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Impact of Infections on Micronutrient Deficiencies in Developing Countries

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Introduction

Despite numerous advances and improvements in child health globally, malnutrition still remains a major problem [1, 2]. A large proportion of cases of malnutrition occur in South Asia [3], which also harbors almost three quarters of the global burden of low birth weight infants [4]. In other parts of the world high rates of HIV threaten to reverse all the gains made by child survival programs, with worsening malnutrition [5]. Such overt forms of malnutrition, however, do not reflect the true global burden of malnutrition, as a large proportion of the hidden burden of malnutrition is represented by widespread single and multiple micronutrient deficiencies.

The relationship between micronutrient deficiencies, such as vitamin A deficiency, and increased risk of childhood infections and mortality is well established [6]. Vitamin A supplementation is now recognized as an important public health intervention among young children in areas of endemic vitamin A deficiency. Other micronutrient deficiencies such as zinc and iron deficiency are also recognized to be widespread in developing countries and associated with increased risk of morbidity [7] and mortality [8].

A number of factors may influence micronutrient deficiencies in developing countries. These include poor body stores at birth as a consequence of maternal intrauterine malnutrition, dietary deficiencies and high intake of inhibitors of absorption such as phytates and increased losses from the body (fig. 1). To illustrate, in the case of iron deficiency, in addition to poor dietary intake and inhibitors of absorption [9], increased intestinal losses following parasitic infestation may also be an important cause of iron deficiency anemia [10]. Overall, although the effects of poor intake and increased micronutrient demands are well described, the potential effects of acute and chronic infections on the body's micronutrient status are less well appreciated. Even more

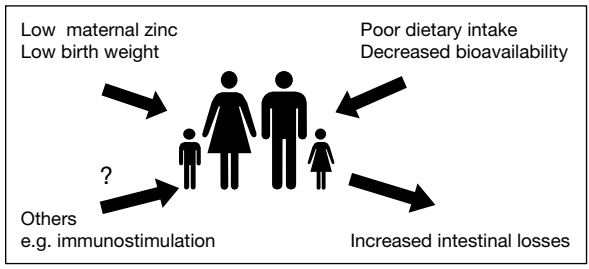

Fig. 1. Pathogenesis of zinc deficiency. Determinants of body zinc status.

obscure is the potential effect of immunostimulation and intercurrent infections on the micronutrient distribution and homeostasis.

The potential bidirectional effect of micronutrient deficiencies on infections and immunity are increasingly being appreciated. The effect of micronutrient deficiencies on immunity and burden of infections in developing countries is well described. This review will, however, focus on the potential effect of infections on the homeostasis and body status of important micronutrients such as iron, zinc and vitamin A.

Biological Plausibility of the Effect of Infections on Micronutrient Deficiencies

A number of epidemiological studies support the close association of infections with micronutrient deficiencies. These include findings of lower serum concentrations of zinc with increasing burden and duration of diarrhea [7] as well as lower serum concentrations in patients with HIV infection [11]. Although children with hypovitaminosis A and low serum concentrations of vitamin A have higher rates of associated infections, the specific contribution of infection to vitamin A deficiency cannot be discounted. This relationship between low serum vitamin A and severity of disease has been observed with the increasing severity of HIV infection [12]. However, several infections are directly associated with an increased risk of micronutrient deficiency. These include diseases such as measles which have been directly implicated with unmasking as well as triggering vitamin A deficiency [13]. Therefore the association of relatively higher rates of micronutrient deficiencies with infectious diseases may be reflective of both an increased predisposition to infections in deficient populations as well as a direct effect of the infection itself on the micronutrient status indicators [14]. Low serum concentrations of micronutrients have frequently been described in subclinical infections. The levels also appear to be lowest where there are the highest levels of inflammatory

proteins. High serum concentrations of C-reactive protein and haptoglobin are known to be related to the density of malarial parasites in Tanzanian children [15], with correspondingly lower concentrations of plasma retinol. The levels of plasma retinol among apparently healthy children in Ghana were also found to be lowest in those with raised markers of acute inflammation [16]. The effects of infection on blood indicators of micronutrient status may be more marked in areas with widespread malnutrition. To illustrate, in an evaluation of the comparative effects of malnutrition and coexisting malaria on serum antioxidant levels in Nigeria, the reduction in plasma β-carotene concentrations was significant with malaria rather than malnutrition [17].

There may be a physiological reason or benefit for the observed effects of infection on micronutrient status and indicators. The reduction in circulating zinc may reduce available zinc for microbial metabolism during infection [18]. Recent evidence suggests that Zn^{2+} may also play a role in reducing cellular oxidative stress and thus the acute changes seen in the circulation may represent a physiological compartmental change by an intracellular shift in zinc [19]. These data thus provide evidence of a similar advantage to that achieved by reducing iron levels during the course of bacterial infection.

Impact of Infection on Indicators of Micronutrient Status

For many micronutrients it is unclear whether the best currently available indicators truly measure the current body status. The reasons for these are manifold. Firstly the alteration in the serum concentration of a particular micronutrient may not reflect the true body status but be an adaptive phenomenon. In other instances, the changes may be transient and related to the severity of coincidental infections.

In an evaluation of the relationship of concomitant infections with serum concentrations of zinc, copper and ferritin among Peruvian children, Brown et al. [20] concluded that the effect was variable and differed by nutrient, nutrition status of the population, and the severity of infections. Figure 2 indicates the mean serum zinc concentrations among a cohort of children under 36 months of age undergoing nutrition rehabilitation for persistent diarrhea in Karachi. No impact or reduction in plasma zinc was seen, except among those with the most severe infections, e.g. sepsis or pneumonia requiring systemic antibiotics. It has thus been suggested that this acute effect of coincidental infections on serum zinc levels may not be significant at a population level in developing countries and that serum concentrations of zinc can be used as a robust measure of population zinc status regardless of concomitant subclinical infections [21].

Reduced levels of circulating free iron as well as increased levels of iron-binding proteins are seen with infections [22] and may represent a physiological adaptation to reduce free iron availability to circulating pathogens. The

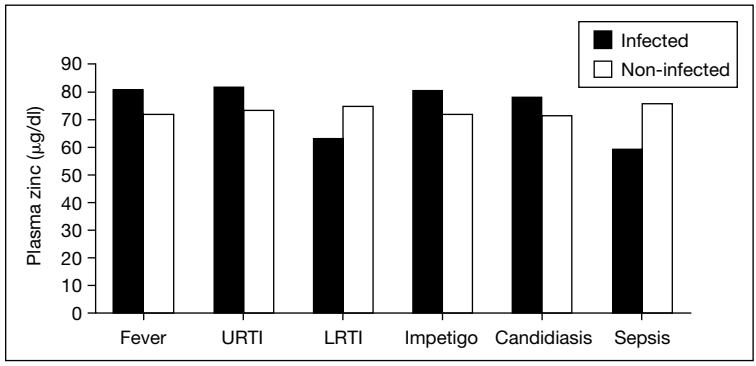

Fig. 2. Relationship of plasma zinc and coincidental infections. URTI = Upper respiratory tract infections; LRTI = lower respiratory tract infections.

effect of infections on serum ferritin, an important indicator of iron stores, is in the opposite direction and serum concentrations are elevated in the presence of infection [23]. Thus estimation of serum ferritin alone may lead to underestimation of the true prevalence of iron deficiency in subjects with high rates of coincidental infections and morbidity. However, this problem can be overcome if the ratio of the serum transferrin receptor and serum ferritin is assessed and the concentration of the serum-transferring receptor does not change during infection [24].

The most striking effects of infections on micronutrient status have been seen in volunteer human experiments assessing the serum levels following injection of low dose endotoxin. Gaetke et al. [25] studied the effect of endotoxin administration in 12 volunteers and demonstrated that serum cytokines went up and zinc concentrations decreased with the injections. However, no effect was seen on serum albumin or albumin-zinc binding.

There is some evidence at a cellular level of a reduction in liver retinol-binding protein (RBP) synthesis in animals injected with endotoxin [26]. RBP, if not bound efficiently to transthyretin (TTR), could also move to the extravascular space or be lost in the urine [27] following endotoxin challenge. It has also been suggested that it may be possible to use the RBP/TTR ratio as a measure of vitamin A status in the presence of acute inflammation [24]. These findings indicate that inflammation-induced hyporetinemia does not necessarily imply a loss of vitamin A, but rather represents a redistribution of tissue vitamin A brought about by a reduced hepatic synthesis of RBP.

The aforementioned acute changes in serum concentrations of zinc and iron may not accurately reflect the changes in the body status of these micronutrients, and there is thus considerable interest in exploring other measures. Earlier studies employed intravenously administered radioactive isotopes such as ^{65}Zn to study a multi-compartment model of zinc kinetics [28].

These techniques have now been largely supplanted by stable isotope studies. Stable isotopes of zinc namely ^{68}Zn and ^{67}Zn have been used to study zinc homeostasis in a six-compartment model [29]. Although some information is available to indicate the high turnover rates of acute phase proteins during infection [30], to date no zinc kinetic studies have evaluated whole body and compartment changes in zinc metabolism during infections using stable isotopes.

Pathogenesis and Mechanisms of Micronutrient Deficiency with Coexisting Infections

There is little information on the short-term compartment changes in micronutrients such as iron, zinc and vitamin A. However, other mechanisms underlying net body losses and homeostasis are well described. It is possible to elucidate the mechanism of alteration in micronutrient status and consequent deficiency from other direct studies and observations.

The gut has a special role in the pathogenesis and severity of micronutrient deficiencies.

The association of helminthic infections, especially hook worms, with iron deficiency in young children is well established and largely relates to direct intestinal losses [31]. Although the association between diarrheal disease-control programs and malnutrition or growth rates has been questioned, in many parts of the world there is a close relationship between the two. In particular prolonged and recurrent episodes of diarrhea, frequently in association with HIV infection, are a frequent cause of morbidity and micronutrient deficiency. In recent years, the association of increased micronutrient losses such as those of zinc and copper with acute diarrhea has been well recognized [32]. These findings may explain the high rates of subclinical zinc deficiency among children with frequent and recurrent bouts of diarrhea, and may be particularly marked among children with persistent diarrhea.

Figure 3 indicates data from sequential metabolic and trace element balance studies among 20 children with persistent diarrhea who underwent inpatient nutritional rehabilitation in Karachi. These findings indicate significant intestinal losses of zinc during diarrhea, which frequently exceed the dietary intake leading to a net negative balance during the diarrheal episode. These trends reversed with recovery from diarrhea and nutritional rehabilitation over a 14-day period. These data support the continued use of zinc supplements during the nutritional rehabilitation.

In addition, it is also recognized that children with shigellosis can lose a significant amount of vitamin A in the urine, thus further aggravating preexisting subclinical vitamin A deficiency [33]. As already illustrated, the risk of micronutrient deficiency in infancy and early childhood can be compounded several fold by the presence of low body stores from birth as in low birth

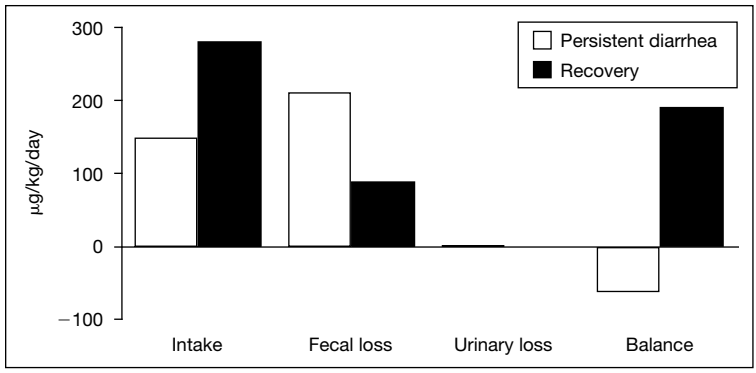

Fig. 3. Metabolic balance of zinc in children (n = 20) with persistent diarrhea and after recovery.

Table 1. Suggested regimen for micronutrient supplementation in infectious illnesses during infancy and childhood

Disorder	Recommended micronutrient	Dose	Duration
Acute diarrhea	Zinc	10 mg/day	7–10 days following diarrhea
Persistent diarrhea and malnutrition	Zinc	10–20 mg/day	14–30 days
	Vitamin A	50,000–100,000 units	Once only
Pneumonia	Zinc	10 mg/day	10–14 days
Pneumonia in malnourished children	Vitamin A	50,000–100,000 units	Once only
Measles	Vitamin A	200,000 units daily	2 days

weight infants, and further aggravated by poor breast-feeding and complementary feeding practices [34].

Conclusions and Implications

The aforementioned data indicate that although coincidental infections may lead to transient alterations in serum concentrations of micronutrients, the physiological basis and significance of these changes are unclear. However, available information from large scale surveys indicate that these transient alterations may not be significant at a population level and thus serum or

plasma estimation of micronutrients can be used as an adequate measure of micronutrient status in such populations.

On the other hand the important contribution of some infections to aggravation of micronutrient deficiencies in at-risk populations cannot be ignored. Increased losses of micronutrients such as vitamin A and zinc during infectious illnesses such as diarrhea are important contributors to micronutrient deficiencies. This may be particularly marked with prolonged diarrhea and dysentery and lead to clinically significant deficits and overt micronutrient deficiency.

Given that the epidemiological association between micronutrient deficiencies, such as zinc and diarrhea, is well established, supplementation strategies are logical in endemic areas. The growing body of evidence on the key role of zinc supplementation in accelerating recovery from diarrheal illnesses in developing countries supports its use in public health strategies in endemic areas. The association of measles with overt and subclinical vitamin A deficiency is also recognized, and administration of vitamin A to all such malnourished and at-risk children forms a corner stone of such management strategies. Table 1 summarizes some of the suggested regimens for supplementation with key micronutrients during specific childhood infectious illnesses, in which a contributory relationship of the disorder to precipitating or unmasking the micronutrient deficiencies is recognized.

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Discussion

Dr. Abrams: When we look at zinc the problem is that unless you are severely zinc deficient you don't tend to drop your serum zinc very much and so that the stable isotope in the kinetic studies pick up more subtle changes than the serum zinc does. So all these studies tend to underestimate or can potentially underestimate the role of

mild deficiencies. The problem is that it is not easy to make stable isotope kinetics, and attempts to do so have not been terribly successful. So I think some isolated groups that may be relatively small are needed. But one can look at all sorts of different markers, always different things, with complete kinetic studies in mild deficiency states, and that is the kind of gap that hasn't been done at this point.

Dr. Bhutta: I couldn't agree more. The important issue, the challenge that I made is really to take the science where it matters. I believe that with some of this stable isotope work we at least have the tools to study populations in representative groups in depth, where there is the reality, the real-life concomitant burden of infections and morbidity which are such an important part of the equation. So I would very much like to see that work being done. Unfortunately according to what I have read in the literature, much of this is induced by necessity and it is also not in association with populations that are largely the focus of public health intervention programs. So we don't quite know what the relationship of all body zinc status measurements in developing countries is with other simpler markers that may be available.

Dr. Hurrell: You mentioned the influence of infection on the loss of micronutrients, how to redistribute them, but you didn't mention absorption. Is there any influence of infection on micronutrient absorption?

Dr. Bhutta: From a few studies that have been done there are data available on vitamin A in terms of the association of diarrhea with a reduced uptake. There are a lot of data available on the iron which I have not touched upon in terms of reduced absorption of iron with chronic enteropathy, and the difficulty in that literature is how do you dissociate that with chronic anemia of infections. There is very little work on bioavailability as well as absorption in children with persistent diarrhea aside from our own work. I think there are probably only 3 groups which have looked at zinc balance studies in diarrhea, one is Castillo-Durán et al. [1] in Chile. We have done studies on this and I think there has been some preliminary work at the International Centre for Diarrheal Disease Research, Bangladesh (ICDDRB), but it has not been able to sort out whether or not there is reduced absorption in a situation with persistent diarrhea and enteropathy and it is probably implicit that children with enteropathy and reduced mucosal surface area would have induced absorption. So to summarize I think your point is valid, I am just not aware of studies which have looked at that very specifically in terms of either mechanisms of absorption or net fluxes.

Dr. Castillo-Durán: Can you comment on the potential use of zinc in oral rehydration solutions (ORS) as a potential new tool for the treatment of diarrhea?

Dr. Bhutta: I think there are two issues with zinc. First of all I think the public health community needs to unravel the zinc story and to really decide if we are looking at zinc deficiency in isolation or is zinc deficiency in most instances out of the multiple micronutrient deficiency scenario? If the latter is the case then I think we really need to look at combined approaches of micronutrient supplementation. Currently the thinking on zinc supplementation during diarrhea and prevention is also to look at a delivery system of zinc which may be in the form of either dispersible zinc tablets or a large scale zinc supplementation trial such as the one that has been started in Bangladesh. There isn't a whole lot of support for using zinc in ORS because you need to use such a large amount of it, and also the evidence is that oral rehydration therapy with ORS intake goes down so rapidly as the diarrhea recovers that you would not be able to replete a deficient state unless you were able to continue ORS intake for several days unnecessarily. So I think the tendency is really to use a different vehicle like a tablet, or to look at additional approaches such as supplementation or fortification.

Dr. Zlotkin: Thank you for your really clear talk on the impact of infection on micronutrient status. As you spoke I realized that perhaps our strategy or focus on the

strategy of providing micronutrients is naïve because as you quite clearly said the effect of infection on micronutrient status is impacted in a number of ways: there can be stool loss; anorexia can be associated with infection and therefore not as much is eaten; there can be a number of impacting factors which affect micronutrient status. In our sessions we really are talking about a strategy for a nutritional approach looking at ways to prevent infections, for example, immunization might be a way to improve micronutrient status: access to clean water; access to ORS; access to health care providers, and perhaps antibiotics. Although they are not directly related to micronutrient status they are certainly very directly related to the general strategy that one has to think about. What you have done is to emphasize that we have to be broad in our thinking, we cannot only approach this as nutritionists but have to think of the infection in the whole scenario.

Dr. Bhutta: I couldn't agree more. In paper 2 of the Bellagio series [2] where we looked at the impact of interventions on reducing child mortality, this is precisely the point that was made that nutrition strategies need to be intertwined with additional strategies to reduce the burden of diarrhea, the burden of respiratory infections, the burden of measles. I think unless you reduce and eliminate measles in developing countries you are going to be chasing the vitamin A requirement for the next 20 years. So I couldn't agree more; we should be looking at integrated strategies where there are additional measures to pure nutrition strategies.

Dr. Gebre-Medhin: The most striking message that I take to heart is the very strong association between deficiency and protein energy malnutrition. As you very well know more than anybody, the average child between 1 and 5 years has something like 7 infections per year. In most instances these infections have a viral character where C-reactive protein (CRP) is not of very much use. Could you comment on other indicators or infections other than CRP which is a very non-robust thing. The second comment is that if you look at Swedish children and their vitamin A status, at any given time there would be something like 15 or 20% who could be looked upon as being vitamin A deficient, but if they are followed up they bounce back again every month. The infection goes away, they bounce back in their status, which shows the very strong impact of this low-grade infection that is possible to correct. I would like to have your views on that.

Dr. Bhutta: I think the second point you made is indeed very important because I personally do not know the answer to it. But on the basis of what we see in the field, I believe that immunostimulation, as a response to chronic low-grade infections in these children, some of which are viral, some of which are bacterial and produce a state of chronic inflammation, is a real entity. In the slide I showed the kind of environment that many of these children are living in in developing countries with recurrent burdens of diarrhea, and therefore the impact of these short-term and also sustained bouts of infection on full body status micronutrients is very poorly recognized. We know from work done years ago what happens in terms of recurrent infections and stunting. I think if you could extrapolate, expand on what happens to micronutrient status in a child with recurrent infections and a continuous background of infection, you would see something quite analogous to what we see in asthma and inflammation, a state of continuous inflammation in some of them. Now whether or not we will be able to reverse that with micronutrient interventions singly or in combination, I personally don't think it is possible. I think there need to be additional measures to reduce the burden of infections themselves. Coming back to your first question as to what can be done in viral infections when CRP does not seem to be elevated. There has been a lot of work with other indicators as you know, and currently Thurnham et al. [3] are looking at whether or not some kind of adjustment for vitamin A can be developed using markers. The indicators that have been developed are alpha 1-antichymotrypsin

(ACP) and α_1-glycoprotein (AGP), you can take your pick and they all behave almost the same. CRP seems to be better in certain hands, and ACP and AGP in others. None of them are exceptional. CRP is simple to do, certainly ACP and AGP are beyond the reach of most laboratories in developing countries. So to answer your question I think there is a lot of work to be done in this area to develop better indicators for an acute phase response than we currently have. I believe CRP is probably the one that has been studied most.

Dr. Tolboom: I have two questions. One on the methodology to measure morbidity. You showed a slide in which a recent cough was associated with a decline in vitamin A; but a cough itself doesn't do that. Have you got an answer to that; is that a problem of methodology?

Dr. Bhutta: Very good question and clearly. When a survey is going to be done at the national level in a population, the community health workers and physicians need to be trained before hand using a tool. In the morbidity complex that I showed, standard definitions were used. For recent morbidity from acute respiratory infections we used indicators that the parents would be able to relate back in terms of recall. This was a 2-week recall period and clearly simple things were used such as 'did your child have a cough'. So what I said was that recent cough is really the only measure that we have from that 2-week recall period, and it does not represent pneumonia.

Dr. Tolboom: Then there was a point about chronic infestations, particularly parasitic diseases. Do you think there is some relationship between, for example, giardiasis that is quite common in most developing countries in 20–25% of schoolchildren, and micronutrient status?

Dr. Bhutta: Again an excellent question and the answer to that is I have looked at the literature in preparation for this as to what is known about helminthiasis in general and micronutrient status. Absolutely nothing has been prospectively studied and done on zinc status in giardiasis or helminthiasis, there is some work available from the National Institute of Nutrition on helminthiasis and vitamin A absorption which indicates that perhaps a high helminth load may be associated with vitamin A deficiency. Obviously there is a whole body of literature on helminthiasis and iron status and again, to illustrate, the most recent publication in that area is 13 years old. So it is not a focus of attention and fertile ground for much greater work.

Dr. Ribeiro: Congratulations on your presentation. Based on your clear summary of the correlation of zinc losses in diarrheal disease, are you convinced that there is a broad place for zinc supplementation during the acute phase of diarrhea and during persistent diarrhea treatment?

Dr. Bhutta: We are part of the International Zinc Nutrition Consultive Group Steering Committee. Clearly in public health sectors there is now growing recognition of the need for zinc interventions. Now whether that takes the shape and form of intervening in any specific diarrheal syndromes has really not been resolved. Most of us are of the opinion that you tend to see zinc deficiency in relation with diarrhea in settings where there is a preexisting background of zinc deficiency. So ideally we should be in the highest category which is really infancy and children who have had hyper-diarrhea, with a view to preventive strategies to replete body zinc and therefore treat all diarrheal episodes with some form of zinc. It is logical because to try and sort out in public health settings which episode will get zinc and which episode will not is very difficult. Again the logic is that if you repeatedly treat episodes of diarrhea in public health settings with perhaps 2-week courses of zinc, then you may be able to improve status over time. Actually the most exciting data on this have come from the ICDDRB and Black's work [4] in which zinc supplementation was given through community health workers for diarrheal episodes and they were able to show a difference in mortality. But more importantly they were able to show a reduction in antibiotic

prescribing at a community level. So when you are giving zinc there may be additional benefits beyond an impact on diarrhea, there may be benefits on nutrition, and certainly if you can reduce antimicrobial prescribing by physicians for diarrhea that may have a huge impact on antimicrobial resistance. So as they say, watch this space, because I hope we will have consensus on what might be the best strategies within a year.

Dr. Gibson: I just wanted to make a comment in response to Dr. Zlotkin's view about a more holistic approach, and just a comment about a program that has been undertaken by World Vision Canada [5] in which they have a very comprehensive micronutrient health program that has been running in Tanzania, Zambia and Malawi. In their comprehensive micronutrient health program which included immunization, fortification, supplementation and health education, they have certainly shown some very impressive results in terms of reducing stunting and reducing anemia and improving infant mortality.

Mr. Parvanta: Thank you very much for that stimulating presentation. I just have a question on the issue of the Pakistan survey. In most population surveys the way the sample sizes are calculated are determined based on the expected prevalence of the deficiency one is looking for and so on. Given what you said about the impact of infections on prevalence estimates, in most surveys nobody accounts really for that in estimating the sample size. Do you have any suggestions on what type of an estimate one could make on the expected prevalence of infections and so on, assuming you even had CRP or AGP or something of that sort.

Dr. Bhutta: That is an excellent question and I wish we had been at this state when we were designing the survey. By the way the survey of a population of 140 million is on a preschool cohort of 6,000 children, so even that was a logistic challenge. It is not provincially representative, so most national surveys have to compromise the ideal with the pragmatic. Now if I was to review this whole thing again, and I wish I had believed that by just accepting that there would be a 20% prevalence of subclinical infections, which is more or less what we found, not subclinical but real infections, we would have adjusted the sample size for that. So in developing countries when looking at high burdens of morbidity, some of those data may not be interpretable on the basis of coexisting infections. Until we get a robust measure using these measures you need to adjust for that. So maybe the 20% adjustment is what I would recommend, at least in the South Asian context. I can't speak for HIV prevalent populations.

Mr. Parvanta: So with the adjustment you are also saying that you would obviously have to have some indicators of infection status afterwards?

Dr. Bhutta: Yes, which we do.

Dr. Lönnerdal: I would like to come back to one of the questions brought up and I think you were fairly cautious in your response. One of the things you missed earlier was when I talked about iron supplementation. I think that in any developing country there would be a significant proportion of infants who will have iron deficiency and anemia and will certainly benefit from iron. But what we have seen is that, in those who actually do have a satisfactory iron status, there could actually be a negative effect, and therefore the cost-benefit or the whole reason of what you should do becomes a little bit more murky. I would like to take this a little bit further and talk about the set of experiments we did in infant rhesus monkeys. We used this model because we can be a little bit more aggressive about what we can do in this setting than in human infants. We used infant formula and fed them for 4 months: one group was fed regular formula; another formula containing probiotics, and a third group was fed formula with extra zinc. We induced enteropathogenic *Escherichia coli* infection in these infant monkeys, which of course we cannot do in human populations. The outcome was that the probiotics group had a reduced severity and duration of the *E. coli* infection.

What we also found was that the group with probiotics and zinc had a significantly worse outcome when it came to diarrhea than the control group. What we retrospectively found was that of course the zinc status of these infants was satisfactory when they were infected. So if they received extra zinc when they had an infection the outcome was actually worse; possibly the extra zinc stimulated bacterial growth or whatever. But again a little bit of caution, in any given population we may have 40 or 50% of the infants that are zinc-deficient and still have some 50–60% that perhaps are zinc-adequate. The question is if you aggressively treat with zinc in order to reduce the severity of diarrhea, then what happens with the group that actually is satisfactory from the beginning?

Dr. Bhutta: Very important question and I think therefore as zinc intervention trials become more skilled, it is very important that we get precise answers to the question, what happens to zinc-replete or zinc-sufficient infants when there is a large zinc supplementation trial. There is a zinc mortality study that is coming to closure very soon and we will have the data from those large-scale studies. So far in the work that we and others have done on zinc supplementation in large-scale population settings, we have not seen evidence of either biochemical zinc sufficiency or levels which may reach toxic levels. So we have not seen it, but that is not to say that it may or may not exist. Your question on probiotics is a very interesting one. I would like to refer you back to one of the earliest publications on zinc and diarrhea that we did [6], and one of the interesting things that we found in infants with persistent diarrhea supplemented with zinc was that when their breath hydrogen excretion was measured, the pattern was very high. We don't understand why this is, but it was like the bacterial overgrowth that you saw and a pattern which was similar to bacterial overgrowth. The conjecture at that time was that zinc is doing something in the gut that we need to be aware of. Having said that I think one of the most exiting opportunities that we have now is to look at probiotics and prebiotics in susceptible populations, both as a means of reducing the burden of diarrhea and potentially improving micronutrient absorption through the gut, and status thereof. So I think some of the studies really need to be done in terms of addressing micronutrient status in populations with diarrhea.

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