated under the conservative assumption of zero within-subject correlation. For Sandström et al. [5], the SD was estimated under the assumption that the range represents the mean ± 2 SD.

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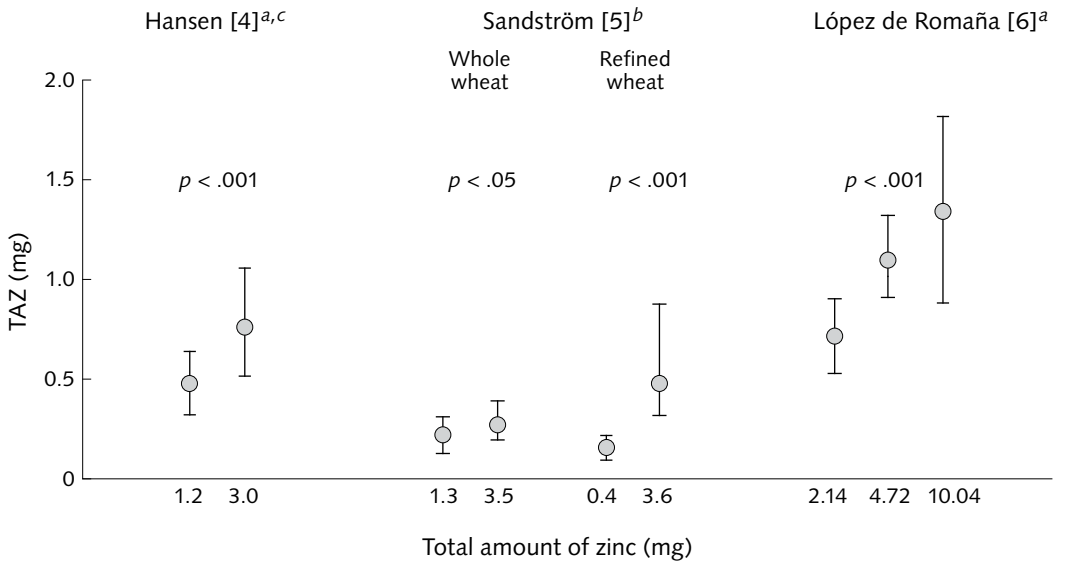

FIG. 2. Effect of the level of zinc fortification of wheat products on total absorption (TAZ) of zinc in studies of adults [4, 5] and preschool children [6]

a. Results are shown as mean ± SD.

b. Results are shown as mean (range).

c. For the purpose of this review, we calculated *p* values from the published results using two-way ANOVA, under the conservative assumption of zero within-subject correlation.

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are progressively less as the level of zinc fortification or total zinc intake increases. A similar conclusion has been reported from a recent study of varied doses of zinc supplements [7].

Bioavailability of different chemical forms of zinc. Several zinc compounds are generally regarded as safe (GRAS) for human consumption and are, therefore, available for use in food fortification. Zinc oxide, which is the cheapest GRAS zinc compound, is insoluble at neutral pH, so concerns have been raised about its bioavailability from fortified foods. Three well-designed tracer studies have been published in which zinc absorption was compared for foods fortified with either zinc oxide or zinc sulfate in amounts to provide the same level of zinc fortification. There were no differences in zinc absorption by healthy US adults from the two zinc compounds when they were provided in either a high-phytate, whole-wheat porridge or a low-phytate bread prepared from yeast-treated, refined-wheat flour [8]. Similarly, in studies conducted in Indonesian schoolchildren, there were no differences in zinc absorption from zinc-fortified, refined-wheat dumplings when the two chemical forms of zinc were compared [9], and in a study carried out among Mexican women, there were no differences in zinc absorption from maize tortillas fortified with either form of zinc [10]. In summary, as shown by the results presented in **figure 3**, the available evidence suggests that there is no difference in zinc absorption by school children or adults when either zinc oxide or zinc sulfate is used to fortify common cereal staples. Whether this is also true for infants and young preschool children is not known.

One other study is available in which zinc absorption was measured from a wheat-soy-milk porridge that was fortified with a mixture of micronutrients that contained either zinc sulfate or zinc methionine [11]. There were no significant differences in fractional absorption of zinc from the respective diets, even though the zinc methionine-containing fortificant mixture also contained other putative enhancers of zinc absorption, as described below.

Effect of phytate on zinc absorption from zinc-fortified foods. Inositol phosphate (phytic acid or phytate) is the chemical form in which phosphorus is stored in plant seeds, such as cereal grains and legumes, that are used for human consumption. The phosphate groups in phytate can form strong and insoluble complexes with divalent cations such as zinc, and because the gastrointestinal tract of humans lacks any significant phytase activity, phytate-bound minerals are excreted in the stool. Several studies have been conducted to measure zinc absorption from high- or low-phytate foods that have been fortified with zinc. Although the phytate contents were not necessarily the only differences between the high- and low-phytate meals in each of the studies described below, it seems likely that the phytate:zinc molar ratio of the meals was the major factor accounting for the observed differences in FAZ.

In one study of Swedish adults, which was described above, bread was prepared from either whole-wheat

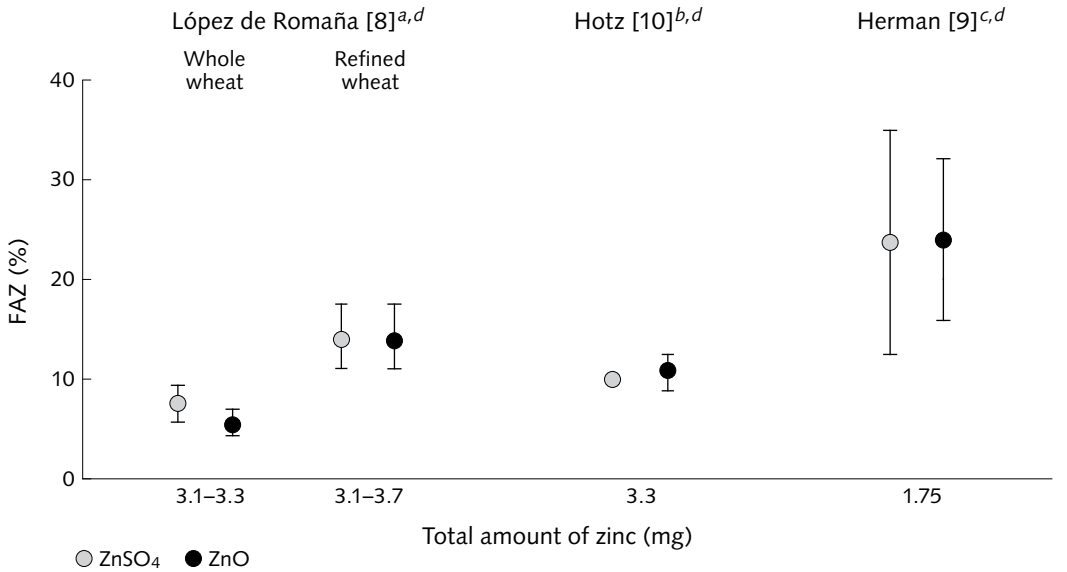

FIG. 3. Fractional absorption of zinc (FAZ) from foods fortified with ZnO or $ZnSO_4$ in studies of adults [8, 10] and children [9]
a. Results shown as geometric mean (95% CI).
b. Results shown as mean (95% CI).
c. Results shown as mean ± SD.
d. No significant differences by type of fortificant within study.
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flour or 72% extraction refined wheat [5], and zinc chloride was added to each type of bread to produce a final zinc content of 3.5 to 3.6 mg/meal. As shown in **figure 4**, FAZ from the zinc-fortified, low-phytate, refined-wheat bread was significantly greater than that from the zinc-fortified, phytate-containing, whole-wheat bread (13.2% vs. 8.2%). Similarly, in the aforementioned study by López de Romaña et al. [8], FAZ was approximately twice as great from a refined-wheat, yeast-fermented bread than from a whole-wheat, unfermented porridge (**fig. 4**), even though their zinc contents were in the same general range (3.1 to 3.7 mg of zinc per serving). In a recent study among healthy Swedish adults, Fredlund et al. [12] investigated the impact of the addition of various amounts of phytate to white wheat bread rolls on zinc absorption. The zinc content was adjusted to 3.1 mg by adding zinc chloride. The investigators found a progressive decrease in zinc absorption with increasing amounts of added phytate. Thus, each of the foregoing studies found that phytate inhibits zinc absorption from zinc-fortified foods, although the total amount of zinc absorbed from phytate-containing foods is greater when the foods are fortified with zinc than when they are not fortified.

Zinc absorption from beverages fortified with zinc. The recent development of sweetened or flavored beverages fortified with MMN provides an alternative method for delivering these nutrients. Zinc absorption from one such beverage and the effect of simultaneous consumption of a low-phytate meal were studied in Peruvian schoolchildren [13]. One serving of the beverage contained 3.75 mg of zinc as zinc gluconate, and the meal contained an additional 1.1 mg of zinc and 13.6 mg of phytate (final phytate:zinc molar ratio of the combined beverage and meal ~0.3:1). The mean FAZ values from the beverage alone (22.8 ± 7.6%) and from the beverage and the meal together (24.5 ± 10.7%) were not significantly different. These results are not unexpected because of the low phytate content of the meal and the fact that, for the purpose of the absorption study, additional zinc was included in the beverage to ensure that the same total amount of zinc was consumed as when the beverage and the meal were taken together.

Potential enhancers of zinc bioavailability from fortified foods. Several studies indicate that the metal-chelating compound ethylenediaminetetraacetic acid (EDTA) facilitates iron absorption from individual foods and mixed diets [14–17], and additional research has been completed to determine whether EDTA also enhances zinc absorption from foods fortified with both zinc and EDTA.

In a study of 24 Sri Lankan schoolchildren [18], the mean FAZ was significantly greater (13.5 ± 6.0% vs. 8.8 ± 2.0%, $p = .037$) when 9.6 mg of Na_2H_2EDTA was added to a local food that was prepared from 25 g of rice flour (containing 0.9 mg of iron and 0.9 mg of zinc) and fortified with 1.5 mg of iron as ferrous sulfate and 1.5 mg of zinc as zinc oxide (EDTA:zinc molar ratio, ~1.4:1; phytate:zinc molar ratio ~1:1) than when

EDTA was not added, as shown in **figure 5**.

By contrast with the foregoing results, Hotz et al. [10] found no difference in zinc absorption from meals containing several foods, including a 64-g maize tortilla that was fortified with zinc oxide alone, zinc oxide plus Na_2H_2EDTA, or $Na_2ZnEDTA$ in a study of 42 adult Mexican women. The maize flour incorporated in the tortilla was fortified at a level of 40 mg of zinc/kg to provide an additional 1 mg of zinc per tortilla, and the whole meal (including beans, tomato sauce, milk, and coffee) contained a total of 3.3 mg of zinc, with a phytate:zinc molar ratio of ~17:1. The molar ratio of EDTA:zinc in the tortilla was 0.5:1, although the final ratio in the meal was ~0.2:1. Possible explanations for the discrepant conclusions of the two studies are the dissimilar ratios of EDTA:zinc that were used or the differences in the phytate:zinc ratios of the respective diets.

A third study of US adults [11], which was described briefly above, compared zinc and iron absorption from a wheat-soy-milk porridge that was fortified to provide an additional 10 mg of each mineral per serving with either ferrous sulfate and zinc sulfate or $Na_2FeEDTA$, zinc methionine, ascorbic acid, and citric acid (EDTA:zinc molar ratio, ~1.2:1). The latter fortificant mixture was designed in an attempt to maximize mineral uptakes from the porridge. The final porridges contained either a low (192 mg) or a high (392 mg) amount of calcium. Except for a marginally significant, small negative impact of the higher calcium intakes, there were no significant differences in zinc absorption with any of the diets, possibly because the relatively high level of zinc intake reduced FAZ to such an extent (~7% FAZ overall) that any smaller effects of the other factors would no longer be detectable.

In summary, there is evidence of an enhancing effect of EDTA on zinc absorption from zinc-fortified foods from just one of the three available studies, although the possible modifying effect of different molar ratios of enhancer:zinc and phytate:zinc on the impact of potential enhancers of zinc absorption needs further study.

Effect of cofortification with other micronutrients on zinc absorption from zinc-fortified foods. It is conceivable that cofortification with other micronutrients could affect zinc absorption from the zinc-fortified food. However, with the exception of the study of folic acid [4] and the study of calcium [11] that were described above, we could not locate any other reports of research that specifically isolated the effect of other

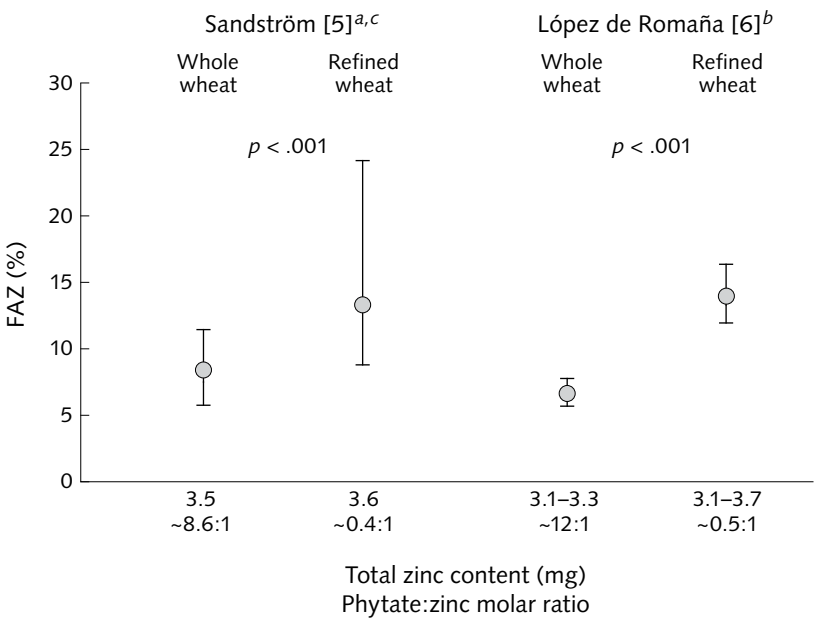

FIG. 4. Fractional absorption of zinc (FAZ) from whole-wheat and refined-wheat products fortified with zinc [5, 8]

a. Results are shown as mean (range).

b. Results are shown as geometric means (95% CI).

c. For the purpose of this review, we calculated *p* values from the published results using two-way ANOVA. The SD was estimated under the assumption that the range represents the mean ± 2 SD.

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fortificants on zinc absorption from zinc-fortified foods. Most studies investigating the impact of other micronutrients on zinc absorption only considered the effects of these other fortificants on the intrinsic zinc content of the food.

Summary of tracer studies of zinc absorption from zinc-fortified foods. In summary, the results from the available sets of tracer studies indicate that the additional zinc provided in fortified foods can contribute positively to the total amount of absorbed zinc. Multiple studies indicate that FAZ declines progressively with increasing levels of added zinc. However, the total amount of absorbed zinc increases in relation to the amount consumed. There does not appear to be any significant difference in zinc absorption from zinc oxide or zinc sulfate, regardless of the phytate:zinc molar ratio of the meal. However, it appears that high-phytate meals depress zinc absorption from zinc-fortified foods. With the possible exception of Na_2H_2EDTA, there is no other evidence that putative enhancers of zinc absorption confer major benefits for zinc absorption from zinc-fortified foods, although the amount of relevant available information is still quite limited. There is also too little information regarding the effects of fortification with other micronutrients on zinc absorption from cofortified foods to permit informed judgments on this issue.

Section 2

Does zinc fortification affect biochemical indicators of zinc status and other zinc-related functions?

Conclusions

The effects of zinc fortification on indicators of zinc status or other potentially zinc-related functional outcomes were analyzed according to the type of food product that was fortified and whether or not zinc was the only nutrient that differed by study group. The results of seven available trials of milk products with or without zinc fortification are inconsistent, although the results suggest that zinc-fortified milk products appear to boost the zinc status of infants and young children and increase the growth of premature infants and malnourished children.

We identified five studies that assessed the impact of zinc-fortified cereal products among preschool-aged or school-aged children. The limited available information suggests that zinc fortification of cereal products might have a positive impact on serum zinc concentration among school-aged children, but not among younger children. There is insufficient information to determine whether zinc fortification of cereal products could enhance growth or reduce morbidity among children at risk for zinc deficiency because of the small number of available studies and the fact that

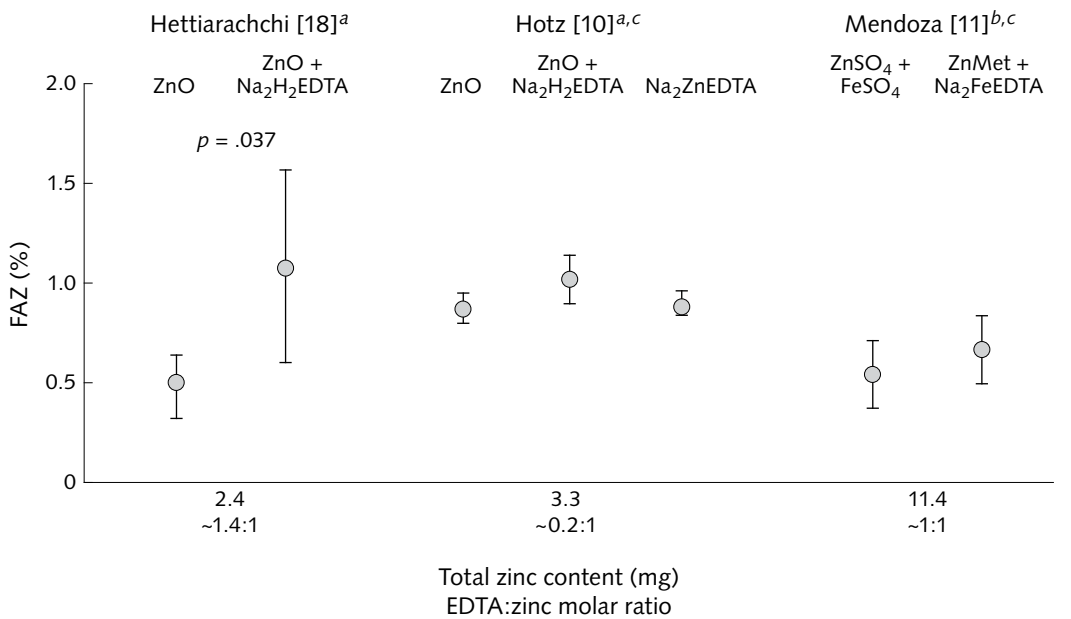

FIG. 5. Effect of EDTA on fractional absorption of zinc (FAZ) in studies of adults [10, 11] and children [18]
a. Results are shown as mean ± SD.
b. Results are shown as mean (95% CI)
c. Groupwise comparisons in studies by Hotz et al. [10] and Mendoza et al. [11] are not significantly different.
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these studies sometimes enrolled children who were not growth-restricted or were too brief to detect such changes.

Eight studies were identified in which the impact of fortifying foods with MMN, including zinc, was assessed. Only one of the six studies that monitored serum zinc concentration found a positive impact on final serum zinc concentration. Only two of the eight studies of MMN-fortified foods found a positive impact on growth, although these effects may not have been due exclusively to zinc. The lack of growth impact in the other studies may be due to methodologic inadequacies, including short study durations, small sample sizes, and study populations that were not sufficiently growth-restricted to benefit from zinc.

In summary, a few studies of zinc fortification have found positive impacts of zinc-fortified foods on serum zinc concentrations and growth, and one study reported a reduction in morbidity from diarrhea; but most studies failed to detect such beneficial effects. The reason for the inconsistent results of these studies is uncertain but may be related to the choice of food vehicles, the age group and zinc status of the study populations, or particular aspects of the study design. Thus, additional research is needed to assess the impact of zinc-fortified products, with or without other micronutrients, in populations known to be at risk for zinc deficiency.

Detailed review of evidence

Overview

Background information on intervention trials with zinc-fortified foods. Evaluations of zinc fortification trials are challenging because of the lack of adequate biomarkers of individual zinc status. Nevertheless, recent analyses indicate that the mean serum (or plasma) zinc concentration of a population responds consistently to zinc supplementation, regardless of the population's initial mean serum zinc concentration [19]. Thus, the change in serum zinc concentration following the introduction of a zinc-fortified food should be able to serve as a useful indicator of the impact of fortification interventions as well. In addition to the change in serum zinc concentration, it is possible to use other functional indicators of zinc responsiveness, such as increased physical growth [20] or morbidity reduction [21], to assess the efficacy of zinc-fortification interventions, although several caveats must be borne in mind. First, zinc must be the only nutrient that differs between the intervention group and the control group to be able to attribute any functional responses specifically to zinc fortification. Second, it is likely that functional responses are useful only if the study population is zinc deficient initially. Thus, the failure of a zinc-fortification program to deliver positive results with regard to functional indicators of zinc status may be due to inadequate consumption of the zinc-fortified food, an insufficient level of fortification, poor bioavailability of the zinc fortificant, or simply the fact that the study population was not initially zinc-deficient. Thus, it is worthwhile in such evaluations to include a separate study group that receives adequate doses of a readily absorbable zinc supplement (rather than a zinc-fortified food) as a positive control.

Bibliographic search. Data sets were identified for this section by using a computerized bibliographic search (PubMed with key words as described above). In addition to the PubMed search, we included three reports of fortification trials that were in press at the time [22–24] and one fortification trial using MMN that had included measurements of serum zinc concentration [25]. Randomized, controlled intervention trials among prepubertal children were considered acceptable for inclusion in the present review either if they involved a zinc-only fortification group or if zinc was given together with other nutrients and zinc status indicators were assessed (specifically, serum zinc concentration, growth, or morbidity). We identified a total of 7 trials using milk or infant formula in young children and 14 trials using cereal products, other beverages, or condiments in prepubertal children. Most of the available information on the impact of zinc fortification interventions was collected during efficacy trials. Very little information is available from large-scale, programmatic interventions. Except for two trials in elderly subjects [26, 27], no fortification trials were identified in which zinc- or MMN-fortified foods were compared with nonfortified foods in other age groups and serum zinc concentration was measured. Because one of these latter trials was not masked, there is insufficient information to warrant inclusion of this age group in the review.

Results of intervention studies with zinc-fortified foods

As indicated above, a total of 21 controlled studies were identified in which a zinc-fortified product was compared with the same product that did not include additional zinc. These studies examined a variety of food vehicles. In 10 of these studies (5 studies of milk products or infant formulas and 5 studies of cereal products), zinc was the only nutrient that differed between study groups. In two studies of infant formula, both zinc and copper were added to the study-group formula but not the control-group formula. In the remaining nine studies, zinc was one component of a MMN fortification premix, and the foods under study were fortified with either all of the nutrients in the premix or none of them. Because zinc was just one micronutrient among several others in these MMN-fortified foods, it is not possible to attribute any consequences of these latter interventions specifically to zinc. Nevertheless, we examined the impact of these MMN-fortified products on serum zinc concentration

and other potentially zinc-related outcomes, such as growth and morbidity, when relevant information was available.

Efficacy trials of zinc-fortified infant formulas or other milk products in which zinc was the only nutrient that differed between intervention and control groups. Of the seven trials of milk-based products, zinc was the only micronutrient that differed between groups in five studies, and in two studies the treatment group received both additional zinc and copper [28, 29] (**table 1**). Three studies were completed in healthy, full-term infants [28, 30, 31], three were conducted in preterm infants [29, 32, 33], and one was conducted in severely malnourished infants [34]. Apart from the study of malnourished children, which took place in Chile, the remaining studies were completed in North America, Europe, or Japan. The sample sizes of these studies were generally small, ranging from 20 to 68 total subjects, thereby limiting their power to detect significant differences in growth outcomes. The zinc concentration of the milk formula that was provided to the term infants ranged from 1.0 to 1.8 mg/L in the control groups and from 3.2 to 5.8 mg/L in the intervention groups; the respective milk zinc concentrations were similar in one of the three studies of preterm infants but were considerably greater in the fortified milks provided in the two other studies, as described below.

Four of the seven studies of zinc-fortified milk found a significantly greater increase in the final serum zinc concentration among children who received the higher zinc-containing products, but three of the studies did not. These differences among studies could not be explained by the clinical characteristics of the subjects, the level of zinc fortification, or the presence of other micronutrients in the formulas.

Two of the three studies of healthy, full-term infants found no significant changes in length or weight among the groups that received the zinc-fortified product compared with the control groups [28, 31], but the children included in these trials had no preexisting evidence of growth restriction. Similarly, in one study of low-birthweight infants, in which the infants' birthweights ranged from 1,800 to 2,500 g and the milk zinc concentrations were either 1.4 or 4.1 mg/L in the two study groups, there were no groupwise differences in the increments in length, weight, or head circumference [32]. By contrast, the two studies of very-low-birthweight premature infants who were provided with zinc-fortified infant formulas containing either 6.7 or 5 mg/L versus 10 or 11 mg/L of zinc, respectively, found a significantly greater change in height-for-age z-score (HAZ) [33] or change in length [29] compared with the control group. There were no groupwise differences in the final growth increments of the malnourished Chilean children at 60 days, but those who received the zinc-fortified preparation had significantly greater height increments after 30 and 45 days of treatment than did the control group [34].

In summary, the results of these trials are inconsistent, although more than half of the studies found a positive impact of zinc-fortified milks on serum zinc concentration. Two of the three trials in premature infants and the one trial in severely malnourished children found a positive effect of zinc-fortified milks on physical growth, but no growth impact was detected among healthy term infants, probably because the control formulas had adequate zinc contents for the term infants, who had no prior growth restriction.

Efficacy trials of zinc-fortified cereal products in which zinc was the only nutrient that differed between the intervention and control groups. Results are available from five controlled studies of zinc-fortified, cereal-based food products [6, 18, 22, 35, 36] (**table 2**). These five studies were carried out in both industrialized and lower-income countries, and they included a broad range of products. The first study compared the effects of providing a ready-to-eat breakfast cereal with or without additional zinc (3.75 mg of zinc added per 28-g serving, as zinc oxide) to healthy US schoolchildren for a period of 9 months [35]. On the basis of the number of servings consumed per week and the size of the serving, children in the group that received the zinc-fortified cereal were estimated to receive 2.57 mg of additional zinc per day during the course of the study. Children in the zinc group had a significantly greater (i.e., less negative) change in plasma zinc concentration than those in the control group. There were no significant differences between study groups in any growth increments, but the children were not underweight or stunted initially, so they would not be expected to exhibit a growth response to zinc.

A study was conducted among 7- to 11-year-old Turkish schoolchildren with low initial serum zinc concentrations (< 65 μg/dL) who were provided bread with or without zinc fortification for a period of 13 weeks [36]. The paper published from this study describes the level of fortification as "2 mg/kg/day elemental zinc acetate," which seems to refer to the zinc intake per kilogram body weight of the child, not to the zinc concentration of the bread. Because the report provides no information on the children's body weights or the amounts of bread consumed, it is not possible to calculate the level of zinc fortification or the amount of additional zinc that was consumed. Children who received the zinc-fortified bread had significantly greater increases in serum zinc and alkaline phosphatase concentrations at the end of the intervention period than their counterparts who received the nonfortified bread.

Two studies were completed among Peruvian children to assess the effects of adding zinc to iron-fortified wheat flour. In the first study [6], which was described above in the section on tracer studies, children 3 or 4 years of age received two meals that contained wheat

TABLE 1. Efficacy trials of zinc fortification of infant formula: Impact on zinc-related indicators (serum zinc concentration, height, weight, morbidity) and possible adverse effects on iron and copper status

Country, year [reference] author	Study design	Age	Inclusion criteria	Sample size[a]	Duration (mo)	Group	Zinc content in infant formula (mg/L)
USA, 1976 [30][c] Walravenes	Double-blind, controlled. No statement on randomization	6 days	Full-term, healthy infants	68	6	Zinc	5.8[d]
						Control	1.8
Japan, 1984 [31] Matsuda	Placebo-controlled. No statement on randomization and blinding	1 mo	Full-term, healthy infants, normal birth weight	39	5	Zinc	3.2[d]
						Control	1.0
Finland, 1994 [28] [c] Salmenperä	Randomized, controlled. No statement on blinding	2–3.5 mo	Full-term, healthy infants	32	12	Zinc	5.1[i]
						Control	1.1
Austria, 1985 [32] [c] Haschke	Placebo controlled. Alternative assignment. No statement on blinding	6 days	Male, LBW, preterm infants	20	3.8	Zinc	4.1
						Control	1.4
Canada, 1993 [33] Friel	Randomized, double-blind, controlled	1 mo prior to home discharge	Preterm, very LBW infants	50	12[l]	Zinc	11[d]
						Control	6.7
Spain, 2003 [29][n] Diaz-Gomez	Randomized, double-blind, controlled	~ 4 wk	Preterm, very LBW infants	36	6	Zinc	10[i]
						Control	5
Chile, 1992 [34][n] Schlesinger	Double-blind, controlled. No statement on randomization	7.6 ± 2.6 mo	Malnourished infants	39	3.5	Zinc	15[d]
						Control	3.2

HAZ, height-for-age z-score; LBW, low-birthweight; NS, not significant; WAZ, weight-for-age z-score

a. Sample size includes total number in control and fortification groups.

b. Results are shown as mean ± SD for serum zinc, hemoglobin, and serum copper concentrations and as geometric mean (range of 1 SD) for serum ferritin concentration.

c. Mean serum zinc concentration estimated from graph.

d. Infant formula was also fortified with iron (and in some cases other micronutrients) in both groups.

e. No significant differences in final serum zinc concentration between groups.

f. No significant difference in final concentrations.

g. Final concentrations are not adjusted for initial concentration.

h. *P* value for the difference in final serum zinc concentration between groups.

Initial serum zinc concentration (μg/dL)[b]	Final serum zinc concentration (μg/dL)[b]	Change in height	Change in weight	Morbidity	Final hemoglobin concentration (g/L)[b]	Final serum ferritin concentration (μg/L)[b]	Final serum copper concentration (μg/dL)[b]
—	72 (NS)[e]	18.2 ± 0.5 cm/6 mo (p < .05)	4,282 ± 104 g/6 mo (p < .03)	—	—	—	124.2 ± 5.8 (NS)[f,g]
—	76	17.0 ± 0.5 cm/6 mo	3,867 ± 170 g/6 mo	—	—	—	112.3 ± 6.1
73 ± 16	103 ± 17 (p < .01)[h]	1.0 ± 0.4 mm/day (NS)	26.4 ± 8.3 g/day (NS)	—	—	—	111 ± 31 (NS)[f,g]
68 ± 12	78 ± 12	1.0 ± 0.4 mm/day	26.5 ± 7.0 g/day	—	—	—	124 ± 21
87	At 6 mo: 85 ± 3 (p = .03)[h]	No significant difference in change in length[j]	No significant difference in change in weight[j]	—	—	—	—
90	At 6 mo: 65 ± 4			—	—	—	—
110	106 (p < .05) [h]	1.2 ± 0.2 mm/day (NS)	27.7 ± 7.8 g/day (NS)	—	—	—	No significant difference in serum copper concentration at any time[k]
120	75	1.2 ± 0.2 mm/day	31.0 ± 8.8 g/day	—	—	—	
83 ± 26[m]	95 ± 17 (NS)[e]	0.087 ± 0.087 ΔHAZ/12 mo (p = .004)	−0.06 ± 0.06 ΔWAZ/12 mo (NS)	—	—	—	—
67 ± 14	93 ± 30	−0.027 ± 0.13 ΔHAZ/12 mo	−0.11 ± 0.13 ΔWAZ/12 mo	—	—	—	—
69 ± 20	119 ± 37 (p = .01)[h]	1.04 ± 0.07 mm/day (p = .036)	180 ± 24 g/wk (NS)[o]	—	122 ± 11 g/L (NS)[f,g]	—	—
78 ± 24	87 ± 30	0.99 ± 0.07 mm/day	174 ± 32 g/wk	—	122 ± 9 g/L	—	—
127 ± 36	122 ± 28 (NS)[e]	0.62 ± 0.23 mm/day (NS)	24.9 ± 6.3 g/day (NS)	Significantly more diarrhea episodes in zinc than in control group	118 ± 7 (NS)[f,g]	15.6 (7.4–32.8) (NS)[f,k]	155 ± 28 (NS)[f,g]
153 ± 55	118 ± 38	0.58 ± 0.26 mm/day	25.8 ± 10.2 g/day		121 ± 8	12.6 (6.0–26.5)	149 ± 29

i. Infant formula provided to zinc group was fortified with zinc and copper. Amounts of other micronutrients were equal for both groups.
j. Results not provided.
k. Results are presented in graph only.
l. Study duration was 12 months, but infants consumed infant formula for 6 months only.
m. Initial serum zinc concentrations were significantly different between groups (p = .041).
n. Additional information provided by author.
o. Final body weight was significantly different between groups (p = .01).

TABLE 2. Efficacy trials of zinc fortification of cereal products in which zinc was the only nutrient that differed between intervention and control groups: Impact on zinc-related indicators (serum zinc concentration, height, weight, and morbidity) and possible adverse effects on iron and copper status

Country, year [reference] author	Study design	Age	Sample size[a]	Duration (mo)	Fortified food	Additional zinc in zinc group	Study groups	Initial serum zinc concentration (μg/dL)[b]
USA, 1979 [35] Hambidge	Randomized, double-blind, controlled	2–7 yr	96	9	RTE cereal	2.57 mg/day	Zinc	80 ± 2[c]
							Control	
Turkey, 1998 [36] Kiliç	Randomized, double-blind, controlled	7–11 yr	24	3	Bread	2 mg/kg/day	Zinc	61 ± 4
							Control	59 ± 3
Peru, 2005 [6] López de Romaña	Randomized, double-blind, controlled	3–4 yr	41	2	Biscuits/ noodles[j]	0, 3, 9 mg/ day	Zinc 9 mg/ day	78 ± 16
							Zinc 3 mg/ day	77 ± 13
							Control	71 ± 9
Peru, 2007 [22] Brown	Randomized, double-blind, controlled	6–8 mo	178	6	Wheat porridge[j]	~3mg/day	Zinc	78 ± 15
							Control	79 ± 15
Sri Lanka 2004 [18][l] Hettiarachchi	Randomized, controlled. No statement on blinding	6–10 yr	49	1	Rice flour[j]	1.5 mg/day	Zinc	78 ± 13
							Control	79 ± 17

NS, not significant; RTE, ready-to-eat

a. Sample size includes the total number in the control and zinc fortification groups.

b. Results shown as means ± SD.

c. Mean serum zinc concentration at baseline for both groups combined.

d. *P* value for change in serum zinc concentration. Final mean and SD estimated from change in serum zinc concentration.

e. Not significant, but population not moderately or severely stunted or underweight.

f. No significant difference in change in concentration over study period.

g. *P* value for the difference in final serum zinc concentration between groups.

products (biscuits and noodles) fortified with iron and 0, 3, or 9 mg of zinc for a period of ~7 weeks. Despite the documented increase in their total zinc absorption, as described above, there were no significant groupwise differences in their change in serum zinc concentration or physical growth, although the period of observation may have been too short to detect such changes.

In the second study from Peru, infants who were initially 6 to 8 months of age were randomly assigned to one of three groups, each of which received an iron-fortified wheat-based porridge and a liquid multivitamin supplement for 6 months to assess the effect of the mode of delivery of additional zinc on their serum zinc concentrations, growth, and morbidity [22]. One group had zinc added to their porridge (at a level to provide ~3 mg additional zinc per day, as zinc sulfate), the second group received the same amount of zinc in the liquid vitamin supplement, and the third group received no additional zinc. Mothers were instructed to give the vitamin supplement apart from meals. Notably, only the group that received additional zinc as a supplement demonstrated a significant increase in serum zinc concentration compared with the other groups, and there were no significant differences in growth or morbidity between study groups, possibly because the study population was not zinc-deficient.

In the final study, Sri Lankan children who were enrolled in the aforementioned tracer study of the

Final serum zinc concentration (μg/dL)[b]	Change in height	Change in weight	Morbidity	Change in hemoglobin concentration (g/L)[b]	Change in serum ferritin concentration (μg/L)[b]	Change in serum copper concentration (μg/dL)[b]
77 ± 14 ($p < .05$)[d]	0.176 ± 0.03 mm/day (NS)[e]	7.97 ± 2.33 g/day (NS)[e]	—	—	—	−13.7 ± 5.5 (NS)[f]
71 ± 14	0.170 ± 0.03 mm/day	7.45 ± 1.67 g/day	—	—	—	−6.2 ± 4.8
82 ± 9 ($p = .0001$)[g]	—	—	—	No significant difference in final concentration between groups[h,i]	30.4 ± 9.7 (NS)[h]	85 ± 16 (NS)[h]
63 ± 3	—	—	—		24.1 ± 11.7	94 ± 16
82 ± 10 (NS)[k]	2.9 ± 1.3 cm/2 mo (NS)[k]	1.0 ± 1.2 kg/2 mo (NS)[k]	—	14.2 ± 11.4 (NS)[k]	−4.3 ± 16.6 (NS)[k]	—
76 ± 8	3.0 ± 1.4 cm/2 mo	1.1 ± 1.1 kg/2 mo	—	17.2 ± 17.8	−8.3 ± 26.3	—
78 ± 12	2.9 ± 1.3 cm/2 mo	1.5 ± 1.2 kg/2 mo	—	23.4 ± 14.4	3.6 ± 37.6	—
77 ± 11 (NS)[g]	6.9 ± 1.1 cm/6 mo (NS)	1.3 ± 0.5 kg/6 mo (NS)	No significant difference in diarrhea prevalence and incidence	−0.12 ± 1.08 (NS)[f]	−15.3 ± 43.4 (NS)[f]	−5.1 ± 37.2 (NS)[f]
76 ± 14	7.0 ± 1.1 cm/6 mo	1.3 ± 0.5 kg/6 mo		−0.26 ± 1.05	−14.3 ± 27.4	9.6 ± 33.7
84 ± 17 (NS)[m]	—	—	—	7.5 (NS)[f,n]	0.5 (NS)[f,n]	—
80 ± 17	—	—	—	3.7	3.6	—

h. Authors provided final values only after 3 months of supplementation. Change in concentration could not be calculated. No significant difference between groups.
i. Results for hemoglobin concentration were unclear.
j. The food vehicle was also fortified with iron (and in some cases other micronutrients) in both groups.
k. No significant groupwise difference in treatment effects.
l. Results combined for the two groups receiving zinc and the two control groups.
m. Change in serum zinc concentration tended to be greater in the group receiving zinc than in the group without zinc ($p = .065$).
n. Changes in hemoglobin and serum ferritin concentration are presented as means without SD.

effects of iron, Na_2EDTA, and zinc fortification of rice received the respective study meals for a total period of 30 days [18]. The children in the two groups who received the zinc-fortified rice snack (which provided 1.5 mg of additional zinc per day, as zinc oxide) had a marginally greater increase in their serum zinc concentrations ($p = .065$) at the end of the study.

In summary, the results of these controlled trials of zinc-fortified cereal products carried out in infants and young children are inconsistent. In some of the studies, but not all, there were significantly greater changes in serum zinc concentration among the groups that received the zinc-fortified product. The differences among studies could not be explained by the amount of zinc in the product; but, interestingly, the two test products that were included in the positive studies did not contain added iron, whereas all of the products used in the studies without significant impact were cofortified with both iron and zinc. Thus, it is possible that the iron in these products interfered with the utilization of zinc. However, some of the aforementioned studies of infant formulas that were cofortified with both iron and zinc did find a positive impact on serum zinc concentration, so this hypothesized effect of iron fortification on zinc uptake was not apparent with the milk-based products.

None of the three available studies of cereal-based products in which growth outcomes were reported

(**table 2**) identified a positive impact of zinc fortification on children's growth. However, the children enrolled in these studies may not have been zinc-deficient, and in some cases the period of observation was too short to detect growth differences or the children were not sufficiently growth-restricted to expect such differences to occur. Thus, additional studies of zinc-fortified cereal products should be carried out over a sufficiently long duration in populations at risk for zinc deficiency.

Efficacy trials of products fortified with MMN, including zinc, in young preschool-aged children. Several studies have evaluated the effects of fortification of different food products with MMN, including zinc, among young children [23, 25, 37–42] (**table 3**). Although it is not possible to attribute any consequences of these interventions specifically to zinc, their impact on serum zinc concentration or other potentially zinc-related outcomes can be assessed. A total of seven studies of MMN-fortified foods were conducted among children in Ghana [37], South Africa (two studies) [38, 42], Ecuador [23], Mexico [25], China [39], and India [40, 41]. This set of studies examined a broad range of MMN-fortified products, including cereal-based complementary foods, milk, other beverages, and condiments. The first five of the cited studies included measurements of serum zinc concentration as one of the study outcomes, and the latter two measured either children's growth [39] or growth and morbidity [40, 41] as the only potentially zinc-related outcomes. Five of the studies [25, 37–41] compared the fortified product with the same product to which no micronutrients were added; one of the South African studies [42] and the study in Ecuador [23] compared the MMN-fortified product with the usual home diets provided by the family.

In the Ghana study [37], children were randomly assigned to one of four treatment groups at 6 months of age. For the current review, only the two groups that received the same cereal–legume preparation ("Weanimix"), with or without added micronutrients, were considered. Weanimix was prepared by mixing roasted and ground maize, soybeans, and groundnuts; the MMN mixture added to the fortified product for younger children provided an additional 143 mg of zinc as zinc oxide per kilogram dry weight, along with additional iron, copper, calcium, phosphorus, potassium, B vitamins, and vitamins A and C. Children who received the fortified product consumed ~7 mg more zinc per day than those in the comparison group. Surprisingly, there were no differences between groups with regard to their final serum zinc concentrations, and there were no differences in growth increments, although the population was not severely stunted or underweight. The lack of impact on serum zinc concentration may have been due to high concentrations of zinc-absorption inhibitors in the food ingredients.

Faber et al. [38] examined the effects of MMN-fortified maize porridge among South African infants 6 to 12 months of age. Families were given two sachets containing 25 g of finely ground dry maize meal per day for a period of 6 months, with the expectation that the infants would consume up to 40 g per day. The fortified product contained a total of 3 mg of zinc as zinc sulfate per 40 g dry weight, as well as iron, copper, selenium, B vitamins, and vitamins A, C, and E. Children in the control group were given the same product without added micronutrients. At the end of the intervention period, children in the fortification group had greater increases in hemoglobin and serum ferritin concentrations, but there were no differences in the change in serum zinc concentration compared with those in the control group. There were no fortification-related differences in growth increments, but the population was not underweight or severely stunted.

A study in China [39] enrolled children 6 to 13 months of age who were randomly assigned by village to receive 17-g of either a fortified or non-fortified "weaning rusk" prepared from wheat flour, sugar, and vegetable oil once daily for a period of 3 months. The fortified product contained 3 mg of zinc as zinc gluconate along with iron, calcium, B vitamins, and vitamins A and D. Children in the control villages received the same rusk without added micronutrients. Children who consumed the fortified rusk maintained their hemoglobin levels, whereas hemoglobin concentrations declined among those in the control group, indicating that children were able to utilize the iron and other micronutrients in the MMN-fortified product. Although serum zinc concentrations were not determined, there were no differences in the growth increments of children in the respective study groups. However, the rates of stunting were very low in both groups.

In a recently published study from Mexico, 10- to 30-month-old children from low-income families received 400 mL per day of whole cow's milk fortified with vitamin A, vitamin C, folic acid, ferrous gluconate, and zinc oxide, which provided an additional 5.3 mg of zinc per day [25]. After 6 months of intervention, anemia prevalence declined significantly from 41.4% to 12.1% among the children who received the fortified milk, whereas there was no change in those who received the nonfortified milk (30% and 24% at baseline and 6 months). There was also an improvement in iron status, as reflected by significantly lower transferrin receptor concentrations in the fortified group. In contrast, there were no groupwise differences in mean serum zinc concentrations.

In a community-based, double-blind, individually

randomized trial in a periurban setting in India, 1- to 3-year-old children received either fortified milk (n = 315) or control milk (n = 317) for 1 year. The fortified milk provided an additional 7.8 mg of zinc and 9.6 mg of iron, along with other micronutrients. Although final serum zinc concentrations were not reported, two possibly zinc-related functional outcomes were significantly affected. Children in the fortified-milk group had significantly greater changes in HAZ (mean difference, 0.19; 95% CI, 0.12 to 0.26; $p < .001$) and weight-for-age z-scores (WAZ) (mean difference, 0.24; 95% CI, 0.11 to 0.36; $p < .001$) [41]. Moreover, the children in the fortified-milk group had significantly fewer episodes of diarrhea [40]. However, because of the study design, it is uncertain whether these results were due specifically to zinc.

In the second study from South Africa [42], Oelofse et al. randomly assigned 46 children 6 to 12 months of age to receive an infant cereal (60 g dry weight per day) fortified with 5.6 mg of zinc as zinc sulfate and iron, calcium, phosphorus, potassium, iodine, B vitamins, and vitamins A, C, D, and E for a period of 6 months or to a control group that was not provided any complementary food. Preliminary dietary studies indicated that most children in the study area consumed a "comparable cereal" that apparently was fortified only with iron and a smaller amount of vitamin A, but none of the other nutrients. There were no significant differences between study groups in the change in hemoglobin or serum zinc concentrations or change in weight or length, although the children in the study population were not severely stunted or underweight and the sample size was fairly small.

Lutter et al. [23] evaluated the impact of the Ecuadorian national food and nutrition program, which provided a fortified complementary food to selected children 6 to 12 months of age living in poor communities. In a longitudinal, quasi-experimental design, children in the program communities were compared with their counterparts in control communities who did not receive any complementary food from the program. The children in the program communities received 6.5 mg of additional zinc along with other micronutrients in each daily ration. After 13 months of intervention, 14% of the children in the program group (n = 171) and 24% of the children in the control communities (n = 150) were underweight (p = .02). Children in the program communities consumed significantly more energy, protein, fat, iron, zinc, calcium, magnesium, phosphorus, B vitamins, and vitamins A, C, and E than children in the control communities, a difference that was due to the fortified complementary food provided by the program. There was no significant difference between study groups in the mean final serum zinc concentration.

In summary, serum zinc concentrations of young, preschool-aged children did not respond to zinc-containing, MMN-fortified, cereal-based complementary foods or MMN-fortified milk in the five studies that measured this outcome. Two of the studies (one of which examined a milk product, the other of which assessed a cereal-based porridge) detected a positive impact on growth. However, as discussed above, these results may not be due specifically to zinc. Moreover, because the children in most of the other study populations were not growth-restricted, it is not possible to judge whether the inclusion of zinc in the MMN premix could have had a positive impact on growth in those cases. Only the study from India reported morbidity outcomes, and the investigators found reduced diarrhea rates among the children who received the fortified product.

Efficacy studies with MMN-fortified foods in school-aged children. Two intervention trials provided MMN-fortified foods, including zinc, to school-aged children. However, one of the studies was excluded from this analysis because a single serving of a flavored beverage was provided apart from meals, so the study was not considered a true fortification trial [43].

One study examined the impact of a zinc-containing, MMN-fortified seasoning powder among schoolchildren in northeastern Thailand [24, 44]. The powder was added to rice or noodles during a midday meal served at the schools. The fortified powder contained 5 mg of zinc as zinc sulfate and iron, iodine, and vitamin A. Control children received the same seasoning powder without added nutrients. At baseline, there was no difference in the percentage of children with low serum zinc concentrations (< 65 g/dL), but children who received the fortified product had higher mean serum zinc concentrations at the end of the study than the control children, and the former were less likely to have low serum zinc concentrations (< 65 g/dL) (27.5% vs. 34.7%; odds ratio, 0.63; 95% CI, 0.42 to 0.94; p = .024) [44]. Children in the fortification group also had higher final hemoglobin concentrations, after adjustment for baseline values, and greater urinary iodine concentrations. In addition, the intervention reduced the incidence of respiratory-related illnesses (rate ratio, 0.83; 95% CI, 0.73 to 0.94; p = .004) and diarrhea (rate ratio, 0.38; 95% CI, 0.16 to 0.90; p = .027) [24]. A positive impact of the fortified product was also found on short-term cognitive function assessed by visual recall test.

By contrast with the foregoing results obtained with MMN-fortified foods in young children, serum zinc concentrations increased significantly in the one available study of schoolchildren who received a MMN-fortified seasoning powder with meals. The reason for these differences between the two sets of studies

TABLE 3. Efficacy trials of zinc and micronutrient fortification in which serum zinc concentration or other potentially zinc-related outcomes were measured

Country, year [reference] author	Study design	Age	Sample size[a]	Duration (mo)	Fortified food	Additional zinc (mg/day)	Other micronutrients added to fortified food
Ghana, 1999 [37] Lartey	Randomized, controlled. No statement on blinding	6–12 mo	104	6	Maize-soy-ground-nut porridge	~7	Iron, copper, calcium, potassium, phosphorus, vitamins A, B_1, B_2, B_3, B_6, and B_{12}, folic acid, vitamin C
South Africa, 2005 [38] Faber	Randomized, single-blinded, controlled	6–12 mo	361	6	Maize porridge	3	Iron, copper, selenium, vitamins A, B_2, B_6, B_{12}, C, and E
China, 1993 [39] Liu	Cluster-randomized, controlled. No statement on blinding	6–13 mo	226	3	Wheat flour weaning rusk	3	Iron, calcium, vitamins A, B_1, B_2, B_3, and B_{12}, folic acid, vitamin D
Mexico, 2006 [25] Villalpando	Randomized, double-blind, controlled	10–30 mo	130	6	Milk	5.3	Iron, vitamin A, vitamin C, folic acid
India, 2007 [40] Sazawal, [41] Juyal	Randomized, double-blind, controlled	1–3 yr	633	12	Milk	7.8	Iron, copper, selenium, vitamins A, C, and E
South Africa, 2003 [42] Oelofse	Randomized, controlled with nonintervention group, not blinded	6–12 mo	46	6	Cereal porridge	5.6	Iron, calcium, phosphorus, potassium, iodine, vitamins A, B_1, B_2, B_3, B_6, and B_{12}, folic acid, pantothenic acid, biotin, vitamins C, D, and E
Ecuador, 2008 [23] Lutter	Quasi-experimental	6–12 mo	321	10–13[d]	Cereal porridge	6.5	Iron, calcium, magnesium, phosphorus, vitamins A, B_1, B_2, B_3, B_6, and B_{12}, folic acid, vitamins C and E
Thailand, 2006 [44] Winichagoon [24] Manger	Randomized, double-blind, controlled	5–13 yr	569	7.8	Seasoning powder	5	Iron, iodine, vitamin A

HAZ, height-for-age z-score; MMN, multiple micronutrients; NS, not significant; RR, rate ratio between study groups; WAZ, weight-for-age z-score

a. Sample size includes the total number in the control and fortification groups.

b. All control groups received the same food product as the intervention group without added micronutrients, except for the studies by Oelofse et al. [42] and Lutter et al. [23], in which the control groups received the usual, self-selected home diet.

c. Plus–minus values are means ± SD.

d. Blood collection at 10 months. Duration of intervention trial was 13 months.

e. *P*-value for the difference in final serum zinc concentration between groups.

f. Children who received the fortified powder were less likely to have low serum zinc concentrations (< 65 μg–dL) at the end of the study (27.5% vs. 34.7%, $p = .024$).

Group[b]	Initial serum zinc concentration (μg/dL)[c]	Final serum zinc concentration (μg/dL)[c]	Change in height[c]	Change in weight[c]	Morbidity
MMN	102 ± 28	94 ± 22 (NS)	7.0 ± 1.4 cm/6 mo (NS)	1.3 ± 0.5 kg/6 mo (NS)	No significant difference in diarrhea, fever, respiratory infection
Control	101 ± 21	101 ± 28	7.0 ± 1.2 cm/6 mo	1.2 ± 0.6 kg/6 mo	
MMN	67 ± 13	69 ± 14 (NS)	7.0 cm/6 mo (95% CI, 6.7 to 7.3) (NS)	1.6 kg/6 mo (95% CI, 1.4 to 1.7) (NS)	—
Control	67 ± 14	69 ± 12	7.1 cm/6 mo (95% CI, 6.9 to 7.4)	1.6 kg/6 mo (95% CI, 1.4 to 1.7)	—
MMN	—	—	3.8 ± 0.2 cm/3 mo (NS)	0.71 ± 0.04 kg/3 mo (NS)	—
Control	—	—	3.3 ± 0.14 cm/3 mo	0.75 ± 0.06 kg/3 mo	—
MMN	—	83 ± 6 (NS)	—	—	—
Control	—	86 ± 7	—	—	—
MMN	—	—	Significantly greater changes in HAZ (mean difference, 0.19; 95% CI, 0.12 to 0.26; $p < .001$)	Significantly greater changes in WAZ (mean difference, 0.24; 95% CI, 0.11 to 0.36; $p < .001$)	4.46 ± 3.8 diarrhea episodes per child ($p < .05$)
Control	—	—			5.36 ± 4.1 diarrhea episodes per child
MMN	79 ± 12	85 ± 9 (NS)	10.0 ± 1.5 cm/6 mo (NS)	2.1 ± 0.9 kg/6 mo (NS)	—
Control	69 ± 16	74 ± 12	10.1 ± 2.1 cm/6 mo	2.1 ± 1.2 kg/6 mo	—
MMN	72 ± 28	89 ± 27 (NS)	No impact on linear growth retardation	12% were underweight in fortified group and 24% in control group ($p < .001$)	—
Control	68 ± 26	86 ± 23			—
MMN	67 ± 15	73 ± 11 ($p = .011$) [e,f]	No impact on growth: difference in means, 0.10 cm; 95% CI, −0.05 to 0.25 (NS)	No impact on weight gain: difference in means, −0.06; 95% CI, −0.24 to 0.13 (NS)	Reduced incidence of respiratory-related illnesses (RR, 0.83; 95% CI, 0.73 to 0.94; $p < 0.01$) and diarrhea (RR, 0.38; 95% CI, 0.16 to 0.90; $p < .05$)
Control	66 ± 15	71 ± 11			

is uncertain, but they may be related to the age of the subjects or to the nature of the fortification vehicles. It is possible, for example, that inhibitors of zinc absorption in the cereal-based complementary foods limited zinc uptake from these products and thereby prevented changes in serum zinc concentration, although this would not explain the lack of impact in the Mexico study, in which zinc was provided in milk. It is difficult to interpret the functional responses to these interventions, for a variety of reasons, as discussed above, and additional studies of the functional impact of zinc fortification will be needed in populations that are known to have a high risk of zinc deficiency.

Section 3

Does household-level fortification with MMN (including zinc) affect indicators of zinc status and zinc-related functions?

Conclusion

There is little available information on the impact of household-level food fortification with zinc-containing supplements on serum zinc concentration and zinc-related functional outcomes. There is just one available efficacy trial in which children received one of two different formulations of micronutrient powders, which did or did not contain zinc; this trial did not find any significant differences between treatment groups in final serum zinc concentration or change in height or weight. The other four available studies compared zinc-containing MMN products with a placebo or another type of household-level fortification product, so it is not possible to attribute any results specifically to zinc. Only one of these studies, which provided MMN crushable tablets, found a significant impact on the final serum zinc concentration, and none of the randomized, controlled trials found any impact on growth. Nevertheless, the results of the only available evaluation of a large-scale program showed a significant reduction in the stunting rate after 2 years of distribution of MMN powders to young Mongolian children aged 6 to 35 months compared with baseline. Although it is not possible to attribute the reduction in stunting prevalence to zinc alone, or even to the intervention per se, zinc may have contributed to the observed reduction in stunting.

On the basis of the limited available evidence, it is not possible to conclude whether zinc deficiency can be controlled through current approaches using household-level fortification. There is a need for further evaluation of the efficacy and effectiveness of home-fortification products in zinc-deficient populations, with regard to specific zinc-related outcomes.

Detailed review of evidence

Overview

Various approaches have been suggested for household-level fortification of complementary foods, by which supplemental MMN are added to these foods just prior to consumption, so as to enable infants and young children to achieve adequate micronutrient intakes [45]. Available home fortification products may be MMN powders (e.g., Sprinkles®), crushable tablets (e.g., Foodlets®), or lipid-based nutrient supplements (e.g., Nutributter®), which are added to food at the household level, usually just prior to serving. Although each of these products contains a broad array of micronutrients, most studies have only investigated the potential of these formulations to control iron deficiency and anemia [46, 47]; little is known about their impact on the status of other micronutrients. We have reviewed the available literature on the impact of home fortification products on zinc-related outcomes.

Bibliographic search

Data sets were identified by using a computerized bibliographic search (PubMed), with key words: 1) zinc, Sprinkles; limiting for human; and 2) home fortification, zinc; limiting for human trials. A total of 17 articles were identified, of which only 1 efficacy trial compared the impact of MMN powders with and without zinc [48]. Three additional efficacy trials compared a zinc-containing MMN powder formulation with a placebo-control group [49, 50] or with treatment groups that received other home fortification products [51]. In these latter three studies, any impact on growth or morbidity cannot be attributed specifically to zinc. We have further included one efficacy trial in which crushable tablets containing MMN were provided in food. This study was part of a multicountry evaluation, the International Research on Infant Supplementation (IRIS) trials. Only in the study included here [52] was it explicitly stated that the crushable tablets were mixed with complementary foods, so this trial can be considered as an evaluation of household fortification. In addition to the articles identified in the PubMed search, we included an evaluation of a large-scale program that was part of a comprehensive nutrition program implemented in Mongolia by World Vision and the government of Mongolia [53].

Results of intervention studies of zinc-containing household-level fortification products that reported potentially zinc-related outcomes

Table 4 provides a summary of randomized, controlled household-fortification trials that included the measurement of serum zinc concentration or other zinc-related outcomes. Results are available from just one randomized, double-blind efficacy trial using MMN powders in which zinc was the only nutrient

that differed between groups. This community-based study was completed to simulate the potential impact of a home-based fortification program in 6- to 18-month-old, moderately anemic Ghanaian children to determine the effect of adding zinc to an iron-containing powder [48]. The final product, which was added to the local maize-based porridge, contained 80 mg of iron and 50 mg of ascorbic acid, with or without 10 mg of zinc as zinc gluconate. At the end of the 2-month period of intervention, most children in both groups had recovered from anemia, although there was a slightly greater percentage of children in the zinc-plus-iron group who remained anemic (37.1% vs. 25.2%, $p < .05$). Although there were no significant differences in final mean serum zinc concentrations, significantly fewer children in the zinc-plus-iron group had a serum zinc concentration < 70 μg/dL than in the iron group (22.6% vs. 36.1%, $p < .05$). There was no significant groupwise difference in the children's growth. A previous meta-analysis of the effect of zinc supplementation on children's growth found positive response to zinc only among those studies that enrolled children whose initial mean HAZ was less than approximately −1.5 [20]. Because the mean HAZ values at baseline were −1.81 and −1.70 in the two groups in the Ghana study, a beneficial impact of additional zinc on the children's growth would have been expected, although the 2-month study period may have been too short to detect such an effect. Moreover, the micronutrient powders provided in this study contained a very high dose of iron (80 mg/sachet), which might have interfered with zinc absorption.

One recent study evaluated the impact of different formulations of MMN powders in a double-blind, placebo-controlled trial in Cambodian children [49]. At 6 months of age, infants were randomly assigned to receive either MMN powders with 12.5 mg of iron, 5 mg of zinc, 50 mg of ascorbic acid, 300 μg of vitamin A, 7.5 μg of vitamin D_3, and 150 μg of folic acid; powders with only 12.5 mg of iron and 150 μg of folic acid; or placebo powders for a period of 12 months. There was no impact of either of the two types of supplement on the children's growth, but the sample size was just marginally adequate ($n = 65$ per group) to be able to detect such differences. Moreover, the stunting prevalence at baseline was only 6%, indicating that the study population may not have been sufficiently growth-restricted to benefit from zinc supplementation. However, the stunting rate increased to 27% over the study period of 12 months, and the final mean HAZ of all groups was −1.55 ± 0.74. Regrettably, serum zinc concentration was not measured.

Another three-cell study, which was carried out among 6- to 12-month-old Pakistani children, investigated the impact of a MMN powder (including 5 mg of zinc) (MMN group) or the same formulation of micronutrients (including 5 mg of zinc) and heat-inactivated lactic acid bacteria (MMN-LAB group) versus a control group that received a placebo and no lactic acid bacteria for 2 months [50]. Children who suffered from a diarrhea episode within the past 2 weeks were randomly assigned to one of the three groups ($n = 25$ per group). Fieldworkers visited homes twice weekly to collect information on the number of diarrheal stools, days of diarrhea, dysentery, and other morbidity symptoms. After adjustment for age, the mean longitudinal diarrhea prevalence was significantly lower in the multiple-micronutrients group (15 ± 10% child-days) than in the placebo group (20 ± 19% child-days) ($p < .007$). Surprisingly, the MMN-LAB group had a higher prevalence of diarrhea (26 ± 20% child-days), which was not significantly different from that in the placebo group ($p = .28$). These results are puzzling because both MMN groups received zinc, so it appears either that the addition of lactic acid bacteria eliminated any beneficial effect of zinc (or the other micronutrients) on diarrhea prevalence or the morbidity results were unrelated to the MMN supplements. Neither serum zinc concentration nor final anthropometrics were evaluated.

A randomized intervention trial compared the efficacy and acceptability of MMN powders, crushable tablets, and a lipid-based nutrient supplement added to home-prepared complementary foods in Ghana [51]. The products differed in their energy and nutrient contents, but all contained iron (powder, 12.5 mg; tablet, 9 mg; lipid-based supplement, 9 mg) and zinc (powder, 5 mg; tablet, 4 mg; lipid-based supplement, 4 mg). At the end of the intervention, infants with the same eligibility criteria for the trial, but not randomly selected for the intervention, were examined as a nonintervention group for a cross-sectional comparison. The prevalence of anemia (32%; hemoglobin < 100 g/L) and iron deficiency (28%; ferritin < 12 μg/L) were significantly higher in the nonintervention group at 12 months than in the three treatment groups (average of 15% and 10% for anemia and iron deficiency, respectively) [51]. However, there was no impact of any of the three treatments on serum zinc concentration compared with the nonintervention group. At 12 months, the weight-for-age z-score (WAZ) and length-for-age z-score (LAZ) adjusted for sex and maternal height were significantly greater only in the group that received the lipid-base nutrient supplements (WAZ = −0.40 ± 1.1, LAZ = −0.14 ± 1.0) compared with the group that received crushable tablets (WAZ = −0.88 ± 1.1, LAZ = −0.44 ± 1.0). There was no significant difference between the lipid-based nutrient-supplements group, the MMN-powders group (WAZ = −0.53 ± 1.1, LAZ = −0.40 ± 1.0) and the nonintervention group (WAZ = −0.74 ± 1.1, LAZ = −0.40 ± 1.0). Since all three treatment groups received zinc, the greater growth rate in the lipid-based nutrient-supplements group cannot be explained by zinc alone.

TABLE 4. Efficacy trials of home fortification products that included zinc and assessed the impact on zinc-related indicators (serum zinc concentration, change in height, change in weight, morbidity)

Country, year [reference] author	Study design	Age (mo)	Sample size[a]	Duration (mo)	Group	Product formulation	Additional zinc (mg/day)	Initial serum zinc concentration (μg/dL)[b]	Final serum zinc concentration (μg/dL)[b]	Change in height[b]	Change in weight[b]	Morbidity
Ghana, 2003 [48][c] Zlotkin	Randomized, double-blind, controlled	6–18	239	2	MMN powders with zinc	80 mg iron, 10 mg zinc, 50 mg ascorbic acid	10	94 ± 29	87 ± 25 (NS)[d]	−0.11 ± 0.61 ΔHAZ/2 mo (NS)	−0.20 ± 0.55 ΔWAZ/2 mo (NS)	NA
					MMN powders without zinc	80 mg iron, 50 mg ascorbic acid	0	92 ± 29	81 ± 22	−0.05 ± 0.31 ΔHAZ/2 mo	−0.15 ± 0.79 ΔWAZ/2 mo	NA
Cambodia, 2006 [49][c] Giovannini	Randomized, double-blind, controlled	6	191	12	MMN powders	12.5 mg iron, 5 mg zinc, plus multivitamins	5	NA	NA	−0.77 ± 1.50 ΔHAZ/12 mo (NS)	−0.32 ± 0.62 ΔWAZ/12 mo (NS)	NA
					Iron, folic acid powders	12.5 mg iron, 150 μg folic acid	0	NA	NA	−0.65 ± 1.25 ΔHAZ/12 mo	−0.20 ± 0.39 ΔWAZ/12 mo	NA
					Placebo powders	No micronutrients	0	NA	NA	−0.85 ± 1.61 ΔHAZ/12 mo	−0.28 ± 0.53 ΔWAZ/12 mo	NA
Pakistan, 2006 [50] Sharieff	Randomized, double-blind, controlled	6–12	75	2	MMN powders	30 mg iron, 5 mg zinc, plus multivitamins	5	NA	NA	NA	NA	15% ± 10% child-days of diarrhea (p = .007)
					MMN-LAB powders	30 mg iron, 5 mg zinc, plus multivitamins and LAB	5	NA	NA	NA	NA	26% ± 19% child-days of diarrhea
					Placebo powders	No micronutrients	0	NA	NA	NA	NA	26% ± 20% child-days of diarrhea

Ghana, 2007 [51][e] Adu-Afarwuah	Randomized, only research assistant blinded, not controlled	6	313	6	MMN powders	12.5 mg iron, 5 mg zinc, vitamins A and C, folic acid	5	75 ± 33	61 ± 16 (NS)[f]	7.9 cm/6 mo (NS/significant)[g]	1.39 kg/6 mo (NS/significant)[g]	No significant differences in morbidity between groups
					Crushable MMN tablets	9 mg iron, 4 mg zinc, plus additional micronutrients	4	65 ± 19	64 ± 17	7.8 cm/6 mo	1.35 kg/6 mo	
					Lipid-based nutrient supplement	9 mg iron, 4 mg zinc, plus additional micronutrients and energy (108 kcal/day)	4	67 ± 18	61 ± 17	8.3 cm/6 mo	1.57 kg/6 mo	
South Africa, 2005 [52][h] Smuts	Randomized, double-blind, controlled	6–12	107	6	Daily MMN crushable tablet	10 mg iron, 10 mg zinc, plus additional micronutrients	10	71 ± 14	78 ± 16 (significant/NS)[i]	2.4 ± 0.6 mm/wk (NS)	56.6 ± 25.8 g/wk (NS)	No significant differences in morbidity between groups
					Weekly MMN crushable tablet	20 mg iron, 20 mg zinc, plus additional micronutrients	2.9	74 ± 15	78 ± 14	2.5 ± 0.5 mm/wk	50.0 ± 21.3 g/wk	
					Placebo crushable tablet	No micronutrients	0	75 ± 14	73 ± 10	2.4 ± 0.6 mm/wk	51.6 ± 27.7 g/wk	

HAZ, height-for-age z-score; LAB, heat-inactivated lactic acid bacteria (*Lactobacillus acidophilus*); MMN, multiple micronutrients; NA, not available; NS, not significant; WAZ, weight-for-age z-score

a. Sample size includes the total number in the control and fortification groups.

b. Plus–minus values are means ± SD.

c. Change in HAZ and WAZ per group derived from baseline and final values.

d. Not significant, but incidence of low plasma zinc concentration was significantly lower in zinc group than in control group ($p < .05$).

e. Study also included a nonintervention group. Eligible children not randomly selected for intervention were included in the nonintervention, control group for a cross-sectional study at 12 months of age.

f. No significant difference in final serum zinc concentrations among the three treatment groups and a nonintervention group at 12 months of age.

g. Infants in the group receiving lipid-based nutrient supplements gained significantly more weight and length than those in the group receiving crushable tablets ($p < .05$); the difference from the group receiving MMN powder was not significant.

h. Results for the fourth intervention group (daily iron alone) are not presented here. Total sample size is the total number for the three groups whose results are presented here.

i. Change in serum zinc concentration was significantly different between the group receiving daily MMN and the placebo group.

Efficacy trials of the crushable tablets were carried out in 6- to 12-month-old infants in four countries [54]. Only the results of the South African study are presented here, since it was the only study that used the household-fortification approach, in which the mothers were instructed to crumble and mix the tablets with porridge [52]. We further limit the presentation of the results to just three of the four study groups, because one group received only iron. One of the three groups that are included in the present comparison received a daily MMN crushable tablet (daily MMN), which contained 13 nutrients at the level of one adequate intake [54]. Another group received a weekly MMN crushable tablet containing the same 13 nutrients, but at double the dose, followed by six daily placebo tablets during the remaining days of the week (weekly MMN). The third group received only placebo tablets. There were no differences in growth or morbidity between the supplemented groups and the placebo group during the 6 months of the study. However, the stunting prevalence at baseline was only 10.7%, indicating that the study population may not have been sufficiently growth-restricted to benefit from supplementation. Serum zinc concentration increased significantly in the daily MMN group compared with the placebo group. This increase was not seen in the weekly MMN group, suggesting that a weekly dose of 20 mg of zinc may not have been sufficient or that the serum zinc concentration returned to baseline levels during the interval between the last weekly dose and the time of specimen collection.

The effectiveness of a large-scale distribution program with MMN powders has been evaluated as part of a comprehensive nutrition intervention implemented in Mongolia by World Vision and the government of Mongolia [53]. The program included distribution of MMN powders (40 mg of iron, 10 mg of zinc, 400 IU of vitamin D, 600 IU of vitamin A, 50 mg of vitamin C, and 150 μg of folic acid) free of charge to more than 14,000 children 6 to 35 months of age. Other program components were provision of iron syrup to anemic children 36 to 59 months of age and iron–folate tablets to pregnant and lactating women, community-based nutrition education, and social marketing. A survey was conducted at baseline and approximately 2 years after program implementation. Eighty-eight percent of eligible children in the intervention areas received MMN powders for a mean duration of 13 months. The prevalence of anemia in children 6 to 35 months of age decreased from 55% at baseline to 33% at follow-up. The prevalence of stunting was significantly reduced from 23% at baseline to 18% at follow-up ($p < .01$), and the prevalence of wasting remained low (1% at baseline, 1.5% at follow-up). Because of the pre-post design of this intervention, it is not possible to attribute the results to the intervention in general or specifically to zinc.

In summary, there is very limited information available on the specific impact of zinc included in MMN powders and other household-fortification products. The only study that directly investigated the impact of adding zinc to micronutrient powders did not find any effects on children's growth or final serum zinc concentrations. However, this study employed a formulation that had a particularly high iron content (80 mg of iron); thus, it is not known whether different results might occur with the usual formulation of MMN powder, which generally contains 12.5 mg of iron per dose. All other studies compared zinc-containing MMN products with a placebo, so it is not possible to attribute any effect on zinc-related outcomes specifically to zinc. None of the randomized, controlled studies found a positive impact on growth, although the mean initial HAZ ranged from −0.2 to −1.8 across studies, and only one was less than −1.5. This is important to emphasize, because the previous meta-analyses of the effect of zinc supplementation on children's growth found positive responses to zinc only among those studies that enrolled children whose mean initial HAZ or WAZ was less than approximately −1.5 [20]. Nevertheless, distribution of MMN powders along with other nutritional interventions may have contributed to a reduced stunting rate that was observed in a large-scale national program in young children in Mongolia. Further research is urgently needed, as several countries are considering the distribution of MMN powders to young children, both to prevent anemia and to address a broad range of other potential nutrient deficiencies.

Section 4

Are there any adverse clinical effects of zinc fortification or negative effects of zinc fortification on the utilization of other nutrients due to zinc fortification?

Conclusion

With very few exceptions, most of the available studies indicate that there are no adverse effects of zinc fortification on iron absorption or indicators of iron and copper status.

Detailed review of evidence

Overview

Currently available evidence concerning adverse effects of zinc has been derived entirely from zinc supplementation trials [55]. As described in detail in the previous paper on preventive zinc supplementation [56], overt toxicity symptoms, such as nausea and vomiting, occur in adults only at high zinc intake levels of 150 mg/day or more. Similarly, the adverse effect of zinc on copper metabolism, as measured by a decrease in erythrocyte superoxide dismutase activity, has been found only at zinc intakes greater than 50 mg/day in

adults [55]. Some studies in young children that provided 10 mg of supplemental zinc daily, with or without iron, found that zinc supplementation had a small negative effect on iron status in some cases [57, 58], although no overall effect was found in a recent meta-analysis [56]. However, little is known about whether any adverse effects may occur with zinc fortification. The tolerable upper intake level (UL) is defined as the highest average level of daily intake that is likely to pose no risk of adverse health effects for almost all persons in the general population. The UL for zinc has been discussed in detail previously [55, 59]. It is worth emphasizing that the currently recommended UL is based on the amount of total zinc intake per day and does not consider the bioavailability of the zinc. In unrefined, cereal-based diets, for example, the amount of zinc absorbed is estimated to be lower than that for more refined foods because of the inhibiting effects of phytic acid present in the whole-grain products [55]. Thus, the suggested ULs for zinc may need to be reevaluated, taking into consideration the bioavailability of zinc from foods, including zinc-fortified foods. Notably, Arsenault and Brown [60] reanalyzed the data of 7,474 nonbreastfeeding preschool children included in the US Continuing Survey of Food Intakes by Individuals and found that overall 36% of children had zinc intakes that were in excess of the UL. Despite these high intakes, there have been no recent reports of zinc toxicity in US children.

To explore for possible adverse effects of zinc fortification, we reviewed the data available from tracer studies and intervention trials, as described above, to evaluate the impact of zinc fortification on iron absorption and indicators of iron and copper status. For the identification of possible adverse effects of zinc fortification on iron status, zinc must be the only factor that differs between the study groups, so the following review is limited to the subset of relevant studies.

Tracer studies of the effect of zinc fortification on absorption of nutrients

Effect of zinc fortification on iron absorption. The effect of zinc fortification on iron absorption from foods cofortified with both zinc and iron has been examined in several studies. In the study of Sri Lankan schoolchildren, there was no difference in iron absorption from the iron-fortified rice product when 1.5 mg of zinc was added as zinc oxide [18]. Similarly, in the study of Indonesian children who received iron-fortified wheat dumplings with or without added zinc (1.5 mg of zinc added to a 25-g portion of dumplings and tomato puree that contained ~0.25 mg of zinc), there was no effect of zinc fortification on iron absorption when the added zinc was provided as zinc oxide [9]. On the other hand, there was a significant 28% decrease in iron absorption when the zinc fortificant was provided as zinc sulfate. Contrary to these latter results, López de Romaña et al. (unpublished) found no significant differences in iron absorption when a total of 0, 3, or 9 mg of zinc, as zinc sulfate, was added to iron-fortified wheat flour products consumed during two meals. Mendoza et al. [11] also found no differences in iron absorption from a wheat-soy-milk porridge to which zinc was added as either zinc sulfate or zinc methionine in adults. Thus, except for the results of the Indonesian study, and only when zinc was provided as zinc sulfate in that study, the other available results indicate no adverse effects of zinc fortification on iron absorption from cofortified foods, regardless of the chemical form of the zinc used for fortification. In summary, the weight of current evidence suggests that zinc fortification should not adversely affect iron absorption from foods cofortified with both zinc and iron.

Intervention studies with zinc-fortified foods

Effect of zinc fortification on indicators of iron status. Only two of the six studies of zinc-fortified milk products presented information on final hemoglobin concentration [29, 34], and one assessed serum ferritin concentration [34]. In both studies, the treatment group received not only additional zinc, but also additional iron, as compared with the control group. Neither of these studies found any difference in iron status-related outcomes between the two groups (**table 1**).

Of the five trials of zinc-fortified cereal products (**table 2**), three reported measurements of hemoglobin concentration [6, 18, 22] and four provided results for serum ferritin concentration [6, 18, 22, 36]. None of these studies found a significant difference in final hemoglobin concentration or serum ferritin concentration between the zinc-fortified group and the control group. With the exception of the study by Kilic et al. [36], all of the fortified food products provided to both groups in these trials contained additional iron. On the basis of the current evidence, it can be concluded that the addition of zinc to food fortification formulations does not have any adverse effect on iron status indicators.

Effect of zinc fortification on indicators of copper status. Four of the studies of zinc-fortified milks presented data on serum copper concentration [30–32, 34] (**table 1**). One study is not included in the following summary because the treatment group received both zinc and copper in infant formula [29]. None of the studies found a significant difference in final mean serum copper concentrations. Three of the studies of zinc-fortified cereal products provided information on the mean final serum copper concentrations [22, 35, 36] (**table 2**). None of these studies detected any impact of zinc fortification on copper status. Thus, there is currently no evidence for an adverse effect of zinc fortification on copper status.

Section 5

What are the steps in implementing zinc fortification programs and what additional research is needed?

The foregoing review of available scientific evidence indicates that zinc fortification programs have the potential of increasing zinc intake and total zinc absorption in targeted individuals. Although there is still only limited information available regarding the efficacy and effectiveness of zinc fortification, several studies have found a positive impact on serum zinc concentration or potentially zinc-related functional responses with particular zinc-fortified food products in selected age groups. Moreover, studies indicate that there is negligible risk and relatively low cost associated with zinc fortification, so such programs are recommended for implementation in high-risk populations that have appropriate conditions for successful food fortification programs.

The following section will review briefly some of the critical steps required for a zinc fortification program and summarize pending research issues. Program-related considerations have been reviewed previously by WHO [1] and the International Zinc Nutrition Consultative Group (IZiNCG) [55], so these will only be summarized in this section. The major issues are:

» Defining the target population
» Assessing zinc intake and status in the target population
» Selecting the food vehicle(s)
» Selecting the zinc fortificant
» Determining the level of zinc fortificant
» Establishing the regulatory parameters
» Costs
» Monitoring and evaluation

Identifying the target population

The most vulnerable population groups for zinc deficiency are infants, young children, and pregnant and lactating women because of their elevated requirements for zinc [55]. All of these groups probably need special attention, and food fortification products should be chosen specifically to target these population groups and their age-specific requirements [61]. As there is currently no information available on the impact of zinc fortification among pregnant and lactating women and their offspring, research is needed to assess such programs.

In the case of young children, as described above, studies show that zinc fortification can increase dietary intake and total absorption of zinc. However, studies have not yet demonstrated that zinc-fortified complementary foods can positively affect indicators of young children's zinc status, growth, or other potentially zinc-related functional outcomes. Whether household-level fortification products can exert a positive impact on zinc-related outcomes in young children also remains to be proven. Thus, additional research is needed to determine the efficacy and effectiveness of these intervention strategies in different populations and using graded dosages of zinc. When such fortification programs are implemented, it is highly recommended that their impact on children's zinc nutrition and health should be rigorously evaluated.

Although there is very little relevant information, mass fortification has the potential to benefit other segments of the population, such as school-aged children [44], adolescents, women of reproductive age, and possibly the elderly. In any case, further research is needed to evaluate the efficacy and effectiveness of fortification programs in all age groups.

Assessing zinc intake and status in the target population

WHO recommends gathering data on micronutrient deficiencies in a representative sample of the target population to justify the needs for a fortification program [1]. WHO, the United Nations Children's Fund (UNICEF), the International Atomic Energy Agency (IAEA), and IZiNCG jointly recommend three indicators for assessing the population's risk of zinc deficiency [62]. The concentration of zinc in blood plasma or serum is the best available biomarker of zinc status in populations. Chronic inadequate dietary intake of zinc is the most likely cause of zinc deficiency. Hence, estimating the adequacy of zinc intakes through quantitative dietary intake surveys is useful to evaluate the risk of zinc deficiency in populations. Moreover, information on dietary intake is needed to determine the appropriate food vehicles and level of fortification, as discussed below. A third possible indicator of population zinc status is height-for-age, a measure of nutritional stunting usually applied among children less than 5 years of age. Stunting is the best known and easiest to measure of the adverse outcomes associated with zinc deficiency, and relevant data are often already available from previous national health or nutritional status surveys [63]. Stunting prevalence is expressed as the percentage of children less than 5 years of age with height-for-age below the expected range of a reference population (i.e., less than −2.0 SD with respect to the reference median), such as the recently established WHO Growth Standards [64].

The risk of zinc deficiency is considered to be elevated and of public health concern when the prevalence of low serum zinc concentrations is greater than 20% or the prevalence of inadequate intakes is greater than 25%. A stunting prevalence greater than 20% is also considered to be indicative of an increased risk of zinc deficiency. However, since zinc deficiency is not the only factor affecting children's growth, assessment

of serum zinc concentration and dietary zinc intake should be used to confirm the risk of zinc deficiency in countries with high rates of stunting.

Selecting the food vehicle(s)

The selection of food vehicles for fortification programs depends on the target population groups, their dietary pattern, and locally available food-production capability. The assessment of food-intake data not only can be used to assess the risk of zinc deficiency but also is necessary to make an informed judgment regarding which food(s) would be the most suitable vehicle(s) for fortification [1]. In the case of young children, the most suitable products could be processed complementary foods or household-level fortification products, such as powders (e.g., Sprinkles®), crushable tablets (e.g., Foodlet®), and lipid-based nutrient supplements (e.g., Nutributter®). If a fortification program is targeting schoolchildren, other products, such as condiments, snacks, or beverages, may be more suitable, depending on the local food habits. For mass fortification, the selected food vehicle should be consumed regularly by a large segment of the general public in fairly constant amounts throughout the year. Typical products chosen for mass fortification are cereals, vegetable oils, fats, milk, and condiments [1].

The main advantage of mass fortification in comparison with supplementation or targeted fortification is that it requires very little investment because it uses already existing products and delivery mechanisms. To keep the fortification program cost-effective and efficient, the fortification technology should be supported by formal, centralized industries. In general, the low cost of mass fortification can only be realized in industrialized settings, where only a few technically advanced factories produce the foods. The challenge in developing countries is to find a suitable food vehicle that is industrially produced and widely consumed in reasonable amounts by the target population and, ideally, with a narrow range of inter- and intraindividual variation in intake [65].

Selecting the zinc fortificant

Several zinc compounds are listed by the US Food and Drug Administration as generally recognized as safe (GRAS) and can be used for fortification. As described previously by IZiNCG, there is no consensus as to which of the GRAS compounds is the most appropriate for fortification programs [55]. Zinc oxide and zinc sulfate are the GRAS salts that are least expensive and most commonly used by the food industry. Despite theoretical considerations that suggest that zinc may be better absorbed from water-soluble compounds such as zinc sulfate, several studies indicate that zinc is equally well absorbed from cereal products fortified with either zinc sulfate or zinc oxide, as described above. Thus, zinc fortification programs will generally rely on zinc oxide, because of its lower cost.

Determining the level of zinc fortificant

The dietary goal of fortification is formally defined by WHO as the provision of most (97.5%) individuals in the population group(s) at greatest risk of deficiency with an adequate intake of specific micronutrients, without causing a risk of excessive intakes in these or other groups [1]. The WHO guidelines on food fortification describe a detailed procedure to estimate the appropriate micronutrient level for mass fortification programs, using the Estimated Average Requirement (EAR) cutpoint method and the concept of the Feasible Fortification Level (FFL) [1]. Recommendations for zinc fortification of cereal flour have recently been revised on the basis of the population's usual flour intakes, degree of milling (level of extraction), and intakes of zinc and phytate from other dietary sources [66]. A summary table from that document is reproduced herein, for 85% extraction wheat flour and different assumptions regarding the average amounts of flour intake and zinc intake from other sources (**table 5**). For more details on other levels of extraction and the basis for these recommendations, the original document should be reviewed [66].

The amount of a particular micronutrient that can be added to foods is limited by various safety, technologic, and economic constraints, which are all considered in the FFL. In other words, the FFL is the maximum content of micronutrient that can be added and still remain compatible with the food matrix, while the increase in food price stays within an acceptable range. The FFL provides the greatest number of at-risk individuals with an adequate intake without causing an unacceptable risk of excessive intake in the population at large. As a consequence, the maximum content of micronutrients in mass fortification programs is determined by those individuals who eat the food vehicles in the largest quantities (i.e., the upper 5% to 10%) [2].

In the case of zinc, experience has shown that the addition of zinc compounds such as zinc oxide and zinc sulfate to wheat and maize flour at recommended levels is technically feasible and does not alter the organoleptic qualities of the fortified foods [67]. The national wheat and maize flour fortification programs in Mexico and South Africa are examples in which zinc is included in the fortification formulation.

A more critical limiting factor may be safety constraints with regard to zinc fortification. Most individuals receiving food products should keep the total intake (from the natural content of foods, fortified foods, and supplements) of certain nutrients lower than the levels that are recognized as safe (Tolerable Upper Intake Levels, ULs) [55, 59]. In mass fortification, adult

TABLE 5. Amount of zinc fortification of wheat flour (mg of zinc/kg flour) that is necessary to ensure 3.84 mg of absorbed zinc/day, considering different amounts of usual zinc and phytate intakes from sources other than wheat flour and the stated amounts of flour consumption (80% extraction flour)[a]

Flour intake (g/day)	Dietary phytate (mg/day)								
	Dietary zinc from sources other than wheat: 3 mg/day			Dietary zinc from sources other than wheat: 5 mg/day			Dietary zinc from sources other than wheat: 7 mg/day		
	0	500	100	0	500	1,000	0	500	1,000
50	134	229	325	94	189	285	54	149	245
75	96	160	223	69	133	197	42	106	170
100	77	124	172	57	104	152	37	84	132
200	48	72	96	38	62	86	28	52	76
300	38	54	70	32	48	64	25	41	57
400	34	46	58	29	41	53	24	36	48
500	31	40	50	27	36	46	23	32	42
600	29	37	45	26	34	42	22	30	38
700	28	34	41	25	32	38	22	29	36
800	27	33	39	24	30	36	22	28	34

Source: reproduced from Brown et al. [66].

a. Shaded values indicate fortification levels > 100 mg of zinc/kg flour, which is the upper range of zinc fortification for which sensory acceptability has been confirmed.

males, who tend to have the highest levels of consumption of staple foods, have the greatest risk of excessive micronutrient intakes. To avoid excessive intakes in this population group, the level of the fortificant may need to be kept so low that the most vulnerable population groups, such as young children, may not benefit sufficiently from a mass fortification program. In that case, fortification of several food vehicles, targeted fortification, or zinc supplementation should be considered for completing the dietary or nutritional gap of those individuals with low consumption of the fortified food or larger nutritional needs.

Establishing the regulatory parameters

Regardless of whether the fortification intervention is voluntary or mandatory, suitable standards should be established to allow appropriate quality control by the producers and inspection and enforcement by the public sector. Specific levels of the added micronutrients should be stipulated, and the government authorities should check on compliance based on those levels [65]. Two regulatory parameters are essential: the legal minimum level (LML) for all nutrients and the maximum tolerable level (MTL) for those nutrients for which excess consumption is of concern (including zinc).

If, for example, under the circumstances of a particular program in which low-extraction wheat flour has an intrinsic zinc content of 10 mg/kg [2] and a zinc fortification level of 30 mg/kg is proposed, the average final zinc content in the flour is calculated as the sum of these two values, resulting in a value of 40 mg/kg. An acceptable range of final zinc content is determined by subtracting and adding two times the expected coefficients of variation of a satisfactorily operating fortification process, which is ~12%. In this case, the minimum and maximum production parameter for zinc results in a range of 30 to 50 mg of zinc per kilogram of flour. As the loss of zinc during storage and distribution is expected to be minimal, the LML is equal to the minimum production factor (30 mg/kg), and the MTL is equal to the maximum production factor (50 mg/kg) [2].

Costs

Estimating costs is an important step in planning a food fortification program. Estimates must include both the costs to industry (e.g., capital investment and recurrent costs, such as the purchase of fortificant) as well as the public sector costs (e.g., enforcement, monitoring, and evaluation). In the case of mass fortification programs, which tend to rely on staples and condiments as the food vehicles, cost is often the most significant limiting factor. Staples and condiments are consumed frequently and in large amounts, not only by the population directly, but also by the food industry. Even small variations in price can thus have profound consequences, and experience has shown that mass fortification in an open market economy is most likely to be successful when the increase in the price of the fortified product, relative to the unfortified one, does not exceed 1% to 2% [1].

However, the addition of zinc adds very little to the fortification cost and hence, for this nutrient, cost is not a restrictive factor. In a recent review of the current costs to supply the EAR of nutrients to women of reproductive age through food fortification, it was concluded that zinc (as zinc oxide) is one of the least expensive nutrients [2]. Taking the expected

micronutrient loss during production, distribution, storage, and food preparation into account, it is estimated that a woman can receive her entire yearly requirement of zinc through food fortification at an annual cost of US$0.006 to US$0.013.

Monitoring and evaluation

Monitoring and evaluation are essential components of any food fortification program, and detailed guidelines are provided elsewhere [1]. In the context of food fortification, the term "monitoring" refers to the continuous collection, review, and use of information on program-implementation activities, for the purpose of identifying problems and redirecting the program as needed. The ultimate purpose of monitoring a fortification program is to ensure that the fortified product, of the desired quality, is made available and is accessible to consumers in sufficient amounts [1]. Regulatory monitoring includes all monitoring activities at the production level, as well as monitoring at customs warehouses and retail stores. This activity is usually conducted by regulatory authorities as well as by the producers themselves as part of their internal quality-control activities. Additional monitoring activities should also assess whether or not a program is providing appropriately fortified food products in sufficient amounts and at affordable prices to the target population and whether these products are consumed at the household level.

The term "evaluation" refers to the assessment of the impact of a program on the nutritional and health status of the target population. During a program evaluation, zinc intake, serum zinc concentrations, and functional outcomes, such as stunting prevalence, should be assessed in a representative sample of the target population [62]. WHO recommends that program evaluation should only be undertaken once appropriate monitoring has established that the fortification program has been implemented as planned and is operating efficiently [1]. There is currently a lack of information on the effectiveness of zinc fortification programs, and program evaluation is highly encouraged.

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A review of interventions based on dietary diversification or modification strategies with the potential to enhance intakes of total and absorbable zinc

Rosalind S. Gibson and Victoria P. Anderson

Abstract

Dietary diversification or modification has the potential to prevent deficiencies of zinc and other coexisting limiting micronutrients simultaneously, without risk of antagonistic interactions. In this review, we have addressed the following. The first section focuses on strategies with the potential to enhance intake and/or bioavailability of zinc, and includes interventions (with and without nutrition education) based on agriculture, production or promotion of animal-source foods through animal husbandry or aquaculture, and commercial and household processing strategies to enhance zinc absorption. Outcome indicators include intakes of foods or nutrients (although rarely zinc) and, in some cases, zinc status, or zinc-related functional responses. The next two sections address whether dietary diversification or modification can achieve increases in absorbable zinc that are sufficient to enhance zinc status or zinc-related functional responses in breastfed infants and toddlers and in older children and women of reproductive age. Evidence for the impact of dietary diversification or modification on behavior change and on nutritional status in the short and long term, and the possible role of modifying factors (e.g., baseline nutritional status, socioeconomic status, infection, sex, age, and life-stage group) is the emphasis of the next section. The following section highlights the evidence for three potential adverse effects of dietary diversification or modification: aflatoxin contamination from germinated cereals, loss of water-soluble nutrients, and displacement of breastmilk. Finally, an example of a dietary diversification or modification program (Homestead Food Production) developed and implemented by Helen Keller International is given, together with the critical steps needed to scale up dietary diversification or modification for programs and future research needs.

Key words: Dietary diversification, dietary modification, zinc deficiency, zinc intake

Introduction

The increasing recognition of the coexistence of MMN deficiencies has highlighted the importance of dietary diversification or modification as a strategy to prevent deficiencies of zinc and other coexisting limiting micronutrients simultaneously without risk of antagonistic interactions. Through a participatory research process that focuses on building relationships with the community and involving them in their design and implementation, dietary diversification or modification strategies have the potential to be culturally acceptable, economically feasible, and sustainable, even in poor resource settings. Further, such strategies have the added advantage of enhancing the micronutrient adequacy of diets for the entire household and across generations. Several additional non-nutritional benefits may also be achieved through the community-based nature of dietary diversification or modification. These may include empowerment of women in the community, training, and income generation. To be successful, however, a multidisciplinary team of specialists in agriculture, nutrition, epidemiology, rural extension, adult education, psychology, and community health is essential to assist with the design, implementation, monitoring, and evaluation of dietary diversification or modification strategies.

This paper provides a critical review of interventions employing dietary changes—diversification or modification strategies at the community or household level that have the potential to increase the intake of total and/or absorbable zinc. Potential strategies at the level of agricultural production are addressed

Rosalind S. Gibson is affiliated with the Department of Human Nutrition, University of Otago, New Zealand. Victoria Anderson is affiliated with University College, University of London.

Please direct queries to the corresponding author: Rosalind S. Gibson, Department of Human Nutrition, University of Otago, PO Box 56, Dunedin, New Zealand; e-mail: Rosalind.Gibson@stonebow.otago.ac.nz.

elsewhere. Three types of intervention strategies have been included: agricultural interventions, interventions based on the production or promotion of animal-source foods, and strategies at the commercial or household level designed to enhance zinc absorption. Only those interventions that included an indicator of consumption, either at the household or the individual level, are included, although very few of these measured intakes of total or bioavailable zinc. Depending on the evaluation design, outcome indicators measured either changes or differences in intakes of foods or nutrients, and in some cases, serum or plasma zinc, and functional health outcomes such as anthropometry, morbidity, and cognitive development. Tables summarizing the interventions are also provided in this review.

Details of the interventions reviewed were derived from the peer-reviewed literature, websites, and project reports. A primary literature search was performed on PubMed using the following key words: (agricult* OR farm OR garden OR rural development OR fish ponds) AND (zinc* OR "animal source foods" OR "nutritional status" OR anthropom* OR "diet intake" OR "nutrient intake" OR morbidity OR "child growth") and restricted mainly to human studies. This literature search yielded 45 articles. The gray literature search identified 15 additional reports from conference proceedings from the International Vitamin A Consultative Group (IVACG) and others, and websites of the Food and Agriculture Organization (www.fao.org), Helen Keller International (www.hki.org), the International Center for Research on Women (www.icrw.org), the International Food Policy Research Institute (www.ifpri.org), the International Life Sciences Institute (www.ilsi.org), and World Vision Canada (www.worldvision.ca). The important contributions made by Ruel [1] and Bierti et al. [2] through their earlier published reviews and the full report by Bierti et al. [3] kindly provided to the authors by PATH Canada are also acknowledged.

The review is divided into five sections, which address the following questions in relation to interventions based on dietary diversification or modification:

Section 1a: Can agricultural interventions that increase the production, accessibility, and consumption of plant-based foods enhance the intake and bioavailability of zinc?

Section 1b: Can production or promotion of animal-source foods through animal husbandry or aquaculture enhance the intake of bioavailable zinc?

Section 1c: Can processing strategies at the commercial or household level enhance zinc absorption from plant-based diets?

Section 2: Can complementary foods nutritionally improved through dietary diversification or modification strategies have an impact on the zinc status and the health and development of breastfed infants and young children?

Section 3: Can foods nutritionally improved through dietary diversification or modification have an impact on the zinc status and the health and development of children and women of reproductive age?

Section 4a: Do interventions that promote dietary diversification or modification have an impact on behavior change and on nutritional status in both the short and the long term?

Section 4b: Is the impact modified by baseline nutritional status, socioeconomic status, infection, sex, age, and life-stage group?

Section 5: Are there any adverse effects of dietary diversification or modification?

Section 1a

Can agricultural interventions that increase the production, accessibility, and consumption of plant-based foods enhance the intake and bioavailability of zinc?

Conclusion

Agricultural interventions can increase the production, accessibility, and consumption of plant-based foods by household members, provided they also contain a strong nutrition education component. Hence, they have the potential to enhance intakes of zinc and other micronutrients, although the magnitude of the increase in bioavailable zinc that can be achieved depends on the type and amount of plant foods consumed by the household. Interventions that include cereals and legumes have the potential to yield the greatest increase in zinc intake because of their higher zinc content compared with that of starchy roots or tubers, and fruits and vegetables. Nevertheless, the bioavailability of zinc in cereals and legumes will be compromised by high phytate levels unless phytate levels are reduced by other strategies.

Comprehensive agricultural strategies that also include a home gardening component with a focus on vitamin A–rich fruits and vegetables have several additional benefits. There is some indication that zinc absorption might be increased as a result of the interaction between vitamin A and zinc. Further, substantial increases in intakes of other important micronutrients, including nonheme iron (although poorly absorbed), copper, vitamin C (an enhancer of nonheme iron absorption), folate, thiamine, niacin, and dietary fiber, will be provided by a more comprehensive agricultural intervention. Finally, because home gardening projects often involve women, have the potential to generate income, and can be readily integrated with nutrition

education and behavior change strategies, they have all the attributes that have been associated with positive nutrition outcomes.

To date, almost all of the interventions based exclusively on agriculture have focused on improvements in vitamin A intakes or status from provitamin A–rich fruits and vegetables rather than zinc. Hence, the data on increasing intakes of total and/or bioavailable zinc through agricultural interventions alone are negligible.

Detailed review of evidence

Evidence for the first part of this conclusion is based on 10 agricultural interventions, chosen because they all included an indicator of consumption and, in some cases, of production and accessibility. They have also been reviewed by Ruel [1] or Berti et al. [2] and are summarized in **table 1**.

The focus of most of these projects was largely on improving intakes of vitamin A–rich fruits and vegetables, with the exception of a project in Egypt that measured changes in the consumption of cereals and legumes [12]. Increases in intake of provitamin A–rich foods would result in some improvement in bioavailable zinc intakes, albeit small. The zinc content of fresh fruits and green leafy vegetables is low (0.2 to 0.7 mg of zinc/100 g fresh weight), but zinc absorption might be enhanced by the concomitant increases in intakes of provitamin A carotenoids, possibly as a result of the interaction between vitamin A and zinc [15, 16]. By contrast, increased consumption of maize, wheat, and peanuts, as reported in the study in Egypt [12], will lead to greater increases in zinc intakes compared with the levels obtained through fruits and vegetables and will be accompanied by more substantial increases in the intakes of protein, iron, thiamine, niacin, folate, and dietary fiber. However, the bioavailability of zinc (and iron) from these food sources will be poor because of their high phytate content.

In 6 of the 10 agricultural interventions [4, 5, 8, 9, 12, 14], the evaluation design included both intervention and control groups and, in three of these [4, 8,14], an assessment of change from preintervention to postintervention in both groups was reported. However, as emphasized by Ruel [1], the intervention and control groups were sometimes in different geographic areas. Further, data on how the control group was selected and its comparability to the intervention group at baseline were not always provided, making it impossible to establish whether any differences existed between the two groups at baseline and to account for any such differences in the statistical analyses. For the remaining four interventions reviewed [6, 7, 10, 11, 13], the evaluations were largely based on a comparison of preintervention versus postintervention, with no control group. In two of the projects [4, 12], dietary intake data at the household level were used to estimate per capita consumption, which limits the strength of the evidence.

Eight of the agricultural interventions included a nutrition education component [4–11, 14], and all reported increased, or greater intakes of vegetables, vitamin A–rich foods, or intakes of vitamin A, depending on the intervention design. However, the results of three of these projects [6, 7, 13] are hampered by the absence of an appropriate control group. Furthermore, it is not possible to distinguish the effect of the nutrition education from the agricultural production or promotion effect in these projects, with the exception of the project in Kenya [14]. In the Kenyan project, differences in intakes of vitamin A–rich foods (i.e., β-carotene–rich sweet potatoes), with and without nutrition education, were investigated, and the results clearly indicated a benefit of including nutrition education [14]. However, for the two projects listed in **table 1** that did not include a nutrition education component [12, 13], the results were inconsistent. The project in Egypt [12] reported higher intakes of protein and iron per capita in intervention households compared with controls, whereas in the Nepal study [13], the results are difficult to evaluate because no control group was included. Postintervention, vitamin A intakes were still inadequate in mothers and children, despite a reported increase in the number of households producing vegetables.

Section 1b

Can production or promotion of animal-source foods through animal husbandry or aquaculture enhance the intake of bioavailable zinc?

Conclusion

Increasing the production of animal-source foods through animal husbandry or aquaculture in regions where such interventions are culturally appropriate can increase the consumption of animal-source foods in the household, especially when nutrition education or behavior change is a component of the intervention. Their impact on increasing intakes of bioavailable zinc depends on the type and amount of animal-source foods consumed. Depending on the evaluation design, increased consumption of red meat or liver can lead to increased or higher intakes* of bioavailable zinc compared with that obtained from fish or dairy products.

This is because beef, pork, lamb, and liver have a higher content of readily absorbed zinc (3.0 to 6.8 mg of zinc/100 g) than poultry (~1.1 to 2.7 mg of zinc/100 g),

* "Increased intake" refers to situations when pre- and post-intervention data are available; "higher intakes" refer to situations when only post-intervention data exist.)

TABLE 1. Agricultural interventions to increase household production, accessibility, or consumption of plant-based foods

Country, year [reference]	Intervention strategies			Design	Methods	Outcomes	
	Production	Nutrition education	Target groups			KAP + dietary intake	Nutritional status
Bangladesh, 1998 [4] Marsh	Low-cost home vegetable gardens, seed distribution	Nutrition education, processing and cooking methods to optimize nutritional value of food; agricultural training	Poor, near-landless women with children < 5 yr, willing to engage in farming activities	Pre-postintervention Pilot study, 2-yr follow-up Intervention group (1,000 households) vs. control group (200 households) (separate village)	Vegetable production, household dietary intake, anthropometry, morbidity, clinical signs of VAD (night-blindness) Anemia (no baseline measures)	Increased size of plot cultivated, increased variety of vegetables grown, increased vegetable production, year-round availability and intake in intervention households (especially in young children) vs. control Increased decision-making by intervention women[a]	Decreased stunting in intervention (44%–34%) vs. control group (42%–35%) Decreased night-blindness in intervention (2.3%–1.2%) vs. control group (no change) Maternal night-blindness lower in intervention (1.4%) than control group (3.4%) No change in diarrhea prevalence during intervention Anemia prevalence: intervention (24.4%) vs. control (33.2%)[a]
Tanzania, 2000 [5] Kidala et al.	Home vegetable gardens	Promotion of home production, consumption, and appropriate storage of vitamin A–rich foods	Mothers with children aged 12–71 mo living in VAD-prone rural villages	No baseline 5-yr follow-up Intervention households ($n = 75$) vs control households ($n = 71$)	Household KAP survey, 7-day food-frequency recall intake of vitamin A–rich foods Serum retinol Stool analysis (for helminths)	Intervention mothers had better KAP than controls ($p < .02$). 67% of intervention households vs. 32% of control households had home gardens ($p = .001$) 65% of intervention children vs. 37% of control children consumed > 7 vitamin A–rich foods in past week ($p = .001$)	No difference in serum retinol (intervention vs. control children) after controlling for helminth infection
India, 2000 [6] Chakravarty	Home vegetable gardens	Nutrition and health education	Households with young children (age group not specified)	Pre-postintervention household survey No control group	Production of vitamin A–rich foods, weekly consumption of vitamin A–rich foods, clinical signs of VAD	Increased no. of varieties, production, and consumption (> 2× baseline) of vegetables Increased awareness of nutrition needs and symptoms of deficiency	Decreased clinical signs of VAD postintervention

continued

TABLE 1. Agricultural interventions to increase household production, accessibility, or consumption of plant-based foods *(continued)*

Country, year [reference]	Intervention strategies			Design	Methods	Outcomes	
	Production	Nutrition education	Target groups			KAP + dietary intake	Nutritional status
Vietnam, 1995 [7] Ngu et al.	Home vegetable gardens	Nutrition education focused on vitamin A	Mothers in rural Vietnam	Pre-postintervention design (2-yr follow-up) No control group	Maternal KAP, vegetable production, dietary intake (by 24-h recall), clinical signs of VAD (night-blindness, Bitot's spots, corneal scarring)	26% increase in maternal KAP scores Increased production (from 41 to 233 g/person/day) Increased daily intakes of energy (from 1,479 to 1,725 kcal), protein (from 39 to 48 g), and fat (from 8 to 14 g) Increased vegetable intake by children < 5 yr (from 13 to 32 g/day)	Decreased clinical signs of VAD postintervention: night-blindness (from 0.55% to 0.00%), Bitot's spots (from 0.40% to 0.09%), corneal scarring (from 0.06 to 0.00%)[a]
Philippines, 1996 [8] Solon et al.	Urban home gardens and institutional gardens	Promotion of vitamin A–rich vegetables from 3-mo media campaign (e.g., radio gardening lessons)	Parents of children < 14 yr, pregnant and lactating women, schoolchildren	Pre-postintervention Intervention city vs. control city	Vegetable production Consumption of vitamin A–rich vegetables by target group in previous week (food-frequency questionnaire) and day (24-h recall)	Increased consumption of > 3 vegetables/wk Increased percentage of children eating green leafy vegetables daily (from 35% to 42%) Vitamin A intake: 12% increase in intervention children vs. 48% decrease in control city children No change in diet of children < 1 yr	Not measured
Guatemala, 1996 [9] Phillips et al.	Home-gardens (vitamin A–rich crops), seeds, technical assistance	Promotion of vitamin A–rich plant foods	Families with young children	No baseline Intervention households (n = 300) vs. control households (n = 300) Also compared with VAC distribution and fortification	Maternal KAP Dietary intake Percentage adopting gardens Cost-effectiveness of programs evaluated	Maternal knowledge greater in intervention than in control group[a] No. of days consuming vitamin A–rich foods (both preformed vitamin A and vitamin A from garden produce) greater in intervention than in control group ($p < .01$) 87% of intervention households had home garden postintervention	Control children at 3.5-fold increased risk of VAD when home garden had no dark-green leafy vegetables (controlled for SES and age)

Philippines, 1979, 1980 [10] Solon et al., [11] Popkin et al.	Home gardens (vitamin A–rich fruits and vegetables)	Promotion of vitamin A–rich fruits and vegetables Deworming, medical care, pharmacy, sanitation, immunization, health and nutrition education	Children in rural villages	Pre-postintervention No control 2-yr follow-up, intervention vs. VAC vs. vitamin A fortification of MSG ($n \approx 400$)	Intake of vitamin A, height, weight, serum retinol Clinical signs of VAD (xerophthalmia)	Increased vitamin A intake in intervention group from baseline[a]	Improvement in expected weight ($p < 0.05$) in relation to Harvard weight for-height in intervention group Serum retinol: no change in intervention group (from 19.0 to 16.4 μg/dL) vs. MSG group (from 21.0 to 28.5 μg/dL) Xerophthalmia: no change in intervention group, significant decrease in VAC and MSG groups*
Egypt, 1987 [12] Galal et al.	31 types of agricultural interventions to increase productivity of local crops	None	Farmers and their children 0–3 yr Self-selected sample, owned more land and was therefore wealthier and more literate than nonparticipants	Pre-postintervention design Intervention households ($n = 227$) vs. control households ($n = 828$) Subsurvey of children 0–3 yr: intervention ($n = 30$) vs. controls ($n = 22$)	Crop yield (maize, peanut, wheat) Household dietary intake (24-h recall) Subsample: immunizations, anthropometry, mortality, weaning age	Energy intake similar in both groups. Higher per capita intakes of maize, peanuts, wheat, and protein and iron in intervention than in control households[a]	No important differences in anthropometry, mortality, immunizations, or weaning age in subsurvey of 0–3-yr-olds[a]
Nepal, 1995 [13] CARE Nepal	Home gardens, water supply, irrigation, seed distribution	None	Rural households with children 6–60 mo	Pre-postintervention 3-yr follow-up No control group	Anthropometry, "number of years since initiation of kitchen garden" Intake of vitamin A–rich foods (food-frequency questionnaire) (postintervention only)	Increased percentage of households producing vegetables (from 51% to 75%)[a] Insufficient intake by mothers and children both pre- and postintervention	Deterioration of nutritional status of children during study period
Kenya, 1999 [14] Hagenimana et al.	Introduction of β-carotene–rich sweet potato	Women's group meetings: food processing techniques, vitamin A–rich foods	Women and children ($n = 300$) aged 0–5 yr	Pre-postintervention Intervention group vs. control group	Anthropometry, intake of vitamin A (food-frequency questionnaire)	Increased frequency of intake of vitamin A–rich foods in intervention group (from 4.2 to 5.8 food-frequency questionnaire score) vs. control group (from 4.3 to 3.0 food-frequency questionnaire score) when original intake was low ($p < .05$)	Not reported

KAP, knowledge, attitudes, and practices; MSG, monosodium glutamate; SES, socioeconomic status; VAC, vitamin A capsules; VAD, vitamin A deficiency

a. Not tested statistically or p-values not given.

eggs (~1.0 to 1.3 mg of zinc/100 g), dairy products (~0.3 to 1.0 mg of zinc/100 g), or finfish (~0.3 to 0.7 mg of zinc/100 g for flesh only; ~3.2 mg of zinc/100 g for whole, soft-boned fish with bones) [17]. However, very few of these interventions have quantified the changes in intakes of total or bioavailable zinc.

Animal-source foods can also contribute important and varying amounts (per 100 g or per MJ) of vitamin B_{12}, vitamin B_2, readily available iron, calcium, and preformed retinol, depending on the type of animal-source foods consumed. Nevertheless, because of design limitations in the interventions reviewed, such as lack of baseline data that include intakes of total or bioavailable zinc, and appropriate controls, the strength of the evidence for enhancing intake and bioavailability of dietary zinc through production or promotion of animal-source foods is rated as moderate.

Detailed review of evidence

Evidence for this conclusion is based on seven mixed interventions of agriculture combined with animal husbandry or aquaculture (**table 2A**), four interventions based exclusively on the production of animal-source foods (**table 2B**), four in which the consumption of animal-source foods was promoted (**table 2C**), and four nonblinded randomized, controlled trials in which meat-based foods were supplied to the participants (**table 2D**).

Three of the mixed interventions (**table 2A**) [18–22] have been reviewed by Ruel [1] and Bierti et al. [2]. Four additional mixed interventions not reported in these earlier reviews were also examined (**table 2A**) [23–26].

Nutrition education or behavior change was a component of five of the seven mixed interventions, although in two of the projects, consumption was not measured [25, 26], so that the impact of the intervention on consumption of animal-source foods cannot be assessed. In the three interventions in which both nutrition education and consumption were measured [18, 19, 21, 22, 24], increases or higher intakes of animal-source foods or iron were reported in the intervention as compared with the control groups, depending on whether change from pre- to postintervention, or difference.

It is noteworthy that in the two interventions without nutrition education [20, 23], no increases in consumption of fish were reported among the fishpond groups compared with the control groups, even though in the Bangladesh study fish production increased [20]. The sample size of the mixed intervention in Thailand [23], however, was very small (n = 30 children per group) and may not have been adequate to show an effect on fish consumption. In the intervention in Iran [26], no measures of food production or consumption were reported.

It is of interest that the project in northeastern Thailand [21, 22] was initially a home gardening project that focused on ivy gourd, in combination with some nutrition and health education to enhance intake of provitamin A carotenoids. However, the project's focus on community participation led to the construction of fishponds and the introduction of a poultry-raising project to complement the home gardening efforts. As a result, consumption of chickens and eggs increased among primary schoolchildren, who consumed them in a school lunch program. Intakes of vitamin A, iron, or vitamin C also increased in certain life-stage groups, but data on zinc intakes were not reported.

Four projects based exclusively on the production of animal-source foods through small-animal husbandry or aquaculture [27–31] are summarized in **table 2B**. In three of the projects [27, 28, 31], increases in consumption of animal-source foods were also reported. In the Ethiopian study, however, despite an increased intake of animal-source foods overall, 37% of families still consumed no meat, and in households with children, only ~40% of eggs produced were consumed [27]. In the two Bangladesh studies [28–30], neither of which included nutrition education, the animal-source food consumed by the treatment group was fish, especially small indigenous fish species. In the intervention of Nielsen et al. [28] (**table 2B**), the small indigenous fish species were purchased by the intervention households with income generated from the sale of the eggs or chickens produced, whereas in the study by Roos et al. [29, 30], although fish was eaten with a high frequency, the amount consumed was small. Indeed, total fish consumption did not differ between the fish-producing and the non-fish-producing households (**table 2B**).

Recent studies have highlighted the potential of certain indigenous fish species in Cambodia and Bangladesh as rich sources of zinc (e.g., *Esomus longimanus*), as well as of vitamin A and iron (e.g., *Amblypharyngodon mola*) [29, 30], so the impact of the consumption of indigenous fish on intakes of bioavailable zinc warrants attention. Moreover, improvements in intakes of several other important nutrients, such as animal protein and vitamin B_{12}, as well as bioavailable calcium, are likely provided the small indigenous fish species are consumed whole with bones.

In the Malawian project [31], increases in both the production and consumption of guinea fowl, chickens, rabbits, eggs, and goat's milk were observed in the intervention households, as a result of a small-animal revolving fund set up by World Vision. The use of these animal-source foods for household consumption rather than income generation was attributed to the nutrition education component of the World Vision Micronutrient and Health (MICAH) program, but because of flaws in the study design, this conclusion cannot be confirmed [31].

Of the total of 10 (6 in **table 2A**, 4 in **table 2B**)

TABLE 2A. Mixed interventions: Agriculture combined with small-animal husbandry or aquaculture to increase production or promotion of animal-source foods

Country, year [reference]	Intervention strategies			Design	Methods	Outcomes	
	Production	Nutrition education	Target groups			KAP + dietary intake	Nutritional status
Vietnam, 1997, 1998 [18] English et al. [19] English and Badcock	Home gardens, fishponds, and animals Food production and utilization assessed at baseline	Targeted at mothers: iron-, zinc-, vitamin C-, protein-, and carotene-rich foods	Mothers and their preschool children (< 6 yr)	Intervention group (n = 469) vs. control group (n = 251) from 1991 to 1993 Only some measurements taken at baseline	Anthropometry and morbidity at baseline and follow-up At follow-up: maternal KAP, food production, and dietary intakes of households and young children by 24 h recall	Intervention mothers had better KAP than controls[a] Intervention group had higher production of fruit, vegetables, fish, and eggs than control group. Intervention children had higher intakes of vegetables, fruit, energy, protein, vitamin C, vitamin A (100 vs. 60 RE/day), and iron (8.9 vs. 5.4 mg/day) than controls (p < .01)	Decreased stunting (from 50.3% to 41.7%, p = .0007) in intervention vs. no change in control group (from 45.8% to 47.6%) Decreased incidence of ARI (from 49.5% to 11.2%, p < .0001) in intervention group vs. no change in control group Decreased incidence of pneumonia complications (rapid breathing: 15.5% to 0.9%; chest indrawing: 5.2% to 0.2%) in intervention group vs. no change in controls Decreased incidence of diarrhea (from 18.3% to 5.1%, p < .0001) in intervention group vs. no change in controls
Bangladesh, 1998 [20] IFPRI	Vegetable production, fishponds, credit and agricultural training	No	Women, their households and children	Pre-postintervention over 2 yr Fishpond group, vegetable group, control group	Focus group interviews: (consumption measures) Anthropometry, hemoglobin	Increased production but no increased consumption of fish among fishpond group Increased vegetable intake among vegetable group compared with controls	No effect on hemoglobin from either fishponds or vegetable production intervention
Thailand, 1999 [21] Smitasiri and Dhanamitta, w [22] Smitasiri et al.	Seed distribution, training of farmer women, promotion of gardens, fishponds, and chicken raising	Nutrition education and social marketing targeted at women	Pregnant or lactating women, children 2–5 yr, and schoolgirls	Quasi-experimental design Pre-postintervention, 9-mo follow-up Intervention group (n≈500) vs. control group (n≈500)	Maternal KAP, dietary intakes by 24-h recall, biochemical assessment (in schoolgirls)	Intervention group: increased iron and vitamin A KAP at postintervention and vs. controls (p < .01); increased vitamin A intake (all groups) (no change in fat intake); increased iron intake in 2–5-yr-olds, 10–13-yr-olds, and lactating women; increased vitamin C intake in lactating women[a]	Intervention schoolgirls: increased serum retinol (from 22.8 ± 7.0 to 33.7 ± 8.2 μg/dL) vs. no change in controls

TABLE 2A. Mixed interventions: Agriculture combined with small-animal husbandry or aquaculture to increase production or promotion of animal-source foods *(continued)*

Country, year [reference]	Intervention strategies			Design	Methods	Outcomes	
	Production	Nutrition education	Target groups			KAP + dietary intake	Nutritional status
Thailand, 2002 [23] Schipani et al.	Home vegetable gardens, fishponds, small-animal husbandry, fruit orchards	No	Children aged 1–7 yr	Quasi-experimental design Gardening households (n = 30) vs. randomly selected nongardening households (control) (n = 30) Seasonal study (rainy, cool, hot seasons)	Interviews, anthropometry, 24-h recall over 3 seasons, serum retinol, serum ferritin, hemoglobin	No differences between groups in mean intake of energy, protein, fat, total iron, animal iron, plant iron, or vitamin C	No differences between groups in mean hemoglobin, serum ferritin, or retinol (for 3 seasons compared) Weight-for-height, weight-for-age, and height-for-age z-scores tended to be higher in gardening group (NS)
Bangladesh, 2006 [24] Stallkamp et al.	Home gardens Poultry, fish, milking cow	Messages to increase consumption of animal-source foods (eggs, meat, liver, milk)	Mothers and youngest child < 5 yr	Pre-postintervention over 3 yr Intervention households (n = 420) vs. control households (n = 420)	Food-frequency questionnaire: animal-source foods 24-h recall: red/orange/yellow fruits and vegetables, dark-green leafy vegetables Hemoglobin	Increased access, availability, and consumption of plant- and animal-source foods in intervention vs. control group Increased vitamin A intake in intervention children (6–59 mo) and mothers postintervention (p < .05)	12% decrease in anemia among nonpregnant intervention group at postintervention (p < .05)
China, 1994 [25] Ying et al.	Township committees encouraged increased production of green leafy vegetables, poultry, and small livestock	Nutrition training for health workers Media campaign: advocacy for breastfeeding, nutrition, and health	Poor rural, children aged < 6 yr (n = 9,921), subsample (n = 500)	Pre-postintervention 4-yr follow-up No control group	Anthropometry, hemoglobin	Changes in intake not measured	Decreased prevalence of malnutrition: wasting –18%, stunting –2.6%, underweight –11.3% at postintervention[a] Decreased prevalence of anemia at postintervention across all age groups (e.g., 2–3-yr-olds: 37.7% to 27.5%, p < .01)

Iran, 2004 [26] Sheikholeslam et al.	Rural cooperatives, income-generating and loan schemes Promotion of home gardens and animal husbandry	Nutrition, health, and literacy education for mothers Promotion of breastfeeding, complementary feeding, consumption of dairy products and fruits	Children aged 6–35 mo Intervention group: n = 1,695 at baseline, n = 1,149 at endline Control group: n = 1,631 at baseline, n = 1,029 at endline 3 districts: Ilan, Borazjan, and Bardsir	Pre-postintervention Intervention vs. control Follow-up at 3 yr (anthropometry only)	KAP: breastfeeding and child feeding, growth monitoring, family planning, sanitation, drinking water, food production Food-frequency questionnaire for household and child consumption patterns Anthropometry	Changes in KAP and food intake not measured or reported	Decreased prevalence of underweight in intervention group in Borazjan (from 23% to 11%) and Bardsir (from 28% to 14%) ($p < .0001$) Decreased prevalence of underweight in control group in Bardsir (from 24% to 16%, $p < .0001$) Decreased prevalence of stunting across all districts in intervention groups (Ilan: from 25% to 12%; Borazjan: from 41% to 13%; Bardsir: from 31% to 19%; $p < .0001$) Decreased prevalence of stunting in Bardsir controls (from 24% to 16%, $p < .0001$) Decreased wasting only in Borazjan intervention group (from 9% to 4%, $p < .0001$) No statistical testing of differences between intervention and control groups

*Not tested statistically or p values not given.
ARI, acute respiratory infection; KAP, knowledge, attitudes, and practices; NS, not significant; RE, retinol equivalents

TABLE 2B. Animal-source food interventions: Small-animal husbandry or aquaculture to increase production or promotion of animal-source foods

Country, year [reference]	Intervention strategy			Design	Methods	Outcome	
	Production	Nutrition education	Target groups			KAP + dietary intake	Nutritional status
Ethiopia, 2003 [27] Ayele and Peacock	Dairy goats, poultry, home vegetable gardens	Community-based nutrition education to promote increased consumption of goat's milk	Poor, women-headed households (n = 5,500) Children aged 6 mo to 6 yr (n = 39)	Pre-postintervention 2-yr follow-up No control group	Production and consumption of goat's milk, goat meat, and eggs (no. of eggs/household/mo from food-frequency questionnaire and household survey)	Increased milk intake in children* Increased energy, animal protein, total protein, and fat from animal-source foods[a] Some increase in meat intake, but 37% of families still had no meat In households with children, only ~40% of eggs produced were consumed	No measures
Bangladesh, 2003 [28] Nielsen et al.	Semiscavenging poultry production	No	Women of reproductive age and girls from poor rural households	Cross-sectional comparative study Adopters (women [n = 35] and daughters 5–12 yr [n = 35]) vs. nonadopters (women [n = 35] and daughters 5–12 yr [n = 35])	Production, utilization, and consumption of poultry products (24-h recall, qualitative questionnaires)	More eggs per month produced and sold in adopter than in nonadopter households NSD in egg or chicken consumption between the 2 groups Consumption of small fish was higher in adopters than in nonadopters: 58 vs. 39 g fish/person/day (p < .08)	No measures
Bangladesh, 2003 [29] Roos et al., [30] Roos et al.	Homestead fishponds	No	Poor rural households	Observational: fish-producing group (n = 59) vs. non-fish-producing group (n = 25)	7-mo fish production Household fish consumption (5-day recall in 3 seasons) Calcium, iron, and vitamin A intakes determined	No difference in fish intake between groups 98% of households consumed fish on at least 1 of the 5 days surveyed, of which 84% was contributed by small wild indigenous fish Vitamin A–rich "mola" fish contributed 18% of the RDA for vitamin A at the household level	No measures
Malawi, 2005 [31] Radford	Small-animal revolving fund: goats, chickens, guinea fowl, and rabbits	Nutrition education focused on importance of consumption of animal-source foods to prevent anemia	Subsistence farming households	Pre-postintervention Intervention households vs. control households (sample size not reported)	Production, accessibility, and consumption of animal-source foods	Intervention group: increased KAP of animal-source foods to prevent anemia in pregnant women Increased prevalence of small-animal husbandry (from 40% to 72%) postintervention Increased percentage of households consuming produce vs. control group (goat meat, 28% vs. 17%; poultry, 57% vs. 41%; rabbit, 66% vs. 41%; eggs, 68% vs. 51%)	No measures reported

KAP, knowledge, attitudes, and practices; NSD, no significant difference; RDA, recommended daily allowance

a. Not tested statistically or *p*-values not given.

mixed agricultural or livestock production interventions reviewed, 6 included a control group (**table 2A** [18–24]) and **table 2B** [31]). However, even these six projects had limitations in their design and evaluation that compromise the interpretation of the results. Of the remaining four, the two conducted in Bangladesh included comparison groups, which were defined as adopters versus nonadopters [28] (**table 2B**) and fish-producing families versus non-fish-producing families [29, 30], and two were evaluated by a comparison between pre- and postintervention values, with no control group [25, 27] (**table 2A** and **B**).

The five interventions that promoted the consumption (but not production) of animal-source foods through nutrition education and behavior change are summarized in **table 2C**. Of these, three were nonblinded, randomized, controlled trials [33, 35, 36], and two had a quasi-experimentally controlled design with a nonequivalent control group [32, 34]. Three of these interventions targeted women of childbearing age [32, 33, 36]. The two in Peru [32, 33] involved the development and preparation of low-cost, heme-iron-rich meals (e.g., from liver, blood, spleen, and fish) in community kitchens. Consumption of these meals led to increases in intakes of bioavailable iron and vitamin C compared with preintervention levels and to the corresponding changes observed in the control groups. Improvements in intakes of bioavailable zinc (as well as animal protein, vitamin B_{12}, and preformed retinol) probably also occurred but were not measured. In the third, partially blinded, randomized, controlled trial in New Zealand [36], women were encouraged to consume more animal-source foods (as well as to follow other strategies to enhance nonheme iron absorption) through intensive dietary counseling by a research dietician. In this study, intakes of bioavailable zinc were measured, but the data for zinc are not yet published (Gibson RS, personal communication).

The other two interventions summarized in **table 2C** targeted infants rather than women of childbearing age [34, 35]. In the study of infants conducted in China [34], egg yolk was targeted for infant feeding, resulting in the nutrition education and behavior change group feeding more egg yolks (per day) than the comparison group; changes in nutrient intakes, including zinc, were not reported. In contrast, in a very successful effectiveness trial conducted in Peru, intakes of available zinc (and iron) from complementary foods were measured and were significantly increased in children 6 to 18 months old in the intervention compared with the control group [35]. These improvements were attributed to feeding more nutrient-dense, thick complementary foods containing animal-source foods, mainly chicken liver (**table 2C**). This nonblinded trial employed a cluster-randomized design and thus provides strong evidence for the role of animal-source foods, especially liver, in enhancing intakes of bioavailable zinc during complementary feeding [35].

The results of two nonblinded, randomized, controlled trials in which red meat–based foods were supplied to infants (**table 2D)** [38] or schoolchildren [43] by the investigators also provide strong evidence that the consumption of red meat–based foods can enhance intakes of bioavailable zinc; these two trials are summarized in **table 2D**. In both of these trials, the group given red meat had higher [38] or increased [43] intakes of total or available zinc (and iron) than controls. Furthermore, in the study on schoolchildren in Kenya, intakes of vitamin B_{12} and vitamin A also increased in the group receiving red meat compared with the corresponding changes in the control group postintervention. Intakes of calcium, vitamin B_{12}, vitamin A, and riboflavin were also significantly higher postintervention in the group receiving milk than in the control group [43].

By contrast, no improvements in zinc (or iron) intakes were observed in Danish infants [39] given a high-meat diet for 2 months; however, compliance was not reported and the study was small (n = 41). Likewise, when fish powder was supplied to Ghanaian infants [37] or milk was given to Kenyan schoolchildren [43], no improvements in zinc (or iron) intakes as compared with the control group were reported (except for the Ghanaian infants at 7 months of age). These results indicate that red meat, but not milk or powdered fish, can enhance intakes of readily available zinc when consumed by infants and schoolchildren.

Section 1c

Can processing strategies at the commercial or household level enhance zinc absorption from plant-based diets?

Conclusion

There is abundant evidence, based on *in vivo* zinc radioactive or stable isotope studies, that high levels of dietary phytate inhibit zinc absorption (**table 3A**) and that by reducing the phytate content in cereal-based diets through commercially available exogenous phytase enzymes, or phytases naturally occurring in whole-grain cereals, zinc absorption can be enhanced (**table 3B**). Whether zinc absorption can be enhanced through household strategies designed to reduce the phytate content of cereal-based diets (**table 3B**) has not been investigated by isotopic measurements of zinc absorption. Phytate reductions of ~50% have been achieved by soaking pounded maize or maize flour or fermenting maize porridges. Significant increases in zinc absorption have been achieved in men fed meals prepared from maize with a 60% reduction in phytate content compared with absorption from meals made from wild-type maize (**table 3C**), a result suggesting that some improvement in zinc absorption is likely with

TABLE 2C. Animal-source food interventions: Nutrition education and behavior change to promote animal-source foods

Country, year [reference]	Intervention strategy			Design
	Production	Nutrition education	Target group	
Peru, 1998 Carrasco Sanez et al. [32]	Development of recipes and meals high in heme iron, vitamin A, and vitamin C, some of which were consumed by the intervention group	Health and nutrition education in community kitchens Capacity building of local women	Periurban nonpregnant women, aged 15–49 yr	Quasi-experimental design with nonequivalent controls Preintervention (n = 310)/postintervention (n = 189) 1-yr follow-up of intervention group (n = 81) vs. nonmembers as controls (n = 108)
Peru, 2000 [33] Creed-Kanashiro et al.	Development of iron-rich and vitamin C–rich menus	Behavior and nutrition education campaign to increase intake of iron and vitamin C Promote local heme iron sources (chicken liver and blood, spleen, beans) and vitamin C	Adolescent girls aged 12–17.9 yr	Cluster-randomized, controlled trial, pre-postintervention 9-mo follow-up Intervention group (n = 71) vs. nonequivalent control group (n = 50)
China, 2000 [34] Guldan et al.	NA	Nutrition education and counseling visits to increase breastfeeding and quality and quantity of complementary foods from 4 mo of age (e.g., give egg yolk daily after 46 mo)	Infants aged 4–12 mo from rural China	Quasi-experimental design with nonequivalent controls. Pre-postintervention 1-yr follow-up Intervention group (n = 250) vs. control group (n = 245)
Peru, 2005 [35] Penny et al.	NA	Nutrition education to increase intake of thick purees and animal-source foods and increase practice of responsive feeding Demonstrations of complementary food preparation Accreditation system in government health facilities	Birth cohort: infants from a poor, periurban area, followed from 0 to 18 mo	Cluster-randomized trial (nonblinded) Pre-postintervention Intervention (n = 187) vs. control (n = 190) 18-mo follow-up
New Zealand 2001 [36] Heath et al.	NA	Individual dietary counseling to diet group only to increase intake and bioavailability of dietary iron; also provided with a cast-iron fry pan and fruit juice	Premenopausal women (n = 75), aged 18–40 yr with mild iron deficiency[b]	Randomized, controlled trial 3 groups: placebo, iron supplement (50 mg iron/day), diet group (not blinded) 16-wk follow-up

KAP, knowledge, attitudes, and practices; LAZ, length-for-age z-score; NA, not available; NSD, nonsignificant difference; SES, socioeconomic status; TfR, serum transferrin receptor; WAZ, weight-for-age z-score

a. Not tested statistically or p-values not given.

b. Mild iron deficiency is defined as serum ferritin < 20 μg/L and hemoglobin ≥120 g/L, in the absence of infection (i.e., elevated C-reactive protein).

	Outcomes	
Methods	KAP + dietary intake	Nutritional status
Interviews Focus groups 24-h recall Hemoglobin	Improved quality of meals: Increased iron, heme iron, vitamin A, and vitamin C content Increased intake of iron and vitamin C–rich foods Increased intake of heme iron, bioavailable iron, and vitamin C in intervention group vs. controls No increase in vitamin A in intervention group vs. controls	Decreased prevalence of anemia (from 49% to 41%) postintervention ($p < .05$) in intervention group Note: Not compared with "control" group of nonmembers
KAP assessment: food, nutrition, health, and anemia 2 24-h recalls (pre- and postintervention) Hemoglobin, serum ferritin, height and weight measures	Intervention vs. control: Increased heme iron and anemia KAP Increased use of animal-source foods in menus Increased intakes of total iron (from 7.75 ± 3.5 to 9.42 ± 5.0 mg/day, $p < .01$) vs. no change in controls; heme iron (from 0.21 ± 0.17 to 0.66 ± 1.35 mg/day, $p < .01$) vs. no change in controls; vitamin C (from 44 ± 39.6 to 67 ± 45 mg/day, $p < .05$) in intervention group vs. from 41 ± 34.6 to 40 ± 27.6 mg/day in controls [NSD]); absorbable iron (from 0.33 ± 0.16 to 0.43 ± 0.41 mg/day) vs. no change in controls (from 0.35 ± 0.13 to 0.37 ± 0.22 mg/day)	Change in anemia prevalence: from 14.1% to 12.3% (NSD) in intervention group vs. from 14% to 37.5% in control group ($p < .01$) Change in prevalence of iron deficiency (measured by serum ferritin): from 21.1% to 18.5% (NSD) in intervention group vs. from 14% to 25% in control group (NSD)
Weight and length Single 24-h recall; food-frequency questionnaire KAP: Infant feeding and health-related behaviors Hemoglobin measures	Intervention group had greater nutrition knowledge, higher breastfeeding rates (83% vs. 75%, $p = .034$), and better reported infant-feeding practices vs. controls ($p < .05$) Intervention group had greater no. of eggs fed per day to children 4–9 mo than controls	NSD in growth between 2 groups before age of 12 mo; at 12 mo, WAZ in intervention group was –1.17 vs. –1.93 for controls ($p = .004$), HAZ was –1.32 vs. –1.96 for controls ($p = .022$), and prevalence of anemia was 22% vs. 32% for controls ($p = .008$)
Home interviews and observations for SES, hygiene, and feeding practices Weight and length 24-h recalls: intake of complementary foods at 6, 9, 12, and 18 mo Morbidity (over past 24 h) at same visits	Intervention vs. control group: More caregivers received nutrition education (16/31 [52%] vs. 9/39 [24%]; $p = .02$) More infants were fed nutrient-dense thick purees at 6 mo (31% vs. 20%, $p = .03$) Higher intake of energy from animal-source foods at 15 mo ($p = .082$) and 18 mo ($p = .001$) Fewer children failed to meet requirements for energy (8 and 12 mo), iron (8 and 9 mo), and zinc (9 mo) ($p < .05$)	Intervention group had higher housing and hygiene scores, education level, and body weight than controls at baseline: analysis performed without and with adjustment Stunting at 18 mo: intervention 5% vs. control 16% ($p = .02$; adjusted odds ratio, 3.04; 95% CI 1.21 to 7.64) Adjusted mean changes in WAZ and LAZ better in intervention than in control group at 18 mo ($p < .05$)
Iron-specific validated food-frequency questionnaire, serum ferritin, hemoglobin, serum TfR, C-reactive protein	Diet group: increased intake of flesh foods, heme iron, vitamin C, foods cooked in cast-iron cookware ($p < .05$); decreased intake of phytate ($p < .05$) vs. placebo group	Diet group: 26% increase in serum ferritin vs. placebo group (NSD) 22% decrease in serum TfR:serum ferritin ratio vs. placebo group (NSD)

TABLE 2D. Nutritional improvement of home-prepared complementary foods or school lunches from inclusion of animal-source foods

Country, year [reference]	Intervention strategy			Design	Methods	Outcome	
	Production	Nutrition education	Target groups			KAP + dietary intake	Nutritional status
Ghana, 1999 [37] Lartey et al.	No	No	Healthy breast-fed infants ≥ 2.5 kg at birth	Randomized, controlled trial for 6 mo, with 4 treatments—Weanimix (W), W + vitamins and minerals (WVM), W + fish powder (WF), and koko with fish power (KF)—compared with nonintervention group (NI) (n = 464)	Monthly 24-h recalls for 3 days (6–12 mo), 12-h weighed records for 50% of subjects (zinc intake from weighed records). Morbidity, anthropometry, hemoglobin, hematocrit, C-reactive protein, plasma zinc, serum retinol, serum ferritin, TIBC, RBC B-2	No differences in intakes between W, WF, and KF groups except at 7 mo, when zinc and iron intakes were higher in KF than W group (p < .05)	No significant differences among 4 groups in morbidity outcomes, weight and length gain, differences in head circumference, mid-upper-arm circumference, skinfolds, arm fat area, arm muscle area, plasma zinc, hemoglobin, hematocrit, transferrin saturation, or RBC B-2 at any time. WAZ and LAZ scores of NI group were lower (p<0.05) than combined intervention group at 6 and 9 mo of age. Significant increase in percentage with low ferritin between 6 and 12 mo in W, WF, and KF groups but not in WVM group (p < 0.05) Change in plasma retinol was significantly greater in WM between 6 and 12 mo than in other 3 groups combined (0.14 ± 0.3 vs. −0.04 ± 0.3 μmol/L, p = .003) All 4 foods improved growth relative to nonintervention group (p < .001)
USA, 2006 [38] Krebs et al.	No	No	Exclusively breastfed, healthy, infants followed from ~6 to 12 mo	Randomized, controlled trial Pureed beef (n = 46) vs. iron-fortified infant rice cereal (n = 42) as first complementary food at ~6 mo, plus fruits and vegetables as desired 9-mo follow-up	2 3-day diet records/mo for 4 visits (+ zinc intake) Rating scale of infant's acceptance of complementary food Anthropometry, developmental testing (Bayley scale), hemoglobin and hematocrit, serum ferritin, somatomedin, plasma zinc	Mean zinc intake higher in meat than cereal group at 5 and 7 mo (p < .001) Tolerance and acceptance of beef and cereal comparable	Greater increase in head circumference in meat than in cereal group Zinc and protein significant predictors of head growth No biochemical differences between groups Trend toward higher behavior index at 12 mo in meat than in cereal group (p = .08)

Denmark, 1998 [39] Engelmann et al.	No	No	Healthy, term, partially breastfed infants, aged 8 mo (n = 41)	Randomized trial Low-meat group (LMG) 10 g meat/day (n = 20) vs. high-meat group (HMG) 27 g meat/day (n = 21) 2-mo follow-up	Food records: 24-h weighed food record 1/wk Anthropometry, serum zinc, hemoglobin, serum ferritin, TfR, morbidity (frequency of illness)	Despite higher intakes of meat and iron from meat, differences in intakes (mg/day, range) of total iron in HMG (3.1, 0.4–6.2) vs. LMG (3.4, 1.4–6.1) or zinc in HMG (3.3, 0.4–5.2) vs. LMG (3.2, 1.4-5.2) were not significant	Change in hemoglobin: LMG –4.9 (–12.9 to –5.6 g/L) vs. HMG –0.6 (–12.1 to –7.3 g/L) (p = .008) NSD in change in serum ferritin, TfR, or serum zinc No differences in morbidity between LMG and HMG
Kenya, 2003, 2006 [40, 41] Grillenberger et al. [42] Murphy et al. [43] Neumann et al. [44] Siekmann et al. [45] Whaley et al.	No	No	Undernourished schoolchildren aged 5–14 yr (n = 554)	Children randomized by school to 3 treatments—meat (n = 134), milk (n = 144), or energy (n = 148) supplement)—or control (n = 129) group 23-mo follow-up	Physical examination and health status Anthropometry 3 24-h recalls Cognitive, behavioral. and activity measurements Morbidity Biochemical measures: zinc, iron, copper, retinol, folate, vitamin B_2, and vitamin B_{12} At baseline, across all groups and independently of intervention, children with *Entamoeba histolytica* infection had smaller increases in serum iron but greater increases in plasma retinol (p = .01), whereas those with enlarged spleens had smaller increases in plasma vitamin B_{12}	Intakes of calcium, vitamin A, vitamin B_{12}, and vitamin B_2 increased more in milk group than in controls Intakes of available zinc, available iron, calcium, vitamin B_{12}, and vitamin B_2 increased more in meat group than in controls	In all 3 treatment groups, weight gain was greater than in controls (p < .05) No effect of treatments on height, HAZ, WHZ, or body fat Gain in arm muscle area was greater in meat group than in milk, energy, or control groups Among those with lower HAZ (≤ –1.4), those in milk group gained 1.3 cm more height than controls (p = .05) and 1 cm more height than those in meat group (p = .09), but there were no effects on change in HAZ Meat group had greater increase in cognitive performance (especially on Raven's Progressive Matrices) than all other groups No improvements in biochemical zinc, iron, copper, folate, retinol, or vitamin B_2 status Vitamin B_{12} status increased in meat and milk groups compared with energy and control groups

HAZ, height-for-age z-score; KAP, knowledge, attitudes, and practices; NSD, nonsignificant difference; RBC B-2, red blood cell vitamin B_2 (riboflavin); TfR, transferrin receptor; TIBC, total iron-binding capacity; WAZ, weight-for-age z-score; WHZ, weight-for-height z-score

a 50% phytate reduction, the magnitude depending on the composition of the habitual diet, zinc intake, and life-stage group. Reductions in the phytate content of cereal- and legume-based diets will also increase absorption of nonheme iron and calcium. Whether zinc absorption can be improved *in vivo* by the addition of enhancers such as low-molecular-weight organic acids produced during fermentation has not been confirmed by isotope studies; only *in vitro* zinc dialyzability has been measured.

At present, there does not appear to be any evidence of up-regulation of zinc absorption during infancy and childhood to help meet the additional zinc requirements for growth. Hence, for complementary diets containing predominantly plant-based foods, phytate reduction strategies without a concomitant increase in animal-source foods are unlikely to increase the intake of absorbed zinc to a level that meets the desired zinc density for complementary foods set by the World Health Organization (WHO) [46] (**table 3D**). Even for older children consuming predominantly plant-based diets, again a combination of strategies that includes red meat or liver is probably needed to ensure their estimated average requirements for zinc are met [47]. In both cases, however, *in vivo* zinc isotope absorption studies are needed to confirm these conclusions.

Detailed review of evidence

The first part of this conclusion is based on a review of six tracer studies that measured and compared apparent or fractional zinc absorption in low- and high-phytate diets (**table 3A**). Four of these studies were performed in adults [48–50, 52], one was performed in young children [53], and one was performed in nursing rhesus monkeys and nursing rat pups [51]. The results have shown consistently that zinc absorption is significantly lower from the high-phytate than from the low-phytate diets (**table 3A**). Further, the results of two of the three stable isotope studies summarized in **table 3B**, in which dephytinization was achieved through the use of commercial phytase enzymes from *Aspergillus niger* [54], or by naturally occurring phytase enzymes [56], showed increases in zinc absorption in the dephytinized soybean isolate and wheat-soy blend porridge compared with zinc absorption from soybean isolate and wheat-soy blend porridges with their native phytate content. In contrast, in a hospital-based study in school-aged children in Malawi [55], which also used a corn-plus-soy porridge dephytinized with *A. niger*, fractional zinc absorption was increased and endogenous zinc losses decreased only in the children recovering from tuberculosis, but not in the apparently well children. Restriction of this response to the children experiencing catch-up growth suggested that zinc absorption may be up-regulated by ZIP4 in response to the high zinc requirements for catch-up growth [57]. ZIP4 is the principal zinc transporter responsible for regulation of zinc absorption across the apical membrane of the enterocyte [58].

In the three stable isotope studies summarized in **table 3B**, the commercial strategies used completely degraded the phytate content of the cereal-based test meals. However, when household strategies such as soaking, germination, or fermentation are used, only about 50% of the phytate content can be removed [59].

The level of phytate reduction required to yield a marked improvement in fractional zinc absorption (FAZ) in population groups consuming cereal- and legume-based diets will vary with the composition of the cereal-legume blend, zinc intake, life-stage group, and health and nutritional status. The results of two short-term (single-day) stable isotope studies (**table 3C**) [60, 61] reported a FAZ of 30% and 28% in adults fed 60% phytate-reduced maize and a FAZ of 38% in adults fed 80% phytate-reduced maize, compared with levels ranging from 14% to 17% for the corresponding wild-type maize with typical phytate content. Increases in absorption of iron and calcium have also been reported among adults fed phytate-reduced maize [63–65].

Nevertheless, the results of a stable isotope study of zinc absorption in Guatemalan schoolchildren fed normal or low-phytate maize (**table 3C**) [62] have emphasized the difficulty of predicting the increase in FAZ that may occur when maize with a 50% reduction in phytate content achieved through household strategies is used. Although FAZ is known to be positively associated with the extent of phytate reduction (**table 3C**) [61], maize is not the only source of phytate in household diets. Moreover, because FAZ is also dependent on the quantity of zinc ingested, declining with increasing quantities of zinc consumed [57], this effect may counteract any increase in FAZ arising from a reduction in dietary phytate. Indeed, an unexpected increase in zinc intakes may have been responsible in part for the failure to observe any increase in FAZ among Guatemalan children fed low-phytate maize; these results are also summarized in **table 3C** [62].

Interventions employing household phytate reduction strategies (and others) are summarized in **table 3D** [66–73]. The Malawian intervention studies summarized in **table 3D** [70–73] promoted a combination of dietary strategies to enhance both the content and the bioavailability of zinc in the maize-based Malawian diets. These included strategies to increase the content of zinc-absorption enhancers such as organic acids through fermentation, as well as the phytate-reducing strategies noted earlier, in an effort to further enhance the content and bioavailability of zinc. Low-molecular-weight organic acids produced during fermentation have the potential to chelate zinc (and iron), thereby making zinc unavailable for complex binding with

phytate. Moreover, the organic acids generate an optimal pH for the activity of any endogenous phytases in cereal or legume flour [74] and may also lower the gastric emptying rate [75]. The latter effect will thus theoretically increase the exposure of zinc to the proximal intestinal epithelium, thereby potentially increasing zinc absorption. However, the magnitude of the enhancing effect, if any, of organic acids on zinc absorption is unknown. Most of the evidence has been based on *in vitro* dialyzability of zinc [76] and needs to be confirmed by *in vivo* zinc isotope absorption studies.

Zinc absorption may also be improved by increasing the amount of dietary protein, the magnitude of the effect being enhanced when animal proteins from meat [77, 78] or cow's milk or yogurt [79] are components of the diet. Hence, in the Malawian studies [71–73], intake of animal-source foods was also promoted, because theoretically, increasing the content of animal-source foods in predominantly high-phytate plant-based diets should not only increase the content of zinc ingested but also enhance FAZ. However, the magnitude of the increase in FAZ will also depend on the adequacy of the baseline zinc intake, since zinc absorption is dose-dependent [80].

In the studies in rural southern Malawi among infants and young children, zinc absorption was not measured. A quasi-experimental design with a nonexperimental control group was used, and the strategies were promoted through nutrition education and social marketing (**table 3D**) [70–73]. Intakes of available zinc were higher ($p < .001$) in the intervention than the control groups postintervention, both in the study on infants [73] and in the study on young children [70, 71] as a result of higher intakes of animal-source foods (mainly small, whole, soft-boned fish), phytate reduction strategies, and use of germinated cereals to enhance the energy and nutrient contents of the maize-based porridges. Nevertheless, the median intake of zinc in the intervention group for the weanling study did not meet the age-specific WHO estimated needs for infants [46], whereas among the young children, 26% in the intervention group and 44% in the control group had inadequate intakes of zinc at the end of the intervention ($p = .002$) [71]. However, the results of the latter study are limited by the absence of baseline intake data due to unforeseen circumstances, so that no adjustments for baseline differences in intakes could be made [71].

Section 2

Can complementary foods nutritionally improved through dietary diversification or modification strategies have an impact on the zinc status and the health and development of breastfed infants and young children?

Conclusion

Enhancing the complementary diets of breastfed infants and young children by the addition of red meat or liver can have a positive impact on their growth and some aspects of development, even though an improvement in biochemical zinc status per se may not always be evident. Strategies designed to enhance zinc absorption through phytate reduction or use of organic acids alone will not be efficacious in terms of improving zinc nutriture in infants or young children unless a substantial amount of red meat or liver is also included in their diets.

Detailed review of evidence

The first part of this conclusion is based on a review of five interventions that either supplied or promoted the consumption of animal-source foods. Of these, four were nonblinded, randomized, controlled trials conducted in Ghana, the United States, Denmark (**table 2D**) [37–39], and Peru (**table 2C**) [35], whereas the fifth was a study in China based on a quasi-experimental design (**table 2C**) [34]. In the randomized, controlled trials in the United States [38] and Peru [35] and the quasi-experimental study in China [34], significant increases in growth were reported in the intervention compared with the control group, although in the US study, the increase was in head circumference and not length or weight gain, as in the other two studies. In the US [38] and Peru [35] studies, greater increases in intakes of both animal-source foods and zinc in the intervention than in the control groups were also reported. Changes in nutrient intakes were not assessed in the Chinese study [34].

It is of interest that in the two studies on infants in China and Peru (**table 2C**) [34, 35], in which animal-source foods were not supplied but their consumption was vigorously promoted by a strong nutrition education component, marked increases in weight and length gain were reported in the intervention compared with the control groups.

These findings are in contrast to those of the randomized, controlled trial conducted in Danish infants (**table 2D**)[39], but the intervention period of this trial was short (2 months), the number of infants was small (41 in total), and no details of compliance were reported. There were also no improvements in zinc intake or serum zinc in the intervention compared with the control group in the Danish trial. Likewise, in the Ghanaian trial (**table 2D**) [37], no increase in infant growth was reported when fish powder was used to enrich fermented maize-based or cereal-legume-based complementary foods. In this study, although there was no control group, growth was significantly greater in all the treatment groups than in the nonintervention group ($p < .001$).

TABLE 3A. Isotope studies investigating the effect of phytate on zinc absorption

Country, year [reference]	No. and age of subjects	Inclusion criteria	Design	Methods	Results
Sweden,1984 [48] Lönnerdal et al.	58 subjects aged 20–30 yr	Healthy, zinc-replete adults	Adults fed either soy isolate formula (phytate:zinc, 6:1), soy flour (10:1), cow's milk formula (0 phytate), cow's milk + phytate level 1 (6:1), or cow's milk + phytate level 2 (19:1)	Radioisotope technique using whole-body counting of radioisotope ^{65}Zn, 14 days after intake of test meal	Apparent zinc absorption via ^{65}Zn from soy isolate: 14.0% ± 1.0%; soy flour: 8.2% ± 1.5%; cow's milk: 32.2% ± 1.4%; cow's milk + phytate level 1: 15.8% ± 0.8%; cow's milk + phytate level 2: 9.4% ± 0.8%
USA, 1984 [49] Turnlund et al.	4 men aged 25–32 yr	Healthy young men	Crossover design. 63-day metabolic study comparing basal diet, basal diet + α-cellulose (34.4 g/day), or basal diet + phytate (2.34 g phytate/day)	Zinc absorption determined by isotopic ratio of ^{67}Zn/^{70}Zn from complete fecal and urine excretion over 63 days	FAZ 34% in basal diet, vs. 33.8% with addition of α-cellulose (NSD) and 17.5% with addition of phytate ($p < .02$)
Sweden, 1985 [50] Nävert and Sandström	42 adults (17 M, 25 F) aged 20–52 yr	Healthy, zinc-replete, nonpregnant adults without gastrointestinal disorders	Reduction of phytate content of bran by leavening/fermentation of bread containing bran Zinc absorption measured with different phytic acid concentrations 10–14-day metabolic balance period	Bread dough fermented/leavened for 15 min, 45 min, 3 h, or 16 h; phytic acid concentration and phytate:zinc molar ratios determined Apparent zinc absorption from bread measured by whole-body retention of radioisotope (^{65}Zn) 10–14 days after intake of test meal	60% decrease in phytate with 2 h fermentation; maximum decrease (80% –85%) after 2 days of fermentation After fermentation, phytate:zinc molar ratio and FAZ were 16 and 9.6%, respectively, after 15 min; 16 and 11.9% after 45 min; 8 and 14.7% after 3 h; and 4 and 19.8% after 16 h Fermentation of bread containing bran reduces its phytic acid content and increases FAZ
USA, 1988 [51] Lönnerdal et al.	10 infant rhesus monkeys aged 1.5 mo and 30 rat pups, aged 14 days	Nursing infant monkeys and nursing rat pups	Animal models used to study the effect of phytate removal on zinc absorption from soy formula. Crossover design (for monkeys). Animals fed regular soy formula (0.621 mmol/L phytic acid) or dephytinized soy formula (0.067 mmol/L) 14-day washout between test meals	Formula dephytinized through extraction and precipitation process[a] Zinc absorption determined by whole-body counting; 14 days postintubation for monkeys, 6 h for rats	FAZ in monkeys: 27% from regular soy formula vs. 45% from dephytinized soy formula. FAZ in rats: 16% from regular soy formula vs. 47% from dephytinized soy Low bioavailability of zinc from soy formula is a function of its phytate concentration and can be overcome by removal of phytate
Denmark, 1989 [52] Kivistö et al.	33 adults (13 M, 20 F) aged 21–42 yr	Healthy, nonpregnant, zinc-replete adults, without known gastrointestinal disorders	Adults fed crispbread with normal or reduced phytate content (part of larger study on nutritional impacts of extrusion methods). Meals served after overnight fast. 10–14-day metabolic balance period	Crispbread: 20% bran, 60% starch, 10% gluten Apparent zinc absorption from test meal measured by whole-body retention 10–14 days after test meal, oral and intravenous ^{65}Zn	Normal crispbread: phytate:zinc molar ratio, 22; FAZ = 6.2% Phytate-reduced crispbread: phytate:zinc molar ratio, 2.8; FAZ = 17.6% Higher FAZ with reduced phytate content of bran

USA, 2006 [53] Etcheverry et al.	38 children (15 M, 23 F) aged 4–8 yr	Healthy, non-allergy-suffering children, not taking medication or supplements at time of study	Test meal made from either beef or soy protein 1 day test meal, urine collection at 48 h Note: Beef meal had 135 mg phytate per serving, soy meal had 144 mg phytate per serving	Zinc absorption calculated by multitracer stable isotope techniques; ratios of oral (^{67}Zn) and intravenous (^{70}Zn) urinary excretion of zinc tracers 48 h after dosing	Mean ± SD FAZ from beef meal (13.7 ± 6.0%) greater than that from soy meal (10.1 ± 4.1%), p = .047. Even low-phytate soy protein inhibits absorption in comparison to meat; 35% enhanced FAZ from beef protein meal compared with low-phytate soy

FAZ, fractional absorption of zinc; NSD, nonsignificant difference

a. Puski G, Harman GH, Talbott RD, inventors; Mead Johnson and Co, assignee. Process for preparing low phytate soy protein isolate. US Patent 4,697,004. 1987 Sept 29.5p. Int Cl4 A23J1 1/14.

TABLE 3B. Isotope studies investigating the effect of phytate reduction by phytase enzymes on zinc absorption

Country, year [reference]	Subjects	Inclusion criteria	Design	Methods	Results
USA, 2004 [54] Davidsson et al.	9 infants (5 M, 4 F) aged 69–191 days	Healthy, full-term infants, formula-fed since birth	Crossover design. Infants fed regular soy formula (250–300 mg/kg phytate) or dephytinized (< 6 mg/kg phytate) formula in random order Each study included a 10-day washout period followed by a 3-day metabolic balance period	Soybean protein isolate dephytinized with the use of phytase from *Aspergillus niger* Apparent zinc absorption measured by stable isotope ^{70}Zn via 72-h fecal excretion of nonabsorbed stable isotope and by chemical balance technique	Apparent zinc absorption measured by ^{70}Zn isotope: 16.7% from regular vs. 22.6% from dephytinized soy formula (p = .03) (95% CI, 0.9–10.8)
Malawi, 2000 [55] Manary et al.	23 children (11 M, 12 F) aged 3–13 yr	Recruited at local hospital (patients recovering from tuberculosis [n = 14] or with minor injuries, those awaiting elective surgery, or healthy siblings (i.e., well children [n = 9])	Children randomly assigned to either reduced phytate corn-plus-soy diet or standard high-phytate corn-plus-soy diet were served 5 times per day for 37 days, after which the children participated in a 7-day zinc stable isotope study	Corn-soy porridge dephytinized with the use of phytase from *A. niger* Oral and intravenous zinc stable isotopes ^{67}Zn and ^{70}Zn and 7-day urine and stool collections	No significant effect of phytate reduction in well children (n = 9); in children recovering from tuberculosis (n = 14), dietary phytate reduction resulted in higher FAZ compared to high-phytate corn-plus-soy diet (0.41 ± 0.14 vs. 0.24 ± 0.09, p < .05) and TAZ (169 ± 55 vs. 100 ± 46 μg/kg/day, p < .05)
Switzerland,2004 [56] Egli et al.	9 adults (2 M, 7 F) aged 22–34 yr	Healthy, nonpregnant adults, without gastrointestinal disorders, receiving no medication (other than oral contraceptive agents)	Crossover design. Adults were fed processed, dephytinized wheat- and soy-based complementary food (< 0.03 mg/g phytate) or unprocessed wheat- and soy-based complementary food (4 mg/g phytate) 2–4-wk period between foods	Wheat- and soy-based complementary food dephytinized through use of native phytases vs. unprocessed (native phytic acid concentration) Apparent fractional zinc absorption measured by stable isotope ^{70}Zn from 7-day fecal excretion	Significantly higher apparent zinc absorption from dephytinized complementary food than from unprocessed food with native phytic acid concentration: FAZ = 34.6 ± 8.0% vs. 22.8 ± 8.8% (p = .005)

FAZ, fractional absorption of zinc; Phy:Zn, phytate to zinc molar ratio; TAZ, total absorption of zinc

TABLE 3C. Isotope studies investigating the effect of phytate reduction by the use of low-phytate maize on zinc absorption

Country, year [reference]	Subjects	Inclusion criteria	Design	Methods	Results
USA, 2002 [60] Adams et al.	5 adults (2 M, 3 F) aged 24–39 yr	Healthy adults, not consuming supplements at time of study	Crossover design. Adults fed regular maize polenta or low-phytate maize polenta in random order as their only food for 2 days (^{70}Zn for day 1 and ^{67}Zn for day 2)	Polenta prepared from low-phytate mutant maize *(lpa1-l)* (60% phytate reduction) or wild-type, normal phytate-level maize FAZ measured by stable isotope ^{70}Zn and ^{67}Zn from fecal samples collected for 8–11 days (8 samples per subject)	Phytate:zinc molar ratio 17:1 and FAZ 0.30 ± 0.13 for *lpa1-1;* 36:1 and 0.17 ± 0.11 for wild type maize ($p < .005$) Substitution of a low- phytate grain in a maize-based diet is associated with a 78% increase in zinc absorption
USA, 2004 [61] Hambidge et al.	10 adults (6 M, 4 F) aged 21–37 yr	Healthy adults habitually consuming a non-vegetarian diet	Crossover design (different maize eaten each day). Group A: 6 adults fed tortillas prepared from 1 of 2 types of low-phytate mutant maize. Group B: 4 adults fed 1 of 2 types of wild-type maize. Comparison of overall FAZ for each low-phytate and corresponding wild-type in relation to their dietary phytate and phytate:zinc molar ratios Each subject was fed 1 maize hybrid for 1 day and then the other maize hybrid on the following day, the order alternating between subjects for group A and group B. The 2-day test meal study period was followed by a 9-day metabolic balance period	Group A: *lpal-1*-LP (60% phytate reduction), and ND-LP (80% phytate reduction). Group B: corresponding wild-types; *lpal-1-WT* (wild type), and ND-WT All test meals were extrinsically labeled with ^{67}Zn on day 1 and ^{70}Zn on day 2. FAZ was measured by the dual-isotope-tracer ratio technique. A third tracer (^{68}Zn) was administered intravenously during the evening of day 1. Timed urine samples were collected twice daily from days 4 to 9, after administration of 1st tracer	Mean (± SD) FAZ values for tortillas prepared from ND-LP, *lpal-l*-LP, *lpa1-1*-WT, and ND-WT were 0.38 ± 0.07, 0.28 ± 0.04, 0.15 ± 0.07, and 0.13 ± 0.05, respectively. A negative correlation existed between FAZ and dietary phytate and phytate:zinc ($p < .001$) FAZ from maize tortillas is positively related to the extent of phytate reduction achieved with low-phytate hybrids
Guatemala, 2006 [62] Mazariegos et al.	60 children (29 M, 31 F) aged 6–11 yr	Healthy children, family willing to consume randomly assigned study maize provided	Cross-sectional, free-living study. Children randomly assigned to consume either low-phytate maize *(lpa1-1)* (~60% phytate reduction) ($n = 20$), wild-type isohybrid (WT) ($n = 20$), or locally grown maize (L) ($n = 20$) for 10 wk. In final week, 1-day stable isotopic testing by dual isotope ratio technique as in Hambidge et al. [61]	Test day: duplicate diets collected for analysis of zinc and phytate content Note: Low power because of high inter- and intragroup differences in phytate intake	Mean (± SD) FAZ values for *lpa1-1*, 0.32 ± 0.07, vs. WT (wild-type), 0.28 ± 0.07, vs. local maize, 0.29 ± 0.06.(NS) FAZ was not increased by the long-term use of low-phytate maize in children whose major dietary staple is maize, possibly because of unexpected increase in zinc intakes

FAZ, fractional absorption of zinc; *lpal-1*-LP, *lpal-1*-low-phytate; *lpal-1-WT, lpal-1*-wild-type; ND-LP, Nutridense low-phytate; ND-WT, Nutridense wild-type isohybrid; NS, not significant

Additional functional health outcomes that have been investigated include developmental outcomes in the US study (**table 2D**) [38] and morbidity in both the Danish [39] and the Ghanaian [37] studies. The US infants fed pureed beef tended to have a higher behavior index score than their counterparts fed fortified rice cereal [38], whereas no significant differences in morbidity were observed between the groups in the Danish [39] and Ghanaian [37] studies.

It is of interest that improvements in growth, health, or development were not reported in the intervention as compared with the control groups in two randomized, controlled trials of complementary feeding in Sweden and Tanzania (**table 3D**), both of which were based on phytate reduction strategies without any increase in the intake of animal-source foods. In the Swedish trial (**table 3D**) [68, 69], consumption of phytate-reduced products (achieved by a commercial hydrothermal process using endogenous phytases) by Swedish infants from 6 to 12 months of age had no effect on physical growth, development, morbidity, or biochemical zinc status compared with the control group. However, only 22% of the infants overall were zinc-deficient at baseline (serum zinc < 10.7 μmol/L), and the mean dietary phytate:zinc molar ratio, even for the control group, was below that said to markedly compromise zinc absorption (< 10:1). In the trial in Tanzania [66, 67], the high phytate:zinc molar ratio of the complementary food in the treatment group, even after processing, may be responsible, at least in part, for the lack of improvement in growth [66, 67], hair zinc level [66], hemoglobin concentration, or zinc protoporphyrin concentration [67] in the intervention compared with the control group. The results of the Tanzanian study suggest that phytate reduction strategies alone are not sufficient to ensure that intakes of bioavailable zinc (and iron) meet the needs for optimal growth and prevention of anemia in infants fed predominantly plant-based complementary diets.

Section 3

Can foods nutritionally improved through dietary diversification or modification have an impact on the zinc status and the health and development of children and women of reproductive age?

Conclusion

Enhancing the diets of children in the household by the addition of animal-source foods to the diet or promotion of their use, with or without phytate reduction strategies, can have positive impacts on body composition, growth, cognitive functioning, and intakes of bioavailable zinc, depending on the source of animal-source foods, even though improvements in biochemical zinc status may not always be evident (**tables 2D** and **3D**). Such strategies should simultaneously enhance biochemical vitamin B_{12} status, and possibly iron and hemoglobin status, depending on the baseline iron status of the study group. Among women of reproductive age, specific data on zinc status are very limited. Depending on their initial nutritional status, increases in the content of animal-source foods in the diet may in some circumstances increase serum zinc, maintain or even enhance biochemical iron status, improve hemoglobin, and reduce anemia prevalence in young women of reproductive age.

Detailed review of evidence

The conclusion related to children is based on one cluster-randomized, controlled trial (not blinded) conducted on Kenyan schoolchildren (n = 554; median age, 7.4 years) (**table 2D**) [40–45] and one quasi-experimentally controlled study with a nonequivalent control group undertaken on rural Malawian children (n = 281) aged 3 to 7 years (**table 3D**) [70–72]. In both studies [40, 72], improvement in muscle mass (based on anthropometry) was observed in the intervention group, which was reported to have a higher intake of cellular animal protein than the controls, although no improvement in biochemical zinc status was apparent in any group. In the Kenyan study (**table 2D**), improvements in body weight [40] (but not height) and in certain domains of cognitive functioning [45] were also reported in the meat-based intervention group compared with the control group. In contrast, in a subgroup of the Kenyan children with lower baseline height-for-age z-score (HAZ) (≤ −1.4) who received the milk-based snack, an improvement in height was observed compared with the controls [40, 41]. An additional reported positive effect of these interventions was improvement in biochemical vitamin B_{12} status in Kenyan children receiving the meat- or milk-based snack compared with controls [44]; furthermore, in Malawian children, despite a fall in hemoglobin of 7 g/L in the control group, there was no significant change in hemoglobin in the intervention group after 1 year, and the incidence rates of anemia and common infections were lower than in the control group, after adjustment for baseline differences [72]. Increases in intakes of several other micronutrients in the intervention compared with the control group were also apparent in these two studies.

The conclusion for women of reproductive age is based on two randomized, controlled trials (one cluster-randomized trial undertaken in Peru [33] and another trial in New Zealand [36]) and a third trial with a quasi-experimental design, also conducted in Peru (**table 2C**) [32]. All three interventions emphasized nutrition education and behavior change to promote the consumption of animal-source foods. The Peruvian

TABLE 3D. Mixed interventions: Phytate reduction strategies with and without other household methods to enhance content and bioavailability of dietary zinc

	Intervention strategies		
Country, year [reference]	Production	Nutrition education	Target groups
Tanzania, 2006, 2004 [66] Lachat et al., [67] Mamiro et al.	Processed complementary food (PCF) with reduced phytate content: mean (± SD) 660 ± 20 mg/100 g DM prepared from germinated millet, kidney beans, roasted peanuts, mango puree; Unprocessed complementary food (U-PCF) with phytate content: 1150 ± 30 mg/100 g DM; Phytate:zinc molar ratios: 25.8 for PCF vs. 47.5 for U-PCF; mothers added oil to PCF and U-PCF	No	Infants aged 6 mo at baseline (n = 309)
Sweden 2003, 2004 [68, 69] Lind et al.	Phytate-reduced (PR) milk-based cereal drink (MCD) (PRMCD) and phytate-reduced porridge (PRP)	No	Infants aged 6-mo at baseline (n = 300)
Malawi, 2003, 2002, 2005 [70] Gibson et al. [71, 72] Yeudall et al.	Distribution of sunflower, pigeon pea, soybean, and papaya seedlings Solar drying Agricultural training Food preparation and processing to reduce phytate content	Social marketing Face-to-face communication Nutrition education to increase intakes of iron, zinc, and vitamin A and modify food-processing techniques Health education	Children aged 3–7 yr from subsistence farming households
Malawi, 2005 [73] Hotz and Gibson	Food preparation and processing to increase energy and nutrient density and decrease phytate content of complementary porridges	Nutrition education to increase intakes of iron, zinc, and vitamin A Food-processing techniques, health education	Mothers and their breastfed children aged 6–23 mo

AMA, arm muscle area; MUAC, mid-upper-arm circumference; NA, not available

Design	Methods	Outcomes	
		KAP + dietary intake	Nutritional status
Double-blind, randomized, controlled trial. Intervention (n = 157) vs. controls (n = 152) Infants supplied with PCF or U-PCF 6-mo follow-up (at 12 mo of age)	Anthropometry, malaria blood smear, hemoglobin, zinc protoporphyrin, hair zinc, and 24-h recall (on a subsample [n = 137]) Mothers instructed to prepare similar amounts of CF every day (104 g DM/d)	No differences in intakes of energy, protein, fat, or total iron. Intake of soluble zinc was higher in infants consuming PCF than in those consuming U-PCF (0.164 vs. 0.0684 mg/day)	No differences in growth, hemoglobin, zinc protoporphyrin, or hair zinc between 2 groups at 12 mo of age
Double-blind, randomized, controlled trial until 12 mo. Followed to 18 mo 3 groups: commercial MCD and porridge (CC group) vs. PRMCD + PRP (PR group), or milk-based infant formula + porridge (IF group)	6- and 12-mo blood samples: serum ferritin, serum zinc, hemoglobin Monthly dietary assessment (5-day food diaries) Anthropometry: monthly from 6 to 12 mo; bimonthly until 18 mo Bayley Scales of Infant Development. at 7, 13, and 18 mo Morbidity daily	No difference in energy intakes among groups at 6–8 or 9–11 mo Protein, iron, zinc (at 6 mo only), vitamin C, calcium, and phytate intakes differed in IF and MCD groups ($p < .05$) because of different nutrient contents of IF and MCD ($p < .05$)	No differences in serum ferritin or zinc between CC and PR groups Hemoglobin lower (117 vs. 120 g/L, $p = .012$) and anemia prevalence higher (23% vs. 13%. $p = .06$) in IF than in PR group because of differences in daily iron intake in 2 groups No differences between CC and PR groups in growth, development at any time, or morbidity at 6–11 or 12–17 mo of age
Quasi-experimental design with nonequivalent control group 2 intervention villages (n = 200) vs. 1 control village (n = 81) 12-mo follow-up	Focus groups, interviews, observations, anthropometry, 24-h recall over 2 seasons, hemoglobin, hair zinc, malaria slides, morbidity from picture calendars	Intervention parents had greater knowledge of iron, vitamin A, and food sources of iron ($p < .05$); greater use of fermented maize flour ($p < .001$); greater median intake of animal-source foods (especially fish), energy, protein, fat, calcium, zinc, and vitamin B_{12}; lower intake of phytate ($p < .05$); and fewer children with inadequate intakes of protein, calcium, zinc, vitamin B_{12}, and folate than controls Note: there was some leakage of knowledge and practices to controls	No difference in growth in intervention vs. controls at postintervention MUAC and AMA z-score higher in intervention than controls ($p < .001$) postintervention No change in hemoglobin in intervention group, but significant decrease in hemoglobin in controls ($p < .05$) No differences in initial or final hair zinc values between groups Incidence of common illnesses lower in intervention than in control group postintervention ($p < .001$)
Quasi-experimental design with nonequivalent control group Intervention group: 3 villages (n = 69) vs. 1 control village (n = 40)	Focus groups, counseling visits, single interactive 24-h recall	Adoption rates: 25% (enriched porridges) to 10% (soaked, pounded maize) Amount of complementary food (g/day) and intakes of energy, animal protein, vitamin B_2, niacin, calcium, iron, zinc, bioavailable zinc and iron were greater in intervention group than in control group ($p < .05$)	NA

studies were conducted through local community kitchens [32, 33]. Only the New Zealand study measured zinc intakes and serum and hair zinc concentrations, but the results are not yet available. All three interventions measured hemoglobin [32, 33, 36]; two also measured serum ferritin [33, 36], although only the New Zealand study [36] accounted for infection. Of the two interventions that measured serum ferritin [33, 36], the intervention in Peru [33] tended to prevent a decline in iron status compared with the control group, whereas in New Zealand [36], the dietary counseling program resulted in a nonsignificant increase (p = .068) in serum ferritin in comparison with the placebo group. Only one of these three interventions reported an improvement in hemoglobin [32], but these changes were not compared with those in the "control group," making it difficult to draw any conclusion.

Functional health outcomes associated with zinc deficiency were not assessed in the published studies.

Section 4a

Do interventions that promote dietary diversification or modification have an impact on behavior change and on nutritional status in both the short and the long term?

Conclusion

Dietary diversification or modification can have an impact on behavior change and on certain indicators of nutritional status in the short term, most notably increases or greater intakes of animal-source foods and certain nutrients, including zinc, depending on the setting and the study design. Whether the interventions also have an impact on biochemical, anthropometric, or other functional health outcomes, even over the short term, is less clear. The impact depends on the age of the participants, their baseline nutritional and health status, the duration and type of intervention strategies employed, and the setting. The long-term impact was evaluated in so few of the interventions that a statement on this issue cannot be made.

Detailed review of evidence

The most convincing evidence for the impact of behavior change on nutritional status, specifically zinc status, stems from the educational effectiveness trial conducted in Peru by Penny et al. [35]; details are summarized in **table 2C** [35]. The design of this cluster-randomized, controlled trial involving six intervention and six control health facilities meets the criteria for a probability assessment outlined by Habicht and coworkers [81]. The program had a significant effect on behavior change, based on improvements in infant-feeding practices and nutrient adequacy (including zinc) of complementary foods, which in turn resulted in a dramatic improvement in length and weight gain in the intervention compared with the control group at 18 months of age. Moreover, this intervention enhanced the quality and coverage of the existing nutrition education offered, without providing material benefits to the centers, in an attempt to maximize the sustainability of the program, although to date, its long-term sustainability has not been evaluated. Two other randomized, controlled trials conducted in Peru (**table 2C**) [33, 82] and New Zealand (**table 2C**) [36] that targeted adolescents and premenopausal women also showed a positive impact on behavior change, based on an increased intake of animal-source foods, which in turn led to increases in intakes of bioavailable iron and improved biochemical iron indices in the intervention compared with the control group. In the New Zealand trial only, intakes and biochemical and zinc-responsive functional indicators were also measured, and the data are currently being analyzed.

Further evidence supporting the impact of behavior change on nutritional status stems from the results of interventions based on a quasi-experimental design that also included a nutrition education or social marketing component. Some of these interventions promoted the consumption of animal-source foods (n = 2) (**table 2C**) [32, 34], and others promoted their production and consumption through small-animal husbandry or aquaculture, with (n = 3; **table 2A**) [18, 19, 21, 22, 24] or without (n = 1; **table 2B**) [31] agriculture. Two additional studies in Malawi also employed phytate reduction strategies together with the promotion of animal-source foods (**table 3D**) [70–73]. Of the eight interventions, two interventions in **table 2C** [32, 34], two in **table 2A** [21, 22, 24], and one in **table 2B** [31] showed an increase in intake of animal-source foods or total iron and heme iron, and in some cases, promising improvements in nutritional status based on reductions in night-blindness, morbidity, and anemia, and increases in serum retinol, serum ferritin, hemoglobin, growth, and muscle mass in the intervention compared with the control group. Certainly these findings suggest some impact on behavior change and nutritional status. Further, theoretically, because a quasi-experimental design was used, potential confounding factors and biases that might have been responsible for the observed effects can be taken into account in the analyses, allowing a plausibility evaluation to be made [81]. However, in most of these interventions, adjustments for baseline differences between the intervention and control groups were not performed, making it difficult to infer that the results observed were due to the intervention per se. Exceptions are the two studies in Malawi (**table 3D**), which did adjust for most baseline differences, so that the improvements [73] or higher

intakes [71] of animal-source foods and available zinc achieved postintervention, as well as other indicators of nutritional status in the study on children [72], can be associated with the impact of the intervention on behavior change in the short term.

Only two of these projects examined their long-term impact on behavior change and nutritional status, although no indicators of zinc status were included (**tables 1** and **2A**) [5, 21, 22]. Both were participatory projects in which home gardening was promoted and agriculture education was included. Follow-up after 4 to 5 years revealed significantly greater knowledge and practices related to vitamin A nutrition and a positive effect on intakes of vitamin A–rich foods in the intervention group compared with the controls in both projects, demonstrating that dietary changes can be sustained over time. Nevertheless, these dietary changes were not reflected by improvements in serum retinol values among the intervention group in the Tanzania study [5], and no biochemical or functional indicators of vitamin A status were measured in the Thai follow-up study [22].

Section 4b

Is the impact modified by baseline nutritional status, socioeconomic status, infection, sex, age, and life-stage group?

Conclusion

It is likely that the impact of dietary diversification or modification interventions is modified by baseline nutritional status (including birthweight), household socioeconomic characteristics, age, infection, and possibly sex. The evidence for this conclusion, however, is not strong, because many of the interventions have not measured these factors at baseline, and hence whether they have a modifying effect has not been extensively investigated.

Detailed review of evidence

Evidence from micronutrient supplementation studies confirms that the magnitude of the response will be dependent on baseline nutritional status (including zinc status). However, in most of the interventions reviewed here, with the exception of the randomized, controlled trials, no comprehensive assessment of the nutritional status (including zinc) of the intervention and control groups at baseline has been reported. Consequently, no baseline nutritional status variables could be used as covariates in the analysis. Hence, whether the impact of the intervention has been modified by baseline nutritional status is generally unknown.

Exceptions are the randomized, controlled trial in Kenya (**table 2D**) [43] and the cluster-randomized educational effectiveness intervention conducted in Peru (**table 2C**) [35]. In the Kenyan schoolchildren, those with a lower baseline HAZ (≤ -1.4) who received the milk-based snack showed a significant improvement in height compared with the controls [41]. In the Peru trial, the socioeconomic characteristics of the intervention and control households differed at baseline and were subsequently shown to modify the effects on both dietary intake and growth. As a result, differences between the intervention and control groups were assessed after adjustment for these covariates [35].

Infection may modify the response to an intervention through several mechanisms. These include impaired appetite, and thus reduced dietary intakes, and alterations in the integrity of the intestinal mucosa, causing increases in intestinal permeability and reductions in nutrient absorption [83]. However, very few of the interventions promoting dietary modification or diversification (excluding the randomized, controlled trials) measured infection by using a biochemical marker or functional indicators of morbidity, as noted earlier. Exceptions are the Vietnamese study (**table 2A**) [18, 19], in which morbidity due to acute respiratory infection was monitored; the Malawian study of the effect of dietary diversification or modification on children aged 3 to 7 years, in which malaria and morbidity due to common illnesses were assessed (**table 3D**) [70, 72]; and the 5-year follow-up study in Tanzania outlined above, which examined stool helminth infection in relation to serum retinol concentrations (**table 1**) [5].

Unlike serum ferritin, infection reduces serum zinc and retinol concentrations [84]. In the 5-year follow-up of the food-based intervention in Tanzania (**table 1**) [5], the mean serum retinol concentration of children in the intervention area was significantly lower than that of children in the control areas, but after adjustment for helminth infection, the means in the two areas were no longer significantly different. Serum zinc was not measured in this intervention. These findings highlight the importance of taking into account the existence of infection in the interpretation of some biochemical indicators, notably serum ferritin, retinol, and zinc. Indeed, this approach was adopted for the randomized, controlled trial intervention studies conducted on infants in Ghana (**table 2D**) [37] and the United States (**table 2D**) [38] and on schoolchildren in Kenya (**table 2D**) [44], but not in the randomized, controlled trial conducted on adolescents in Peru (**table 2C**) [33]. Indeed, in the trial conducted on Kenyan schoolchildren (**table 2D**), at baseline, across all groups and independently of group assignment, children with *Entamoeba histolytica* infection had a smaller increase in serum iron ($p = .01$) but a greater increase in plasma retinol ($p = .01$), whereas those with an enlarged spleen

had a smaller increase in plasma vitamin B_{12} [44].

Age can also modify the impact of dietary diversification or modification. In the quasi-experimentally controlled study undertaken on Chinese infants (**table 2C**) [34], age had a significant effect on the growth outcome. Before 12 months, there was no difference in growth between the two groups, but at 12 months, infants in the education group had better growth in weight and length ($p < .05$) than the controls, and fewer had moderate and severe malnutrition (weight-for-age z-score [WAZ] or HAZ < –2). Whether these differences in the growth response were due to differences in age or the length of the intervention is not clear.

Section 5

Are there any adverse effects of dietary diversification or modification?

Conclusion

Existing evidence suggests that provided care is taken to ensure that germinated cereals are prepared with microbiologically safe water, free of any environmental contaminants, and stored appropriately, they can be used safely to enhance the energy and nutrient density and reduce the phytate content of unrefined cereal-based porridges without risk of aflatoxin contamination or environmental contamination from the water. Further, although soaking cereal flours may result in some loss of zinc and water-soluble vitamins, such losses are small and are unlikely to override the positive effect of increased zinc absorption arising from the loss of water-soluble phytate from unrefined cereal-diets, provided care is taken to ensure that the soaking water is microbiologically safe and not subject to environmental contamination. Care must be taken, however, to ensure that when dietary diversification or modification strategies are promoted to enhance complementary feeding, displacement of breastmilk is minimized by sustaining optimal breastmilk intake.

Detailed review of evidence

The use of germinated cereals is often promoted as a dietary modification strategy to enhance the energy and nutrient density and reduce the phytate content of unrefined cereal-based porridges used for infant and young child feeding [47]. Concern has been expressed over the susceptibility of germinated cereals to contamination from aflatoxins produced by *Aspergillus flavus, A. parasiticus,* and *A. nomius,* because aflatoxins are potent carcinogens. Plant foods most susceptible to aflatoxin contamination include peanuts, seeds, and cereal grains such as wheat and maize [85]. Indeed, maize in the field and in traditional maize stores in all zones in West Africa is reported to be at risk for aflatoxin contamination [86]. Germinated cereals are vulnerable to aflatoxin contamination because of the extra moisture required for germination and improper drying and storage conditions. Unfortunately, no household strategies exist to decontaminate plant foods once they are contaminated with aflatoxins; heating and fermentation do not destroy them. However, research has consistently shown that if germinated cereals are prepared appropriately, dried, and then stored in a covered container in the household to prevent spoilage by insects, aflatoxin contamination can be avoided [87, 88].

Soaking maize and legume flours to remove water-soluble sodium and potassium phytate by diffusion was one of the phytate reduction strategies used in the Malawian interventions (**table 3D**) [70–72]. Soaking maize flour may result in some loss of zinc and water-soluble vitamins, such as thiamine, niacin, and riboflavin ($n = 6$) [88, 89], but the losses are small and are not expected to exceed the amounts that are lost during cooking or incurred when unrefined maize is milled [59]. If microbiologically unsafe water is used for soaking, there may be cause for concern, although enteropathogenic microorganisms should be destroyed during the cooking of the cereal porridges. Care must be taken, however, to avoid the use of water for soaking that may have been subject to environmental contamination. There is also some evidence that soaking maize flour during fermentation and removal of the excess water may result in loss of water-soluble antimicrobial substances [90].

Care must be taken to ensure that the promotion of dietary diversification or modification to improve complementary diets does not result in excessive displacement of breastmilk. Studies have reported an inverse relationship between the intake of breastmilk and the intake of complementary foods [91, 92], although for infants over 6 months of age the proportion of breastmilk displaced by complementary foods is probably less than that for younger infants. Moreover, it appears that displacement of breastmilk is less with those interventions that are designed to increase the energy density of complementary foods (e.g., by the use of germinated cereals) rather than meal frequency. Nevertheless, breastmilk is not an irreplaceable source of zinc for older infants, because of its low zinc content from 6 months postpartum. The decline in breastmilk zinc content is independent of maternal zinc status [46] and is seldom balanced by an increase in breastmilk intake. Instead, in later infancy, complementary foods are responsible for the increase in total zinc intakes that occur. Unless animal-source foods are also consumed, however, the actual increases in absorbable zinc will be small, because most complementary diets in developing countries are based predominantly on unrefined, high-phytate-containing cereals [93].

Scaling up dietary diversification or modification interventions

There are very few examples of dietary diversification or modification interventions that have been implemented as programs. In 2002, Helen Keller International began a pilot project on Homestead Food Production (HFP) in Bangladesh [94, 95]. This new program integrated animal husbandry into the Helen Keller International home gardening and nutrition education programs that had been ongoing in Bangladesh since 1990. The aim of the new integrated HFP program is to further enhance the production, availability, and consumption of animal-source foods by women and children. Nutrition education is an integral part of the program, and nutrition messages are designed specifically to increase the consumption of eggs, meat, liver, and milk, as well as micronutrient-rich foods from plant sources. This integrated HFP program has now been expanded to other areas within Bangladesh, as well as into Nepal, Cambodia, and, more recently, the Philippines. In all these countries, Helen Keller International works in close partnership with other nongovernmental organizations at the household level to provide overall guidance and training in the integrated HFP program.

To date, Helen Keller International has conducted preassessment and baseline surveys in all the program sites. Cross-sectional data have been collected from HFP program households and from nonprogram households (controls) selected through multistage cluster sampling at baseline and for Bangladesh, Nepal, and Cambodia at 3 to 4 years postintervention [94–96]. Data collected include sociodemographic status, homestead food-production practices (including animal and plant food production), household income generation and expenditure, and nutritional status and food consumption of mothers and their children. Food consumption was assessed by a 7-day food recall [96] or with the use of the Helen Keller International 24-hour vitamin A semiquantitative (VASQ) method to assess vitamin A intakes [97]. In Bangladesh, an indicator of household vulnerability was created based on the existence of anemia (hemoglobin < 120 g/L for nonpregnant women and < 100 g/L for children < 5 years) and a low intake of vitamin A (defined as intake of retinol activity equivalents less than the recommended nutrient intake) of the mother, her youngest child, or both [95].

The results of the evaluation of the HFP program in Bangladesh showed significantly higher intakes of animal-source foods, specifically eggs, poultry, other meat, milk or milk products, and dark-green leafy vegetables, at endline compared with baseline among children aged 6 to 59 months from target households, whereas intakes in the control group were almost unchanged [94, 95]. In addition, more of the target households earned income from gardening and poultry activities, showed improvements in poultry-rearing practices, and had a higher involvement of women in decision making than control households. However, changes in zinc intakes and status per se were not evaluated in this study. Comparable data from the Helen Keller International HFP programs in Nepal and Cambodia have not yet been published. A decline in the prevalence of anemia among nonpregnant women and children from HFP households compared with controls was also reported in Nepal and Bangladesh, but not in Cambodia. Whether this effect is due to an increased consumption of animal-source foods among the target HFP households in Nepal and Bangladesh is uncertain, based on the analyses presented to date [24, 94].

Implications for implementing dietary diversification or modification programs

On the basis of the available scientific evidence reviewed for Section 1a and 1b, dietary diversification or modification strategies employing agricultural interventions, animal husbandry, or aquaculture have the *potential* to increase intakes of total or absorbable zinc, although the magnitude of the increase will depend on the type of intervention, the habitual diet and life stage of the target group, and whether nutrition education or behavior change is included as a component of the intervention. To date, none of the agricultural interventions (Section 1a) and very few of those based on animal husbandry or aquaculture, with or without agriculture (Section 1b), have addressed zinc as a target nutrient, so that data on total or absorbable zinc intakes are very limited. This is unfortunate, because such data could be obtained, together with intakes of food groups and other micronutrients, at no additional cost. In several of the interventions reviewed, postintervention increases or higher intakes were reported of animal-source foods, energy, total protein, and other micronutrients, notably vitamin A, iron, and vitamin C. Whether improvements in intakes of animal-source foods would be sufficient to meet the needs of absorbable zinc for infants and young children is questionable, given their small gastric capacity [46]. For this age group, a combination of intervention strategies, including home fortification of complementary foods with micronutrients, is probably more appropriate in most settings, provided their effectiveness and sustainability have been confirmed and they are readily accessible to poor-resource households.

Increases in absorbable zinc resulting from agricultural interventions alone in persons consuming predominantly unrefined or unleavened cereal-based diets are likely to be negligible (Section 1a), unless strategies that encompass phytate reduction (with or without the inclusion of absorption enhancers) are included. The increase in absorbable zinc that might be achieved through *household* phytate reduction

strategies in individuals consuming unrefined or unleavened cereal-based diets has not been measured by *in vivo* stable isotope studies (Section 1c). However the level of household phytate reduction that can be achieved approximates that achieved with the use of low-phytate maize from plant breeding. Significant increases in fractional zinc absorption from test meals of low-phytate maize compared with that from the corresponding isohybrid wild-type maize with a typical phytate content have been confirmed from stable isotope studies. The magnitude of the increase in fractional zinc absorption depends on the level of phytate reduction and the total zinc intake of the target group.

Interpretation of the results for the interventions reviewed in Sections 1a–c is hampered by limitations in both the intervention design and the quality of the data collected, as well as omission of several key measurements (e.g., data on zinc intakes), emphasizing the importance of conducting rigorously designed interventions based on agriculture, small-animal husbandry, or aquaculture.

Sections 2 and 3 address whether dietary diversification or modification strategies can achieve increases in absorbable zinc that are sufficient to enhance serum zinc or zinc-related functional responses in breastfed infants and young children (Section 2) and in older children and women of reproductive age (Section 3). From the available scientific evidence on infants and young children, inclusion of animal protein (especially liver) in complementary diets appears to be associated with a consistent positive response in growth (in length, weight, or head circumference), which is possibly a zinc-related functional outcome. Whether there is also a response to serum zinc is uncertain, because there are fewer reports of this measurement. The positive effect on growth is consistent with the results of a well-designed effectiveness trial in Peru involving a complementary feeding educational intervention implemented through government health services in areas where food availability was not limited. In this effectiveness trial, the two key nutrition education messages were feeding infants thick purees that contained a source of animal protein, specifically chicken liver, egg, or fish, at each meal. Children in the intervention group had significantly higher intakes of energy from animal sources and of zinc (as well as iron and calcium) at 9, 12, and/or 18 months and a significant reduction in the prevalence of stunted growth at 18 months of age than those in the control group. Serum zinc was not measured in this trial.

For older children, evidence from a limited number of trials suggests that an improvement in a zinc-related functional outcome, body composition (i.e., muscle mass based on mid-upper-arm muscle area), may be achieved through dietary diversification or modification strategies that include animal-source foods either provided or promoted through nutrition education and social marketing, even though no positive response in biochemical zinc status was observed. More rigorously designed efficacy trials in children are needed to confirm these suggestions.

Whether dietary diversification or modification results in a positive impact on biochemical zinc status and zinc-related functional outcomes for women of premenopausal age is unclear. Again, rigorously designed efficacy trials with an appropriate design and sample size, which include measurements of both potential confounders and appropriate outcomes, are needed to ensure that results can be attributed to the intervention.

Evidence for the sustainability (both short-term and long-term) of the behavior change or changes in nutritional status associated with the promotion of dietary diversification or modification was examined in Section 4a. Although some positive effects on behavior change and nutritional status over the short term have been documented, the long-term impact has seldom been evaluated, so no conclusion can be drawn. **Table 4** provides a summary of the interventions reviewed and tabulated in **tables 1** to **3D,** together with a ranking compiled by the authors of the conclusions of each study according to three levels of confidence (high, medium, or low), based on the methods and data presented.

How to achieve dietary diversification and modification interventions

A detailed discussion on how to design and implement dietary diversification or modification strategies to increase intakes of micronutrients, including absorbable zinc, is beyond the scope of this review. Several useful publications on food-based approaches are available [97–100], and the reader is advised to consult these sources for more details. A technical brief that summarizes the dietary diversification and modification strategies for enhancing intakes and bioavailability of dietary zinc has also been prepared by the International Zinc Nutrition Consultative Group (IZiNCG) [101]. Three of the more detailed publications, however, target mainly vitamin A deficiency and include limited information on how to enhance the production, accessibility, or consumption of animal-source foods, a prerequisite for increasing intakes of absorbable zinc to levels likely to be associated with zinc-related functional outcomes. Programs that have integrated animal husbandry with home gardening include the integrated HFP program developed by Helen Keller International, as noted earlier, and the MICAH Program of World Vision Malawi. The latter emphasized the consumption of animal-source foods through the creation of a small-animal revolving fund for rabbits, poultry, guinea fowl, and goats [31]. Community kitchens are another

TABLE 4. Summary of interventions reviewed

Author [reference]	Baseline data for production indicators	Nutrition education or social marketing	Baseline data for nutritional status indicators	Data collected on control population	Dietary intake outcomes	Nutritional status outcomes	Reviewer's confidence in study conclusions
Table 1. Agricultural interventions to increase household production, accessibility, or consumption of plant-based foods							
[4] Marsh	+	+	+[a]	+	+	+ Zinc related	High
[5] Kidala et al.	–	+	–	+	+	+	Medium
[6] Chakravarty	+	+	+[b]	–	+	+	Low
[7] Ngu et al.	+	+	+[b]	–	+	+	Low
[8] Solon et al.	+	+	–	+	+	–	High
[9] Phillips et al.	–	+	–	+	+	+	Medium
[10] Solon et al., [11] Popkin et al.	–	+	+[b]	–	+	+	Medium
[12] Galal et al.	+	–	+[b]	+	+	+	Low
[13] CARE Nepal	–	–	+[b]	–	+	+	Low
[14] Hagenimana et al.	–	+	+[a]	+	+	+	High
Table 2A. Mixed interventions: Agriculture combined with small-animal husbandry or aquaculture to increase production or promotion of animal-source foods							
[18] English et al., [19] English and Badcock	–	+	+[a]	+	+	+ Zinc related	High
[20] IFPRI	+	–	+[a]	+	+	+	Medium
[21] Smitasiri and Dhanamitta, [22] Smitasiri et al.	+	+	+[a]	+	+	+	High
[23] Schipani et al.	+	–	+[a]	+	+	+ Zinc related	High
[24] Stallkamp et al.	+	+	+[a]	+	+	+	High
[25] Ying et al.	+	+	+[b]	–	–	+ Zinc related	Low
[26] Sheikholeslam et al.	+	+	+[b]	+	–	+ Zinc related	Medium
Table 2B. Animal-source food interventions: Small-animal husbandry or aquaculture to increase production of animal-source foods							
[27] Ayele and Peacock	+	+	–	–	+	–	Medium
[28] Nielsen et al.	+	–	–	+	+	–	Medium
[29, 30] Roos et al.	+	–	–	+	+	–	Medium
Table 2C. Animal-source food interventions: Nutrition education or behavior change to promote animal-source foods							
[32] Carrasco Sanez et al.	–	+	+[b]	+	+	+	Medium
[33] Creed-Kanshiro et al.	–	+	+[a]	+	+	+	Medium
[34] Gulden et al.	–	+	+[a]	+	+	+	Medium
[35] Penny et al.	–	+	+[a]	+	+	Zinc related	High
[36] Heath et al.	–	+	+[a]	+	+	+	High

TABLE 4. Summary of interventions reviewed *(continued)*

Author [reference]	Baseline data for production indicators	Nutrition education or social marketing	Baseline data for nutritional status indicators	Data collected on control population	Dietary intake outcomes	Nutritional status outcomes	Reviewer's confidence in study conclusions
Table 2D. Nutritional improvement of home-prepared complementary foods or school lunches from inclusion of animal-source foods							
[37] Lartey et al.	–	–	+[a]	+	+	+ Zinc related	High
[38] Krebs et al.	–	–	+[a]	+	+	+ Zinc related	High
[39] Engelmann et al.	–	–	+[a]	+	+	+ Zinc related	High
[40, 41] Grillenberger et al., [42] Murphy et al., [43] Neumann et al., [44] Siekmann et al., [45] Whaley et al.	–	–	+[a]	+	+	+ Zinc related	High
Table 3D. Mixed interventions: Phytate reduction strategies with and without other household methods to enhance content and bioavailability of dietary zinc							
[66] Lachat et al., [67] Mamiro et al.	–	–	+[a]	+	+	Zinc related	Medium
[68, 69] Lind et al.	–	–	+[a]	+	+	Zinc related	High
[70] Gibson et al., [71, 72] Yeudall et al.	–	+	+[a]	+	+	Zinc related	High
[73] Hotz and Gibson	–	+	+[a]	+	+	–	High

+, present; –, absent
a. Baseline differences between intervention and control groups controlled for in analyses.
b Baseline differences between intervention and control groups not controlled for in analyses.

model that has been successful among women in Lima, Peru, in increasing the consumption of iron-rich foods such as organ meats and thus probably increasing the intake of absorbable zinc; details are given in Carrasco-Sanez et al. [32].

Regardless of the model adopted, all interventions should be designed, implemented, and evaluated by a formative research approach and should include appropriate information, education, and communication strategies to ensure their adoption, sustainability, and effectiveness, as described in Dickin et al. [102]. Details on how to train people in participatory methods are described in Pretty et al. [103]. Methods aimed to strengthen the leadership abilities of community women include the Cornell Modified Community-Based Nutrition Monitoring [104] and the Appreciation-Influence-Control Process [105]. Finally, monitoring and evaluation should be included as an integral component of any intervention program so that its performance and impact on the target group can be established. Selection of the appropriate indicators and design for the monitoring and evaluation to meet the program objectives is critical; details can be found in Habicht et al. [81], Levinson et al. [106], and the IZiNCG Technical Report [107]. Indicators recommended by WHO, the United Nations Children's Fund (UNICEF), the International Atomic Energy Authority (IAEA), and IZiNCG to monitor or evaluate risk of zinc deficiency include dietary zinc intake, plasma or serum zinc concentration, and the prevalence of stunting [107]; further details on the measurement of these indicators are available in IZiNCG Technical Briefs 1, 2, and 3 [108–111].

Future research needs

In the future, measurements of intakes and major food sources of zinc and phytate should be included in all dietary diversification or modification interventions, together with energy, protein, animal protein, and other high-risk micronutrients (e.g., iron, calcium, vitamin A, vitamin B_6, vitamin B_{12}, and riboflavin). These measurements can be included at no additional cost, provided local food-composition data for zinc and phytate are available.

More rigorously designed multicenter efficacy trials in infants, children, and premenopausal women known

to be at high risk for zinc deficiency at baseline (based on a prevalence of stunting greater than 20% and either a prevalence of low serum zinc greater than 20% or a prevalence of inadequate zinc intake of greater than 25%) are needed to confirm whether improvements in both serum zinc and zinc-related functional responses, such as growth, body composition, and morbidity, can be achieved by including animal-source foods, specifically red meat or liver, in their diets. The animal-source foods can be supplied to the participants in the trials or their use can be promoted through nutrition education and behavior change. Care must be taken to collect and analyze all blood samples collected in efficacy trials according to IZiNCG recommended techniques and to include measurements of all potential confounders (including α_1-glycoprotein and C-reactive protein as indicators of infection).

Rigorously designed efficacy trials should be conducted in target groups known to be at high risk for zinc deficiency to examine the long-term impact of dietary diversification or modification on behavior change and on zinc-related functional outcomes. These trials should include the measurement of all confounding factors that may have the potential to modify their impact and subsequent sustainability.

Effectiveness trials with designs similar to that of the trial undertaken in Peru should be undertaken in other settings where stunting rates are greater than 20% to confirm whether nutrition education messages to promote the inclusion of animal-source foods (appropriate to the setting) in complementary diets, and delivered through the local health services within a country, can increase intakes of absorbable zinc and have a positive impact on serum zinc concentrations or stunting and other potentially zinc-related functional outcomes (e.g., morbidity) in young children in countries where access to animal-source foods is not a limiting factor.

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Dietary intervention strategies to enhance zinc nutrition: Promotion and support of breastfeeding for infants and young children

Kenneth H. Brown, Reina Engle-Stone, Nancy F. Krebs, and Janet M. Peerson

Abstract

Breastmilk is the only dietary source of zinc for exclusively breastfed young infants, and it remains a potentially important source of zinc for older infants and young children who continue breastfeeding beyond early infancy. Therefore, we examined available information on breastmilk zinc concentration and total milk consumption to develop estimates of the amount of zinc transferred in breastmilk to children of different ages. Breastmilk zinc concentration declines rapidly during the first few months postpartum and more slowly thereafter. Breastmilk supplies all of the theoretical zinc needs for at least the first several months of life, although the period during which breastmilk alone remains sufficient is uncertain. Breastmilk continues to provide more than half of children's estimated zinc requirements after the introduction of complementary foods, even into the second year of life. Public health programs to promote and support breastfeeding should be included among the strategies to ensure adequate zinc status of young children.

Key words: Breastfeeding, breastmilk, infant, zinc, zinc absorption, zinc requirements

Kenneth H. Brown, Reina Engle-Stone, and Janet M. Peerson are affiliated with the Department of Nutrition and the Program in International and Community Nutrition, University of California, Davis, California, USA; Kenneth H. Brown is also affiliated with Helen Keller International, Dakar, Senegal. Nancy F. Krebs is affiliated with the Department of Pediatrics, University of Colorado School of Medicine, Denver, Colorado, USA.

Please direct queries to the corresponding author: Kenneth H. Brown, Department of Nutrition, University of California, One Shields Ave., Davis, CA 95616, USA; e-mail: khbrown@ucdavis.edu.

Background

Promotion and support of appropriate breastfeeding practices should be considered among the recommended dietary strategies to enhance the zinc status of infants and young children, for two main reasons: breastmilk is an important potential source of bioavailable zinc, and breastfeeding protects against diarrhea [1–3], which causes excessive zinc losses [4]. This paper will provide a summary of available information on the amount of zinc that can be provided to infants and young children through breastfeeding. The paper is divided into three sections, which address the following questions with regard to breastfeeding as a component of dietary intervention strategies to prevent zinc deficiency:

Section 1: How much zinc is transferred in breastmilk to exclusively breastfed infants less than 6 months of age in relation to their physiological requirements for absorbed zinc?

Section 2: How much zinc is transferred in breastmilk to infants and young children less than 24 months of age who are consuming breastmilk in addition to complementary foods?

Section 3: What are the programmatic implications of the answers to the foregoing questions, and what are the remaining research needs?

Section 1

How much zinc is transferred in breastmilk to exclusively breastfed infants less than 6 months of age in relation to their physiological requirements for absorbed zinc?

Conclusions

The mean amount of zinc transferred in breastmilk to exclusively breastfed infants declines rapidly from ~4 mg/day during the first few days of life to ~1.75

mg/day by 1 month. Zinc transfer declines more slowly thereafter to ~0.7 mg/day at 6 months. The adequacy of breastmilk as the sole source of zinc during this period is difficult to determine because of uncertainty in the fractional absorption of zinc (FAZ) from breastmilk and doubts regarding the true physiological requirements for absorbed zinc during this period of life. Nevertheless, using different assumptions for likely FAZ, based on available studies that suggest a mean FAZ of ~0.50 and current estimates of zinc requirements, breastmilk alone is probably adequate for exclusively breastfed term infants for at least 3 months and possibly for as long as 6 months, depending on which sets of estimates are applied and whether zinc accrued by the fetus during gestation is available for infant metabolism.

There is little information from zinc supplementation trials conducted among exclusively breastfed term infants less than 6 months of age, and the results of supplementation trials carried out among a broader age range of breastfed infants are inconsistent. In general, three such trials carried out in industrialized countries failed to demonstrate a consistent functional advantage of providing additional zinc to infants less than 6 months of age, so the amount transferred in breastmilk is presumably adequate during this period. Breastmilk is clearly an important source of highly bioavailable zinc during this period of life and may be adequate as the sole source of zinc for exclusively breastfed term infants until ~6 months.

Detailed review of evidence

Overview of methodologic issues

Milk sampling. Accurate measurement of the amount of zinc transferred through breastmilk from mothers to their nursing children requires first, collection of representative samples of milk for analysis of zinc concentration and second, assessment of the total amount of milk consumed. The total amount of zinc transferred through breastmilk can then be determined by multiplying the milk zinc concentration by the total amount of milk consumed. To develop an appropriate milk sampling protocol for assessing milk zinc concentration, several investigators have examined whether milk zinc concentration varies by the time of day, portion of the feeding, left versus right breast, the gestational age at birth and postnatal age of the child, and the age, parity, and zinc status of the mother. The results of these studies are summarized in the following paragraphs. The single factor that appears to have the greatest impact on milk zinc concentration is the time postpartum. Thus, assessments of other factors affecting milk zinc concentration are interpretable only if the age of the child is held constant. The specific relation between time postpartum and milk zinc concentration is discussed in more detail in the next section.

Studies of milk zinc concentration in relation to time of day have yielded inconsistent results. Four studies found no relation between the time of day and milk zinc concentration [5–8], but three studies found that specimens obtained early in the morning had ~15% greater milk zinc concentration than specimens obtained in the afternoon or evening [9–11]. Thus, it might be advisable to obtain more than one sample during the day to gather more representative information on the total amount of zinc transferred, even though many studies suggest that just one sample might be sufficient.

Two studies examined milk zinc concentrations in different portions of the feeding, and neither found any significant differences in the zinc concentrations of foremilk and hindmilk [5, 12]. Only one study compared zinc concentrations in milk obtained from the left versus the right breast, and no consistent differences were detected [12].

Ten studies are available that compared the zinc concentration in the milk of mothers who delivered at term with that of mothers who delivered prematurely [13–22]. There were no consistent differences in the zinc concentrations of milk obtained from the respective sets of mothers. Other studies of the relations between maternal age and parity and milk zinc concentrations are difficult to interpret because of the failure to control adequately for infant age. Among the subset of studies that did compare results at specific, narrowly defined time periods postpartum, there are no consistent effects of maternal age or parity on milk zinc concentrations [7, 23–26].

As reviewed by Hess and King in this issue [27], maternal zinc status and maternal zinc supplementation do not appear to affect milk zinc concentration. On the other hand, several longitudinal studies have found greater variability in milk zinc concentration between women than within women [10, 28], indicating that individual women have characteristic levels of zinc in their milk, possibly because of genetic factors influencing zinc transport to or within the mammary gland. The public health importance of these interindividual differences in breastmilk zinc concentration is unknown, because no relevant studies are available relating milk zinc concentration to infant growth.

In summary, there are few extraneous factors, other than time postpartum, that consistently affect breastmilk zinc concentration. Thus, it is possible to collect milk samples for analysis of zinc concentration from either breast, from any portion of the feeding, and at just one time or a few times per day, regardless of the age, parity, and nutritional status of the mother.

Pattern of distribution of data regarding milk zinc concentration and milk volume. To calculate the range of total zinc consumption from breastmilk by children of different ages, information is needed on the

distribution of data concerning milk zinc concentration and total milk intakes during each age interval of interest. Few studies have reported information on the distribution of milk zinc concentration at different ages, so we reanalyzed data from a previously published study that collected milk samples from 71 infants at 2 weeks of age and again monthly from 1 through 7 9 months of age [28]. As shown in **figure 1**, these data indicate that the distribution of milk zinc concentration is skewed somewhat to higher values at each age, although the data can be normalized by logarithmic transformations.

We also examined the results of seven previously completed studies of breastmilk intake to assess the age-specific distribution of milk volumes [28–34] and concluded that milk intake was generally normally distributed in all studies (Shapiro-Wilk statistic > 0.98 in all cases).

Studies of breastmilk zinc transfer to exclusively breastfed infants under 6 months of age

Identification and selection of studies. Data sets containing information on breastmilk zinc concentration were identified through a computerized database search (PubMed, accessed September 12, 2007) using the keywords "breast," "milk," "zinc," and "infant" and no additional limits, which yielded 320 bibliographic citations. The titles were screened for relevance, and the full texts of the articles were obtained when possible. Additional data sets were located from the lists of citations included in the articles found during this initial search, through papers cited in a previously published review [35], and by contacting other experts in the field. Using this search strategy, we identified a total of 69 publications from 64 different studies that reported data on breastmilk zinc concentration.

From the set of 69 publications that were retrieved, we selected only those studies that presented data from mothers of healthy, term infants and provided information on both milk zinc concentration and time postpartum. Despite the conclusions of the methodologic review presented above, for the sake of caution we excluded studies that enrolled preterm infants or children with specific illnesses, unless data were reported separately for unaffected children. We also excluded studies of mothers who were receiving zinc

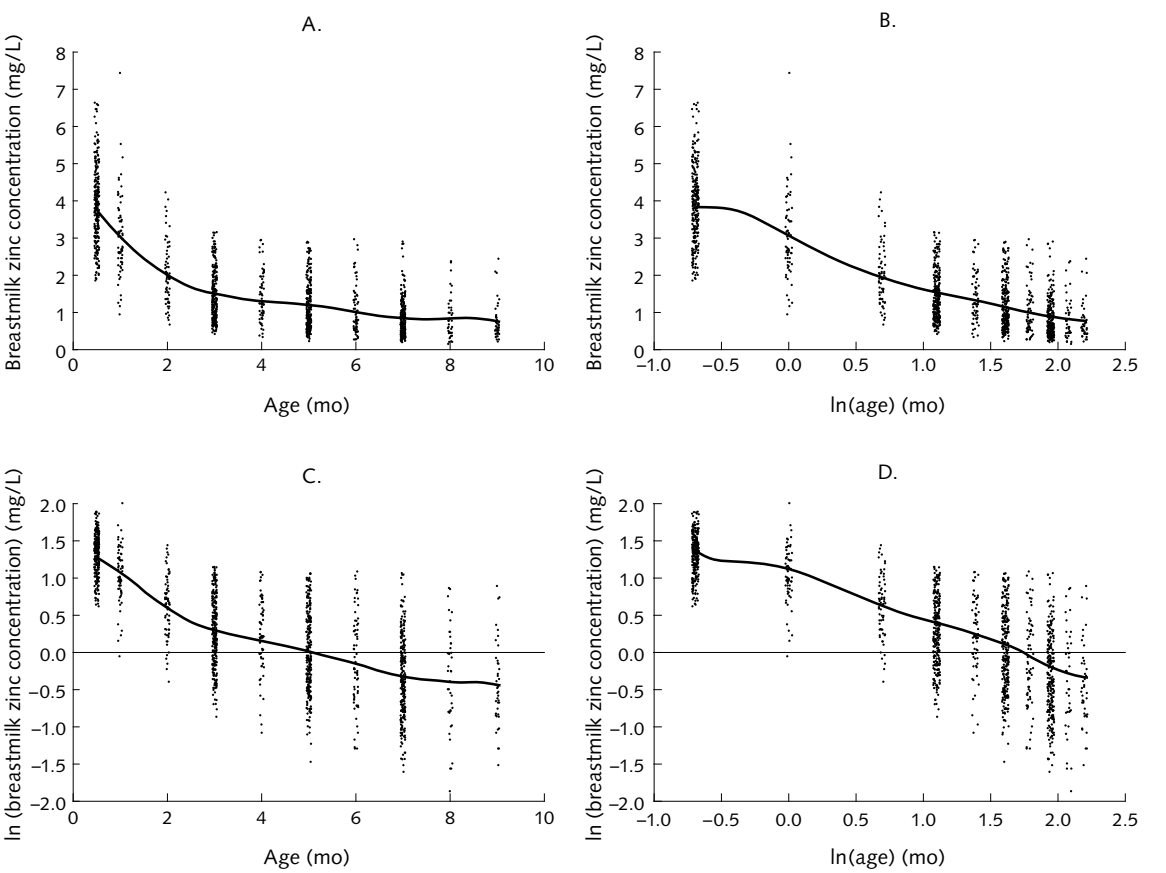

FIG. 1. Distribution of breastmilk zinc concentration according to time postpartum, and effects of different mathematical transformations of data. Data provided by Krebs et al. [28]

supplements, unless the authors specifically stated that there was no effect of supplementation on milk zinc concentration or data from nonsupplemented women were presented separately. We also excluded studies of women who were receiving other mineral-containing supplements or other medicines. These exclusions resulted in a final set of 33 studies that were included in the combined analysis, as shown in **table 1** [5, 7, 13–18, 21, 23, 26, 28, 36–61]. The studies that were excluded from the analysis are shown in **table 2** [6, 8–11, 19, 22, 24, 25, 62–86], along with the reason(s) for the exclusion.

Milk zinc concentration according to infant age. To express the data on milk zinc concentration from different studies in consistent units, we applied conversion factors of 65.4 g of zinc per mole and 1.03 g of breastmilk per milliliter [35], and all results are presented as milligrams of zinc per liter of milk. The data available from all 33 acceptable studies are summarized in **figure 2** and **table 3**. Each point in the figure represents the mean milk zinc concentration reported for the midpoint of a particular age interval in a given study. The data across studies are remarkably consistent, showing a relatively high concentration of zinc in colostrum and early transitional milk, a rapid fall in milk zinc concentration during the first 1 to 2 months postpartum, and a slower decline thereafter. Tests of various transformations of breastmilk zinc concentration and age indicated that a log–log relationship including a quadratic term for log(age) provided the best fit for the relationship between milk zinc concentration and time postpartum ($r^2 = 0.88$, $p < .0001$).

Amount of milk consumed according to infant age. Information on the amount of milk consumed by children of different ages was extracted from the publication "Complementary feeding of young children in developing countries" by the World Health Organization (WHO) and the United Nations Children's Fund (UNICEF) [87]. This document provided separate information for exclusively breastfed infants less than 6 months of age and for non-exclusively breastfed children less than 24 months of age, as shown in **table 4**.

Total zinc transfer to exclusively breastfed infants less than 6 months of age and relation to zinc requirements

To estimate the total amount of zinc transferred in breastmilk, we completed the following simulation exercise. Breastmilk zinc concentration was assumed to follow a log-normal distribution, based on the information described above [28]. Because only sample means and standard deviations were available from the individual studies used to estimate milk zinc concentrations, the means and standard deviations of log(breastmilk zinc concentration) were estimated from the untransformed summary data by using the method of moments [88]. The relationships between

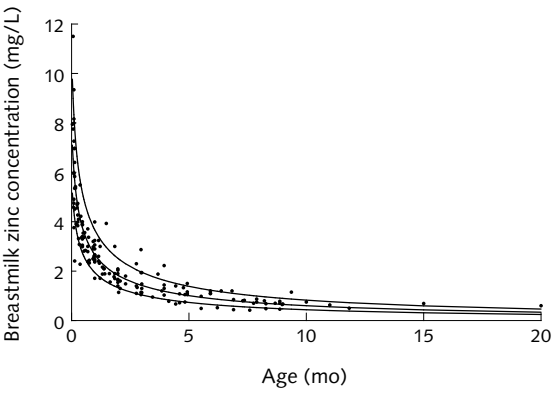

FIG. 2. Breastmilk zinc concentration according to time postpartum, all studies. Ln(Zn concentration) = 0.975 – 0.501*ln(age) – 0.063*ln(age)^2

infant age and the means and standard deviations of log(breastmilk zinc concentration) and of breastmilk intake were estimated with regression analysis. Finally, we generated 1,000,000 simulated data points, assuming that age was uniformly distributed between 0 and 6 months for exclusively breastfed children and between 0 and 18 months for partially breastfed children, and that log(breastmilk zinc concentration) + log(breastmilk zinc concentration)^2 and breastmilk volume were normally and independently distributed at each age. We then multiplied individual simulated values for breastmilk intake and milk zinc concentration to estimate total zinc transfer.

The results of the simulations of the total daily amount of zinc transferred in breastmilk according to child age are shown in **figure 3** for exclusively breastfed infants less than 6 months of age. The mean amount of zinc transferred in breastmilk declines from ~4 mg/day during the first few days of life to ~1.75 mg/day

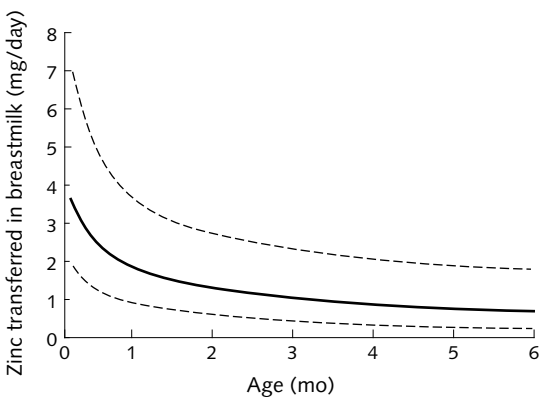

FIG. 3. Simulated mean and 95% prediction interval of daily zinc transfer in breastmilk to exclusively breastfed infants according to infant age

TABLE 1. Publications included in the data analysis

Country, year [reference] author	Study group	Health and zinc status of mothers	Zinc concentration in milk according to stage of lactation (mg/L)	Description of milk sample	Growth or zinc status of infant
Argentina, 2001 [14] Ronayne de Ferrer	n = 20 mothers with term milk (mean gestational age of infants, 39.5 wk; range, 38–41 wk; mean birthweight, 3,303 g) and n = 24 middle-class mothers with preterm milk (mean gestational age of infants, 30.9 wk; range, 27–35 wk; mean birthweight, 1,440 g)	Apparently healthy	Mean ± SEM, term infants only: Days 2–5: 6.96 ± 0.69 Days 6–10: 4.27 ± 0.51 Days 11–15: 3.36 ± 0.31 Days 16–30: 2.44 ± 0.11	Full breast sample collected by manual or pump expression between 10 and 12 am	Infant growth and zinc status not reported
Brazil, 1989a [41] Donangelo (excluded data from 31 to 280 days because of wide range of infant ages)	n = 93 mothers of low SES with normal and uncomplicated gestations, both partially breastfeeding and exclusively breastfeeding. Mean maternal age, 25.9 ± 5.6 (SD) yr; mean birthweight, 3,277 ± 476 (SD) g	Mothers received iron, vitamin B_{12}, and/or folate during pregnancy (but not lactation). 11% of mothers had serum zinc below cutoff for low zinc for adult women	Mean ± SEM, exclusively breastfeeding mothers: Days 1–5: 5.94 ± 0.55 (n = 17) Days 6–30: 2.84 ± 0.37 (n = 13) Days 31–280: 1.65 ± 0.38 (n = 10) Mean ± SEM, partially breastfeeding mothers: Days 31–280: 1.26 ± 0.19 (n = 21)	5–10 mL collected by manual expression between 9 and 10 am before the infant was fed	Infant growth and zinc status not reported
Brazil, 1998 [15] Trugo	n = 28 mothers with term milk (mean birthweight, 3,380 ± 53 [SD] g) and n = 7 mothers with preterm milk (mean birthweight, 1,810 ± 47 [SD] g) at 1–5 days postpartum; n = 9 mothers with term milk (mean birthweight, 3,160 ± 29 [SD] g) and n = 15 mothers with preterm milk (mean birthweight, 1,700 ± 39 [SD] g at 6–36 days postpartum). All of low SES	All low SES; none received supplements. No information on maternal health and zinc status	Mean ± SD (range), term infants only: Days 1–5: 7.26 ± 3.03 (1.3–13.7) (n = 22) Days 6–36: 2.79 ± 1.14 (1.2–4.2) (n = 8)	5–10 mL of milk collected by manual expression between 9 and 10 am before the infant was due to be fed	Infant weights were adequate for gestational age. Infant zinc status not reported
Brazil, 2003 [21] da Costa	n = 50 mothers of term infants (37–41 wk gestation) > 20 yr of age, with at least 2 yr interparturition period; no supplements	Maternal health and zinc status not reported	Mean ± SD: Days 1–7: 6.97 ± 2.82 (n = 48)	Manual expression of 1.5 mL of colostrum from both breasts between the 1st and 7th days of lactation	Infant growth and zinc status not reported

Burundi, 1995 [53] Robberecht	n = 5 exclusively breastfeeding middle-class women (mean age, 32 yr) with infants aged 0–10 mo. Uncomplicated pregnancies	Healthy; zinc status not measured specifically	Mean ± SD: Days 2–4 (colostrum): 3.76 ± 0.51 (n = 1, 8 samples) Days 5–14: 3.08 ± 0.62 (n = 1, 10 samples) 1 mo: 2.47 ± 1.17 (n = 3, 12 samples) 2 mo: 1.64 ± 0.06 (n = 2, 6 samples) 4 mo: 1.30 ± 0.06 (n = 2, 5 samples) 10 mo: 0.75 ± 0.02 (n = 2, 6 samples)	Breastmilk samples from 5 lactating women [54]	Infant growth and zinc status not reported
Canada, 1982 [18] Mendelson	n = 10 mothers of term infants (mean gestational age, 39.0 wk; range, 38–40 wk; mean ± SD birthweight, 1,143 ± 305 g) and n = 14 mothers of preterm infants (mean gestational age, 28.7 wk; range, 26–33 wk; mean ± SD birthweight, 3,361 ± 550 g) All appropriate for gestational age	Normal pregnancy; maternal health and zinc status not reported	Mean ± SD, term infants only: Days 3–5: 5.35 ± 1.2 (n = 8) Days 8–10: 4.1 ± 0.65 (n = 8) Days 15–17: 3.37 ± 0.6 (n = 7) Days 28–30: 2.6 ± 0.65 (n = 6)	Complete 24-h expression with breast pump	Infant growth and zinc status not reported
Canada, 1999 [20] Friel	n = 19 mothers 20–35 yr of age with full-term infants	Healthy, nonvegetarian; zinc not specifically measured	Mean ± SD: Days 2–3: 4.58 ± 0.52 (n = 17) Day 8: 3.99 ± 0.88 (n = 16) Day 15: 3.54 ± 0.74 (n = 16) Day 22: 3.37 ± 2.06 (n = 15) Day 28: 2.5 ± 0.94 (n = 15) Day 36: 2.32 ± 1.41 (n = 15) Day 43: 1.89 ± 0.65 (n = 15) Day 50: 1.56 ± 0.96 (n = 15) Day 61: 1.14 ± 0.55 (n = 12)	Full breast expression by electric pump during a regular feeding of the child between 10 am and 2 pm; a 15-mL aliquot was taken	Infant growth and zinc status not measured
China, 2007 [60] Xiang	n = 41 term, exclusively breastfeeding mother-infant pairs in rural China. Group I: n = 18 at 1 mo (mean maternal age, 26.6 yr). Group II: n = 23 at 3 mo (mean maternal age, 27.4 yr)	Mean maternal zinc intake, 7.5 mg/day; mothers apparently healthy	Mean ± SD: Group I (1 mo): 3.24 ± 0.23 Group II (3 mo): 1.94 ± 0.17	5-mL milk samples were expressed manually at 5 time intervals (8–10 am, 10 am–12 pm, 12–2 pm, 2–4 pm, and 4–6 pm) after the breast had been suckled by the infant for 2 min	Weights and lengths were in normal ranges at birth, 1 mo, and 3 mo. Infant zinc status not reported

continued

TABLE 1. Publications included in the data analysis *(continued)*

Country, year [reference] author	Study group	Health and zinc status of mothers	Zinc concentration in milk according to stage of lactation (mg/L)	Description of milk sample	Growth or zinc status of infant
Finland, 1979 [57, 58] Vuori	n = 27 mothers aged 20–35 yr (mean, 28 yr)	Healthy, well-nourished, all were primiparous mothers of term infants	Mean: Wk 2: 4.0 (n = 20) Wk 3: 3.0 (n = 23) Wk 4: 2.5 (n = 22) Wk 5: 2.4 (n = 25) Wk 6: 2.1 (n = 17) Wk 7: 1.9 (n = 18) Wk 8–10: 1.3 (n = 22) Wk 11–13: 1.1 (n = 18) Wk 14–16: 0.95 (n = 11) Wk 17–19: 0.78 (n = 12) Wk 20–22: 0.75 (n = 13) Wk 23–25: 0.49 (n = 10) Wk 26–28: 0.52 (n = 9) Wk 29–31: 0.44 (n = 6) Wk 32–34: 0.42 (n = 1) Wk 35–37: 0.48 (n = 2)	Fore- and hindmilk sample from each feeding (5–7/day in first 3 mo; 3–4/day thereafter) at 1–2-wk intervals up to 2 mo and 3–4-wk intervals thereafter	Infant growth (measured by height and weight) was comparable to reference norms for Finnish infants. Infant zinc status not reported
Germany, 1992 [55] Sievers	n = 10 healthy mothers aged 26–35 yr with term infants	Healthy; zinc not measured specifically	Median (10th, 90th percentile): Day 17: 3.6 (2.4, 4.9) Day 35: 2.6 (1.6, 3.6) Day 56: 1.7 (1.1, 2.8) Day 85: 1.3 (0.8, 1.4) Day 117: 1.2 (0.6, 1.9)	At each feed 2 mL of fore- or hindmilk from nursed breast (n = 2,207 specimens)	Negative zinc balance observed in 11 of 33 breastfed infants; median daily zinc retention was positive in all cases. Infant growth not reported
Greece, 2005 [49] Leotsinidis	n = 180 mothers aged 25 ± 4 (SD) yr with full-term infants, mean birthweight 3,396 ± 480 g	Healthy; zinc status not specifically measured. Anemia prevalence was 41.9%	Mean ± SD: Day 3: 4.91 ± 1.73 Day 14: 2.99 ± 0.92	10–20 mL collected in the morning by manual expression, 2 h after the previous breastfeeding	Infant growth and zinc status not reported
Honduras and Sweden, 2004 [40] Dömellof	n = 263 mother-infant pairs (total for Honduras + Sweden); mothers > 16 yr of age; exclusively breastfeeding at 4 mo. Infants were full term, with birthweight > 2,500 g, no illness	Maternal zinc status not reported. Prevalence of low ferritin (< 12 μg/L) was 32% in Honduras and 12% in Sweden	Mean ± SD at 9 mo: Honduras: 0.7 ± 0.18 (n = 108) Sweden: 0.46 ± 0.26 (n = 86)	10-40 mL collected by manual expression at 9 mo postpartum in the morning > 1 h after feeding	Infant growth and zinc status not reported

India, 1997 [16] Hemalatha (colostrum and transitional milk data not included because day of collection not reported)	Mothers of 68 preterm infants (gestational age at birth not given), 92 term breastfed infants, and 26 term formula-fed infants. All weaned with cereal at 6 mo	Maternal health and zinc status not reported	Mean ± SD, term infants only: Colostrum: 2.27 ± 0.191 Transitional: 2.19 ± 0.297 Mo 1–3: 1.90 ± 0.20 Mo 4–6: 1.50 ± 0.225 Mo 7–12: 1.15 ± 0.15	Full breast expression from the left breast between 10 and 11am	Mean ± SEM plasma zinc (μg/dL): Cord: 118.3 ± 5.07 Mo 3: 76.9 ± 8.85 Mo 6: 74.2 ± 5.12 Mo 9: 83.8 ± 7.84 Yr 1: 114.6 ± 13.02 Infant growth not reported
Italy, 1999 [26] Chierici	n = 22 mothers of appropriate weight for gestational age term infants. Half were randomized to receive vitamin and mineral supplements	Healthy, nonsmokers, nonvegetarians; uncomplicated pregnancy, labor, and delivery	Mean ± SD, placebo group only (n = 11): Day 3: 8.16 ± 2.96 Day 30: 3.9 ± 1.01 Day 90: 2.87 ± 1.23	10 mL of milk was obtained from both breasts with a breast pump before the baby nursed	No difference in gains in infant weight, length, or head circumference between the 2 maternal study groups
Japan, 1982 [23] Higashi (colostrum data excluded because day of collection not specified)	n = 65 mothers 21–37 yr of age (mean, 27.3 yr) with healthy, full-term infants with birthweight > 2,500 g	Mothers judged to have good nutritional status based on clinical examination. Mean serum zinc ranged from 55 to 115 μg/dL	Mean ± SD: Colostrum: 10.39 ± 4.43 (n = 65) Wk 1: 4.56 ± 3.01 (n = 65) Mo 1: 2.66 ± 1.03 (n = 65) Mo 3: 1.14 ± 0.67 (n = 45) Mo 5: 1.05 ± 0.46 (n = 35)	10 mL of milk obtained by manual expression in the morning before the baby was due to be fed	Infant growth and zinc status not reported
Japan, 2005 [61] Yamawaki	n = 1,197 mothers under 40 yr of age (mean, 29.12 ± 3.99 [SD] yr) who did not smoke or use vitamin supplements, with infants with no symptoms of atopy and birthweight > 2,500 g (mean, 3,135 ± 346 [SD] g)	Zinc status not specifically measured	Mean ± SD: Days 1–5: 4.75 ± 2.48 (n = 20) Days 6–10: 3.84 ± 1.39 (n = 38) Days 11–20: 3.37 ± 0.89 (n = 40) Days 21–89: 1.77 ± 1.08 (n = 551) Days 90–180: 0.67 ± 0.8 (n = 476) Days 181–365: 0.65 ± 0.43 (n = 39)	~50 mL of milk obtained at an intermediate time during suckling	Mean birthweight, 3,135 ± 346 (SD) g. Infant zinc status not specifically reported
Nigeria, 1982 [36] Atinmo	n = 20 low-SES mothers with term infants	Maternal health and zinc status not reported	Mean ± SD: Days 2–4: 5.98 ± 1.12 Days 8–14: 5.49 ± 0.73 Mo 1–2: 3.93 ± 0.78	10 mL of milk collected by manual expression at noon just before infant feeding	Infant growth and zinc status not reported

continued

TABLE 1. Publications included in the data analysis *(continued)*

Country, year [reference] author	Study group	Health and zinc status of mothers	Zinc concentration in milk according to stage of lactation (mg/L)	Description of milk sample	Growth or zinc status of infant
Nigeria, 1984 [50] Mbofung	n = 240 low-SES mothers 17–40 yr of age who had given birth to a normal, full-term infant	Mothers judged healthy by clinical examination and medical history; zinc not specifically measured	Mean ± SEM: Days 1–7: 5.83 ± 0.13 (n = 96) Wk 4: 3.2 ± 0.10 (n = 54) Wk 8: 3.0 ± 0.10 (n = 34) Wk 12: 2.3 ± 0.11 (n = 84) Wk 16: 1.88 ± 0.13 (n = 40) Wk 20: 1.08 ± 0.10 (n = 31) Wk 24: 0.98 ± 0.08 (n = 21) Wk 32: 0.82 ± 0.09 (n = 12) Wk 36: 0.68 ± 0.10 (n = 8)	8–12 mL of milk collected between 9 am and noon from the breast due for the next feeding by the mother using manual expression	Infant growth and zinc status not reported
Spain, 1997 [52] Ortega	n = 57 lactating women aged 18–35 yr with healthy infants with normal birthweight and a mean gestational age of 39.4 wk	Comparison of healthy mothers with low zinc intake (< 50% RI; mean serum zinc, 78.5 ± 9.8 μg/dL) and high zinc intake (≥50% RI; mean serum zinc, 87.0 ± 17 μg/dL) (p< .05)	Mean ± SD, mothers with low zinc intake: Days 13–14: 3.05 ± 0.48 Day 40: 1.88 ± 0.41 Mean ± SD, mothers with high zinc intake: Days 13–14: 3.34 ± 0.60 Day 40: 2.16 ± 0.52 8% of mothers with low zinc intake and none with high zinc intake had < 0.75 mg/L breastmilk zinc	5-mL samples collected from each breast pre- and post-feeding between 10 and 11 am	Normal growth No difference in infant growth between groups
Spain, 2001 [56] Silvestre	n = 22 women, well-nourished and healthy with term infants	Well nourished; zinc not measured specifically	Mean ± SD: Days 2–4: 7.99 ± 3.23 Day 14: 3.31 ± 1.06 Day 30: 2.41 ± 0.90 Day 60: 1.40 ± 0.65 Day 90: 1.05 ± 0.71	Foremilk collected from both breasts prior to feeding; collection between 11 am and 4 pm	Healthy; infant growth and zinc status not reported
Turkey, 2005 [13] Ustundag	n = 20 mothers (mean age, 23 ± 1 [SD] yr) of term infants and n = 20 mothers (mean age, 21 ± 2 [SD] yr) of preterm infants (< 37 wk gestational age); all mothers were nonsmokers	Healthy mothers; weight ranged from 50 to 65 kg. Zinc not measured specifically	Mean ± SD, mothers of term infants only: Days 0–7: 2.41 ± 0.28 Days 7–14: 2.28 ± 0.19 Day 21: 2.39 ± 0.20 Mo 2: 2.01 ± 0.18	Milk collected by manual expression within 2 h of the first feeding in the morning between 8 and 11 am	Infant growth and zinc status not reported

UK, 1982 [44] Hibberd	n = 10 mothers of term infants	Healthy; zinc not specifically measured	Mean ± SD: Day 2: 7.75 ± 1.33 Day 4: 4.51 ± 1.32 Day 6: 3.90 ± 1.23 Day 9: 3.32 ± 1.27 Day 16: 2.81 ± 1.16 Day 23: 2.33 ± 1.31 Day 30: 1.71 ± 1.15 Day 37: 1.71 ± 0.92	Complete expression from both breasts at each feeding with the use of an electric breast pump; an aliquot was taken for analysis and samples from each 24-h period were pooled	Infant growth and zinc status not reported
USA, 1979a [59] Vaughan	n = 38 Caucasian women aged 19–42 yr, mothers of term infants; 1–31 mo postpartum (average participation was 4 mo); 22 primiparous and 16 multiparous	"Good or excellent" nutritional status Maternal zinc intake (mg/day): Mo 4–6: 12.7 Mo 7–9: 17.3 Mo 10–12: 16.5 Mo 13–18: 16.1 Mo 19–31: 13.1 Serum zinc concentration (μg/dL): Mo 4–6: 230 Mo 7–9: 220 Mo 10–12: 140 Mo 13–18: 150 Mo 19–31: 220	Mean ± SD: Mo 1–3: 1.60 ± 0.23 (n = 28) Mo 4–6: 1.05 ± 0.15 (n = 39) Mo 7–9: 0.75 ± 0.11 (n = 23) Mo 10–12: 0.63 ± 0.09 (n = 13) Mo 13–18: 0.69 ± 0.18 (n = 28) Mo 19–31: 0.60 ± 0.19 (n = 30) Total: 15.0 ± 4.2	150–200 mL of milk collected once per month at 4-wk intervals. Mothers instructed to collect milk over a 3–5-day period in the morning, afternoon, and evening at random intervals within feeding	Infant growth and zinc status not reported
USA, 1983a [7] Feeley	n = 102 mothers aged 16 to 38 yr with healthy full-term infants; no difficulties during delivery. Middle SES; 97 Caucasian, 3 African-American, 2 Asian-American	n = 39 (38%) took self-prescribed zinc supplements at a dose of 15 mg/day; n = 2 (2%) took self-prescribed zinc supplements at a dose of 25 mg/day No association between use of zinc supplements and milk zinc concentration	Mean ± SD: Days 4–7: 5.36 ± 0.02 (n = 91) Days 10–14: 4.22 ± 0.01 (n = 163) Days 30–45: 2.99 ± 0.01 (n = 158) Means for each stage of lactation are significantly different (p< .05)	30 mL (1/3 foremilk, 1/3 halfway through feeding, 1/3 hindmilk) milk sample obtained early morning and late evening by the mother with the use of manual expression or pump	Infant birthweight ranged from 2.5 to 4.8 kg with a mean of 3.5 ± 0.5 (SD) kg. Infant zinc status not reported
USA, 1983b [43] Garza	Longitudinal study of n = 6 mothers 26–35 yr of age with healthy term infants with appropriate weight for gestational age; and exclusively breastfeeding until beginning of study (5–7 mo postpartum)	Healthy, nonsmoking, limited coffee, tea, and alcohol, no medications. Zinc not specifically measured	Mean ± SEM: Mo 6: 1.2 ± 0.2 (n = 5) Mo 6.5: 1.2 ± 0.4 (n = 6) Mo 7: 1.2 ± 0.3 (n = 6) Mo 7.5: 0.8 ± 0.3 (n = 6) Mo 8: 0.7 ± 0.3 (n = 5) Mo 8.5: 0.8 ± 0.3 (n = 6) Mo 9: 0.7 ± 0.1 (n = 4)	Entire contents of a single breast obtained with an Egnell pump 3 to 4 h after the previous feeding, between 8 am and noon	Infant growth and zinc status not reported

continued

TABLE 1. Publications included in the data analysis *(continued)*

Country, year [reference] author	Study group	Health and zinc status of mothers	Zinc concentration in milk according to stage of lactation (mg/L)	Description of milk sample	Growth or zinc status of infant
USA, 1983c [51] Moser	n = 23 mothers of term infants recruited from private obstetrics clinic. Mean age was 30 ± 2 (SEM) yr; mean education was 15 ± 2 (SEM) yr	Mean ± SEM maternal zinc intake (mg/day): Wk 37 gestation: 9.8 ± 0.6 (n = 23) Mo 1 postpartum: 9.4 ± 0.5 (n = 23) Mo 3 postpartum: 12.8 ± 1.8 (n = 20) Mo 6 postpartum: 9.6 ± 0.7 (n = 19) Mean ± SEM maternal plasma zinc (μg/dL): Wk 37 gestation: 63.8 ± 2.0 (n = 23) Mo 1 postpartum: 79.1 ± 1.7 (n = 23) Mo 3 postpartum: 87.6 ± 2.2 (n = 21) Mo 6 postpartum: 84.4 ± 2.4 (n = 19)	Mean ± SEM: Mo 1: 2.6 ± 0.2 (n = 21) Mo 3: 1.3 ± 0.1 (n = 20) Mo 6: 1.1 ± 0.1 (n = 18)	30 mL collected at the first morning feeding after 6 am containing ½ foremilk and ½ hind milk	Infant growth and zinc status not reported
USA, 1984a [17] Butte	Longitudinal study of n = 8 preterm infants (gestational age, 30–36 wk; mean, 33.9 wk; birthweight, 1,920 ± 70 [SD] g) and n = 13 term infants (gestational age, 37–42 wk; mean, 39.2 wk; birthweight, 2,990 ± 46 [SD] g)	Mothers of term infants 20–35 yr of age who did not consume > 2 servings/day of coffee, tea, alcohol, or carbonated beverages; parity ≤ 2, no routine medications. Maternal health was judged to be good by health history	Mean ± SD, term infants only: Wk 2: 3.4 ± 0.8 Wk 4: 2.9 ± 0.9 Wk 6: 2.1 ± 0.9 Wk 8: 1.9 ± 0.6 Wk 10: 1.8 ± 1.0 Wk 12: 1.4 ± 0.7	Full breast expression with breast pump between 8 and 12 am, at least 2 hours after feeding	Mean birthweight, 2.99 ± 0.46 (SD) kg. Infant zinc status not reported
USA, 1985a [38] Casey	n = 11 mothers aged 26-39 yr. White, middle-class, well nourished. Mean parity was 2.5 (range, 2–4)	Apparently well-nourished, with uncomplicated pregnancy; zinc not measured specifically	Mean ± SD: Day 1: 7.94 ± 5.65 (n = 7) Day 2: 11.5 ± 4.7 (n = 8) Day 3: 9.34 ± 1.97 (n = 9) Day 4: 6.40 ± 0.66 (n = 9) Day 5: 5.42 ± 1.11 (n = 8) Day 8 ± 2: 4.74 ± 1.02 (n =10) Day 14 ± 3: 3.88 ± 0.91 (n =10) Day 21 ± 3: 3.71 ± 1.09 (n = 9) Day 28 ± 3: 2.98 ± 0.78 (n = 8)	5 mL collected from both breasts in the middle of the midmorning feeding (2 min after letdown)	Mean birthweight, 3.33 ± 0.46 (SD) kg (range, 2.70–4.05); mean weight at 1 mo, 3.95 ± 0.33 (SD) kg. Infant zinc status not reported

USA, 1985b [5, 45] Krebs (only data from placebo group included)	Middle-income, healthy lactating women, longitudinal trial, zinc supplement (ZS): *n* = 14, placebo (NZS): *n* = 39. Mean infant age was 3.4 mo	Healthy; some took zinc supplements during pregnancy [47]. NZS: mean dietary zinc intake 10.7 ± 4.1 mg/day	Mean ± SD: Placebo group: Mo 1: 2.65 ± 0.81 Mo 9: 0.67 ± 0.40 Zinc supplement group: Mo 1: 2.83 ± 1.05 Mo 9: 0.82 ± 0.54 (at mo 9, 8 subjects remained in placebo and 4 in zinc supplement group)	5 mL collected by manual expression. Pooled samples from 6 collections/day	Infant growth and zinc status not reported
USA, 1987 [37] Butte	*n* = 45 mothers of healthy, full-term, exclusively breastfed infants aged 0–4 mo	Healthy; zinc not measured specifically	Mean ± SD: Mo 1: 2.37 ± 0.82 Mo 2: 1.55 ± 0.62 Mo 3: 1.13 ± 0.52 Mo 4: 1.03 ± 0.52 Interindividual variability (CV), 0.33 Intraindividual variability (CV), 0.42	24-h alternate breast pooled sample: full breastmilk samples (entire content of 1 breast), at each feeding over 24-h period	Infant growth progressed satisfactorily. Infant length positively associated with zinc intake at mo 1 and 2
USA, 1989 [39] Casey	*n* = 13 well-educated mothers (mean age, 31.9 ± 4.4 [SD] yr), median income > US$35,000, with normal prepregnancy weight and weight gain during pregnancy. Uneventful pregnancy	Healthy mothers, nonsmokers, all multiparous. Dietary assessment: mean zinc intake, 10.9 ± 0.5 (SD) mg/day; 5/13 took supplemental zinc (additional 10–45 mg/day); no effect of supplement reported	Mean breastmilk zinc peaked at 2 days, then declined throughout lactation; decline was nonlinear and more rapid in 1st 2 wk. Higher zinc concentrations at 3, 6, 9, and 12 mo were not associated with supplemental zinc intake Mean ± SD (*n* = 13): Day 7: 4.70 ± 1.20 Mo 1: 2.90 ± 0.70 Mo 12: 0.46 ± 0.30	2–5 mL obtained by manual expression from both breasts at midfeeding in the morning	Infant growth and zinc status not reported

continued

TABLE 1. Publications included in the data analysis *(continued)*

Country, year [reference] author	Study group	Health and zinc status of mothers	Zinc concentration in milk according to stage of lactation (mg/L)	Description of milk sample	Growth or zinc status of infant
USA, 1995 [28] Krebs (pooled data from maternal supplementation trial)	Healthy, lactating women. Prospective, randomized, double-blind, controlled trial; maternal zinc supplementation group (15 mg/day) (n = 40), placebo group (n = 31)	Healthy (plasma zinc was 79.8 μg/dL in both the zinc-supplementation and the non-zinc-supplementation groups, compared with 88.3 μg/dL in the nonlactating control group) Randomized, controlled trial. Maternal zinc supplementation, 15 mg/day. No difference in milk zinc concentration between groups; thus, data were pooled	Mean ± SD (n = 71): Mo 0.5: 3.88 ± 0.99 Mo 1: 3.06 ± 1.12 Mo 2: 2.04 ± 0.83 Mo 3: 1.48 ± 0.65 Mo 4: 1.44 ± 0.66 Mo 5: 1.16 ± 0.62 Mo 6: 1.09 ± 0.67 Mo 7: 0.85 ± 0.51 Mo 8: 0.87 ± 0.58 Mo 9: 0.78 ± 0.51	Milk collection: 1st 29 subjects, 5–10 mL hand-expressed midfeeding from both breasts during 3-day period. 2nd 42 subjects, 3 midfeeding samples/day for 3 days, with > 4 h between samples	Healthy infants. Mean weight-for-age percentile was 62 at 2 mo, 33 at 7 mo, and 25 at 9 mo of age. Mean length-for-age percentile was 43 at 2 wk, 28 at 7 mo, and 26 at 9 mo of age [48]
USA, 1996 [46] Krebs	n = 9 mothers (individually identified as: A–I) of normal, term, exclusively breastfed infants aged 2–5 mo	Healthy; zinc not measured specifically	Mean ± SD for each infant: Mo 2.0: A (1.37 ± 0.2), B (1.96 ± 0.26), Mo 2.3: C (1.01 ± 0.28), D (1.96 ± 0.25) Mo 4.0: E (2.76 ± 0.3), F (1.69 ± 0.32), Mo 4.5: G (1.40 ± 0.21) Mo 4.8: H (1.33 ± 0.15) Mo 5.0: I (1.08 ± 0.14)	5–10 mL expressed at each feeding over 3 days	Mean weight change during 1-wk study period was 20 ± 7.9 g/day. Mean total zinc absorbed was 0.61 ± 0.23 mg/day

RI, recommended intake; SES, socioeconomic status

TABLE 2. Publications excluded from the data analysis

Country, year [reference] author	Study group	Health and zinc status of mothers	Description of milk sample	Zinc concentration in milk according to stage of lactation (mg/L)	Growth or zinc status of infant	Rationale for exclusion
Bangladesh, 1990 [82] Simmer	*n* = 34 lactating mothers in rural area	No information on maternal health or zinc status	All milk extracted from both breasts over 24-h period using mechanical pump; 24-h infant test-weighings performed on 2 days	Mean ± SD: Mo 1: 1.96 ± 0.99 Mo 2–3: 1.25 ± 0.54 Mo 6: 0.93 ± 0.41 Mo 9: 0.73 ± 0.61 Mo 12: 0.54 ± 0.27	No information on infant growth or zinc status	No information on preterm births
Bangladesh, 1996 [9] Hussain	*n* = 34 low-SES mothers in Dhaka in wk 6–36 of lactation. Mean age, 22 yr	2/3 of mothers had BMI < 20	5 mL collected from alternate breasts by manual expression	Mean ± SD: 5–7 am: 2.10 ± 0.83 2–3 pm: 1.74 ± 0.53 10 pm: 1.84 ± 0.69	No information on infant growth or zinc status	Data not presented by stage of lactation
Brazil, 1985 [66, 67] Dorea	*n* = 8 mothers living in a low-income community. Mean age, 22.5 yr	General decrease in WHZ over 0–6 mo lactation but remained > 90% WHZ reference population	~5 mL collected at beginning and end of 1 nursing period at the same time of day	Breastmilk zinc concentration reported as a figure [67]	Infants grew normally during the study period. Breastmilk zinc was a significant predictor of weight gain (p = .0006) and linear growth (p = .02)	Numerical results not reported
Brazil, 1989b [71, 72] Lehti	*n* = 25 Amazonian mothers	Generally inadequate nutritional status	Foremilk of the fullest breast collected by manual expression between 8:30 and 11:30 am	Mean ± SD: Mo 0–1: 2.2 ± 0.9 (n = 37) Mo 1–2: 1.6 ± 0.9 (n = 59) Mo 2–3: 1.4 ± 0.9 (n = 41)	No information on infant growth or zinc status	No information on preterm births
Brazil, 2007 [73] Melnikov	*n* = 117 mothers (mean age, 22.1 yr) and their infants. None of the infants showed malformations or clinically detectable impairment	Mothers were apparently healthy. Breastmilk zinc was not related to maternal age, parity, or history of miscarriage	Manual expression on day 2 postpartum	1.22 ± 0.78 mg% [sic]	No information on infant growth or zinc status	No information on preterm births
China, 2002 [81] Sian	*n* = 18 mothers (mean age, 26 ± 2 [SD] yr) of infants ~2 mo of age (mean age, 48 ± 11 [SD] days)	Mean maternal zinc intake estimated by 3-day diet records, 7.8 ± 1.7 mg/day (n = 18)	5 mL collected by manual expression of midfeeding sample	At ~2 mo postpartum, mean breastmilk zinc concentration was 2.34 ± 0.83 mg/L. Milk zinc output was 2.01 ± 0.97 mg/day	No information on infant growth or zinc status	No information on preterm births

continued

TABLE 2. Publications excluded from the data analysis *(continued)*

Country, year [reference] author	Study group	Health and zinc status of mothers	Description of milk sample	Zinc concentration in milk according to stage of lactation (mg/L)	Growth or zinc status of infant	Rationale for exclusion
Côte d'Ivoire, [70] Lauber	n = 33 mothers in a rural area; 8 were primiparous. 19 infants were aged 1–3 mo and 10 infants were aged 4–15 mo	General health was good and nutritional status was fairly satisfactory	Aliquot taken from full breast expression by electric pump in early morning, from the breast not used to feed the infant the previous night	Mean ± SD: Mo 1: 3.5 ± 0.9 (n = 4) Mo 6: 2.3 ± 0.9 (n = 7) Mo 12: 1.6 ± 0.9 (n = 8) Mo 18: 1.5 ± 1.3 (n = 6)	Weight-for-age declined from mo 5 onward and leveled off at mo 10 Height-for-age remained at 94%–96% of Harvard standards; practically no change in weight-for-height	No information on preterm births
Croatia, 1996 [24] Frković	n = 29 mothers of wide SES range; mean age, 28.9 yr (range, 17–45 yr); mean parity, 1.8	No information on maternal health or zinc status	80 mL collected	Mean (range): Days 2–12: 4.98 (1.69–11.60)	Mean cord blood zinc was 118 ± 21 μg/dL	No information on preterm births
Ethiopia, 2003 [83] Umeta	Rural Ethiopian mothers with infants aged 5–11 mo; n = 253 (83%) agreed to give breastmilk sample	Mothers of stunted and nonstunted children	~15-mL sample collected from right breast, ~60 min after last feeding	Age-adjusted breastmilk zinc (5–11 mo postpartum) of mothers of stunted infants (mean ± SEM): 0.60 ± 0.03 mg/L (n = 92) vs. 0.68 ± 0.02 mg/L (n = 161) in mothers of nonstunted infants (p = .02)	Infants of mothers with low breastmilk zinc were more stunted	No information on preterm births and wide infant age ranges
Finland, 1980 [84] Vuori	n = 28 mothers aged 24–35 yr (mean age, 28 yr). All mothers belonged to 2 highest (out of 3) SES groups. Mean height, 164 cm; mean weight, 54.4 kg prepregnancy, 59.1 kg after delivery, 57.6 kg at 1st survey week (6–8 wk postpartum), 55.5 kg at 2nd survey week (17–22 wk postpartum)	Lowest individual zinc intake was 9.3 mg/day. No correlation between zinc levels in diet and milk. Mean ± SD zinc intake (range): 13.7 ± 2.7 (10.0–19.4) mg/day at 1st survey week, 12.8 ± 2.8 (9.3–18.6) mg/day at 2nd survey week	8-mL aliquots at beginning and end of each feeding during a 24-h period and pooled to one sample	Mean ± SD: 1st survey week: 1.89 ± 0.74 2nd survey week: 0.72 ± 0.44 (p < .001)	No information on infant growth or zinc status	No information on preterm births

Finland, 1994 [25] Salmenperä	*n* = 200 exclusively breastfeeding mother–infant pairs (*n* = 116 exclusively breastfed to 6 mo; *n* = 36 exclusively breastfed to 7–9 mo). Mothers supplemented with 20 mg/day or 40 mg/day zinc or placebo	Healthy, nonsmoking mothers with uncomplicated pregnancy and delivery; full-term healthy infants of appropriate weight for gestational age	Milk was manually expressed; 10 mL of milk was pooled from the beginning and end of each feeding	Median (range) for placebo group (received iron): Days 4–5: 4.75 (3.27–6.9) (*n* = 75) Mo 2: 1.41 (1.1–2.19) (*n* = 77) Mo 4: 0.9 (0.58–1.38) (*n* = 67) Mo 6: 0.67 (0.4–1.13) (*n* = 56) Mo 7.5: 0.61 (0.39–0.97) (*n* = 31) Mo 9: 0.6 (0.38–0.95) (*n* = 14) Mo 10: 0.61 (0.42–0.87) (*n* = 8) Mo 11: 0.43 (0.33–0.57) (*n* = 6) Mo 12: 0.43 (0.33–0.56) (*n* = 5)	No significant difference in serum zinc between exclusively breastfed infants and infants fed complementary foods at 7.5–9 mo. Milk zinc concentration greater in mothers given 40 mg/day zinc than in those given 20 mg/day zinc at 6 and 7.5 mo (*p* = .02)	All women received supplemental micronutrients
Guatemala, 2005 [65] Dhonukshe-Rutten	*n* = 56 women with healthy, exclusively breastfed infants aged 1–6 mo in periurban Guatemala City and low-income rural area	Zinc not specifically measured; 17/42 had intestinal parasites	Full expression with hand pump plus manual expression > 1 h after last feeding from breast not used in last feeding	Mean ± SD: Mo 1: 3.85 ± 0.76 (*n* = 4) Mo 2: 2.38 ± 0.53 (*n* = 14) Mo 3–6: 1.53 ± 0.64 (*n* = 29)	No information on infant growth or zinc status	Women with parasites had higher breastmilk zinc than non-infected counterparts: 2.30 ± 1.10 (SD) vs. 1.80 ± 0.82 mg/L
India, 1980 [6] Rajalakshmi	Cross-sectional study: *n* = 412 low- income urban mothers, *n* = 100 high-income urban mothers, *n* = 208 low-income rural mothers Semilongitudinal study: *n* = 24 low-income mothers	High-income women had greater height, weight, MUAC, and triceps skinfold thickness than low-income women Mean hemoglobin, 13.0 ± 0.15 (SEM) g/dL in low-income group and 12.9 ± 0.26 (SEM) g/dL in high-income group	10 mL collected by manual expression just prior to infant feeding. Day-to-day variation assessed by daily collection for 5–7 days. Diurnal variation assessed by collections at 10 am and 6 pm in 6 women	Breastmilk zinc values according to stage of lactation were available from cross-sectional and semilongitudinal studies, as well as data on diurnal and day-to-day variation in breastmilk zinc	No information on infant growth or zinc status	No information on preterm births

continued

TABLE 2. Publications excluded from the data analysis *(continued)*

Country, year [reference] author	Study group	Health and zinc status of mothers	Description of milk sample	Zinc concentration in milk according to stage of lactation (mg/L)	Growth or zinc status of infant	Rationale for exclusion
India, 1999 [19] Sharda	Mothers of 155 infants of gestational age 26–41 wk and birthweight 550–3,800 g Appropriate for gestational age and small for gestational age not defined; time of collection of milk samples not reported	Maternal serum zinc concentration ranged from 57.5 to 76.5 μg/dL Serum zinc concentration in nonpregnant women was 99.4 ± 14.4 (SD) μg/dL	Milk samples (colostrum) obtained by mechanical expression from both breasts	Mean ± SD: Gestational age 37–41 wk: 5.03 ± 0.078 (n = 5) Term, appropriate weight for gestational age: 5.04 ± 0.072 (n = 3) Term, small for gestational age: 4.98 ± 0.098 (n = 2) Gestational age 26–30 wk: 4.75 ± 0.078 (n = 3) Gestational age 31–33 wk: 4.71 ± 0.098 (n = 11) Gestational age 34–36 wk: 4.75 ± 0.10 (n = 11) Preterm, appropriate weight for gestational age: 4.72 ± 0.12 (n = 17) Preterm, small for gestational age: 4.75 ± 0.072 (n = 8)	Breastmilk zinc lower in preterm than in term infants and in low-birthweight than in normal-birthweight infants No difference in milk zinc concentration between mothers of term infants with different birthweights and mothers of preterm infants with different birthweights Mean ± SD milk zinc: Birthweight 0.5–1.5 kg: 4.75 ± 0.085 mg/L (n = 12) 1.5–2.5 kg: 4.77 ± 1.64 mg/L (n = 14) 2.5–4.0 kg: 5.00 ± 0.14 mg/L (n = 4)	Time of milk collection postpartum not reported
Indonesia, 1998 [68] Gross	n = 92 middle-income mothers in a poor urban area (East Jakarta), 25.4 ± 5.2 (SD) yr of age Mean infant age, 2.4 ± 1.4 (SD) mo (range, 0.1–5.2 mo)	Mean BMI, 22.0; 40% anemic (hemoglobin < 120 g/L); mean plasma zinc, 85.5 ± 24.2 (SD) μg/dL (increased with time postpartum); 29% had low plasma zinc concentrations	Entire milk content from the breast last suckled was collected with a manual pump during 5 consecutive days between 9 and 11 am	Median: 2.7 10th, 90th percentiles: 1.3, 4.7 Range: 0.5–12.8 (n = 91)	Mean HAZ, WHZ, WAZ near 0 and distribution similar to NCHS reference population	Data from wide infant age range presented; values for milk zinc concentration according to infant age not presented numerically
Italy, 1993 [22, 78] Perrone	n = 26 mothers aged 17–37 yr with term infants and 6 mothers with preterm infants. No supplementation during pregnancy or lactation	Apparent good health	Full breast manual expression at the 2nd nursing, 9–11 am	Median ± SD mg zinc/kg dry weight of breastmilk, term infants: Wk 1: 36.4 ± 2.8 (n = 46) Wk 2: 24.2 ± 1.6 (n = 15) Wk 3: 28.6 ± 6.8 (n = 19) Wk 4: 21.7 ± 1.4 (n = 59)	Healthy; zinc status not specifically measured	Data presented as pooled wide range of infant ages; zinc measured as mg per dry weight

Japan, 2003 [69] Honda	*n* = 68 Japanese mothers aged 19–38 yr	No information on maternal health or zinc status	Milk samples collected at 5–8 days postpartum Details of milk collection not specified	Mean ± SD, 5–8 days postpartum: Mother > 35 yr: 5.41 ± 1.44 Mother < 35 yr: 5.90 ± 1.83 Nullipara: 6.27 ± 1.92 Multipara: 5.35 ± 1.5 Cesarean delivery: 5.8 ± 1.58 No Cesarean delivery: 5.83 ± 1.84	No information on infant growth or zinc status	No information on preterm births
Kenya, 2002 [77] Onyango	Mothers of 250 toddlers (mean age, 13.9 mo; range, 12–26 mo) from rural, low-income families	Not supplemented; zinc not specifically measured	Details of milk collection not specified	Breastmilk zinc content not directly measured	No information on infant growth or zinc status	Breastmilk composition taken from another study in The Gambia; no information on preterm births
Kuwait, 2000 [62] Al-Awadi	*n* = 34 mid-upper-class, lactating mothers of term infants living in Kuwait. Maternal age range was 25–40 yr	Healthy; none had plasma zinc below reference values	Manual expression between 6 and 8 am before the infant's 1st feeding	Mean ± SD: Mo 0–6: Kuwaiti: 3.2 ± 0.12 Non-Kuwaiti: 2.4 ± 0.06 Mo 6-12: Kuwaiti: 2.4 ± 0.14 Non-Kuwaiti: 1.9 ± 0.05 Mo 12-18: Kuwaiti: 2.0 ± 015 Non-Kuwaiti: 1.7 ± 0.09	No information on infant growth or zinc status	Pooled data presented from wide range of infant ages
Nepal and USA, 1988 [74] Moser	*n* = 26 Nepalese mothers 2–6 mo postpartum, and *n* = 23 US mothers 3–6 mo postpartum	Mean ± SD plasma zinc (μg/dL): Nepal: 66.7 ± 2.0 USA: 88.3 ± 2.0 ($p < .05$) No significant difference in dietary zinc intake	Early morning, 5–10-mL prefeeding sample	Mean ± SD: Nepal: 1.10 ± 0.098 USA: 1.20 ± 0.098	Mean ± SD infant plasma zinc (μg/dL): Nepal: 68.0 ± 5.9 USA: not determined	Wide age ranges and results not disaggregated by age
Nigeria, 2000 [76] Okolo	*n* = 15 mothers 26.2 ± 3.3 (SD) yr of age and infants 6.12 ± 0.26 (SD) mo of age	Healthy mothers; mean serum zinc, 117 μg/dL	Breastmilk collected at 6 mo of lactation; no information on collection method	Mean at ~mo 6: 1.52	Mean serum zinc concentration was 164 μg/dL. Infant growth not reported	No information on preterm births

continued

TABLE 2. Publications excluded from the data analysis *(continued)*

Country, year [reference] author	Study group	Health and zinc status of mothers	Description of milk sample	Zinc concentration in milk according to stage of lactation (mg/L)	Growth or zinc status of infant	Rationale for exclusion
The Gambia and UK, 1990 [63] Bates	*n* = 56 mothers (mean age, 29.1 yr) in Gambia and *n* = 57 mothers (mean age, 31.8 yr) in UK; stage of lactation varied from 0 to 99 wk postpartum in Gambia and from 0 to 112 wk postpartum in UK	Maternal health and zinc status not reported	1–5 mL expressed between feedings	Mean: The Gambia Mo 1: 4.22 Mo 2: 3.50 Mo 3: 2.78 Mo 4: 1.71 Mo 5: 1.69 Mo 6: 2.15 UK Mo 2: 1.34 Mo 3: 2.06 Mo 4: 0.87 Mo 5: 0.73 Mo 6: 0.73	No information on infant growth or zinc status	Mothers received micronutrient supplements; no information on preterm births
Turkey, 2006 [79] Rakicioğlu	*n* = 21 mothers, 27.3 ± 5.4 (SD) yr, nonsmokers, not taking supplements; infants aged 2–5 mo	Healthy; no chronic diseases	Manual full breast expression after the first nursing period of the day between 9 and 11 am	Mean ± SD: 1.5 ± 0.4 before Ramadan, 1.8 ± 0.3 after Ramadan (p = .001)	Increases in the body length and weight of infants were within the normal NCHS range	No information on preterm births
UK, 1993 [80] Richmond	Mothers of 39 normal, healthy children 17–61 wk of age	No information on maternal zinc status	No information on milk sample collection	Infant zinc intake from milk (geometric mean ± SD): 1.06 ± 2.10 mg/day	No information on infant growth	Zinc concentration of milk not presented. No information on preterm births
USA, 1976 [10] Picciano	Mothers in 6th to 12th wk of lactation (*n* = 50) with healthy, full-term infants. 42 mothers were 20–30 yr old and 8 were > 30 yr. 16 were primiparous and 34 were multiparous	37 of the 50 women were taking supplemental vitamins. "A few supplements contained less than 10% RDA for zinc"	1 40-mL sample/day at early morning feeding for 5 consecutive days (daily period). 2 additional samples at weekly intervals (weekly period) or within 1 day (within-day period)	Mean ± SD: Daily period: 1.68 ± 0.78 Weekly period: 1.59 ± 0.84 Within-day period: 1.58 ± 0.81 Mean: Time of day: morning 1.71, midday 1.54, evening 1.49 (morning value was significantly	Healthy; zinc status not specifically measured	Data not presented by stage of lactation; wide range of infant ages

				and evening values) Age: 20–30 yr, 1.55; > 30 yr, 2.00 ($p < .001$) Parity: 0, 0.39; ≥1, 1.75 ($p < .001$) Lactation history: 0, 1.46; ≥1, 1.75 ($p < .001$)		
USA, 1979b [8] Kirksey	n = 52 Caucasian, middle-SES mothers aged 18–31 yr. 33 were patients at an obstetrical clinic at enrollment; 19 were members of La Leche League (stages of lactation in these women ranged from 1 to 30 mo)	Healthy; zinc intake exceeded 2/3 of RDA (includes supplements); zinc intake from diet was less than 2/3 RDA for 30 women	33 obstetrical patients: 5–10 mL collected after milk letdown at 1st morning feeding on days 3 and 14 postpartum and prior to multivitamin supplementation 19 La Leche League patients: similar to obstetrical patients, except milk collected on 3 consecutive days	Mean breastmilk zinc concentration decreased from 4.72 mg/L on day 3 to 3.20 mg/L on day 14. Correlation between zinc and iron in colostrum: $r = 0.730$. Levels of zinc in milk were similar for supplemented subjects (mean zinc intake, 28 mg) and unsupplemented subjects (mean zinc intake, 11 mg)	No information on infant growth or zinc status	Labels missing from data table
USA, 1982 [86] Zimmerman	n = 34 mothers recruited at hospital (7 preterm infants, 27 full-term infants)	Healthy mothers	5–15-mL samples collected after letdown and before feeding, at 1, 15, and 30 wk (single samples)	Mean: Wk 1: 3.50 Wk 15: 0.850 Wk 30: 0.550 No difference between breastmilk zinc of mothers of preterm and full-term infants	No information on infant growth or zinc status	Sample included 7 preterm infants
USA, 1984b [64] Dewey	n = 46 mothers during mo 7–20 of lactation aged 21–37 yr	Apparently healthy; zinc status not specifically measured	Full breast expression in the morning at the 2nd feeding of the day, either manual or with an Egnell pump	Mean ± SD: Mo 4–6: 0.79 ± 0.47 (n = 38) Mo 7–11: 0.42 ± 0.22 (n = 27) Mo 12–20: 0.33 ± 0.26 (n = 4)	Weight-for-age was at or above the 25th percentile of NCHS standards for 6 of 8 infants at 7 mo and 5 of 6 infants at 11 mo. 4 of 6 infants had decreased gradually in weight for age since mo 7	No information on preterm births

continued

TABLE 2. Publications excluded from the data analysis *(continued)*

Country, year [reference] author	Study group	Health and zinc status of mothers	Description of milk sample	Zinc concentration in milk according to stage of lactation (mg/L)	Growth or zinc status of infant	Rationale for exclusion
USA, 1985c [85] Finley	*n* = 62 mothers with a mean age of 29 yr	Half of the mothers were vegetarian and half were non-vegetarian. Zinc status not specifically measured.	Manual expression of 1 breast in the morning at the 2nd feeding of the day	Data for milk zinc concentration according to month postpartum are presented as a figure	No information on infant growth or zinc status	No information on preterm births
USA, 1988 [11] Karra	*n* = 49 middle-income mothers; mean age, 28.8 yr; mean parity, 1.7	Apparently healthy and adequately nourished; zinc not specifically measured	Milk samples collected at every infant feeding over a 24-h period at 1, 2, 3, 4, 5, and 6 mo postpartum. 10 mL of milk was expressed manually or by breast pump	Data for milk zinc concentration according to month postpartum are presented as a figure	No information on infant growth or zinc status	All women received micronutrient supplements; numerical results not reported
USA, 1990 [75] Moser-Veillon	*n* = 20 lactating women aged 20–36 yr. Double-blind, randomized, controlled trial. Multivitamins (*n* = 10) or multivitamins + 25 mg zinc (*n* = 10). Supplementation from 1 day to 36 wk postpartum	Healthy; mean plasma zinc values ranged from 77.2 to 96.1 μg/dL	Manual expression of half-foremilk, half-hindmilk, in the morning	Mean ± SEM, placebo group: Wk 1: 4.62 ± 0.48 Wk 2: 3.63 ± 0.33 Wk 4: 2.73 ± 0.18 Wk 12: 1.72 ± 0.12 Wk 24: 1.37 ± 0.22 Wk 36: 0.60 ± 0.22	No information on infant growth or zinc status	All mothers received a multivitamin supplement

BMI, body mass index; HAZ, height-for-age z-score; MUAC, mid-upper-arm circumference; NCHS, US National Center for Health Statistics; RDA, recommended daily allowance; SES, socioeconomic status; WAZ, weight-for-age z-score; WHZ, weight-for-height z-score

TABLE 3. Breastmilk zinc concentration according to child's age[a]

Age group (mo)	No. of observations	Mean ± SD milk zinc concentration (mg/L)
< 1	74	4.11 ± 1.50
1 to 2	42	1.91 ± 0.53
3 to 5	24	0.98 ± 0.35
6 to 11	24	0.77 ± 0.22
12 to 21	2	0.64 ± 0.05

a. Data were extracted from studies presented in table 1. Figures for milk zinc concentration represent weighted means of results from all studies that provided information for respective age ranges.

by 1 month. Breastmilk zinc transfer declines further, albeit more slowly, to ~0.7 mg/day by 6 months. The prediction interval displayed in the figure indicates the ranges of values for daily milk zinc intakes that are likely to occur for children at a particular age. Because the available data do not permit adjustment for day-to-day (intraindividual) variability in milk zinc transfer, the portrayed range overestimates the distribution of children's *usual* zinc intakes. Thus, the curves can be used to describe the ranges of daily zinc intakes, but they cannot be used reliably to assess the adequacy of usual zinc intake from breastmilk.

Adequacy of breastmilk zinc transfer in relation to the zinc requirements of exclusively breastfed term infants less than 6 months of age

There are two possible theoretical approaches to assess the adequacy of breastmilk as a source of zinc for exclusively breastfed term infants: to compare usual zinc intake and assumed absorption from breastmilk with the physiological requirements for absorbed zinc, or to test for any functional response after providing supplemental zinc to such infants. The former approach is particularly challenging, for several reasons. First, not only is information needed on the usual total amount of breastmilk zinc consumed at each age, but knowledge is also required regarding the absorption of zinc from this food source. Furthermore, the true physiological requirements for absorbed zinc at each age must be known with some degree of certainty. Although information can be compiled on zinc consumption from breastmilk at different ages (as described above), there is less information on individuals' *usual* zinc intakes or on zinc absorption from breastmilk. Moreover, because the FAZ varies according to the amount of zinc consumed in a particular meal [89, 90], and the amount of milk (and hence zinc) consumed at a single feeding may be extremely variable, the available information on mean FAZ from breastmilk should be considered a rough approximation, at best, of the true value for an individual child.

Two studies have been published on infant FAZ from breastmilk. One study, which reported results from nine exclusively breastfed infants 2 to 5 months of age who received zinc stable isotope tracers along with six feedings per day, found a mean FAZ of 0.54 (range, 0.40 to 0.68) [46]. Another study of 10 non-exclusively breastfed infants 5 to 7 months of age reported a mean FAZ of 0.50 (range, 0.27 to 0.78) when they were fed a total of 250 mL of expressed breastmilk, along with other foods, over the course of three feedings during an 8- to 12-hour study period [91]. Breastmilk zinc was highly bioavailable under the circumstances of these studies, although the actual study conditions were not necessarily consistent with usual breastfeeding practices, so the ultimate implications of these results remain uncertain. In developing countries, the mean frequency of breastfeeding is often greater than 12 times per day [1, 30, 32], so the amount consumed per feeding may be less than that provided in the foregoing studies. In that case, the zinc intakes per feeding would be less, and the FAZ might be even greater than was observed. Because of the broad range of FAZ reported from these studies and the uncertainty regarding the true FAZ from different amounts of breastmilk fed during real-life feeding conditions, we applied assumptions for FAZ values ranging from 0.40 to 0.60 to explore the range of zinc that might be absorbed from breastmilk by infants of different ages, according to these assumptions. The results of these exploratory calculations are shown in **table 5**.

Several expert groups have published estimates of the physiological requirements for absorbed zinc. A WHO committee calculated the amount of zinc that must be absorbed each day to replace endogenous losses, based on data extrapolated from adults, and to allow for infant growth, based on estimates of zinc accretion in newly synthesized tissues of growing infants [92]. The WHO committee estimated that the requirements for absorbed zinc derived by this method are 1.3 mg/day

TABLE 4. Amount of breastmilk transferred from mother to child (g/day) according to child's age and breastfeeding pattern[a]

	Age group (mo)				
Breastfeeding pattern	0–2	3–5	6–8	9–11	12–13
Exclusive breastfeeding	714 ± 131	784 ± 128	—	—	—
Partial breastfeeding	644 ± 159	706 ± 146	674 ± 151	616 ± 172	549 ± 187

a. Data from Brown et al. [87]. Values represent mean ± SD for children from developing countries.

during the first 3 months of life and 0.7 mg/day for the period from 3 to 5 months. The Steering Committee of the International Zinc Nutrition Consultative Group (IZiNCG) accepted the WHO recommendations but further noted that these requirements may be partially offset by hepatic zinc reserves accumulated during gestation [93].

According to the comparisons in **table 5**, breastmilk alone would provide the WHO/IZiNCG estimated mean requirements [92, 93] during the first month of life (without considering the possibility of drawing on hepatic zinc reserves) if the FAZ is greater than 0.50 but would provide less than the mean requirement thereafter. Nevertheless, IZiNCG concluded that breastmilk alone is an adequate source of zinc for approximately 6 months, based on the assumption that some reserves present at birth could be used to meet physiological demands and the fact that supplementation trials have not demonstrated consistent benefits of additional zinc supplementation for exclusively breastfed term infants, as discussed below.

Zinc supplementation trials among breastfed infants less than 6 months of age

Three intervention trials in which breastfed infants less than 6 months of age received either zinc supplements or placebo have been reported from industrialized countries. However, in two of these trials, the period of supplementation lasted until the infants attained 10 to 11 months of age, so the results do not specifically reflect the effect of providing additional zinc to infants less than 6 months of age. In the one trial completed among term infants less than 6 months of age, zinc-supplemented US girls gained 3 g/day more weight than their nonsupplemented counterparts from 4 to 7 months of age, but there were no significant effects on the linear growth of girls or on the weight or length gains of boys [94]. In another study of US term infants 4 to 10 months of age, there was no effect of zinc supplementation on growth or other functional outcomes among either girls or boys [95]. Finally, in a study conducted in France among infants 3 to 11 months old, most of whom were African immigrants, the zinc-supplemented boys had significantly greater weight and length gains after 3 months of supplementation than boys in the placebo group, but there were no significant effects of supplementation among girls [96]. However, these latter findings are difficult to interpret, because differential dropout rates by treatment group may have confounded the results. Moreover, the infants in this trial were receiving nonquantified amounts of other foods, so it is uncertain whether the groups differed only with regard to zinc supplementation. In summary, the results of these three studies carried out in industrialized countries are inconsistent, and none clearly demonstrates a uniformly positive impact of additional zinc on the growth of infants under 6 months of age. Thus, it seems that breastmilk alone is probably an adequate source of zinc for approximately the first 6 months of life for normal-birthweight, term infants in industrialized settings. We could not locate any relevant information from developing countries that was obtained specifically from exclusively breastfed infants.

Section 2

How much zinc is transferred in breastmilk to infants and young children less than 24 months of age who are consuming breastmilk in addition to complementary foods?

Conclusions

The amount of zinc transferred in breastmilk to partially breastfed infants less than 6 months of age is approximately 15% less than that described above for exclusively breastfed infants, because of the smaller volumes of milk consumed by partially breastfed infants. The age-related pattern of change in milk zinc intakes is similar for both groups of infants. After 6 months of age, breastmilk continues to be an important source of zinc, providing ~0.5 mg/day from 6 to 8 months and ~0.4 to 0.3 mg/day thereafter, a time when the estimated physiological requirements for absorbed zinc range from 0.84 to 0.5 mg/day, depending on age and the source of these estimates.

TABLE 5. Estimated daily zinc intake among exclusively breastfed infants less than 6 months of age and estimated total absorbed zinc at three assumed levels of fractional zinc absorption (FAZ), and relation to estimated physiological requirements for zinc, according to infant's age

Age group (mo)	Milk zinc transfer (mg/day)	Amount of absorbed zinc (mg/day), according to assumed FAZ			Estimated average physiological requirements for zinc (mg/day)[a]
		FAZ = 0.40	FAZ = 0.50	FAZ = 0.60	
< 1	2.52	1.01	1.26	1.51	1.3
1–2	1.37	0.55	0.68	0.82	1.3
3–5	0.86	0.34	0.43	0.52	0.7

a. Requirements from WHO and IZiNCG [92, 93]

Detailed review of evidence

Studies of breastmilk zinc transfer to partially breastfed infants less than 24 months of age

Identification of studies and milk zinc concentration by child age. The same process that was described above was used to identify and analyze studies of breastmilk zinc transfer to partially breastfed infants and young children, and the same summary values for milk zinc concentration were used for the specific age intervals, as shown in **figure 2**.

Amount of milk consumed according to child age. As with the preceding analysis, we extracted information on breastmilk volumes from the WHO/UNICEF publication on complementary feeding [87]. For the present analysis, we selected the age-specific information for partially breastfed infants and young children from developing countries, as summarized in **table 4**.

Total zinc transfer in breastmilk to partially breastfed children less than 24 months, and relation to requirements. The amount of zinc transferred in breastmilk to partially breastfed children was estimated using the same simulation procedure described above. The analysis had to be limited to children less than 18 months of age because there was only one data point available for milk zinc concentration from children older than 18 months, and the available information on breastmilk volumes provided a single figure for the entire age range from 12 to 23 months. Because the volume of milk consumed by partially breastfed infants less than 6 months is approximately 15% less than the volume consumed by exclusively breastfed infants, the total milk zinc transfer to the former infants is correspondingly less, although the age-related pattern of change is similar, as summarized in **figure 4** and **table 6**. Even after 9 months of age, breastmilk continues to provide an average of ~0.4 mg/day to partially breastfed infants.

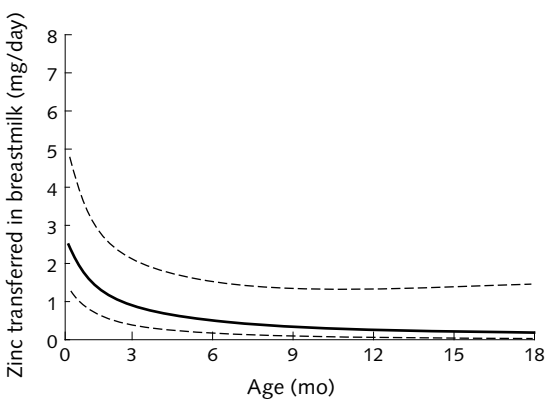

FIG. 4. Simulated mean and 95% prediction interval of daily zinc transfer in breastmilk to partially breastfed children according to age

Assessment of the adequacy of breastmilk zinc transfer in relation to theoretical requirements is even more problematic for partially breastfed children than for those who are exclusively breastfed. Because partially breastfed children are consuming other foods in addition to breastmilk, these other foods may contribute additional zinc and they may affect zinc absorption from breastmilk [98]. Thus, we have not attempted to assess the adequacy of breastmilk zinc transfer at different ages, and we simply present the mean amount of milk zinc that is transferred to demonstrate the potential contribution that breastmilk might provide in relation to these requirements. Clearly, breastmilk provides a potentially important proportion of the daily zinc requirement, even among partially breastfed children in the second year of life.

Section 3

What are the programmatic implications of the answers to the foregoing questions, and what are the remaining research needs?

The foregoing analyses indicate that breastmilk seems to provide an adequate amount of zinc to exclusively breastfed term infants less than 6 months of age. Breastmilk continues to be an important source of zinc for partially breastfed infants well into the second year of life. Thus, breastfeeding should be promoted and supported as an intervention that contributes to the prevention of zinc deficiency. WHO currently recommends that "infants should be exclusively breastfed

TABLE 6. Estimated daily zinc intake among partially breastfed children less than 18 months of age and relation to estimated average physiological requirements for zinc, according to child's age

Age group (mo)	Milk zinc transfer (mg/day)	Estimated average physiological requirement for zinc (mg/day)[a]	
		WHO and IZiNCG	IOM, 2000
< 1	2.18 ± 1.06	1.3	—
1–2	1.18 ± 0.56	1.3	—
3–5	0.73 ± 0.39	0.7	—
6–8	0.51 ± 0.33	0.8	0.84
9–11	0.39 ± 0.31	0.8	0.84
12–17	0.29 ± 0.31	0.8 / 0.5	0.74

a. WHO and IZiNCG provided similar estimates for children < 12 months of age, although IZiNCG assumed that breastmilk was adequate for exclusively breastfed infants < 6 months of age, so the estimates in the table refer to non-exclusively breastfed infants [92, 93]. At 12 to 17 months of age, the two estimates provided by WHO and IZiNCG differ. The US Institute of Medicine (IOM) does not provide estimates of physiological requirements for zinc for children < 7 months of age [97].

during the first 6 months of life. Thereafter they should receive nutritionally adequate and safe complementary foods while breastfeeding continues up to 2 years of age and beyond" [99]. Thus, these infant and young child feeding recommendations are consistent with the current conclusion that breastfeeding should be promoted as an important dietary source of highly bioavailable zinc.

Information is available on how best to promote and support appropriate breastfeeding behaviors [100], and a detailed discussion of these issues is beyond the scope of the present review. Briefly, attention must be devoted to advocacy, policy formulation, health curriculum reform and in-service training of health service personnel, and the development of suitable community-based organizations to provide information, counseling, personal support, and referral when necessary [101].

Additional research is needed on the zinc requirements of infants and young children and the bioavailability of zinc from breastmilk when fed in usual amounts, either alone or with other foods. This information is needed to help determine both the period for which breastmilk alone is an adequate source of zinc and how much additional zinc is needed from complementary foods. Information is also needed on the period of adequacy of breastmilk for providing zinc to low-birthweight infants. Finally, different approaches for promoting optimal breastfeeding behaviors require further evaluation in the context of large-scale programmatic interventions.

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The potential to improve zinc status through biofortification of staple food crops with zinc

Christine Hotz

Abstract

Biofortification is an agricultural strategy that aims to increase the content of select micronutrients, including zinc, in staple food crops such as rice, wheat, maize, pearl millet, and others. When consumed among zinc-deficient populations, zinc-biofortified staple foods should improve the adequacy of zinc intakes and hence reduce the risk of dietary zinc deficiency. Several conditioning factors will contribute to the potential for this strategy to meet its goal, including the additional amount of zinc that can be bred into the staple crop food, the amount of zinc that remains in the staple crop food following usual processing methods, and the bioavailability of zinc from the staple crop food in the context of the usual diet. Reduction of the phytate content of cereals with the use of agricultural techniques is a potential complementary strategy for improving the bioavailability of zinc. The feasibility of biofortification to result in a meaningful increase in the adequacy of population zinc intakes and to reduce the consequences of zinc deficiencies still needs to be determined through efficacy trials. At the program level, the ability to widely disseminate biofortified crop varieties and the willingness of farmers to adopt them will also affect the magnitude of the impact of this strategy.

Key words: Agriculture, biofortification, diet, phytate, staple foods, zinc

The author is affiliated with HarvestPlus and the International Food Policy Research Institute, Washington, DC.

Please direct queries to the author: Christine Hotz, HarvestPlus and International Food Policy Research Institute, c/o the Micronutrient Initiative, 180 Elgin St., Suite 1000, Ottawa, ON K2P 2K3, Canada; email: c.hotz@cgiar.org.

Introduction

Biofortification is an intervention strategy under development with the goal of increasing the content of select micronutrients, including zinc, in the edible portion of staple food crops by agricultural, agronomic, or genetic means. When consumed, biofortified staple foods would lead to improved adequacy of zinc intakes and hence a reduced risk of dietary zinc deficiency, among those who currently have high rates of inadequate intakes. At present, the potential for biofortification with zinc, iron, and provitamin A carotenoids is primarily being evaluated or explored in some of the world's most important staple food crops for the poor, including rice, wheat, maize, cassava, beans, and sweet potatoes [1].

Biofortification of staple food crops can be achieved through the following processes: conventional breeding, by selecting for genotypes with the highest micronutrient content observed for that crop; use of genetic modifications, such as gene insertions or induced mutations; and use of agronomic practices, such as application of zinc-containing fertilizer.

The focus of this strategy is to increase the adequacy of micronutrient intakes among rural, agriculturally based populations that already produce and consume the staple food vehicle. As is the case for universal staple food fortification, biofortification is intended to contribute to the prevention of micronutrient deficiencies, reaching all household members. Unlike traditional fortification, biofortification does not require that the food vehicle be centrally processed. Therefore, this strategy has the potential to fill the gap in coverage by fortification, as it can be more accessible to those who consume staple foods from local or self production. On the other hand, the time required to develop the biofortified crops and the available natural variation of certain micronutrients in different crops may not permit as great an increase in micronutrient content

as fortification may be able to achieve, and greater effort would be required to add MMN through biofortification than is the case for fortification. Transgenic approaches for increasing micronutrient contents in staple crops may enable greater enhancements in the contents of several micronutrients, but there are many regulatory and safety issues that need to be addressed before that option can become widely available. Also, to reach a sufficient number of farmers, dissemination of planting materials for biofortified varieties will depend on the effectiveness of seed systems and agricultural extension activities, which in some countries are still rather limited.

Research and development activities for zinc-biofortified crops are ongoing, and conventionally bred crops may be available for testing and release as approved varieties within the next 5 years.

This paper is divided into four sections, which address the following sets of questions in relation to biofortification of staple food crops with zinc:

Section 1: Can the achievable level of increased zinc content contribute to meeting zinc requirements, taking into account bioavailability, in a large proportion of the vulnerable population?

Section 2: What are the major factors potentially modifying the impact of zinc-biofortified staple food crops?

Section 3: Can biofortified staple foods improve population zinc status and associated health outcomes?

Section 4: What are the risks of consuming zinc-biofortified foods?

Section 1

Can the achievable level of increased zinc content contribute to meeting zinc requirements, taking into account bioavailability, in a large proportion of the vulnerable population?

Conclusions

Achievements in conventional breeding for zinc-rich staple food crops suggest that there is adequate breeding potential for several crops, including rice, maize, wheat, and pearl millet. If assumptions are met for daily intake of the staple food, bioavailability of zinc from the diet, and amount of zinc lost during processing and cooking, the additional amount of zinc achieved through biofortification in these crops would contribute approximately 40% of the absorbed zinc requirement for nonpregnant women and for children 4 to 6 years of age. Adoption rates or "coverage" of zinc-biofortified crops and their consumption by different age groups will need to be assessed in large-scale implementation trials but may be expected to range from 30% to 60% in Asia (e.g., rice and wheat) and from 20% to 40% in Africa (e.g., maize).

Detailed review of the evidence

To assess the feasibility of biofortification to result in a meaningful increase in the adequacy of population zinc intakes, there are three main issues that need to be considered: What are the levels of additional absorbed zinc intake that need to be achieved to contribute meaningfully to human zinc requirements? Can those levels be achieved through the possible biofortification processes? If minimum target levels for zinc contents of staple foods are achieved, will they reach the target population at risk?

What are the levels of additional absorbed zinc intake that need to be achieved to contribute meaningfully to zinc requirements?

The amount of additional zinc in various food crops that will result in a biologically meaningful improvement in population zinc status needs to be established through research. The theoretical optimal amount of additional zinc to be added through biofortification would be calculated to cover the deficit between actual zinc intakes and the intake amount that would minimize the proportion of the population with inadequate zinc intakes. This is the approach that has been recommended for the design of universal food fortification programs [2]. However, because biofortified staple foods are still being developed and zinc content is being increased incrementally over time, there is presently less flexibility in catering to specific needs of populations than there may be with commercial food fortification. For this reason, minimum target levels have been set as preliminary goals for the first phase of development of biofortified staple foods. The proposed minimum target level for zinc has been set to provide an additional amount of bioavailable zinc in the food supply that is equivalent to ~40% of the physiological requirement for absorbed zinc for nonpregnant women and nonbreastfed children 4 to 6 years of age [3, 4] among populations with high intakes of the staple food of interest.

The latter target increment makes assumptions about the usual amount of staple food intake per day; the loss of zinc from the seed, root, or tuber during processing (e.g., milling) and cooking; and the bioavailability of zinc from the staple food in the context of the usual diet [3]. The specific assumptions used in setting these minimum, generic breeding targets for zinc contents in various crops are summarized in **table 1**.

For the appropriate development of biofortified staple foods, each of these assumptions will need to be verified and adjusted accordingly in populations targeted for biofortification. Dietary intake data from

a range of rural, low-income populations at risk for dietary zinc deficiency are lacking, so quantification of usual, individual intakes of zinc and of the targeted staple foods is required. The losses of zinc with cooking of staple foods is typically very low (e.g., 0% to 10%; [5]), but losses with milling of grains at different extraction rates can be high (**table 2**) and must be quantified. Losses of phytate should be measured together with zinc losses, since both the total zinc content and the phytate:zinc molar ratio determine the amount of absorbable zinc [4]. The assumed 25% bioavailability of zinc is only an average figure, and since the target levels, and presumably the biological impact of dietary zinc interventions, will depend on the net amount of zinc absorbed, zinc absorption from biofortified crops should be measured directly. Zinc bioavailability is particularly important to confirm for children, since the absorption of zinc from different staple food-based diets has not frequently been quantified in children of different ages and it cannot be directly predicted from the equations derived from studies in adults [4, 6]. The potential modifying effects of milling in relation to zinc content and bioavailability are discussed in Section 2.

Another approach to assessing the adequacy of different increments in the zinc contents of staple foods is through modeling with dietary intake data. One such analysis was conducted with the use of dietary intake data from the National Nutrition Survey of Mexico of 1999 [7] and proposed zinc requirements [4]. The analysis examined the effect of increasing the content of zinc in maize from 18 to 33 μg/g dry weight on the adequacy of bioavailable zinc intakes. Bioavailability was estimated with the use of a prediction equation [4], and the zinc content and phytate:zinc molar ratio of the diets and estimated absorbable zinc were compared with the age-specific physiological requirement for absorbed zinc to determine adequacy. At the level of maize consumption in the population, the model predicted a decrease in the prevalence of inadequate zinc intakes from 28% to 15% among rural preschool children and from 43% to 19% among rural women. With the use of this approach, appropriate target levels for a specific population could be determined by assessing the simulated impact of different levels of

TABLE 1. Assumptions used to set minimum target levels for increased zinc content of biofortified staple food crops[a]

Staple food[b]	Group	Estimated Average Requirement for zinc[c]	Staple food intake (g/day)	Average zinc content of crop (mg/kg)	Additional zinc content to be achieved (mg/kg)	Bioavailable zinc (as percentage of physiological requirement[d])
Whole maize Whole wheat Polished rice Cassava	Nonbreastfed children 1–3 yr	2	100	Whole maize: ~30 Whole wheat: ~30 Polished rice: ~16 Cassava: ~10	+ 8	34
	Children 4–6 yr	4	200			43
	Nonpregnant women ≥ 19 yr	7	400			39
Sweet potato Common beans	Nonbreastfed children 1–3 yr	2	50	Sweet potato: ~9 Common beans: ~32	+ 17	36
	Children 4–6 yr	4	100			46
	Nonpregnant women ≥ 19 yr	7	200			41

a. For all staple crops, zinc losses during processing were assumed to be 10% and zinc bioavailability was assumed to be 25%.
b. For maize, rice, wheat, and beans, intakes are expressed on a dry weight basis; for cassava and sweet potato, intakes are expressed on a fresh weight basis.
c. The Estimated Average Requirements for unrefined cereal-based diets or refined vegetarian or mixed diets recommended by the International Zinc Nutrition Consultative Group (IZiNCG) [4] are given.
d. The physiological requirements for absorbed zinc presented by IZiNCG [4] were used.

TABLE 2. Zinc contents of whole cereal grains and milled products

Cereal	Zinc content (μg/g dry weight)	Cereal	Zinc content (μg/g dry weight)	Cereal	Zinc content (μg/g dry weight)
Maize		Wheat		Rice	
Whole	21.0	Whole	37	Brown	28
High extraction	17.3	85% extraction	19	Polished	17
Low extraction	8.0	70% extraction	12		
Degermed	4.4				

Sources: Welch and Graham [32], Ferguson et al. [33], US Department of Agriculture [34], and International Minilist/WorldFood Dietary Assessment System, 2.0 (University of California, Berkeley, CA, USA).

additional zinc on the prevalence of inadequate intakes of bioavailable zinc.

Can those levels be achieved through the possible biofortification processes?

At present, it is estimated that the minimum target level for zinc in adapted varieties of rice, maize, wheat, and pearl millet is achievable through conventional breeding within the next 2 to 5 years [8]. For other crops, such as yams and cassava, sufficiently high levels of zinc content may not be achievable through conventional breeding in a measurable time frame because of limited natural genetic variation in zinc content. For some crops, such as beans and potatoes, adequately increased zinc content may be achievable, but the time frame for doing so cannot yet be estimated.

Although proof-of-concept has been achieved for genetic modification to synthesize β-carotene and increase iron content through synthesis of ferritin protein, the use of biotechnology to increase the zinc content of staple foods is still at an exploratory stage [9]. Research is still required to identify genes involved with the plants' uptake and translocation of zinc and its deposition into the edible portion of the grain, root, or tuber. Information is accumulating on zinc transporters and how they function in different plant tissues throughout their development [10, 11].

Some progress has been made with the use of biotechnology that may affect zinc nutrition indirectly by improving its bioavailability. Staple food crops, such as maize, have been modified by mutagenesis to achieve a reduced grain content of phytic acid [12]. Progress has also been made with the use of transgenic approaches to express heat-stable phytase from microbial sources in the endosperm of wheat [13]. Although plenty of evidence exists to indicate that reduced phytate intakes relative to zinc intakes result in increased efficiency of absorption of zinc [4, 14], Mazariegos and colleagues [15] recently concluded that zinc absorption was not significantly greater among children consuming a diet based on a low-phytate maize mutant (~60% reduction) than among children consuming an isogenic wild-type maize, possibly because of greater than expected variation in total phytate intakes, inadequate statistical power, and differences in the total zinc content of the diet, which independently affects the fractional absorption of dietary zinc. There is as yet no clear evidence available from controlled trials for the benefits to zinc status from long-term intake of diets in which only the phytate content is reduced.

Zinc-containing fertilizers for wheat production in areas with zinc-deficient soils, such as Turkey, are already in use as a means of improving crop yields [16]. The application of zinc in soil fertilizers or foliar sprays can, in some situations, also result in increased zinc content of the grain. For example, the grain zinc concentrations of bread wheat, durum wheat, triticale, and barley were found to increase by 39% to 77% with soil zinc application in either rain-fed or irrigated fields [17], and the concentration of zinc in rice paddies increased up to 2.3-fold with soil zinc application in Pakistan [18]. In Bangladesh, foliar fertilization with zinc resulted in grain zinc content that was 2.6 times greater in rice and 2 times greater in wheat than in controls [19]. Further, these increments were estimated to result in a doubling of the total zinc intake of Bangladeshi children. Assuming a 43% increase in the zinc content of milled rice due to soil zinc application, Gibson and colleagues recently estimated that the total zinc intake of Thai schoolchildren at high risk for zinc deficiency would be nearly doubled from 4.8 to 8.6 mg/day with treatment, on the basis of their usual intakes of rice and zinc [20]. However, variation in grain zinc concentration in response to zinc fertilizer application will occur with different crops, genotypes of the same crop, and environmental conditions [16], so the benefits of these techniques for human zinc nutrition cannot be extrapolated to all situations.

If minimum target levels for zinc contents of staple foods are achieved, will they reach the target population at risk?

To reach large segments of populations vulnerable to zinc deficiency, biofortified staple food crops must be adopted by farmers for regular production and by consumers for regular consumption. This requires several conditioning factors. First, the strategy must tap into available mechanisms for disseminating new seed or planting materials to farmers, such as through public extension systems, private seed companies, or nonprofit or nongovernmental organizations involved in rural extension. Adoption of the seed will require that the biofortified varieties have acceptable traits, such as adequate (if not improved) yield, pest and disease resistance, and preferred physical and organoleptic properties. If these latter conditions are met, adoption of zinc-biofortified staple foods should not require a strong behavior change component. In general, it is reasonable to expect that these latter conditions are or will be met for zinc-biofortified crops [21].

Adoption rates for new crop varieties can vary widely by region; for example, conservative estimates of adoption rates of 30% to 60% have been assumed for Asia, where seed systems are better developed, and 20% to 40% for Africa, where seed systems are less well developed [22]. Where adoption rates are typically lower, additional investment in interventions through nutrition education, farmer education, and seed systems support may be required to assure adequate coverage in a reasonable length of time. Coverage for zinc-biofortified staple food crops will need to be determined through the evaluation of large-scale interventions to determine the percentage of the population that produces or purchases biofortified foods, the percentage

of production represented by the biofortified variety, and, ultimately, the amount of biofortified varieties consumed by the target population that is at risk for zinc deficiency. A pilot intervention to introduce β-carotene–rich sweet potato in Mozambique as a biofortified staple crop included an assessment capturing many of these elements [23]. Similar research on a larger scale will need to be carried out for zinc-biofortified crops in a variety of settings; the potential coverage and impact may only be known in the next 5 to 10 years.

Section 2

What are the major factors potentially modifying the impact of zinc-biofortified staple food crops?

Conclusions

The effects of milling degree on the potential usefulness of zinc biofortification in grains require further study. Quantification of the zinc and phytate contents of whole and refined biofortified grain products and the absorption of zinc from them should be measured in human studies. It is possible that loss of zinc with milling may be offset by the parallel loss of phytate and a commensurate increase in zinc bioavailability. Nonetheless, maximum benefits of biofortification may be observed if more of the additional grain zinc can be deposited into the starchy endosperm. Another possible strategy for positively affecting the impact of zinc biofortification is a concomitant decrease in phytate content. Some experience with low-phytate mutant cereal crops suggests that it is possible to decrease grain phytate content substantially, but the agronomic viability of these mutants and the impact on zinc status after long-term consumption still require assessment. The potential impact of host or environmental factors affecting intestinal health and either zinc absorption or intestinal losses of endogenous zinc needs to be quantified. If these conditions are highly prevalent in populations that could benefit from biofortification, the additional amount of zinc achieved through biofortification may need to be higher, or the impact of zinc-biofortified foods may be conditional on their treatment or prevention.

Detailed review of the evidence

In cereal grains, rather large concentrations of zinc typically occur in the outer layers (e.g., aleurone) and the germ. As a result, large proportions of the total grain zinc content are lost during milling; some examples of the content of zinc in grain and their milling products are shown in **table 2**. If the process of biofortification results primarily in increased amounts of zinc in the portions of the grain lost in milling, the benefits of the strategy may be more limited for those populations that consume milled grains. Efforts of breeders and plant scientists need to focus on increasing micronutrient content of the starchy portion of the grain (endosperm) to have maximum benefit for the widest range of populations.

On the other hand, milled grain products will have a lower content of phytate, the primary plant component that inhibits zinc absorption in humans. Therefore, although a lower amount of additional zinc may be achievable in the starchy endosperm, what is there will be more bioavailable. For example, using a recently published trivariate model for estimating zinc absorption from diets with varying levels of zinc and phytate [14], Hambidge and colleagues [6] predicted that the same amount of additional zinc would be absorbed from biofortified wheat flour whether it was whole or of 85% extraction, based on biofortified and control wheat produced in Mexico by the Center for the Improvement of Maize and Wheat (CIMMyT). A human study of zinc absorption using these wheat products is in progress to verify the predictions.

Strategies for reducing the phytate content of zinc-biofortified cereal grains may help to increase their impact on zinc intake adequacy. Induced mutations in several crops, including maize, wheat, and barley, have led to varying degrees of decrease in grain phytate content [24–26]. Although an increase in zinc absorption with decreased phytate content of maize from such mutants was well demonstrated among US adults [27], the increase in the amount of absorbed zinc following the longer-term consumption of the reduced-phytate maize mutants consumed with typical diets was not significantly greater than that for diets with the wild-type control maize or a local maize variety with a naturally higher zinc content [15]. The long-term effects of reduced phytate intake on zinc absorption and excretion should be reassessed in further studies. Some of the low-phytate mutant crops were shown to have reduced yields [24], and this would need to be overcome in order for these mutants to be further tested and released as varieties.

Other factors that may alter the efficacy of zinc-biofortified wheat include any of those that limit the ability of individuals to maintain zinc homeostasis [4]. For example, potentially common conditions, such as frequent diarrhea, intestinal parasitic infections, or tropical enteropathy characterized by increased intestinal permeability [28, 29], could lead to increased intestinal losses of endogenous zinc [30] and hence increased requirements for dietary zinc. Such factors could therefore limit the benefit of any food-based intervention, including zinc fortification. It is uncertain to what extent poor intestinal health caused by any of those conditions may affect zinc absorption and zinc homeostasis [4]; research in this area is needed.

Section 3

Can biofortified staple foods improve population zinc status and associated health outcomes?

Conclusions

There is no direct evidence yet available to demonstrate whether or not zinc-biofortified staple foods can have an impact on zinc status and associated health outcomes.

Detailed review of the evidence

The availability of zinc-biofortified staple crops is still limited, and thus randomized, controlled efficacy trials of their impact on zinc status and associated health outcomes have not yet been conducted [3]. The first of such trials are expected to be conducted within the next 3 to 5 years.

Section 4

What are the risks of consuming zinc-biofortified foods?

Conclusions

At the modest, physiological levels of increased zinc content presently achievable through biofortification, it is unlikely that there is any substantial risk of toxic intakes of zinc as a result of biofortification. It is also unlikely that an increased zinc content of staple foods would result in changes to their organoleptic qualities that could lead to rejection of biofortified varieties that might be introduced.

Detailed review of the evidence

The increased content of endogenous zinc in staple foods is not expected to cause any changes to the color, texture, or organoleptic qualities of these foods, and therefore the additional zinc may be considered as "invisible" to the population [21]. This is in contrast to biofortification with provitamin A, which causes distinct changes in color and other organoleptic properties. Nonetheless, high-zinc lines in many cases will need to be backcrossed into local or improved varieties in order to maintain all of the locally preferred agronomic and organoleptic qualities of the crop [31]. Ultimately, the preferences of farmers and consumers need to be addressed when new varieties are to be released.

As described above in relation to assessing the appropriateness of different target increments in the zinc content of staple foods, simulations based on existing dietary intake data can be used to estimate the impact of biofortification on the prevalence of intakes that might exceed the upper limit for zinc. The simulation model using data from the National Nutrition Survey of Mexico of 1999 [7] predicted that only 3% and 0% of preschool children and women, respectively, would have total zinc intakes exceeding the proposed upper limit for zinc [4] after the biofortification of maize. Nonetheless, to fully assess the prevalence of high zinc intakes in a population, it also would be necessary to conduct similar modeling for other subgroups, such as adolescent boys and adult men, because their intakes of staple foods are usually higher than those of women and preschool children, and hence they are more likely to achieve levels of zinc intake that could exceed the upper limits. Ultimately, direct studies of the potential toxicity of zinc biofortification, such as through assessment of the effect of excess zinc on copper status, would be necessary to confirm the true risk of toxicity; the upper limits for zinc intakes were derived from studies of zinc intakes from supplements and may not be relevant to intakes of zinc from food sources [4].

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Galvanizing action: Conclusions and next steps for mainstreaming zinc interventions in public health programs

Kenneth H. Brown, Shawn K. Baker, and the IZiNCG Steering Committee*

Abstract

This paper summarizes the results of the foregoing reviews of the impact of different intervention strategies designed to enhance zinc nutrition, including supplementation, fortification, and dietary diversification or modification. Current evidence indicates a beneficial impact of such interventions on zinc status and zinc-related functional outcomes. Preventive zinc supplementation reduces the incidence of diarrhea and acute lower respiratory tract infection among young children, decreases mortality of children over 12 months of age, and increases growth velocity. Therapeutic zinc supplementation during episodes of diarrhea reduces the duration and severity of illness. Zinc fortification increases zinc intake and total absorbed zinc, and recent studies are beginning to confirm a positive impact of zinc fortification on indicators of population zinc status. To assist with the development of zinc intervention programs, more information is needed on the prevalence of zinc deficiency in different countries, and rigorous evaluations of the effectiveness of large-scale zinc intervention programs should be planned. Recommended steps for scaling up zinc intervention programs, with or without other micronutrients, are described. In summary, there is now clear evidence of the benefit of selected interventions to reduce the risk of zinc deficiency, and a global commitment is urgently needed to conduct systematic assessments of population zinc status and to develop interventions to control zinc deficiency in the context of existing public health and nutrition programs.

Key words: Dietary diversification, dietary modification, research needs, zinc deficiency, zinc fortification, zinc intervention, zinc supplementation

Kenneth H. Brown is affiliated with the Department of Nutrition and the Program in International and Community Nutrition, University of California, Davis, California, USA; Kenneth H. Brown and Shawn K. Baker are affiliated with Helen Keller International, Dakar, Senegal.

Please direct queries to the corresponding author: Kenneth H. Brown, Department of Nutrition, University of California, One Shields Ave., Davis, CA 95616, USA; e-mail: khbrown@ucdavis.edu.

* The other members of the Steering Committee of the International Zinc Nutrition Consultative Group (IZiNCG) are Zulfiqar A. Bhutta (Department of Paediatrics and Child Health, the Aga Khan University, Karachi, Pakistan), Omar Dary (A2Z/The USAID Micronutrient and Child Blindness Project, Washington, DC, USA), Rosalind S. Gibson (Department of Human Nutrition, University of Otago, Dunedin, New Zealand), Christine Hotz (HarvestPlus and International Food Policy Research Institute, Washington, DC, USA), Janet C. King (Children's Hospital Oakland Research Institute, Oakland, California, USA), Bo Lönnerdal (Department of Nutrition and Program in International and Community Nutrition, University of California, Davis, California, USA), Juan A. Rivera (Nutrition and Health, National Institute of Public Health, Cuernavaca, Mexico), Marie T. Ruel (Food Consumption and Nutrition, International Food Policy Research Institute, Washington, DC, USA), Emorn Wasantwisut (Institute of Nutrition, Mahidol University, Salaya, Thailand), and Sonja Y. Hess (IZiNCG Executive Officer; Department of Nutrition, and the Program in International and Community Nutrition, University of California, Davis, California, USA).

Background and summary of the evidence

This second technical document prepared by the International Zinc Nutrition Consultative Group (IZiNCG) provides information on the range of intervention strategies that can be considered for control of zinc deficiency and systematic reviews of current knowledge regarding their efficacy and effectiveness. The results of these analyses confirm a beneficial impact of preventive zinc supplementation for reducing the incidence of selected childhood infections and increasing children's physical growth [1], and of therapeutic zinc supplementation for reducing the duration and severity of diarrhea [2]. In particular, preventive zinc supplementation reduces the incidence of diarrhea by approximately 27% among young children over 12 months of age and decreases the incidence of acute lower respiratory tract infections by approximately 15%. Preventive zinc supplementation may also reduce

the incidence of malaria, but the number of available studies is still relatively small, so more research is needed to confirm this outcome. Overall, zinc supplementation reduces child mortality by approximately 6%. This impact of preventive zinc supplementation is restricted to children over 12 months of age (in whom the mortality reduction is approximately 18%) and possibly to small-for-gestational-age infants. Preventive zinc supplementation also increases linear growth and weight gain of young children, thereby contributing to reduced rates of stunting and underweight. Importantly, available studies show that preventive zinc supplements provided in recommended amounts do not have adverse effects on the status of other micronutrients or cause any detectable functional abnormalities. Thus, preventive zinc supplementation is both safe and efficacious, contributing positive benefits for achieving the United Nations Millennium Development Goals number 1 (reduction of poverty and hunger, as measured by rates of underweight and stunting) and number 4 (reduction of child mortality).

The present analysis confirms that provision of therapeutic zinc supplementation as adjunctive treatment reduces the duration of acute diarrhea by 0.5 days and that of persistent diarrhea by 0.7 days [2]. The results therefore support the recommendation by the World Health Organization (WHO) and the United Nations Children's Fund (UNICEF) to include therapeutic zinc supplementation in diarrhea control programs [3]. Insufficient information is available to determine whether zinc supplementation might also enhance the treatment of acute and chronic respiratory infections, and available studies found no impact of therapeutic zinc on the outcome of malaria treatment [2].

Preventive zinc supplementation has also been examined among pregnant and lactating women [4]. An earlier meta-analysis concluded that zinc supplementation during pregnancy reduced the rate of preterm births by 14% [5]. However, there were no consistent effects on complications of labor and delivery or on infant birthweight. Reports from studies in Peru and Bangladesh noted reduced rates of postnatal infant infections [6, 7], and the one study that examined infant growth throughout the first year of life found increased growth beginning at 4 months and continuing through 12 months of age among infants whose mothers received supplemental zinc during pregnancy [8]. However, there is still too little information to allow definitive conclusions to be drawn on these postnatal infant outcomes. Available information suggests that maternal zinc supplementation during lactation does not affect breastmilk zinc concentration [4], although most of these studies were conducted among presumably well-nourished women in industrialized countries, so more information is needed from undernourished women. Studies among lactating women have not reported on maternal health, so information is needed on such outcomes.

Less information is available on the impact of zinc fortification programs and of dietary intervention strategies, although the available evidence suggests that both of these approaches should enhance zinc status. For example, studies indicate that zinc fortification can increase zinc intake and total zinc absorption [9]. However, data are still lacking to demonstrate a similar positive impact of zinc fortification on young children's zinc status, and additional information is needed on the effectiveness of large-scale fortification programs, among both children and adults. Information is also needed on the impact of point-of-use multiple micronutrient (MMN) fortificants on zinc status and other zinc-related health outcomes.

A number of dietary intervention strategies have the potential for improving zinc status. Breastmilk is an important potential source of zinc for infants and young children, and current international guidelines for the promotion of breastfeeding should be viewed as an appropriate component of programs to support adequate zinc nutrition of young children [10]. Other interventions to increase the availability, accessibility, and consumption of animal-source foods or to increase the zinc content of plant-source foods or to increase zinc absorption from these foods should all enhance the consumers' zinc status [11]. However, rigorous evaluations of large-scale dietary approaches are still lacking. In the future, biofortification holds promise as a sustainable approach to improve the zinc content and/or bioavailability of staple food crops [12].

To summarize, there is good evidence supporting the beneficial impact of zinc interventions, especially of zinc supplementation and, to a lesser extent, zinc fortification, on zinc status and zinc-related functional outcomes. Moreover, recent reviews of major strategies to reduce childhood morbidity and mortality have highlighted the importance of controlling zinc deficiency. For example, the *Lancet* series on maternal and child undernutrition concluded that zinc deficiency is responsible for approximately 4% of the worldwide morbidity and mortality burdens of young children [13]. Similarly, the recently revised Copenhagen Consensus on the best ways of advancing global welfare cited zinc supplementation as one of the top-ranked interventions for reducing malnutrition and improving welfare overall [14].

On the basis of these sets of information, it is reasonable to ask why national governments and international agencies are not investing more in programs to reduce zinc deficiency. Although there are multiple possible answers to this question, the most likely explanations seem to be the lack of comprehensive information on the prevalence of zinc deficiency, the limited programmatic experience in the delivery of zinc interventions, the lack of guidelines from international agencies on the need to control zinc deficiency, and possibly the

inherent inertia of under-resourced health systems and donor agencies to implement new programs, especially when total funding for nutrition programs is relatively stagnant.

To address these formidable challenges, efforts are urgently needed to include assessment of zinc status in national nutritional status assessment surveys, to promote increased understanding of the importance of zinc deficiency and to motivate greater interest in controlling this problem, and to conduct operational research to determine how best to deliver zinc-intervention programs in the context of ongoing nutrition and health activities. The following paragraphs summarize some of the major issues that need to be addressed to permit scaling up and mainstreaming of zinc interventions. Because information on the implementation and impact of these programs is still rudimentary, it will be important to incorporate into the design and implementation of these programs rigorous monitoring and evaluation systems so that they can be critically assessed and modified as necessary.

Collecting information on population zinc status

Objective information on population zinc status is urgently needed for countries that have been identified as having an elevated risk of zinc deficiency. Recommendations are now available on the best approaches for assessing population zinc status [15]. To minimize the cost of collecting information on population zinc status, opportunities should be sought to include zinc-related information in already scheduled health or nutritional status surveys to be conducted in representative samples of national populations. This information can be used to assess the need for specific zinc interventions and for targeting these activities, and as a baseline to assess the future impact of such programs.

Mobilizing interest in zinc nutrition

There has been a recent resurgence in global attention to the problem of malnutrition in general and of micronutrient deficiencies in particular, as is evidenced, for example, by the creation of the REACH initiative (Ending child hunger and undernutrition initiative [http://endingchildhunger.blogspot.com]) and the development of the new multipartner 10-year strategy for reduction of vitamin and mineral deficiencies [16]. Moreover, the aforementioned *Lancet* series on maternal and child undernutrition [13, 17] and the Copenhagen Consensus publication [14] have captured global interest and can be used as powerful advocacy tools. There are multiple global, regional, and national forums addressing nutrition and child survival issues. These opportunities should be seized to sensitize policy makers and program managers to the importance of mainstreaming zinc into child health and nutrition interventions. IZiNCG technical documents and a one-page summary of major advocacy points concerning zinc nutrition are available on the internet (www.IZiNCG.org) to assist with these communication tasks.

To motivate further discussions and actions at the national level, it would be helpful to enlist existing national nutrition stakeholders' groups or to help catalyze formation of such groups where they do not exist. Examples of important partners to include in such national stakeholders' groups are representatives of the public health community, consumer groups and other civil society organizations, industry, and donor agencies, as well as individual politicians, academicians, and journalists.

Implementing and scaling up preventive zinc supplementation

Given the growing evidence and heightened awareness concerning the positive impacts of preventive zinc supplementation in children, there is an urgent need to address specific operational issues that are currently impeding programmatic progress. The present review identified several key operational issues [1], including the optimal dosage range for preventive zinc supplementation, with or without other micronutrients, for particular target groups within the population; the appropriate frequency and duration of supplementation; and the types of products that can be used to deliver zinc, such as dispersible tablets, multimicronutrient powders (e.g., products like Sprinkles), and lipid-based nutrient supplements. The review also identified a number of existing delivery platforms, within both the public and the private sectors, that could be exploited for delivering preventive zinc supplements to children, because the currently ongoing activities provide reasonably frequent and reliable contacts with the target groups and high levels of coverage. Examples of potential delivery platforms that should be assessed for the feasibility of adding preventive zinc supplementation are listed below. The choice of which platform(s) is(are) most appropriate in a particular setting needs to be made at the country level.

- Twice-yearly vitamin A supplementation, which increasingly is being integrated into semiannual events for child survival, and expanded with multiple additional product-delivery components;
- Growth monitoring and promotion programs, if these are sufficiently well organized and utilized to support and justify additional interventions;
- Community-based or community-directed distribution programs, such as are being adopted, for

example, to distribute preventive and therapeutic medicines for certain parasitic diseases;
» Social marketing through private-sector distribution channels

Further, in view of the apparent impact of preventive zinc supplementation in reducing mortality in small-for-gestational-age infants and the multiple special needs of these infants, there are compelling reasons for designing programs to identify and provide special support to low-birthweight infants, including preventive zinc supplementation.

As summarized above, there is less consistent evidence for a beneficial impact on pregnancy outcomes when preventive zinc supplementation is given to pregnant women. However, in view of the possible benefits of zinc supplementation for reducing the risk of premature delivery, the possible positive impact of zinc supplementation on infant birthweight among undernourished women, and the possible benefits for infant postnatal health, as well as the lack of reported adverse effects, zinc should be included in maternal supplements given during pregnancy in populations at risk for zinc deficiency.

Implementing and scaling up therapeutic zinc supplementation

As indicated in the present series of papers [2], recommendations already exist for including zinc supplementation as adjunctive therapy during episodes of acute and persistent diarrhea [3]. These revised guidelines take into account two new approaches: the new formulation for oral rehydration solutions containing lower concentrations of glucose and salt, and providing children with 20 mg of supplemental zinc per day for 10 to 14 days (10 mg per day for infants less than 6 months old). Despite the global consensus on these recommendations, progress in mainstreaming these revised guidelines has been slow. To help countries revise their national diarrhea programs, useful implementation guidelines have been developed in several languages by the Zinc Task Force* [18–20]. As a first step in promoting inclusion of zinc in diarrhea treatment, existing diarrhea program guidelines, professional practices, and domiciliary treatment tendencies should be assessed. Information is also needed on care-seeking behaviors and household procurement of medicines and oral rehydration therapy. This information can be used to develop training guidelines for health professionals, a communication plan for informing and motivating potential beneficiaries, and a strategy for procuring and distributing zinc supplements. These activities can be used to revitalize national diarrheal disease control programs at the same time that zinc treatment is introduced into the national diarrhea treatment protocol. Increased efforts are particularly important in Africa and South-East Asia, where deaths due to diarrhea remain very high [21].

Implementing and scaling up zinc fortification

Implementation of fortification programs requires a number of steps, which have been described in detail elsewhere [22]. Multiple vehicles for mass and targeted zinc fortification are available, and guidelines have recently been published on recommended levels of zinc fortification of cereal flour [23]. The recommended level of zinc fortification of cereal flour depends on the population's usual intake of the cereal product(s), the degree of milling (hence, the intrinsic zinc and phytate contents of the flour), and the zinc and phytate contents of the remainder of the diet. For cereal flour fortification programs that are already operating in countries at risk for zinc deficiency, it is important to review current norms and to add or adjust zinc levels in line with the new guidelines. For cereal flour fortification programs currently being planned in countries with an elevated risk of zinc deficiency, zinc should be included in the fortification premix at appropriate levels.

Implementing and scaling up dietary interventions

Ultimately, the most desirable approach to eliminate zinc deficiency will be to ensure universal access to diets with adequate zinc content and bioavailability. There is considerable experience in designing and implementing programs to promote increased household food production through home gardens and small-animal production, including poultry, fish, and small ruminants, but there is limited experience in taking such programs to scale [24]. These approaches have the added benefit of addressing multiple nutrient shortfalls, not just zinc deficiency, and of providing other economic advantages to the household [11]. For greatest success in terms of nutritional outcomes, household food production interventions should be linked to effective behavior change communication interventions and focus on women, so that the products will be used by the household members to address specific nutritional problems of the most vulnerable members of the family. Although improved household production of zinc-rich foods, particularly those foods derived from animal sources, is expected

* The Zinc Task Force was formed as a collaborative working group among UNICEF, WHO, the Johns Hopkins Bloomberg School of Public Health, and the US Agency for International Development (USAID), with support from the Bill and Melinda Gates Foundation, to accelerate the adoption of zinc for diarrhea management.

to enhance the consumers' zinc status, there have been few attempts to conduct rigorous evaluations. Thus, it is still not possible to state which specific dietary approaches are most likely to mitigate the problem of zinc deficiency.

Research needs

There are a number of remaining knowledge gaps concerning zinc nutrition, assessment of zinc status, and the efficacy, effectiveness, and cost of zinc intervention programs, which need to be addressed to permit more rapid and extensive implementation of zinc intervention programs. The most important research needs were described in the individual sections of this document, so they will be reviewed only in general terms in this concluding paper. Readers are also referred to a recent publication that applied a new research priority-setting methodology to review zinc-related research needs for public health programs [25].

A recurring theme in many recent documents on zinc nutrition is the need for novel biomarkers of zinc status that are reliable, low-cost, and feasible for field implementation. Nevertheless, consensus has been reached on possible approaches to assess population zinc status [15], using currently available indicators, so the most pressing research need for public health programs is to apply the existing techniques so as to generate more information on the prevalence and major risk factors of zinc deficiency in different countries. Whenever nutrition status surveys are conducted in countries that have been identified as having an elevated risk of zinc deficiency, the possibility of including indicators of zinc status should be considered.

On the topic of preventive zinc supplementation, the major research questions deal with optimal dosage regimens (amount, frequency, and duration of supplementation), appropriate combinations of micronutrients, and the effectiveness of available delivery platforms that might be exploited for distributing the supplements. Information is also needed on the efficacy of preventive supplementation for reducing mortality among low-birthweight infants. Many of the same dosage and delivery issues pertain to therapeutic zinc supplementation, although more information is also needed from efficacy trials for particular diseases, such as acute lower respiratory infection and tuberculosis, and for selected age groups.

A great deal of research is still needed regarding the impact of maternal zinc supplementation during pregnancy on both maternal and infant health outcomes. In particular, studies should focus on the optimal time of initiating supplementation (pre- or periconceptual vs. later in pregnancy), maternal factors, such as body weight and zinc status, that may modify the response to zinc supplementation, and possible adverse effects of supplementation. Longer-term outcomes for child health also should be explored. The effect of zinc supplementation of undernourished women on their breastmilk zinc concentrations should be studied further.

The efficacy and effectiveness of zinc fortification of different food vehicles should be determined for different age and sex groups. Recent guidelines on appropriate levels of zinc fortification of cereal flour [23] should be assessed in the context of ongoing programs to confirm their adequacy. Greater attention also should be devoted to the design and evaluation of the nutrition and health impacts of dietary interventions designed to enhance zinc status. Such interventions may include increased production and consumption of animal-source foods, food processing to reduce phytate content, and strategies to increase the zinc content of staple foods, for example, through biofortification or better agronomic practices.

Conclusions

In summary, there is now clear evidence of the benefit of selected interventions to eliminate or reduce the risk of zinc deficiency. Such interventions, if implemented at scale and attaining high coverage, would have a direct impact on achieving several Millennium Development Goals, including reducing child morbidity, mortality, and restricted growth. A global commitment is urgently needed to conduct systematic assessments of population zinc status and to develop and mainstream interventions to control zinc deficiency in the context of existing health and nutrition programs.

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List of Contributors

IZiNCG Steering Committee

Kenneth H. Brown, M.D. (guest editor, author, IZiNCG internal reviewer)
Professor
Department of Nutrition, and Program in International and Community Nutrition
University of California
Davis, California, USA
and Africa Regional Advisor in Nutrition and Child Survival
Helen Keller International
Dakar, Senegal

Sonja Y. Hess, Ph.D. (guest editor, author, IZiNCG internal reviewer)
Research Nutritionist
Department of Nutrition, and Program in International and Community Nutrition
University of California
Davis, California, USA

Shawn K. Baker, M.P.H (author, IZiNCG internal reviewer)
Regional Director for Africa
Helen Keller International
Dakar, Senegal

Zulfiqar A. Bhutta, M.D., Ph.D. (author, IZiNCG internal reviewer)
Professor and Chair
Department of Paediatrics and Child Health
The Aga Khan University
Karachi, Pakistan

Rosalind S. Gibson, Ph.D. (author, IZiNCG internal reviewer)
Research Professor
Department of Human Nutrition
University of Otago
Dunedin, New Zealand

Christine Hotz, Ph.D. (author, IZiNCG internal reviewer)
Nutrition Coordinator
HarvestPlus, and International Food Policy Research Institute
Washington, DC, USA

Janet C. King, Ph.D. (author, IZiNCG internal reviewer)
Senior Scientist
Children's Hospital Oakland Research Institute
Oakland, California, USA
Professor Emeritus, University of California at Berkeley and Davis

Bo Lönnerdal, Ph.D. (author, IZiNCG internal reviewer)
Professor
Department of Nutrition, and Program in International and Community Nutrition
University of California
Davis, California, USA

Juan A. Rivera, Ph.D. (author, IZiNCG internal reviewer)
Director
Nutrition and Health
National Institute of Public Health
Cuernavaca, Mexico

Omar Dary, Ph.D. (IZiNCG internal reviewer)
Food Fortification Specialist
A2Z/The USAID Micronutrient and Child Blindness Project
Washington, DC, USA

Marie T. Ruel, Ph.D. (IZiNCG internal reviewer)
Director
Food Consumption and Nutrition Division
International Food Policy Research Institute
Washington, DC, USA

Emorn Wasantwisut, Ph.D. (IZiNCG internal reviewer)
Senior Advisor
Institute of Nutrition
Mahidol University
Salaya, Nakhon Pathom, Thailand

Other contributors

Victoria P. Anderson, M.Sc. (author)
Research Assistant
Department of Human Nutrition
University of Otago
Dunedin, New Zealand

Reina Engle-Stone (author)
Research Assistant
Department of Nutrition, and
Program in International and Community Nutrition
University of California
Davis, California, USA

Batool A. Haider, M.D. (author)
Epidemiologist and Senior Instructor (Research)
Department of Paediatrics and Child Health
The Aga Khan University
Karachi, Pakistan

Nancy F. Krebs, M.D. (author)
Professor
Department of Pediatrics
University of Colorado School of Medicine
Denver, Colorado, USA

Janet M. Peerson, MS (author)
Statistician
Department of Nutrition, and
Program in International and Community Nutrition
University of California
Davis, California, USA

Josh Jorgensen (research assistant)
Research Assistant
Department of Nutrition, and
Program in International and Community Nutrition
University of California
Davis, California, USA

K. Ryan Wessells (research assistant)
Research Assistant
Department of Nutrition, and
Program in International and Community Nutrition
University of California
Davis, California, USA

Ricardo Uauy, M.D., Ph.D. (Coordinator of external review)
President of the International Union of Nutritional Sciences (IUNS)
Professor of Public Health Nutrition
London School of Hygiene and Tropical Medicine
London, UK
and Institute of Nutrition University of Chile INTA
Santiago, Chile

Nita Dalmiya, M.P.H. (external reviewer)
Nutrition Specialist
Nutrition Section
UNICEF
New York, USA

Ian Darnton-Hill, M.B.B.S., Ph.D., M.P.H. (external reviewer)
UNICEF Special Adviser to the Executive Director on Ending Child Hunger and Undernutrition
UNICEF, New York, USA
and REACH: Ending Child Hunger and Undernutrition Inter-Agency Taskforce
World Food Programme
Rome, Italy

Olivier Fontaine, M.D. (external reviewer)
Medical Officer
Department of Child and Adolescent Health and Development
World Health Organization
Geneva, Switzerland

Dominic Schofield, M.B.A. (external reviewer)
Nutrition Specialist (Food Fortification)
Nutrition Section
UNICEF
New York, USA