Interactions between Micronutrients: Synergies and Antagonisms

Bo Lönnerdal

Department of Nutrition and Program of International Nutrition, University of California, Davis, Calif., USA

Introduction

There is growing awareness that the micronutrient status of large segments of populations is inadequate and causing adverse effects on infants and children. Iron, vitamin A and iodine deficiencies are common and the long-term consequences are well known. Many programs have been and are being launched to prevent and treat such micronutrient deficiencies. However, whereas some micronutrients have mutually beneficial or synergistic effects, i.e. providing more of one micronutrient will improve the status/metabolism of another, other micronutrients interact negatively with each other, such that providing too much of one micronutrient will impair the status of another micronutrient. It is therefore important that there is a thorough knowledge of micronutrient interactions when designing and interpreting programs to combat micronutrient deficiencies. Without such knowledge, unexpected and unfortunate outcomes may be experienced and, perhaps less serious, the evaluations of interventions in different settings/populations may become difficult or impossible. Meta-analysis of studies performed by different researchers in various settings/populations may be a powerful tool to evaluate outcomes of interventions; however, if the underlying micronutrient status is highly variable and the outcome is dependent upon this, a ‘true’ picture of the global situation may be difficult to obtain.

Iron and Zinc

When it was recognized that suboptimal zinc nutrition may be common in large segments of the population, various strategies to prevent zinc deficiency were considered. Since zinc is stable in water solution and non-toxic, oral
Micronutrient Interactions

Supplements would be one possible avenue. However, in areas where zinc deficiency may be expected, iron deficiency is frequently common, and iron supplements may be used. Therefore, Solomons and Jacob [1] evaluated the effect of oral iron on zinc absorption by giving zinc and ferrous sulfate to human subjects in different molar ratios. They found that iron lowered zinc uptake as measured by the increase in plasma zinc at a molar ratio of 2:1. This obviously could be a concern if iron and zinc were to be given together. However, very large amounts of iron and zinc were given because of the method used (plasma area-under-the-curve) and it is conceivable that these two elements would only compete with each other when given in water solution and possibly not when food is present. This was investigated by Sandström et al. [2] who studied zinc absorption at physiological intakes using radioisotopes. When excess iron was added (25:1 ratio), zinc absorption from a water solution was inhibited significantly, whereas no effect was observed when they were given in a meal (fig. 1). As the inhibitory effect was abolished when histidine (chelator of zinc) was added, it was believed that when iron and zinc are chelated to their ‘normal’ ligands, resulting from digestion of foods, they will be absorbed via different pathways and no interaction would occur. A study by Rossander-Hulthén et al. [3] showed that similar results were obtained for iron absorption in humans when excess zinc was added, i.e. an inhibitory effect of zinc on iron absorption was found when they were given in water solution, whereas no effect was seen if they were given with a meal (fig. 2). These studies strongly suggested that iron and zinc may interact when given as supplements, but that this would not occur when they are given as food fortificants.

Two recent studies on iron and zinc supplementation of Indonesian infants [4, 5] show that antagonistic interactions between iron and zinc in fact do

**Fig. 1.** The effect of iron:zinc ratio on the absorption of zinc in human healthy adults as measured when the elements were given in water, water with histidine (dietary zinc chelator) or in a standardized meal. Adapted from Sandström et al. [2].
occur when they are given as supplements (drops). In a study by Dijkhuizen et al. [4] infants were given iron alone (10 mg/day), zinc alone (10 mg/day), both elements together (10 + 10 mg/day) or placebo from 4 to 10 months of age. Supplementation significantly reduced the prevalence of anemia, iron deficiency anemia and zinc deficiency. Iron supplementation did not negatively affect plasma zinc concentrations, and zinc supplementation did not increase the prevalence of anemia. However, iron supplementation combined with zinc was less effective than iron supplementation alone in reducing the prevalence of anemia (20 vs. 38% reduction) and in increasing hemoglobin and plasma ferritin concentrations. There were no differences in growth among the groups, and the growth of all groups was insufficient to maintain their z scores for height-for-age and weight-for-height, showing that overcoming these micronutrient deficiencies is not sufficient to improve growth performance in these infants.

In the study by Lindh et al. [5], the infants received the same treatments, but from 6 to 12 months of age. After supplementation, the iron group had higher hemoglobin and serum ferritin than did the iron+zinc group, indicating an effect of zinc on iron absorption (table 1). The zinc group had higher serum zinc than did the placebo group, whereas this was not the case for the iron and iron+zinc groups, suggesting an effect of iron on zinc absorption. Thus, supplementation with iron+zinc was less efficacious than single supplements in improving iron and zinc status, with evidence of a negative interaction between iron and zinc when the combined supplement was given. In this study, significant effects on growth were observed.

The reason why different results for growth were obtained in these two studies, which were performed in similar populations, is not known, but the different ages for the introduction of the supplements is one possibility. A stable isotope study by Domellöf et al. [6] showed that at 6 months (and

---

**Fig. 2.** The effect of added zinc on iron absorption in healthy human adults as measured when the elements were given in water solution or in a standardized meal. Adapted from Rossander-Hulthén et al. [3].
presumably at lower age), iron absorption was immature and that there was no downregulation of iron absorption with increased iron status. At 9 months, however, regulation of iron absorption appeared to be similar to that in adults, i.e. iron-replete infants absorbed much less iron than iron-depleted infants. Experiments in experimental animals show that regulation of iron transport mechanisms is immature at young age, but matures in late infancy [7]. Thus, competition for absorptive pathways may be more pronounced during late infancy. Further studies using stable isotopes are needed to elucidate the interaction between iron and zinc at different ages.

A crucial question is whether the interaction between iron and zinc has any functional consequences. In the study by Lindh et al. [5], weight-for-age was significantly higher in the zinc-supplemented group than in the other groups, and psychomotor development (Bayley Scales of Infant Development) was significantly higher in the iron-supplemented group than in the placebo group. Thus, the combination of iron and zinc supplements did not improve growth or development as compared to placebo. Iron supplementation has recently been shown to adversely affect the growth and morbidity of Honduran and Swedish infants [8] and to cause a redistribution of vitamin A (lower plasma retinol, higher liver retinol) in Indonesian infants [9]. Whether these consequences are a direct effect of iron or an indirect effect on zinc metabolism is not yet known. However, these observations may have considerable implications when designing programs for micronutrient interventions. It should be emphasized that, similar to adults, there does not appear to be an interaction when formulas or weaning foods are fortified with iron or zinc [10, 11].

### Iron and Copper

Iron and copper are known to compete for absorptive pathways, although the mechanism behind this negative interaction has not been delineated.
Although most studies on this interaction have been done in experimental animals, there are some human studies suggesting that this may be an underestimated nutritional problem. Haschke et al. [12] showed that copper absorption in infants was significantly lower from infant formula fortified with a high level of iron (10.2 mg/l) than from formula with a low level of iron (1.1 mg/l). In another study, feeding infants a formula with a higher level of iron (7 mg/l) resulted in a significantly lower concentration of ceruloplasmin, or ferroxidase I, the major copper-binding protein in serum [10]. In a recent study of breast-fed infants given iron supplements (1 mg/kg/day), the activity of erythrocyte Cu,Zn-superoxide dismutase (SOD), which has been suggested as a long-term marker of copper status, was significantly lower than in unsupplemented infants [13]. Thus, copper status may be compromised by excessive iron supplementation. Impaired copper status has been shown to compromise immune function [14] and the decreases in ceruloplasmin and Cu,Zn-SOD may affect iron metabolism and the defense against free radicals negatively. Whether these observations had any functional consequences in the infants is not known, but should be studied further.

**Iron and Ascorbic Acid**

It is well known from a multitude of studies that ascorbic acid has an enhancing effect on iron absorption. This may be due in part to the reducing effect of ascorbic acid, keeping iron in the absorbable ferrous (+II) form, and in part to a weak chelating effect of ascorbic acid, also helping to keep iron soluble. Although it is known that the iron intake of several populations is adequate but inhibitory compounds limit the bioavailability of iron, there have been relatively few studies exploring this synergistic effect in fortification programs. Derman et al. [15] showed that fortifying infant formula with ascorbic acid significantly enhanced iron absorption in human adults, but studies on infants fed formulas with various levels of ascorbic acid were not done. Davidsson et al. [16] evaluated the addition of ascorbic acid to a Peruvian school breakfast meal and found that iron absorption was significantly enhanced in a dose-dependent manner. It is likely that the addition of ascorbic acid can overcome the inhibitory effect of phytic acid in the meal as has been shown previously [17]. Whether this enhancing effect of ascorbic acid on iron absorption is sustainable in populations with diets high in phytate was explored by Garcia et al. [18]. In a food-based community trial in Mexico they found that ascorbic acid from lime juice twice daily (50 mg/day) for 8 months did not improve the iron status of iron-deficient women, although a stable iron study showed an enhancing effect on iron absorption [19]. Although further long-term studies are needed on ascorbic acid fortification, it is possible that the initial enhancing
effect of ascorbic acid on iron absorption is downregulated by homeostatic mechanisms.

Iron and Vitamin A

Studies on children in Central America showed that low plasma retinol concentrations were correlated to low hemoglobin, serum iron and transferrin saturation values [20]. These observations were made when iron intake was adequate; no correlation was found when dietary iron was low. In a study on experimental vitamin A deficiency in human adult volunteers, Hodges et al. [21] found that hemoglobin values decreased in a pattern similar to that of plasma vitamin A and that during repletion with vitamin A, hemoglobin values increased with plasma vitamin A. Studies in experimental animals have shown that liver and spleen iron increase concomitantly with the decrease in serum iron and hemoglobin [22]. When following the absorption of iron (with the radioisotope $^{59}$Fe) in vitamin A-deficient rats [23], no difference in iron absorption or iron turnover was observed between vitamin A-deficient and control animals. The incorporation of $^{59}$Fe into red blood cells, however, was significantly lower in the vitamin A-deficient animals than in controls. Consistent with this observation was the increased accumulation of $^{59}$Fe in the liver of vitamin A-deficient animals. Thus, it appears that the mechanism of interaction between vitamin A and iron is an impairment in the mobilization of iron from the liver and/or incorporation of iron into the erythrocyte. Consequently, in order to optimize the outcome, it appears important to normalize vitamin A status in populations being given additional iron. Studies in some populations [24] have shown that supplementation with both iron and vitamin A increases hemoglobin concentrations to a greater extent in anemic pregnant women than does iron supplementation alone (table 2), although the magnitude of this effect varies considerably.

A synergistic effect between vitamin A and iron on iron absorption has been suggested by one group of investigators [25]. Both preformed retinol and
β-carotene were shown to have a positive effect on the absorption of radioiron from single meals in adult human volunteers. The mechanism between such an interaction remains elusive, but it was suggested that a physicochemical interaction between iron and vitamin A occurs, which results in a complex being formed, enhancing iron absorption. However, a recent thorough study in human subjects did not find an enhancing effect of vitamin A on iron absorption [26]. These authors suggested that it may only occur in populations with low vitamin A status and that the effect in that case may be more related to the repletion in vitamin A status than a direct effect on iron absorption. Whether the enhancing effect of vitamin A on iron absorption in itself is biologically relevant in human populations or a consequence of the experimental method used also remains to be elucidated.

**Zinc and Vitamin A**

Zinc deficiency is known to impair vitamin A metabolism; thus, interventions intended to prevent or treat vitamin A deficiency may vary in efficacy depending on the subject’s zinc status. It was found early that experimental animals fed low zinc diets had low levels of serum retinol and that these levels could not be increased by large supplements of retinyl acetate. Smith et al. [27] showed that rats fed a low zinc diet had markedly reduced levels of plasma retinol as compared to zinc-adequate animals, in spite of similar and adequate levels of vitamin A in the diet. They suggested that the mobilization of vitamin A from the liver was impaired by zinc deficiency. In a subsequent study [28], they showed that whereas plasma protein concentrations were lower in zinc-deficient animals, concentrations of retinol-binding protein (RBP) was dramatically reduced. Thus, the lower levels of circulating vitamin A may be due to a decrease in liver RBP synthesis. Studies in non-human primates fed a diet marginally low in zinc showed that plasma retinol was positively correlated to plasma zinc concentrations, and plasma RBP concentrations were also positively correlated to plasma zinc [29].

There have been few studies on the interaction between zinc and vitamin A in humans. In several conditions manifested by low zinc status in humans, such as alcoholic cirrhosis and protein-energy malnutrition, zinc supplementation sometimes but not always resulted in improved vitamin A status [30]. Similarly, some studies on preterm infants [31] and children [32] have shown positive effects on serum retinol, RBP and conjunctival epithelium, but not all studies show such effects. Christian et al. [33] showed that zinc potentiated the effect of vitamin A in restoring night vision among night-blind pregnant women, but this only occurred in women with low initial serum zinc concentrations. Thus, careful assessment of both initial zinc status, which is notoriously difficult, and vitamin A status before evaluating the effect of zinc on vitamin A status may be required.
Iron and Riboflavin

Riboflavin deficiency in human adults is known to result in low hemoglobin levels. When riboflavin deficiency was induced in human volunteers, the subjects got anemia, which was restored when riboflavin status was restored [34]. Studies in the Gambia have also shown that iron given with riboflavin supplements was more efficient in restoring hematologic parameters than iron given alone [35, 36]. Studies in experimental animals showed that the activity of NADH-FMN oxidoreductase was low in riboflavin-deficient animals and that this may be the underlying mechanism for the abnormal iron metabolism observed [37]. A study by Powers [38] showed that ariboflavinosis was more rapidly induced in young animals than in adult ones and that NADH-FMN oxidoreductase activity was dramatically reduced in riboflavin-deficient animals as compared to controls (2.9% of controls). Since iron stores, as measured by ferritin, were low in the riboflavin-deficient animals, it was possible that iron absorption was impaired. Adelakan and Thurnham [39] demonstrated such an impairment by showing that $^{59}$Fe appearance was slower and lower in riboflavin-deficient animals than in controls. More unabsorbed $^{59}$Fe remained in the gastrointestinal tract of riboflavin-deficient animals, supporting the idea of impairment in the uptake phase of iron. Taken together, these studies show that NADH-FMN oxidoreductase activity is low in riboflavin-deficient animals and that iron absorption is impaired, possibly because NADH-FMN oxidoreductase is involved in the mobilization of iron from ferritin. Thus, in riboflavin-deficient animals the release of iron (temporarily stored in mucosal ferritin) may be reduced significantly and more iron than normal would be lost by mucosal sloughing.

Further, assimilated iron may be diverted to the erythroid marrow at the expense of repleting iron stores. This is supported by a study in pregnant and lactating women in the Gambia [36] in which riboflavin given in addition to iron resulted in a significant increase in circulating plasma iron and in iron stores, relative to placebo. Additional studies in experimental animals [40] showed that riboflavin deficiency is associated with an increase in small intestine crypt depth and a twofold increase in the rate of crypt cell production. Thus, although there may be a contribution from turnover of enterocytes with an increased iron content, enhanced iron loss associated with riboflavin deficiency is likely due to an accelerated rate of small intestine epithelial turnover.

Iron and Iodine

A synergism between iron status and the efficacy of iodine fortification and supplementation in human populations was recently discovered. Zimmermann et al. [41] reported that in children with goiter, the therapeutic
response to orally given iodized oil was lower in children with iron deficiency anemia than in iron-replete children. Further, iron treatment of goitrous children with iron deficiency [42, 43] improved their response to iodized salt (table 3) or oil. It is known from studies in experimental animals that iron deficiency impairs central nervous system control of thyroid metabolism [44] and modifies nuclear triiodothyronine binding [45]. In human subjects with iron deficiency anemia, plasma concentrations of thyroxine and triiodothyronine are decreased, the conversion of thyroxine to triiodothyronine is lower and concentrations of thyrotropin are elevated [46, 47]. Thus, it appears likely that key steps in iodine metabolism are iron-dependent and that adequate iron status is a prerequisite for effective iodine treatment of goiter. Further studies in cells and experimental animals are needed to better define the molecular mechanisms behind the effect of iron deficiency on thyroid hormone metabolism.

### Conclusions

Supplementation and fortification with single micronutrients has led to highly variable outcomes, making evaluations of the relative efficacy of each type of intervention difficult. It is likely that the results have been confounded by underlying deficiencies of other micronutrients. Thus, synergistic effects of providing two or more micronutrients may be expected. On the other hand, several studies have shown that supplementation and fortification with multiple micronutrients also have had mixed success, and in some cases adverse
effects of multinutrient supplementation as compared to single-nutrient supplementation [48]. In this case, interactions between micronutrients are likely, i.e., one or more of the added micronutrients is already adequate and further amounts may interfere with the absorption or metabolism of another one. Far better knowledge and awareness of micronutrient interactions and their underlying mechanisms are needed to institute improved interventions without negative side effects.

References

Micronutrient Interactions


Discussion

Dr. Specker: I am curious, you talk about iron fortification or supplementation and its relationship to copper deficiency, but I guess I was not playing close enough attention. Do you think that it is a short- or a long-term effect?

Dr. Lönnerdal: I think it is a long-term effect. The copper absorption study was conducted during a short period of time but the study in which we saw the effects was a long-term fortification study [1]. So I think it is a long-term effect rather than an acute one. There may also be an acute effect, but I think here the concern should be about the long-term outcome because again when we give iron we tend to do it for the long-term. We want to have a program in place where we give iron as a supplement or as a fortificant, but very few studies have looked at copper status.

Dr. Barclay: In Indonesia, was that a supplementation study or a fortification study?

Dr. Lönnerdal: That was a supplementation study, iron drops or zinc drops or a combination.

Dr. Pettifor: Your study on iron supplementation and the reduction in growth, do you have any idea of the cause for this reduction of growth? Was it an anorexia issue, or was it an intake problem?

Dr. Lönnerdal: No, it was not an intake problem, we did not see an effect on that. I can only speculate. We are doing some secondary data analysis right now looking in particular at the Honduran cohort. We were surprised that we saw it in Honduras actually because we didn't think that we had the statistical power to detect this but we did. My guess right now is that we are impairing zinc status, and we know that zinc, certainly zinc deficiency or even marginal zinc deficiency, reduces IGF-I, and there is a potential link to linear growth. So possibly it could be an indirect effect via zinc. There is the possibility that other micronutrients could be affected but zinc and growth are ultimately connected, and again we know that iron certainly does have an effect on zinc as we saw in these studies. It will also be interesting to complete the iron absorption study we published [2], and that is what I referred to earlier together with Dr. Abrams; we saw no downregulation at a young age but we saw it in older infants. In the same cohort we actually have stable zinc and stable copper absorption results, but we have not completed the data calculation yet. Hopefully, these results will aid in our interpretations.

Dr. Abrams: Has anyone thought to give iron in the morning and zinc in the evening, or some approach like that?

Dr. Lönnerdal: That was my conclusion from these studies but I have not pursued it yet. I think that is what we need to try because supplementation can be very effective, and in many settings it may be the only practical way to prevent or treat deficiencies. I believe it will be necessary to take the supplements on separate occasions, like morning/evening (red pill, blue pill or whatever to try to distinguish them), but my feeling is that gastric emptying needs to be complete before the next one is given. It may also be possible to give them on alternate days. The problem may be that for zinc you cannot skip too many days because we don't have any stores for zinc. For iron there has been discussion about whether we can give weekly instead of daily supplements; would it be possible to give it every other day? We need to have some new approaches: either on the same day but spread apart or alternate days or something like that.
Dr. Delgado: What is your opinion on the delivery interactions of micronutrients in the special condition of catch-up growth in premature babies? A better evolution of catch-up growth in premature babies supplemented with zinc has recently been shown.

Dr. Lönnerdal: I haven’t really worked on that myself. There are many studies that show a very positive effect of zinc on catch-up growth [3]. I think we need to go back and look a little bit at interactions because the hematologists really like to give a lot of iron to these premature babies to make them catch up when it comes to hematology. Many pediatricians caring about the growth of the infants like to give zinc. So there are all kinds of possibilities for interactions. In reference to what Dr. Abrams eluded to, we really don’t know how effective infants are when it comes to turning iron into hemoglobin at a young age. We have done radioisotope studies on infant rhesus monkeys showing similar results as stable isotope studies in human infants [4]. When infants are young they are not very good at incorporating iron into hemoglobin. The question is what do they do with the iron in the case that they are not putting it into hemoglobin? It would be nice if they put iron into ferritin because that is a fairly innocuous form of iron, but I am not quite sure they are doing that judging from our human data. So the question is: if you have iron floating around in the system and not making ferritin or hemoglobin, what is it doing? I think we need to go back and look critically at those studies because zinc researchers have usually looked at the zinc effect, the iron researchers have looked at the iron effect, and very few studies have looked at them together, which is a bit surprising to me.

Dr. Zlotkin: We recently completed a study with Sprinkles which I call a fortification methodology because it is a supplement added to food. This was a double-labeled stable isotope study in which we actually gave the $^{50}$Fe intravenously and the $^{57}$Fe orally. There were 3 groups and each group received 30 mg iron and 50 mg ascorbic acid: 1 group had no zinc; the 2nd group had 5 mg, and the 3rd group had 10 mg zinc. The question we asked was: does the provision of zinc as a fortificant impact on iron absorption? What we saw was a significant impact of 10 mg but not of either 0 or 5 mg. This has not been published yet but for the sake of this discussion, again as a fortificant.

Dr. Lönnerdal: I think that the ratio between iron and zinc is very critical. I prefer fortification because I think it is a much more biologically safe way of providing minerals like iron and zinc. We have done a fortification study and we know how much iron can be added and how absorbable is it. It may be safer but it is not as effective as one would like.

Dr. Zlotkin: The dietary reference intake (DRI) process has recently come up with upper limits or upper levels, and for zinc the level recommended is in fact lower than the 10 mg that you used in your studies in Indonesia. There has been a lot of discussion and controversy around the relatively low upper limit that was defined for infants under the age of 2 years, and I believe the upper limit was something like 6 mg/day, between 5 and 6 mg/day. Any comments on whether or not the potentially adverse effects you might be seeing have anything to do with going above the upper limit?

Dr. Lönnerdal: Not really, I think that actually the safe level is quite a bit higher, and we know that from our Swedish studies, for example. The formula zinc level has been 10 mg/l for quite some time, but when it was increased, there was more iron so the iron problem was taken care of first. Then the zinc content was balanced in order to not interfere with iron, but at the same time making sure that there was enough zinc. This example was a formula with a very high phytate content. It is not that I don’t believe in the DRI but I think that we can overcome the problems by carefully considering the dietary matrix and the ratio between iron and zinc.

Dr. Lozoff: We just completed a large trial on preventing iron deficiency anemia in Chile and found no effect of iron supplementation on growth in that study [5]. There were over 1,600 babies between 6 and 12 months old who were supplemented. In this
Micronutrient Interactions

situation there was a lot of iron deficiency in the first place. But in Honduras there may be a different effect.

**Dr. Lönnerdal:** We saw no overall effect on growth in Honduras, but when we split them up by initial iron status, it was found.

**Dr. Lozoff:** This is one of the things that we haven’t done yet. These babies were all breast-fed, but should we look at those who are exclusively breast-fed separately? There is another thing I wanted to ask you about. A new study, recently published in the *Journal of Pediatrics* [6], reports iron supplementation to totally breast-fed babies in Canada beginning at 1 month of age. In this small study, an increase in hemoglobin was found in those given iron both at 6 and 12 months, but no differences in growth. A very low dose of iron was given (7.5 mg/day). If prevention with such a low dose of iron is used, would there still be concern about interactions or growth?

**Dr. Lönnerdal:** In the Swedish and Honduras studies we also used 1 mg/kg/day, definitely a very modest level. We have also seen some studies that used designs similar to ours with the iron/zinc combination, but at different sites [7], and when the amount was adequately small no effect was found. To find an effect on growth of course a large sample size is needed, and we found an effect at that level when the groups were larger. More studies need to be done on that. In a population like the Canadian one I would be hesitant to give iron starting at 1 month of age. Even though I believe that iron is good for us, it is a very toxic element and I think we have underestimated the negative effects of iron. A balanced approach should be used.

**Dr. Mannar:** In your studies on the use of ascorbic acid in fortified foods in which you found no effect of ascorbic acid, I was wondering whether one of the possible reasons could be that ascorbic acid is lost during food processing?

**Dr. Lönnerdal:** It was not my study, it was a study by Garcia et al. [8]. It was prepared with freshly made lime juice and consumed as a drink, so it was not part of the food preparation. They also analyzed the vitamin C content.

**Dr. Hurrell:** I was quite surprised by the efficacy study of Garcia et al. [8] showing no effect of ascorbic acid on iron status. I suppose the drinks were fed with the meal. Perhaps one of the explanations could be that the phytic acid content of the diet was higher than the meal which was tested as a single meal absorption study. If we consider the various studies which have been made on the molar ratios of ascorbic acid to iron needed to overcome the inhibitory effect of phytate, when the phytic acid content is high, what is now recommended is a 4:1 molar ratio of ascorbic acid to iron, that is 12:1 by weight. So if the diet contains 10 mg iron you would need to have something like 120 mg ascorbic acid in order to expect an increase in iron absorption. I think in the study by Garcia et al. $2 \times 25$ mg of ascorbic acid was fed. I would imagine that this is not a high enough level. I don’t know how high the phytic acid was in their meals, but perhaps this is an explanation for why they failed to show an effect of ascorbic acid on iron status.

**Dr. Lönnerdal:** It could be and I can check with Dr. Allen. In the 8-month study they used meals of similar composition to the one used in the stable isotope study. Of course in the stable isotope study the meals were very well defined, but in the free-living setting they were typical Mexican meals. Just as there are no really typical American or Swedish meals, I don’t think there is a typical Mexican meal, the diet varies. It could be that there was regularly more phytate than in the defined meal; this is quite possible. It is also potentially possible that when vitamin C faces the intestinal mucosa, the iron-regulatory mechanism adapts to this situation just as it does for calcium. However, more studies are needed.

**Dr. Zlotkin:** I just want to get back to your point of iron supplementation versus fortification. Again the American/Canadian DRI of iron for infants at about 1 year of age is 11 mg/day. That is the current recommended dose. The assumption is that that would be not as a supplement but as the iron found in food, and in fact when you look
at the intake of iron from any data from America the intake of iron is around this range or even higher for the 1-year-old in America today. Again, although I can’t recall that anyone has specifically done placebo-controlled trials looking at growth, we really do define our growth expectations internationally based on that population. So I just want to make sure and be clear on what you are saying. You are not suggesting that an intake of 10 or 11 mg/day from food as recommended in the DRI would have specifically negative effects on growth, you are specifically talking about when it is given as a supplement at a young age, and that is perhaps under 6 months of age.

Dr. Lönnerdal: Exactly, I think it is only as a supplement. There are many studies showing that when iron is in the diet these effects are not seen at all.

Dr. Castillo-Durán: One of my concerns is whether this interaction also remains in the condition of disease. For instance in the studies from India or other countries, all the children have diseases, parasitic infections and so on. Does this interaction remain on the same level or is it higher? Do you have any information about this confounding variable which is important for many countries?

Dr. Lönnerdal: I think you are hitting on something very sensitive here and I think you are right, and Dr. Semba will talk more about this. For example, if this is a zinc-mediated effect certainly growth is affected and I would certainly expect immune function to be affected, and then again these effects could be expected for a variety of diseases. We were very close to seeing a significant effect on infection, both upper respiratory disease and diarrheal disease, but statistical significance was not reached for that. In a larger cohort it would perhaps have been seen. But in studies like those in Honduras and Sweden, all infants with acute infection were omitted from the studies by analyzing C-reactive protein. There were not many; in neither Honduras nor Sweden did we find many with elevated C-reactive protein.

References
