Impact of Micronutrient Deficiencies on Behavior and Development

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Introduction

A variety of micronutrients affect infant behavior and development [for a recent review see, 1]. Here we focus on deficiencies of iron or zinc, which are among the most common single nutrients disorders in the world.

Iron Deficiency

A comprehensive review of studies on the behavioral and developmental effects of iron deficiency was published in 2001 [2]. That article and a Cochrane review [3] concluded that the association between iron deficiency in infancy and poorer behavioral/developmental outcome is strong, but causal connections remain to be proved. Thoughtful cautions have also been noted about interpretation [4]. Though still not definitive, some recent studies strengthen the causal link, and the results are consistent with current understanding of iron's role in the developing brain. We will emphasize these studies, summarizing previous research only briefly to lay the foundation. Unless otherwise indicated, studies assessed iron status by multiple measures in addition to hemoglobin. Most studies used case-control designs, comparing infants with iron-deficiency anemia to those with better iron status, and some included controlled trials of iron treatment. Most included reasonable

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assessments of environmental and potentially confounding factors. The very few randomized trials of preventing iron deficiency will be specifically identified. It is important to note that most studies have focused on healthy full-term infants without other health or nutritional problems. Researchers have studied such populations by design in order to assess the effects of iron deficiency without the confounding effects of malnutrition, infectious diseases, etc.

Mental Development

Mental outcomes in most studies have been global tests of development, such as the Bayley Scales of Infant Development [2]. In almost all the case-control studies, mental development test scores averaged 6–15 points lower among iron-deficient anemic infants, even after control for background factors, an important consideration since iron-deficient anemic infants may have a variety of disadvantages in background. In general, iron therapy did not correct the lower test scores in the majority of iron-deficient anemic infants. A major exception was a study in Indonesia [5] in which iron-deficient anemic infants dramatically improved in test scores after iron therapy. Despite the consistency of finding lower pretreatment scores, global test scores provide little indication of what iron deficiency might be doing to the developing brain.

Of the few available preventive trials, a recent large study in Chile was the only one to find effects of iron supplementation specifically on mental functioning. Infants supplemented with iron between 6 and 12 months (n = 1,123) were compared to a no-added-iron group (n = 534) [6]. There were no differences in overall mental or motor test scores, but infants in the no-added-iron group showed differences on the Fagan Test of Infant Intelligence. On this test of visual recognition memory and novelty preference, there were no differences in novelty preference but the infants in the no-added-iron group looked longer. Longer looking times are considered to be an indicator of less efficient high-speed information processing and predict later IQ better than global tests of infant development [6].

One recent case-control study also examined specific cognitive processes. This study used event-related potentials to assess recognition memory among infants of diabetic mothers, who are at high risk for being born with brain iron deficiency [7]. These infants showed poorer recognition memory than control babies on age-appropriate measures, from birth to 8 months [8]. This study is unique in its use of neuroimaging with event-related potentials and a strong developmental cognitive neuroscience perspective.

There is evidence that differences associated with iron-deficiency anemia in infancy are long-lasting. Most follow-up studies have been at early school age (4–8 years), with generally uniform results: children who had iron-deficiency anemia in infancy tested lower than peers in overall mental functioning [2]. In the longest follow-up to date [9], 11- to 14-year-old children in Costa Rica who had been treated for severe, chronic iron deficiency in
infancy still tested lower in arithmetic and writing achievement than their peers who had had good iron status in infancy. Twice as many had repeated a grade, and 3 times as many had been referred for special education or tutoring. They also had poorer performance on some tests of specific cognitive functions (tachistoscopic threshold, spatial memory, and selective attention).

Another important study of long-term outcome was conducted statewide in Florida [10]. Anemia in infancy, based on hemoglobin screening in the Women, Infant, and Children program, was associated with special education placement at age 10 years, based on the criteria used by the Florida Department of Education for mild or moderate mental retardation. Although this study was limited in that hemoglobin was the sole measure of iron status, it is exceptional in relating anemia in infancy (presumably due to iron deficiency) to mental retardation or special education placement among school-aged children in an entire population.

The above long-term follow-up studies involved infants who were identified as having iron deficiency in the infant/toddler period. Only one study has related a measure of iron status at birth (cord ferritin level) to later development (at 5 years) [11]. Children with cord ferritin levels in the lowest quartile received lower scores for language ability, fine-motor skills, and tractability.

**Social/Emotional Development**

Virtually every study that has examined infant affect or social/emotional behavior has found differences in iron-deficient anemic infants compared to those with better iron status [2]. In case-control studies, infants with iron-deficiency anemia were typically rated as being more wary/hesitant, fearful, unreactive to usual stimuli, solemn, unhappy, and/or easily fatigued during developmental testing. In studies in Guatemala and Costa Rica, infant behavior was also coded and rated from videotape during play and developmental testing. Infants with iron-deficiency anemia stayed closer to their mothers, made fewer attempts at task items, showed less pleasure and delight, were less playful, were more wary/hesitant, etc. [12]. In the Costa Rica study, the only study to date that included observations in the home, daily spot observations over a 3-month period showed that iron-deficient anemic infants were more likely to be asleep, irritable, carried, doing nothing, in bed, not interacting with anyone, etc. [12].

Two of the preventive trials reported differences in the affective domain as well. In a study in the UK in which infants were supplemented with iron from 7 to 18 months [13], there were no differences between supplemented and un-supplemented infants at the conclusion of the trial. However, at 24 months, supplemented infants did not show the decline in global development quotient that was observed in babies who did not receive iron. An examination of the test’s subscales revealed that the only significant difference was the personal/social subscale. In the preventive trial in Chile [6], a greater proportion of infants who did not receive iron showed no social interaction, no
positive affect, and no social referencing throughout a 45-min test session. Among the few babies who cried, more of the no-added-iron group could not be soothed by words or objects; they had to be held or they could not be soothed at all. Fewer of them were considered to be ‘unadaptable’. In this rating, being ‘unadaptable’ means protesting when a toy is taken away. Since this is quite normal behavior for infants who are engaged with an object, it appears that the babies who did not get iron were less involved with test objects.

Affective and social/emotional differences were still observed in former iron-deficient children in the Costa Rica follow-up at 11–14 years [8]. Their parents and teachers rated their behavior as more problematic in several areas, agreeing in increased concerns about anxiety/depression, social problems, and attention problems.

Motor Development
Among studies that included an assessment of motor development, most found that infants with iron-deficiency anemia received lower motor test scores, averaging 9–15 points lower [2]. An important population study in the UK found that a hemoglobin level of <95 g/l at 8 months predicted poorer locomotor development at 18 months [14]. Several studies have observed little or no improvement in motor test scores after iron therapy or improvements only in a minority of iron-deficient anemic children who showed the most dramatic hematologic response to iron. The above-mentioned study in Indonesia was again an exception – motor score deficits were completely corrected with iron therapy [5].

Among the preventive trials, one conducted in Canada [15], with iron supplementation between 2 and 15 months, showed lower motor scores in the unsupplemented group at 9 and 12 months. In the preventive trial in Chile [6], infants who did not receive iron crawled somewhat later, on average, than iron-supplemented infants. At 12 months, a greater proportion of the unsupplemented group was rated as tremulous.

In long-term follow-up studies, differences in motor test scores have been observed, up to more than 10 years after iron treatment [9, 16, 17]. Differences in visual-motor integration years after the period of iron-deficiency anemia have also been observed in 2 studies that have included such a measure [16, 17].

Spontaneous Motor Activity and the Sleep/Wake Cycle
Despite compelling reasons from animal studies to expect differences in spontaneous motor activity in the iron-deficient anemic infant, only one project has assessed this directly. Using activity meters on the baby’s ankle, spontaneous motor activity was compared between Chilean infants with iron-deficiency anemia and a non-anemic group. In the course of a year of iron treatment, the formerly iron-deficient anemic infants showed reduced motor
activity during waking before and after a nap in a neurophysiology laboratory [18]. A different pattern was observed in the home. During the period of iron deficiency, there was increased motor activity (leg movement) in virtually every phase of the sleep/wake cycle throughout a 24-hour period [19]. Most differences in the home disappeared after iron therapy.

In the same Chile study, polysomnographic recordings were obtained during a spontaneous daytime nap in the laboratory in infancy and an overnight sleep study at 4 years. There were multiple differences in measures of the sleep/wake cycle in infancy and/or at follow-up. Findings included differences in REM latency, duration of REM episodes and their pattern through the night, the duration of slow wave sleep episodes early in the night, both the isolated REMS and non-isolated REMS indices and inter-REM intervals [20, 21]. Thus, iron-deficiency anemia appears to alter key components of the internal temporal order within the 24-hour cycle.

**Sensory Development**

A few recent studies have examined sensory development in iron-deficient anemic infants [22]. In the neurophysiology component of the study in Chile, 6-month-old infants with and without iron-deficiency anemia were studied with auditory brainstem responses (ABRs) during a spontaneous nap in the laboratory [23]. There was slower transmission throughout the auditory pathway (longer latency for the wave I–V interval) among the babies with iron-deficiency anemia. The differences became even bigger after a year of iron therapy and correction of anemia. Children who had participated in the study as infants were assessed at 4–5 years of age with ABRs and visual evoked potentials [22]. The formerly iron-deficient anemic group showed longer ABR and visual evoked potential latencies. The magnitude of effects was large: 1–1.2 SD.

**Effects among Infants and Toddlers Who Are Not Healthy and Well-Nourished**

One recent study of undernourished infants in Indonesia found that those who received an energy supplement plus iron-containing micronutrients walked at an earlier age, had higher mental and motor test scores, were more motorically active, and showed more mature social-cognitive and emotional regulatory behaviors. This multifaceted project, although involving a relatively small sample, was noteworthy in its comprehensive approach and development of a model that encompassed affective, motor, and mental effects [24].

A large double-blind, placebo-controlled trial of iron supplementation and anthelmintic treatment was conducted in Zanzibar, where malnutrition was widespread and malaria omnipresent [25]. Infants and preschoolers assigned to iron for a 12-month period improved more in language development than those assigned to placebo. Among children 12–36 months of age, iron supplementation also improved motor development, but the effect was apparent
only in children with baseline hemoglobin concentrations of <90 g/l. Since so many of the world's children live in settings where generalized undernutrition and infectious diseases occur along with iron deficiency, more research in such settings is urgently needed.

Postulated Mechanisms

Some of these findings can be interpreted in light of the current understanding of iron's role in the developing brain. Iron is required by every cell in the body and is thus involved with many processes. So far, the most relevant to behavior and development are iron's role in myelination, neurotransmitter function, and neuronal metabolism [26, 27]. The findings most tightly linked to alterations in myelination are slower transmission in the auditory and visual systems. Both of these sensory systems are rapidly myelinating during the period of iron deficiency, and they are sensory systems critical for learning and social interaction. It is also likely that there are other intracerebral effects, given that so many brain systems are myelinating during this period. Thus, impaired myelination could underlie other poorer outcomes. For instance, longer looking times on the Fagan Test, long-lasting differences in visual-motor integration, and later crawling/walking might be consistent with altered myelination.

With regard to alterations in neurotransmitter functioning, the dopamine system has been best studied [26]. Among many functions, dopamine plays a major role in systems of behavioral activation and inhibition and the degree to which individuals experience inherent reward. The affective changes in iron-deficient anemic infants (wariness, hesitance, absence of positive affect), their lack of social referencing, and the observation that toys can be taken away from them without protest all seem to make sense in this context. Dopamine's role in extraneous motor movement is also well-established, and the observations of tremor would fit. In addition, there is a strong association between iron deficiency and periodic leg movements or restless leg syndrome in older children and adults. The increased leg movements observed in the Chilean infants during the period of iron-deficiency anemia might be an infant equivalent or precursor [19]. In addition, differences between home and laboratory suggest that the iron-deficient anemic children respond differently to context – with a reduction in motor activity after the stress or unfamiliarity of the laboratory.

Recent work in the animal model has documented iron's role in neuronal metabolism and shown that there are differential effects on the developing hippocampus and other parts of the brain required for cognitive functioning [27]. The findings of poorer recognition memory in infants at high risk for iron deficiency (due to maternal diabetes) appear to fit with an effect of iron deficiency on the hippocampus and related components of the central nervous system [7]. However, the findings need to be replicated among infants with dietary iron deficiency.
To summarize, there seems to be delayed or mistimed sensory input in at least 2 sensory systems in early iron-deficiency anemia. These changes, together with other cognitive, motor, and affective differences, may mean that the iron-deficient baby seeks and/or receives less stimulation. Over time this may result in reduced input from the physical and social environment, which, in animal models, has been shown to have secondary effects on brain structure and function.

**Zinc Deficiency**

There are no clear biomarkers to identify zinc deficiency [28]. Therefore, investigators rely on responses in randomized zinc supplementation trials among children thought to be zinc-deficient. In contrast to studies of iron deficiency, most of which involved well-nourished, healthy babies, research on zinc deficiency has generally focused on children at risk of poor growth. Recent studies among nutritionally at-risk infants have demonstrated the beneficial effects of zinc supplementation on infant mortality [29] and on multiple indicators of health, including growth [30], diarrhea [31, 32], and pneumonia morbidity [31]. An early observational study from Egypt reported an association between maternal micronutrient intake and infants’ developmental skills at 6 weeks and 6 months of age [33, 34], suggesting that zinc may play a critical role in the development of infants’ mental and motor skills. However, reviews of the randomized trials of zinc supplementation conducted in the past decade have concluded that there are inconsistent findings regarding the relationship between zinc supplementation and child development [35, 36].

**Mental Development**

Mental development has been examined in at least 6 randomized trials of zinc supplementation. They were conducted among very low-birth-weight infants in Canada [37], small-for-gestational age infants in India [35], and low-income infants in Chile [38], Brazil [39], and Bangladesh [40, 41]. In the Bangladesh trials, zinc-supplemented infants had mental scores that were 3–6 points higher than unsupplemented infants at 12 months, regardless of whether zinc supplementation was administered to mothers during pregnancy [41] or directly to the infants [40]. None of the other trials found differences in mental development related to zinc supplementation.

**Social/Emotional Development**

Two trials assessed infants’ social/emotional development related to zinc supplementation. Zinc-supplemented infants in Brazil were more cooperative than unsupplemented infants, based on behavioral observations conducted by examiners [39]. In a trial among infants born small-for-gestational age in
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India, there were no differences in orientation or emotional regulation related to zinc supplementation [35]. However, when mothers were asked about their infants’ behavior, mothers of zinc-supplemented infants with a birth weight of <2,500 g were more likely to report that their infants were irritable than were mothers of unsupplemented infants [35].

**Motor Development**

Very low-birth-weight infants in Canada who received formula with a higher concentration of zinc (11 versus 6.7 mg/l) for the first 5 months of life had better scores in motor development than those who received the lower concentration of zinc [37]. In Chile, low-income infants who received zinc daily for 1 year had higher scores in motor quality (gross and fine motor movement and control), but there were no differences in the children's overall motor scores [38]. In India, infants born small-for-gestational age who received zinc supplements for the first 9 months of life had marginally higher motor scores at 6 months [35], but there were no differences at 10 months.

**Spontaneous Motor Activity**

Three trials that examined infant activity all found increases related to zinc supplementation. In Peru, fetuses of mothers who received zinc supplementation during pregnancy were more active during prenatal sonograms, in comparison to fetuses of control mothers [42]. In India, low-income toddlers who received zinc daily for 6 months had more vigorous activity during play compared with control toddlers [43]. In Guatemala, stunted toddlers who received zinc daily for 7 months had more functional activity during play compared with control toddlers [44]. Thus, findings related to zinc supplementation and spontaneous motor activity among infants at high risk for zinc deficiency are relatively consistent.

**Methodological Considerations**

Most of the studies examining the impact of zinc supplementation on child development used global measures of development (e.g., Bayley Scales of Infant Development) and were conducted with careful controls for environmental and potentially confounding variables. Although the Bayley Scales are well-standardized and include explicit instructions for administration, they may not capture subtle differences in information processing.

Infants who experience intrauterine growth retardation are likely to become zinc-deficient, because they have limited hepatic stores of zinc and increased requirements for catch-up growth [45]. Children with low consumption of bioavailable sources of zinc (e.g., animal products) are also at risk for zinc deficiency. However, undernourished children are also at risk for other micronutrient deficiencies that have been associated with developmental delays, especially iron and B₁₂ [46], emphasizing the importance of examining the interrelationships among micronutrients.
Postulated Mechanisms

Zinc deficiency may be particularly relevant to early development because zinc is present in all cells, playing fundamental roles in cell division and maturation and in the growth and function of many organ systems, including the central nervous system [47]. In the central nervous system, zinc is concentrated in the synaptic vesicles of specific glutaminergic neurons. Zinc serves as a neurotransmitter, passing into postsynaptic neurons during synaptic events. Zinc is thought to be essential for nucleic acid and protein synthesis, processes that may be disrupted by zinc deficiency [48]. However, none of the measures used in studies of human infants/toddlers can be easily or directly linked to these central nervous system processes.

Conceptual Framework for Developmental/Behavioral Effects of Early Nutrient Deficiencies

There is ample evidence that nutrient deficiencies are more likely to occur in disadvantaged environments, which themselves have adverse effects on children. There is also clear indication that nutrient deficiencies have direct effects on central nervous system development. Research examining the impact of iron and/or zinc deficiencies on children's development has often hypothesized a direct effect, through changes in neuroanatomy or neurotransmission, for example. However, it is also possible that behavior changes associated with micronutrient deficiencies alter the caregiving that the child receives, thereby compromising the child's development even further. For example, if a micronutrient-deficient child is unable to elicit or to benefit from nurturant interactions from a caregiver, that child may be denied the enrichment that is known to promote early development. The result could be a child who experiences the brain changes that have been associated with micronutrient deficiency, together with limited environmental input. Over
time, these combined influences may result in poorer behavioral and developmental outcomes (fig. 1). This process, whereby poorer outcomes in infants and toddlers with nutritional deficiencies are partially mediated through caregiving behavior, is known as functional isolation [49]. Future research should consider how the caregiving system is related to child development and whether it mediates the effects of micronutrient deficiencies.

References

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28 Hambidge M: Biomarkers of trace mineral intake and status. J Nutr 2003;133:948S–955S.
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Discussion

Dr. Pettifor: I have a question about the preventive trial in Chile in which there were some subjects on iron and others without iron. What percentage of those who were not on iron actually developed clinical iron deficiency? If one looks at iron deficiency as being a continuum was there a cutoff point where it could be said that a certain level of iron status was needed before they develop any of the changes that you noted, or was it a continuous progression from those who were severely iron deficient to those who were quite iron replete?

Dr. Lozoff: In the Chile study [1], the unsupplemented group was given milk or vitamins in identically marked cans or bottles, so the study was double-blind. There was no added iron. Chile and Costa Rica at the time and Brazil are countries where babies are often given unmodified cow's milk quite early. Even mixed with breast-feeding, there is a lot of iron deficiency under those conditions. Like other studies, we found that 23% of the unsupplemented group had iron deficiency anemia and an additional 31% or so had iron deficiency without anemia. Overall more than 50% of the unsupplemented group had iron deficiency by the strict criteria of at least 2 of 3 abnormal iron measures, and all iron parameters were worse. This means that the entire unsupplemented group had a poorer iron status. We looked for a continuum of effects but didn't observe any. What we found was that the whole unsupplemented group did worse in behavior and development. Thus, in a study where infants were randomly allocated, there was no suggestion of a cutoff point.

The earlier community study in Costa Rica [2] had been designed to try to answer that question in a non-experimental study. With children at every level of iron deficiency, we analyzed the Bayley test scores. In that study we concluded, and Walter et al. [3] in Chile reached pretty much the same conclusion, that iron deficiency needed to be severe enough to cause anemia before lower mental and motor test scores were seen. I was comfortable with that for a long time, but I think this could be revisited for the following reasons. These were effects on a global test of development. As we move into more sensitive measures, such as nerve conduction and other things, it is an open question whether there are effects of iron deficiency without anemia. In fact we are looking at that right now in a program project grant from the US National Institutes of Health (I should add that all of the work I reported was supported by the National Institutes of Health). What is this program project grant? It is a cross-species project with human infants, two developing monkey projects, and a rodent model. Across these species we are trying to develop behavioral measures that link them and then go progressively to the brain in the animal models. We are also specifically looking for effects of iron deficiency without anemia using very sensitive measures and also trying to identify which behaviors can be corrected by iron treatment and which not.

Dr. Pettifor: Looking at all the factors that influence the development of children in early life and trying to relate the percentage effect of genetics, of assessing data on iron deficiency, etc., how much do you think is the overall effect of iron deficiency on mental development?

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**Dr. Lozoff:** All of the results I showed are statistically significant after controlling for everything that we could. We measured mothers' IQ, the home environment, socioeconomic status, child's growth, etc. and controlled for all these factors. But that is statistical control. One of the very special things about the Chilean study, the preventive trial, is that children were randomly assigned to receive high or low iron or no added iron. By that design the children of mothers with lower IQs and worse environments were equally likely to be in the different groups, and they were. In addition, we controlled for all of those things statistically. So the behavioral differences are most likely due to lack of iron.

In the analysis of the Costa Rica study, we gave all these factors a chance to account for variability and then asked how much variance was left over that could be attributed to iron deficiency anemia. Background factors accounted for 3–8% of the variance, and iron deficiency anemia accounted for an additional 5–8%. That doesn't fully answer the question, however, because these things co-occur. By statistical control, for instance, you remove the shares effects.

**Dr. Endres:** As you described this relationship almost too beautifully to be believed, I just have a very simple question. Were the psychologists blinded to the study?

**Dr. Lozoff:** Absolutely, neither the parents nor the psychologists knew the child's iron status. In fact, the mothers were not told the baby's blood results until the end of the study, so it was always double-blind. Even though the results are dramatic and I think they are important, it is not as though you would see a child in your waiting room and say that there is something wrong with that baby. They are very carefully screened so that nobody was sick, and in fact if you look at the developmental test scores, there was virtually nobody below the normal range. So we are finding differences within the normal range. This relates to another point. Much research on iron deficiency has carefully focused on such otherwise healthy children. We don't know much about the effects of iron deficiency among children in less optimal health. Yet around the world in developing countries many children have a variety of health problems. Studies with otherwise healthy infants help us understand the effects of iron deficiency apart from generalized undernutrition or other illnesses or infections, so it gives a conservative estimate of what iron is doing.

**Dr. Gebre-Medhin:** I think we have no doubt about the fact that iron deficiency anemia in an infant is a very serious matter. A child with verified iron deficiency is a very sick child so this is a very important area for discussion. Now my question is partly a follow-up on Dr. Endres' question, what about the parents: do they communicate with the psychologist; do they tell them whether the child has been iron medicated or not? Was this an experimental study in which you were responsible for the distribution of iron and treatment, or was this an observational retroactive prospective study? The reasons that lead children to iron deficiency anemia may be one thing, but seeking care in the hospital will dramatically disaggregate them into very different groups. How was that issue controlled?

**Dr. Lozoff:** The first question is could mothers have alerted the testers in some way or the other. There are a couple of observations here. I don't agree that most children with iron deficiency anemia are very sick. I don't know if you noticed the percentages of those showing altered behavior, but the highest percentage was for 'no social referencing': 18% of the unsupplemented group compared to 8% of the children who received iron [1]. That means that over 80% of the children who were in the unsupplemented group did check in with their mothers. So it is not that every iron-deficient child or every child who is not getting iron shows this behavior. We have no indication from available studies that there is something affecting every baby or something easy for the mothers to report. In Costa Rica, I asked the pediatrician to indicate who she thought was iron deficient based on her interaction and examination, and it was about 50–50 whether she could guess. That is my most direct indication that even a highly skilled pediatrician, when asked to pick out these children, could not do it, let alone the mothers.
Your second question is were the studies observational or intervention. The earlier studies I talked about are almost universally case-control studies. However, children were identified not in a clinic setting but in neighborhoods. The studies tested the children and then compared those with iron deficiency anemia with those with better iron status. In Costa Rica, it was a door-to-door study: knock on the door and ask whether there was a child between 12 and 23 months. If the baby was completely healthy and full-term, they were invited to enter the study. So they did not come to the doctor seeking attention. We closely supervised the iron therapy and have continued to follow them. Nonetheless, Costa Rica was an observational study in that whatever factors might go along with iron deficiency were there. We statistically controlled them but nonetheless they are there.

Now we come to the Chile study and why the Chilean study had its design. We made sure no child with iron deficiency anemia went into the preventive trial, and then they were randomly allocated to get high iron or low iron, or high or no added iron. So this study is different from most earlier ones. The children were not simply turning up with iron deficiency later on. Rather, iron status was a result of randomization. I told you that 23% of the children in the no-added-iron group had iron deficiency anemia. The figure for the iron-supplemented group was 3%. There is no question that the study design produced groups with extremely different iron status.

Dr. Abrams: I want to put my hand up as a neonatologist here. What we do every day in the nursery is we care for premature infants who would otherwise be in utero and allow them to have hemoglobins of 7–10 without problems and leave them that way for several months without transfusing them. In this general change over the last 10 years in neonatology, I was wondering if you have any data or any comments about whether or not this is a very good idea from a developmental perspective and if any one has ever looked at this issue in in utero terms?

Dr. Lozoff: The question relates to the premature infant and the low hemoglobins we allow them to have. Dr. Georgieff is a neonatologist very involved with iron deficiency these days, and he would be a wonderful person to address this question. A concern around the premature infant and the perinatal period is that iron regulation is not fully developed. This means that iron toxicity may be more of a risk then that it is later on. Iron deficiency is not good for babies’ brains, but excess iron might also occur in the very immature brain. We certainly need more research on these issues.

Dr. Guesry: Could you say something about the critical age threshold because earlier studies seem to say that if one wants to avoid neurological sequelae the correction of iron deficiency anemia should take place before 1 year of age. The latest study, the one that you mentioned in Indonesia, seems to say that, even after 1 year of age [4], correction of the anemia could also correct neurological symptoms.

Dr. Lozoff: The Costa Rican children averaged 17 months of age; in that study the children were between 12 and 23 months. So all of them were after the first year of life. In Chile, the period of supplementation was between 6 and 12 months. You have brought up an important point that I can’t emphasize enough: when looking at different studies, attention must be paid to the age at which they are performed. Timing questions are very challenging in the human: timing, duration and severity of iron deficiency are intimately intertwined. A baby who gets iron deficient earlier is likely to have iron deficiency longer and more severely. Thus, from observational studies in the human it is almost impossible to separate timing, duration and severity. Now in the Chile study we know with confidence that no baby had iron deficiency anemia for longer than 6 months, because we tested their blood at 6 months and those with iron deficiency anemia were pulled out. Then we tested their blood again at 12 months. So the maximum duration of iron deficiency anemia can only have been 6 months. In fact, it is probably less. Infants started on the different supplements at 6 months, and so it was probably not until 8, 9, 10 months that some developed iron deficiency anemia.
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This is a very short time period. I actually think this is why we did not see differences in the Bayley test in the Chilean study: the babies had iron deficiency for such a short time. Timing is a question that we are looking at in the program project grant using animal models, because timing, duration and severity can be controlled experimentally. So again it is humbling how long it takes to get answers to some of these very important questions.

_Dr. Bloem:_ Were these studies carried in the urban or rural areas?

_Dr. Lozoff:_ All of the studies I'm involved in have been in urban settings. They involved working class communities – lower to lower middle class. The mothers in Chile and Costa Rica averaged about 9 years of education. Everybody was literate, and health was excellent in both these countries with national health care systems. So they are in much better conditions than most children in the world.

_Dr. Bloem:_ How much do you think lead toxicity has influenced your results?

_Dr. Lozoff:_ Both of these countries have used unleaded paint for many decades. In Costa Rica we measured lead in all the children; the lead levels averaged about 10 µg/dl. This was in the early 1980s, when the US cutoff was 25 µg/dl. Lead levels were controlled in all of the results. It is very interesting that in Costa Rica there was no negative correlation between lead and child development at those levels [5]. In Chile we didn't have enough money to do lead levels on everybody. With the good graces of the CDC we were able to test for lead in a sub-sample. We sent bloods from infants with the very highest erythrocyte protoporphyrin values and some of the normal values. Then one of my colleagues, Dr. Pino, got a grant in Chile that covered more lead testing. All combined, we were able to do leads on about 330 children. Again, for Chile there was no negative relation between lead and poor development [1], but we couldn't control for lead levels in all analyses because we didn't have them for everybody. The mean lead level was about 8. Chile was in the process of implementing unleaded gasoline at the time. We found that there was a further drop in the lead level of infants over the last 18 months of the study. At the start of that time the average was about 8.3 µg/dl and at the end it was 5.9 µg/dl [6]. So the lead levels of infants seem incredibly sensitive to an environmental intervention like the switch to unleaded gasoline.

_Mr. Parvanta:_ How do children who are somewhat delayed in development, like this second child who was not really reacting, impact on what is the reaction of the mothers of these children?

_Dr. Lozoff:_ The nature of child development is transactional. An alteration in the child's behavior will have an effect on caregivers and vice versa, and there are cascades of transactional effects. What was interesting to me is that these testers, who just met these babies, modified their behavior. That seems appropriate. If you see a baby who is looking hesitant, you back off. I presume that the mothers would have that same kind of reaction and appropriately and adaptively buffer their children from unhappier reactions to the world. So it may not be that the mothers are responding inappropriately. However, if the caregiver behavior is sustained over time, there could be an adverse impact in terms of the baby's experiences of the world. In another unpublished study involving Indian children at about 3 years of age, the children came into an observational room in a familiar neighborhood clinic. A mat was placed on the floor, and toys were given to the child. The initial few minutes were only for the mother, the child, and the toys, and there were no differences in behavior in children with iron deficiency anemia. Then an examiner came in with a new toy covered by a box, removed the box, and walked out. That is all that happened, and under these conditions children with iron deficiency anemia were less likely to touch the toy or smile. So it appears that the simple perturbation of something unfamiliar happening was sufficient to alter behavior. These results allow me to make another point. In the familiar environment I am not sure that the altered behavior would be very obvious.
In developmental research, you often have to push the system, you have to stress the child a bit before you will see whatever is going on. The India study is an example. It was just the tiniest push – a little unfamiliarity of a box covering a toy or a new person coming in – was sufficient to make the behavioral differences apparent. So I completely agree with you that we must consider not only the babies’ behavior but also what impact that has on the caregiving environment or we will not be really understanding how poorer outcome happen and might be sustained.

**Dr. Pettifor:** About the issue of the global problem of iron deficiency and ways of trying to address the problem: if one looks at the major problems of infants from 6 months to 2.5 years in many developing countries, they are huge, yet iron supplementation programs are poor basically as far as implementation and compliance are concerned. If you were going to look at this problem, how would you attempt to address it on a global scale, and is it something that should be placed up there right at the top with all the other problems in the developing world?

**Dr. Lozoff:** I know others are going to be talking about this. Asking mothers to give babies iron drops for months may not work very well, even though under controlled circumstances, a benefit can be shown. The United States is an example of tremendous success in improving the iron status in the population in a different way [7, 8]. Up until about 1970, 20–25% of poor children in the United States also had iron deficiency anemia. At that point a number of things started happening: infant formula became fortified with iron; infant cereals became fortified with iron; breast-feeding was encouraged; a lot more ascorbic acid was added to the infant diet. And iron deficiency anemia went down to 3%. Canada now does the same, as does Chile. Although these are examples of effective interventions, the challenge for the developing world is how to improve infant iron status without interfering with breast-feeding. I am going to defer this to other speakers. I will just simply say that this is a problem that can be solved, but I am not saying that improving iron status is easy or simple.

**Dr. Barclay:** From the data currently available, is it possible to say which would be a most effective strategy in preventing the type of deficiencies you are talking about? Would iron supplementation or fortification of food be the best way to go?

**Dr. Lozoff:** My role in this area has been on the ‘why does it matter’ side of things. Other speakers are better for answering these questions.

**Dr. Lönnerdal:** Considering the strong interaction between the caregiver and the infant, I have a question concerning the findings of Murray-Kolb et al. [9] regarding the behavior and emotional scale of pregnant women. The worst case here would be an iron-deficient anemic mother interacting with an iron-deficient anemic child where this interaction could be seriously affected. Have you looked at the iron status of the women?

**Dr. Lozoff:** In Costa Rica and Chile we measured mothers’ iron status. In Costa Rica it is absolutely amazing, there were only about 2 mothers with iron deficiency anemia and 8 with iron deficiency. Thus, at least 20 years ago, the diet in Costa Rica was perfectly good for the adult. But the mothers’ blood was obtained when the children averaged 17 months of age. We don’t have the mothers’ blood during pregnancy. For Chile we have mothers’ blood only for some infants and only at 12 months of age. Typically the peak period for iron deficiency is 6–24 months. However, an increasing number of people are interested in the prenatal iron deficiency question. Dr. Lönnerdal is referring to a new study by Corwin et al. [10] in which they looked at maternal depression and mood and found a relationship with the mother’s iron status. In the adult literature there have been no consistent findings about iron deficiency and mood, but such a link makes sense. Of course, as Dr. Lönnerdal points out, having a mother who is iron deficient and depressed is unlikely to be good for the baby.

**Dr. Specker:** Did you find any infants who didn’t respond to the iron supplementation?

**Dr. Lozoff:** In Costa Rica, we went into the home at least 5 days/week and placed the medication into the child’s mouth. 93% of the children responded to iron with an
increase in hemoglobin of 10 gl or more or normalization of all iron measures. No child had iron deficiency anemia after 3 months, none. In some cases, however, the biochemistry indicators were vastly improved but not fully corrected. Thus, in that study I can say that non-response was not a problem. In Chile, there were 2–3 children who did not respond to iron and it seemed that something else was going on.

References
