Zinc deficiency in children with environmental enteropathy—development of new strategies: report from an expert workshop[1–4]

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ABSTRACT

Zinc deficiency is a major cause of childhood morbidity and mortality. The WHO/UNICEF strategy for zinc supplementation as adjunctive therapy for diarrhea is poorly implemented. A conference of experts in zinc nutrition and gastrointestinal disorders was convened to consider approaches that might complement the current recommendation and what research was needed to develop these approaches. Several key points were identified. The design of novel zinc interventions would be facilitated by a better understanding of how disturbed gut function, such as environmental (or tropical) enteropathy, affects zinc absorption, losses, and homeostasis. Because only 10% of zinc stores are able to be rapidly turned over, and appear to be rapidly depleted by acute intestinal illness, they are probably best maintained by complementary regular supplementation in a primary prevention strategy rather than secondary prevention triggered by acute diarrhea. The assessment of zinc status is challenging and complex without simple, validated measures to facilitate field testing of novel interventions. Zinc bioavailability may be a crucial factor in the success of primary prevention strategies, and a range of options, all still inadequately explored, might be valuable in improving zinc nutrition. Some therapeutic actions of zinc on diarrhea seem attributable to pharmacologic effects, whereas others are related to the reversal of deficiency (ie, nutritional). The distinction between these 2 mechanisms cannot be clarified given the insensitivity of serum zinc to identify subclinical deficiency states. Why zinc seems to be less effective than expected at all ages, and ineffective for secondary prevention of diarrhea in children <12 mo of age, remains unclear. It was concluded that a reframing of the current recommendation is warranted with consideration of how to better optimize and deliver zinc and whether to provide a complementary public health primary prevention zinc strategy. This requires careful consideration of the zinc product to be used as well as strategies for its delivery. *Am J Clin Nutr* 2014;100:1198–207.

INTRODUCTION

A series of expert workshops in zinc nutrition and gastrointestinal disorders, especially acute childhood diarrhea, commenced in Seattle, WA, in September 2012 with the following purposes:

- Identifying those issues that, if resolved or better understood, would lead to more effective products and delivery strategies for addressing the global health problems associated with zinc deficiency
- Assessing whether there might be additional options for delivery of zinc supplementation that would complement the existing WHO/UNICEF Joint Statement on Clinical Management of Acute Diarrhea (1)

The current WHO/UNICEF Joint Statement on Clinical Management of Acute Diarrhea recommends 10–20 mg zinc/d (dependent on age) for 10–14 d for reduction in diarrhea duration and severity (the therapeutic benefit) and prevention of subsequent episodes (the "secondary prevention" benefit) (1, 2). There is no WHO/UNICEF "primary prevention" strategy directed toward healthy children at risk of zinc deficiency as a public health approach.

At the initial workshop, the topics of zinc bioavailability, mechanisms and sites of absorption, zinc homeostasis, biomarkers for zinc and zinc-related diseases, environmental enteropathy (EE)[5] and effect of zinc deficiency on the gut, the epidemiology of zinc deficiency, the evidence for zinc therapy

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[5] Abbreviations used: EE, environmental enteropathy; EFZ, endogenous fecal zinc; EZP, exchangeable zinc pool; IP, inositol phosphate; L:M, lactulose:mannitol ratio.

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of diarrhea, and global policy were considered. This article summarizes the outcomes of these discussions, focusing especially on the areas in which our understanding of zinc nutrition, homeostasis, and therapy is incomplete. To stimulate further research into these areas, we provide an overview of zinc as a nutrient and the current WHO/UNICEF recommendations, followed by suggestions as to how one could proceed to address those gaps in our understanding that limit development of strategies that might complement existing recommendations.

ZINC POLICY

Zinc deficiency is a major cause of childhood morbidity and mortality in developing countries (3, 4). Deficiency itself is common, although its prevalence varies in different countries, as judged from differences in plasma zinc between populations (5). Zinc supplements reduce the incidence of diarrhea and probably mortality (6). Thus, the 2008 Copenhagen consensus meeting concluded that zinc supplementation, together with vitamin A supplementation, is the most cost-effective strategy for advancing child welfare (7).

The UNICEF/WHO strategy for diarrheal control lists 7 points of importance, including poor sanitation, contaminated food supply, inadequate toilet facilities, and lack of access to vaccination and oral rehydration therapy as being important issues to address for global child health (2). Although zinc is included in that strategy, it is listed only in the context of treatment and secondary prevention. "Secondary prevention" refers to strategies applied after a disease has occurred so as to reduce disease severity and prevent subsequent disease (1, 2). Zinc therapy to treat acute diarrhea has been part of WHO/UNICEF recommendations since 2004 (1). It recommends that an episode of acute diarrhea should trigger daily administration of 10–20 mg zinc (depending on age) for 10–14 d, a reactive medicalized strategy dependent on a functional medical system, accessible products in public and private sectors, and the willingness of mothers to comply with administration to children who will usually be without symptoms for the latter three-quarters of this therapeutic period. In this policy, the indications for zinc therapy remain narrow, even though zinc has the potential to benefit a range of other health outcomes, including other infections and growth (6, 8). The WHO has not yet produced a guideline or strategy for zinc as a primary prevention program. Some of the evidence for impact on other health outcomes is inconsistent, and even for diarrhea there is evidence from randomized controlled trials that zinc administered as a micronutrient powder fails to prevent episodes of diarrhea (4, 9).

Notwithstanding the evidence base underpinning this strategy for diarrhea (10, 11), the zinc component of the oral rehydration solution–zinc treatment strategy is poorly implemented. Globally, adherence to the recommended usage of zinc for 10–14 d after an acute episode of diarrhea is only 3–5% (12), although other data show better usage in resource-intensive programs such as in Bangladesh (13). Nonimplementation is considered to be a major part of the problem. Speakers considered that there may be reasons to explain this low uptake at all levels, from product design, through dosage schedule, adverse events such as vomiting, provider confidence in the benefit, consumer education and acceptance, unpleasant taste, as well as delivery channel inadequacy, which is a subject of continued effort and investment.

ZINC NUTRITION

Zinc is a ubiquitous trace element, essential for growth and development. It is required for >300 metalloenzymes and proteins (14–17) that have diverse roles including the integrity and function of epithelia (14, 18). The major organ system of zinc exchange with the environment is the gut (18, 19). Zinc is primarily absorbed by a saturable process in the proximal small bowel but also more distally through entero-enteric reabsorption that diminishes losses (20). The amount of zinc ingested and the quantity of phytate in the diet are the major determinants of absorption efficacy. Zinc homeostasis is maintained predominantly by modulating endogenous zinc excretion by the intestine (21). In young children, zinc deficiency is associated with poor health, including impaired growth, possibly impaired intellectual development, and increased risk of diarrhea and pneumonia (22, 23).

Two generalized dietary patterns are major factors in the etiology of dietary zinc deficiency, one in which inhibition of absorption predominates and bioavailability is the issue and the other in which the zinc content of the diet is deficient (24). Dietary inhibitors are contained in cereals and legumes that are high in phytate, a potent inhibitor of zinc bioavailability, whereas deficient diets are based on starchy roots and tubers that have low zinc content. Where zinc deficiency is prevalent, such as in developing countries, these patterns are accompanied by a low intake of a range of other micronutrients and minimal animal source protein.

Host factors contributing to deficiency involve compromised absorption and excess loss. Frequent episodes of an acute illness, such as diarrhea, compromise zinc status due to zinc losses. Fecal zinc losses increase significantly during an episode of diarrhea in infants and are associated with negative zinc balance (25). The high prevalence of EE in developing countries, contributed to by poor water quality and sanitation, increases the risk of zinc deficiency (26), as suggested by the close correlation between global distribution of surrogate measures of EE, such as intestinal permeability, and measures of zinc deficiency (27, 28). Thus, it is likely that acute events in susceptible children can tip the scale of marginal deficiency to more significant deficiency; hence, the basis for WHO/UNICEF recommendations for zinc supplementation in response to an attack of diarrhea (2).

Although the evidence for the effectiveness of zinc therapy for diarrhea is particularly strong and has provided the impetus for formalization of WHO recommendations focusing on diarrhea, it seems counterintuitive for policy aimed at correcting a global micronutrient deficiency relevant to a broad range of health outcomes to depend primarily on a therapeutic strategy for one acute clinical condition, diarrhea, during which zinc is being lost and its conservation is compromised.

It was felt that an additional strategy would be to complement the current therapeutic strategy used to treat diarrhea with a primary prevention strategy implemented in the context of a public health approach. The aim of primary prevention is to prevent the disease from occurring (2). In considering the global prevalence of zinc deficiency and current recommendations for zinc supplementation, the workshop identified 6 issues as being important for advancing both product development and delivery of a primary prevention supplementation strategy on a global scale. These covered several aspects of zinc nutrition (bioavailability, store

depletion and homeostasis, and assessment of zinc status), the host challenge presented by EE, and issues for efficacy (mechanisms of action and reasons for ineffectiveness).

EE and zinc

There is evidence that EE, a condition characterized by morphologic changes in the gut of inhabitants of developing countries (29), is associated with perturbations in zinc homeostasis (26). EE, also originally known as tropical enteropathy, is characterized by intestinal inflammation, partial villous atrophy, and epithelial cell degenerative changes leading to loss of absorptive surface (29). Functional disturbances in EE include reduced absorption, increased turnover of intestinal cells, increased mucosal permeability, and generalized activation of the innate and adaptive immune system (29). Alterations in the microbiome as found in EE may arise from a variety of causes including frequent enteric infections and probably contribute to intestinal dysfunction in affected children (30). Frequent enteric infections and EE are important in the pathogenesis of growth-faltering and its consequences in children in developing countries (31), acting through poorly understood mechanisms that may potentially include a zinc-deficient status.

Whether zinc deficiency contributes to the development of EE, or whether it is EE that contributes to zinc deficiency, is not completely understood. It was felt to be likely that there is an aggravating, cyclic relation.

Several observations point to a close link between EE and disturbances in zinc nutrition. Disordered zinc homeostasis has been observed in children considered to have EE. These children showed an inability to conserve endogenous zinc losses [so-called endogenous fecal zinc (EFZ)], which can amount to 6 mg/d, during periods of inadequate bioavailable zinc intake (18, 26).

In healthy adults, EFZ correlates with the exchangeable zinc pool (EZP). With moderate dietary depletion (from 9.6 to 3.8 mg/d for 3 wk), EFZ falls together with a rapid 30% decrease in the EZP (32). In some children with EE, this relation is lost, with a decrease in EZP not correlating with a concurrent reduction in EFZ, suggesting an inability to preserve zinc homeostasis through conservation of endogenous zinc (26).

In children living where EE is endemic, EFZ loss is positively correlated with the lactulose:mannitol (L:M) ratio, the best noninvasive marker we currently have of increased mucosal permeability (26). The altered intestinal permeability associated with elevated L:M ratios may reduce dietary zinc absorption. Despite these perturbations of intestinal permeability, an elevated baseline L:M ratio does not prevent an increase in plasma zinc in response to zinc supplementation at a 5-mg/d dose (33).

The regulation of EFZ and its dysregulation in disease remains to be more fully explored. Whether it is possible to reduce endogenous zinc losses into the lumen in children with EE, or to increase conservation of endogenous zinc from the lumen by improving its bioavailability and hence recovery by absorption, is unknown. Endogenous zinc lost into the small intestinal lumen is available in the more distal gut for reabsorption, but its bioavailability and absorption in these more distal regions have not been adequately explored. There is no reduction in EFZ in children with EE when dietary phytate is reduced, meaning that phytate does not seem to influence zinc bioavailability (34). If, however, EFZ excretion is not fixed and it can be made bioavailable, then there is opportunity to improve zinc reabsorption provided that absorption in the colon is possible. Colonic absorption of zinc has been observed in rodents (35). These observations point to the importance of considering not just zinc intake but zinc losses as being a crucial part of the zinc balance equation.

Key recommendations are as follows:

- Studies of zinc deficiency and strategies for its reversal should occur in the target population, namely children with or likely to have EE, given the disturbances seen in zinc homeostasis in this setting. This demands study of novel interventions in the field where resources and infrastructure are minimal.
- The few observations available to this point suggest that a much greater understanding of the nature of EFZ and the reasons for its apparently obligatory loss of zinc in large amounts might identify a new strategy for improving zinc status in these children, either by reducing this loss or by improving bioavailability and hence reabsorption.

Conclusions: The design of novel zinc interventions will be facilitated by a better understanding of how disturbed gut function, such as that seen in EE, affects zinc absorption, losses, and homeostasis.

The nature and adequacy of zinc stores

Much of our knowledge with regard to zinc storage and compartmentalization at the organ and cellular level is derived from zinc kinetic studies using zinc stable isotopes as tracers combined with biospecimen collection and occasional tissue sampling. Modeling these data (32, 36) points to the presence of several zinc pools that are of limited total size (1.5–2 g/70 kg person). The EZP includes zinc in plasma, liver, pancreas, and possibly intestinal lumen and turns over within 3 d (32). It comprises ~10% of total body zinc (37). The slowly exchanging zinc pools appear to be located mainly in muscle and bone. The proportion between muscle and bone varies between neonates and adults (38), pointing to the need to be careful in extrapolating results in adults to small children.

EZP is directly influenced by certain conditions; its size is positively correlated with dietary zinc intake, body size, the amount of zinc absorbed, and fecal losses (32, 39–41). Although the size of the EZP falls with severe zinc depletion (40), it has not yet been proven to be a sensitive indicator of marginal zinc status (18, 42), which we predict might be the case because it represents only 10% of total zinc. Furthermore, EZP is not more closely linked to clinical signs of deficiency than is plasma zinc (18). More work is needed to identify which zinc pools are sensitive to changes in zinc intake and bodily stress, or to zinc loss, especially in children who are at risk of zinc-deficiency–related disorders. Once identified, research that addresses how to replenish these pools would be simplified.

Zinc storage capacity seems to be highly vulnerable to reduced dietary intake or increased losses due to illness. By using plasma zinc, a component of the EZP, as an indicator of zinc stores, plasma zinc begins to fall within a week of placing healthy adults on severe zinc depletion; overt clinical symptoms such as skin rash become evident soon after (18). Fecal zinc losses are high during an episode of diarrhea in infants, and losses fall within 1 wk as recovery proceeds (25). Despite these losses, plasma zinc does not change over 7–10 d in infants with gastroenteritis (43),

which suggests, in the face of presumed excessive zinc losses, that either the gut can compensate and replace the labile pool or that plasma zinc is not a sensitive indicator. Alternatively, acute phase effects on plasma zinc mask its potential usefulness (44). The gut is considered to be the primary site of zinc homeostasis because of its role in absorption of dietary (exogenous) zinc and in loss of secreted/excreted endogenous zinc (19). Zinc transporters in the gut are subject to homeostatic regulation. Molecular studies have shown that the zinc transporter Slc39a4 (ZIP4), on the apical surface of enterocytes, is upregulated in response to low dietary zinc intake in rodents (45). The only data available in humans show downregulation of zinc transporter 1 (ZnT1) and ZIP4 during zinc supplementation (46). Given such changes in zinc transporters, one might expect to see enhanced absorption of zinc in individuals with low zinc status. This has not been seen (47) and warrants further study in humans with zinc deficiency. Several explanations could account for a failure to observe increased absorption in zinc deficiency. The study design might not have accounted sufficiently for provision of bioavailable zinc to a regulated site of absorption. Zinc might be conserved by enhanced absorption of endogenously secreted zinc, which would not be observed when studying absorption of exogenous zinc. Finally, zinc deficiency itself might compromise absorption through either reduced absorptive capacity or losses through increased permeability.

The fact that gut integrity and function is compromised by acute diarrheal illness, general malnutrition, and EE—the latter being near ubiquitous in children in developing countries—considerably complicates the design and interpretation of studies addressing zinc absorption and homeostasis in vulnerable children.

Because exchangeable zinc stores are vulnerable and rapidly depleted by acute intestinal disease, it raises the question as to whether zinc stores are best maintained by regular interventions, whether they be pharmacologic, nutritional supplements, or dietary, as part of a public health strategy (ie, primary prevention) rather than an occasional medicalized therapeutic and secondary prevention strategy triggered by acute diarrhea in which we attempt to replenish stores during an acute period of zinc depletion (25).

Key recommendations are as follows:

- Further stable isotope studies and kinetic modeling should be undertaken in vulnerable children with a view to better defining those zinc pools that are sensitive to changes in zinc intake, bodily stress, zinc deficiency, or zinc loss.
- Further study of zinc absorption during zinc deficiency is warranted. Defining the best zinc-based interventions that sustain adequacy of zinc pools will inform a strategy aimed at sustaining an adequate zinc status.

Conclusions: Zinc stores are limited and rapidly depleted by acute intestinal illness; they seem likely to be best maintained in vulnerable populations by regular supplementation in a public health (primary prevention) strategy rather than a therapeutic and secondary prevention strategy triggered by acute diarrhea.

Assessment of zinc status

There is no simple means to identify subclinical zinc deficiency or to relate health outcomes to changes in zinc status. It has been said that lack of a reliable, responsive, and specific indicator of zinc status drives us to extremes: extremely large trials looking for population effects and extremely small trials that use stable isotope techniques that are cumbersome to use and expensive to perform. This may be due to the many roles of zinc in cellular metabolism together with the lack of a single definable storage compartment or ligand (eg, ferritin for iron). The Biomarkers of Nutrition for Development (BOND) initiative is seeking to identify biomarkers of zinc exposure, status, function, or effect (of an administration) (48), but in the interim we remain dependent on testing zinc interventions with the use of clinical indicators, and the consequent high cost and complexity of study.

Plasma zinc

Plasma zinc has been the most used as a marker. Although plasma zinc <40 μg/dL is 71% sensitive for clinical zinc deficiency (49), it is insensitive in an individual for early/marginal zinc depletion and does not correlate well with clinical status above this concentration (5). For instance, the responsiveness of acute diarrhea to zinc therapy is not predicted by plasma zinc concentrations (50). Plasma zinc is, however, a useful indicator of the zinc status of a population and its risk for zinc-related morbidity (51, 52). Unfortunately, plasma zinc readily decreases in response to a meal and is metabolically redistributed by endotoxemia, infection, and carcinoma (44). Thus, plasma zinc may represent a transit compartment for zinc being mobilized from the labile pool. As such, it might reflect deficiency at steady state, but as in many clinical situations a steady state is not necessarily present. This could account for the absence of a close correlation of plasma zinc with the labile pool size (53). Serum iron also does not correlate with acute dietary intake, so the problem may not be unique to zinc (54).

Emerging markers

Clues to potentially useful simple measures come primarily from depletion studies conducted in initially healthy zinc-replete adults and where evidence for depletion has depended on a progressive decrease in plasma zinc values, sometimes accompanied by overt symptoms of zinc deficiency. Several studies have highlighted dematin, thymulin, IL-2, uroguanylin, and A20 [a zinc-finger transactivating factor that inhibits the expression of certain cytokines (55)] as possible markers (55–58). These markers have potential because of their ease of measurement and relation to depletion and/or perceived pathogenesis of zinc-deficiency disorders. Studies examining the response of these to repletion are limited, and the relevance of such measures to health outcomes in vulnerable children (whether acutely ill or not) remain largely unexplored. Field-friendly assay technologies for these markers are also lacking.

The BOND initiative (48) suggests that so-called functional markers, such as linear growth, weight gain, changes in immune function and/or mucosal integrity, taste acuity, amounts of zinc-dependent enzymes and proteins, and biomarkers of oxidative stress, inflammation, or DNA damage, might be useful. But most are subject to influences other than zinc deficiency, and their utility in the field has limitations.

Marker validation

The workshop participants considered that a fundamental barrier to validation of markers able to detect marginal deficiency states was the absence of a reliable measure of zinc nutritional

status. Dual-isotope studies of zinc kinetics and homeostasis, together with compartmental modeling of bodily zinc compartments, provide some guidance (59, 60), as discussed above. However, these studies are expensive, are usually logistically complex depending on which outcomes are being assessed, and, with few exceptions, have been performed most often in healthy adults. Few studies have applied these informative methods to vulnerable children, and it was observed at the workshop that data on which to base compartmental modeling are lacking for children of any population, especially for those aged up to 3–5 y. Even kinetic measures such as the EZP, a seemingly good candidate for defining zinc "status," are not consistently linked to health outcomes and have rarely been correlated with changes in the simple measures listed above. Plasma zinc itself lacks dynamic responsiveness and is insensitive to changes in EZP (53). It was felt that free plasma zinc might be an exception to this, but there are no studies testing this possibility.

Key recommendations are as follows:

- A simple field-friendly measure for assessment of zinc status would benefit our understanding of the health impact of zinc deficiency, would greatly facilitate the testing of novel interventions in the field by reducing the need for time-intensive studies measuring health outcomes such as growth, and would complement trials of novel zinc interventions.
- Validation of the putative markers referred to above by comparison to dual-isotope measures of zinc pools and to relevant health outcomes should be the next priority. This will require large-scale, well-resourced studies in populations of children with marginal zinc deficiency assessed before, during, and after zinc intervention strategies.

Conclusions: A thorough assessment of zinc status by current methods is complex. For now, the simplest measure is plasma zinc, which is 71% sensitive for clinically apparent deficiency [ie, skin rashes, hair loss, and loss of taste acuity (18)] at a cutoff of 40 μg/dL (49). It is insensitive for marginal (subclinical) deficiency. Thus, it is difficult to identify zinc deficiency in association with acute diarrhea and to know how to monitor efficacy of replacement in terms of zinc nutrition.

Zinc bioavailability

Zinc in the gut lumen derives from 2 main sources: exogenous (diet, supplements, etc) and endogenous (18, 24). It was acknowledged by participants that poor bioavailability of dietary zinc is one reason for zinc deficiency (24), and as a consequence, improving bioavailability might improve the likelihood of success of primary prevention strategies in which increases in zinc intake will be modest at best, perhaps just a few milligrams per day.

Dietary zinc and its bioavailability

The intestinal luminal environment and the chemical nature of dietary zinc are keys to its bioavailability. The state of luminal zinc is complex, and the dynamic exchange between any free or bound luminal zinc and zinc transporters regulating uptake has received relatively little attention (20). Furthermore, dietary phytate is the major inhibitory luminal ligand and is present at amounts 10- to 30-fold higher (mole for mole) than zinc (59). The Miller equation (a saturation response model of zinc absorption as a function of dietary zinc and phytate) predicts that 90% of the uptake of exogenous zinc is explained by zinc and phytate concentrations in the lumen (24, 59), which are dependent primarily on dietary intake. These calculations are, however, derived from observations in healthy adults. No such data are available for children at risk of zinc deficiency.

There are 3 broad strategies for reducing the effect of luminal phytate: reducing the amount in the diet, hydrolyzing phytate in foods before ingestion or in the lumen after ingestion, and providing a stronger competing ligand that does not prevent absorption via zinc transporters.

A reduction in the dietary phytate:zinc ratio unquestionably improves zinc bioavailability (61). This can be achieved by general improvement in dietary quality with less emphasis on high-phytate grains for energy and nutrition or else by agricultural methods designed to reduce grain phytate content. The practicality of these approaches in a public health strategy remains to be shown.

Phytate hydrolysis is a strategy under consideration. Phytate [inositol-6-phosphate (IP6)] is a strong ligand at IP4-6, but only binds weakly at IP2 (62, 63). There is clear evidence that phytate is broken down progressively to IP1-2 in the gut, but most of this occurs in the colon as a result of bacterial action (human mucosa has very little inherent phytase activity) (64). A number of options are available to achieve phytate breakdown and improve zinc bioavailability at proximal intestinal sites. It is possible to activate naturally occurring food phytases during food preparation, although this requires careful education of mothers (65). One can also feed microbial phytases (common in animal food programs), which are stable in the stomach in contrast to food phytases, and achieve partial phytase hydrolysis with subsequent improvement in bioavailability of exogenous zinc (66). This strategy has already generated favorable results in the field setting (67).

The opportunity for providing a competing luminal ligand is uncertain. A high-quality diet that includes quality protein sources and fruit and vegetables aids bioavailability (68) but only to a small degree. These modest improvements are possibly attributable to competition with phytate. There is potential to use EDTA to compete with phytate (63, 67), but its value and safety in zinc nutrition have not been thoroughly evaluated. Phytate also affects iron absorption, with inhibition being maximal at a ratio of 1:10 (69). Thus, iron and zinc must compete with each other for phytate at their normal luminal concentrations. If iron intake is low, that leaves more phytate available to bind to zinc, and vice versa. This dichotomy complicates the luminal picture considerably.

In animals, rodents in particular, there is evidence that feeding microbially fermentable carbohydrate, particularly resistant starch, improves zinc balance and zinc content of bone (70, 71). This might result from improved bioavailability of zinc in the colonic lumen, allowing for absorption in that organ. Whether this can occur in humans is uncertain, and the applicability of rodent studies of zinc deficiency to humans is unclear.

Bioavailability of zinc tablets

Therapeutic doses of zinc have traditionally been 10 or 20 mg/d depending on age, significantly in excess of daily intake and recommended requirements ($\sim$5–6 mg for young children) (5). It seems likely that this dose overcomes the effect of luminal phytate to some degree because of its clinical efficacy. However, zinc absorption is halved when tablets fed to healthy adults are

taken with a meal compared with fasting (72), and this effect might be due to reduced bioavailability.

Endogenous zinc

Zinc also enters the lumen from endogenous sources as elemental zinc bound to proteins. Endogenously derived luminal zinc is composed of pancreatic enzymes, shed cells (in which zinc will be bound to intracellular proteins including storage proteins such as metallothionein), and plasma proteins (73). Ten percent of plasma proteins are turned over in the gut each day, carrying zinc with them (74). The capacity of endogenous zinc to exchange with exogenous zinc and the bioavailability of endogenous zinc are uncertain. Given the origin of some endogenous zinc from shed cells and plasma proteins, reabsorption at more distal sites would be necessary for it to be a useful contributor to zinc nutrition. In healthy adult subjects, most exogenous zinc seems to be absorbed in the duodenum and jejunum (75). Studies in rodents show zinc absorptive capacity in the ileum and colon (76–78), but preliminary data in humans have not confirmed this finding. Yet, zinc transporters are distributed throughout the human small and large intestine (79).

Whereas reduction in the dietary phytate:zinc ratio improves the bioavailability of exogenous zinc (61), this is not the case for endogenous zinc in children in Malawi with EE who were likely zinc deficient. Dual-isotope studies showed that excessive losses of EFZ were not reduced by phytate reduction in the diet (34).

If losses of endogenously derived zinc are so crucial to zinc homeostasis in these children, and given that some consider this loss obligatory (18), then there is a need to better understand why endogenous zinc is not bioavailable. Explanations could include the chemical nature of endogenous zinc (the state of hydration may vary considerably between elemental zinc and zinc salts), compartmentalization within the lumen, or protection by endogenous ligands (zinc is bound to protein by amino acid ligands, usually histidine, and water molecules are often involved) (20).

Understanding and improving the factors influencing the bioavailability of both exogenous and endogenous zinc may well be crucial to developing a robust public health primary prevention strategy to enhance zinc nutrition, particularly because the amount of extra zinc being made available will be small if dietary strategies are relied on as the source of zinc.

Key recommendations are as follows:

- Sampling of luminal contents of children, especially those with EE, will provide information on the chemical status of luminal zinc and potentially aid in our understanding of how to improve bioavailability.
- Undertaking such studies while feeding different forms of zinc will allow us to determine whether the Miller equation (the saturation response model of zinc absorption as a function of zinc and phytate concentrations) is relevant to such children.
- Strategies aimed at further evaluating the benefit of improving phytate hydrolysis in the gut should be explored. This should be combined with studies addressing whether zinc can be absorbed at more distal gut sites (ileum and colon) in which phytate is likely to be hydrolyzed and zinc bioavailability can most likely be improved.

Conclusions: Zinc bioavailability may be a crucial factor in the success of public health primary prevention strategies, and a range of food-based options, all still poorly pursued in terms of feasibility, may be valuable in improving zinc status.

Zinc mechanisms of action

Zinc has many biological functions, and it is likely that its health benefits depend on a multiplicity of actions. Yet, understanding which particular actions relate to the beneficial effect of zinc therapy on diarrhea (the most-studied health disorder relevant to zinc) is lacking.

The mechanism or mechanisms of action of zinc in improving acute diarrhea might be pharmacologic or nutritional. Understanding which applies is important and has relevance to understanding whether a public health primary prevention zinc strategy has a real place. These 2 classes of action are readily conceived as follows:

1) A nutritional mechanism would apply if reaching a threshold state of sufficiency is adequate to maximize zinc-dependent actions and reduce risk of acute diarrhea. That is, zinc administration is solely (or largely) correcting a micronutrient zinc deficiency. Here, replenishment of zinc pools in the face of zinc losses, and time taken to recover zinc-dependent processes, will drive rapidity and adequacy of response.

2) A pharmacologic mechanism would apply in which its benefits are dose-dependent over a wide range and independent of a diagnosable deficiency state. Evidence for a pharmacologic role of zinc would be the demonstration of efficacy of zinc treatment in patients without evidence of zinc deficiency.

The difficulty in separating these 2 possibilities is created by the insensitivity of current tests, specifically plasma zinc, for detecting subclinical zinc deficiency. Meta-analyses show that responsiveness to zinc in acute diarrhea is not dependent on plasma zinc concentrations (50), but this needs study with adequate power and attention to sample collection before a clear conclusion can be reached.

Understanding which mechanisms are linked directly to improved clinical outcomes will greatly improve our capacity to test new zinc interventions and interpret clinical responses (or the lack of).

Effect of zinc deficiency on the gut

Patients with acrodermatitis enteropathica, an inherited disorder of zinc absorption, sometimes suffer from diarrhea (80, 81). Studies in rodents show that zinc deficiency per se can have profound effects on the intestinal structure; zinc deficiency induces changes in intestinal morphology, including decreases in villous height and crypt depth, inflammatory cell infiltration of the lamina propria, and breaches of intestinal mucosa (82).

Zinc deficiency is associated with the following alterations in intestinal function and so may relate to diarrhea and its exacerbation in a number of ways.

1) Zinc deficiency intestinal ion transport (83). Net sodium and water transport is decreased and the effect of cholera toxin is enhanced even though glucose transport is unaffected. This effect may be due in part to increased

uroguanylin expression, which, in turn, increases cyclic guanosine-5′-monophosphate and chloride secretion.

2) Zinc deficiency alters intestinal permeability (84, 85). A consistent finding of zinc deficiency is alteration in tight junction function with increased intestinal permeability, which is a characteristic of EE and reflected by an increase in the L:M ratio.

3) Zinc deficiency alters mucosal immune function (86), perhaps including effects on oxidative stress or other immune mechanisms (87).

These effects on intestinal structure and function might contribute to the risk of diarrheal events and also might, if rapidly reversible, account for the early "antisecretory" rapid therapeutic effect of zinc on an acute episode of diarrhea (11). In the context of correcting nutrient deficiency, zinc's mechanism of action would seem to be to reverse these effects. However, the evidence that zinc therapy/supplementation directly reverses these dysfunctional events associated with zinc deficiency is not well studied and is sometimes conflicting. For instance, the capacity of zinc to normalize the L:M ratio in EE has not been consistently shown (26) despite its association with altered permeability (84, 85). However, zinc deficiency may not be the only factor leading to increased intestinal permeability in EE.

Zinc-deficiency–independent actions

That zinc might have a therapeutic role in the treatment of acute diarrhea is suggested by in vitro studies that show that zinc directly affects intestinal ion transport regardless of zinc status (83), as follows:

- Zinc increases sodium/hydrogen exchanger isoform 3 activity in Caco-2 cells, thus enhancing sodium absorption.
- Zinc inhibits chloride secretion (cyclic AMP, nitric oxide, and possibly Ca^{2+} stimulated), most likely as a consequence of blocking basolateral potassium channels.
- Zinc does not affect adenylate cyclase, cystic fibrosis transmembrane conductance regulator, sodium-potassium-ATPase, or sodium-potassium-chloride cotransporter.
- The effects of zinc on second messenger systems (eg, cyclic AMP, cyclic guanosine-5′-monophosphate, calcium, nitric oxide) are not uniform, suggesting that zinc might not be equally effective in all infective diarrheas due to variations in the effect of different pathogens on these second messengers.

Key recommendations are as follows: studies aimed at assessing the mechanism for the action of zinc should ideally include

- a study of the efficacy of zinc therapy in acute diarrhea in children without zinc deficiency;
- studies that relate clinical response to zinc status and to administered zinc dose;
- studies examining the effect of zinc on functional markers and improved health outcome in an effort to determine whether they are temporally credible and predictive of health benefit; and
- dose-ranging studies to include both replacement and pharmacologic ranges, which would be informative.

Conclusions: There is a lack of understanding of how zinc acts in the varied clinical settings associated with zinc-deficiency–related disorders; some actions may be attributable to the pharmacologic effects of zinc, whereas others seem to be dependent on reversal of its deficiency (ie, nutritional). Without a simple method to identify subclinical deficiency, we will not advance our understanding of the mechanisms.

Limited effectiveness in practice

Although evidence supports the effectiveness of zinc for treatment of an acute attack of diarrhea (11), the consistency of benefit and magnitude of effect are less than what would be desirable given the 13–20% reduction in severity of diarrhea (88). Meta-analyses have failed to clarify why the effect is constrained to this degree.

Heterogeneity in response to zinc therapy for an acute attack is evident in the meta-analyses (50). For instance, some studies showed that girls are less responsive than boys to zinc therapy for a number of acute infections, not just diarrhea (89). There is also some evidence that certain etiologies for diarrhea might not be zinc responsive, eg, *Escherichia coli* and rotavirus (90). This would limit effectiveness on a regional basis depending on the etiologies. It is possible that the pharmacologic actions of zinc might not apply to all relevant epithelial transport disorders, consistent with a pathogen-dependent effect on these transport systems (90).

The secondary prevention effect of zinc therapy (86) for reducing subsequent attacks of diarrhea is also subject to heterogeneity (91). Children <12 mo of age do not receive significant protection after 10-d zinc therapy for an acute attack (91). Does this point to a failure of zinc therapy over 10–14 d to replenish zinc pools or to different confounding factors such as a change in etiology, or is it something unrelated to zinc? At this point, one can only speculate as to the reasons. Perhaps, zinc provided during breastfeeding maintains adequacy of stores to 12 mo of age.

Heterogeneity of response could result from a number of additional factors:

1) Zinc response might only occur in the context of zinc deficiency, and acute diarrhea is obviously subject to other predisposing factors.

2) A pharmacologic action of zinc might be conditional on as-yet-unidentified covariates related to factors such as diet, disease processes, pathogenesis of diarrhea, and concomitant illness (eg, malaria and HIV/AIDS).

3) Either the dose, dosing schedule, or duration of dosing is suboptimal.

4) The zinc product might be variably accepted in different contexts of use.

5) Polymorphisms in zinc-dependent proteins might relate to the impact of supplementation.

6) Other common nutrient deficiencies, such as vitamin A, folate, and iron, might interact with zinc and/or require correction before efficacy of zinc can occur.

The meta-analyses show the benefits of zinc are not as great as might be hoped for, being no greater than 20% for treatment of acute diarrhea and of similar benefit for secondary prevention (11, 88, 91, 92). At the population level, these benefits are worthwhile, but they may be limited because zinc is only part of

the answer, the intervention is not optimized, or because we have failed to address the confounding factors. A better understanding of the causes of heterogeneity of the responses to zinc might give clues to improving the intervention and how to better deploy the zinc strategy to where it is most beneficial.

Key recommendations are as follows:

- There is a real need for better clinical trial design to identify those factors that cause heterogeneity, particularly those modifiable factors that predict response and those, such as presence and degree of zinc deficiency or aggravating nutritional disorders of other micronutrients that relate to apparent failure, at an individual level. Studies should collect and be powered to test the effect of these confounders on health outcomes. These factors might assume considerable importance for primary preventive strategies because dose and dosing schedules might need modification in populations in whom these modifiers are prevalent.
- Studies aiming at prevention (especially primary prevention) of acute diarrhea should incorporate dose-ranging studies, short-term (7–14 d) compared with long-term or intermittent therapy, and study populations in whom iron and vitamin A status is defined.

Conclusions: It remains unclear why zinc intervention seems less effective than expected at all ages and ineffective for secondary prevention of diarrhea in children <12 mo of age. Resolving the reasons might lead to improved approaches to zinc supplementation.

CONCLUSIONS

It is apparent that current WHO/UNICEF recommendations to use zinc supplements as treatment of diarrhea, although somewhat effective for reducing severity, are limited in their effectiveness. Several factors could account for this: the challenges of creating effective delivery channels, potential underlying biological limitations, and/or incomplete scope of the strategy in terms of it not addressing primary prevention. The current WHO/UNICEF Joint Statement strategy, in focusing on only one consequence of zinc deficiency, namely acute diarrhea, fails to directly embrace other areas in which zinc might be effective. This strategy clearly fails to address several fundamental problems, namely poor dietary availability and bioavailability and the impact of rapid zinc loss during diarrhea on limited zinc stores that cannot be simply and accurately monitored. As a consequence, it runs the risk of closing the door to public health primary prevention strategies directed at populations at risk of zinc deficiency that seek to maintain an adequate zinc status rather than to replenish at times of compromised balance.

Experts identified issues that, if resolved or better understood, might lead to more effective products and delivery strategies for addressing the global health problems associated with zinc deficiency. A substantial number of opportunities for improving how we address zinc deficiency on the global scene were also identified. These opportunities relate to adding primary prevention to the global strategy, issues regarding general zinc nutrition (dietary zinc sources and bioavailability, the size and vulnerability of zinc stores, assessment of status), the additional challenges presented by gastrointestinal compromise in the vulnerable population, and limitations to effectiveness of zinc as a therapeutic or secondary prevention intervention. Recommendations have been proposed as to how each of these might be addressed.

These recommendations are not put forward with the intention of holding up progress with the existing WHO/UNICEF Joint Statement for Clinical Management of Acute Diarrhea. However, it must be acknowledged that the existing strategy does not include any primary prevention aspects to be applied as a public health approach to reach children before they get sick. Furthermore, other health benefits are not directly targeted. The International Zinc Nutrition Consultative Group recommended in 2009 (93) that zinc supplementation programs should be considered for children in countries with an elevated risk of zinc deficiency, with the goals of reducing their incidence of diarrhea, pneumonia, and possibly other infections; reducing mortality; and of increasing growth velocity and thereby reducing their risk of nutritional stunting and underweight.

These observations lead to the conclusion that there needs to be consideration of a reframing of strategy from the existing treatment and secondary prevention approach to include primary prevention, given the significant health benefits to be gained. The development of a primary prevention strategy requires careful consideration of the zinc product to be used as well as strategies for its delivery. The development of such strategies will be greatly aided by the identification of simple and accurate measures of preclinical zinc deficiency.

Consequently, it was felt that global strategy could comprise not only the existing zinc strategy to complement oral rehydration solution and be applied during an acute episode of diarrhea but also a primary prevention strategy implemented in the context of a public health approach. The issues raised in the workshop suggest that we do not yet have the ideal product (or suite of products) suitable for a primary prevention public health strategy that matches the widely diverse delivery channels for application (the delivery needs) and the health outcomes that we wish to improve. The range of primary prevention options is considerable, including regular supplementation with the use of tablets, syrup, or micronutrient powder; inclusion in a multinutrient package; fortification approaches based on agricultural and/or food-processing methods; and methods to enhance the bioavailability of zinc. A thorough consideration of the nature and value of zinc primary prevention products relative to delivery strategies and channels that would use those products is now required.

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