Considering Environmental Factors in Research on Nutrient Deficiencies and Infant Development

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I have become increasingly concerned with the issue of environmental factors over the past 20 years of involvement with clinical trials related to iron deficiency anemia and infant behavior. This concern has been incremental, as each study has taught me lessons about environmental influences. Each study raised new questions, which affected the design of the subsequent study. I review this series of studies to illustrate some of the issues and provide a real-life case study as background for a discussion of environmental factors in clinical trials in infant nutrition. In so doing, however, I want to emphasize that I am approaching this topic as a clinical investigator, not as a statistician or a methodologic expert.

Although a randomized clinical trial is the ideal study design to minimize confounding by environmental influences, the experience with clinical trials related in iron deficiency anemia and infant development illustrates that environmental factors are still of major concern. Before turning to this issue, I briefly summarize the results of the available clinical trials. These studies all used multiple measures to characterize iron status, and most used the Bayley Scales of Infant Development to assess development and behavior. Statistical details are omitted, and only those results at the 0.05 level of statistical significance or better are reported.

All seven of the available studies of iron-deficient anemic infants that included careful definitions of iron status and appropriate comparison groups reported that iron-deficient anemic infants had lower mental test scores than infants with better iron status (1–7), and five showed lower motor scores as well (1,3–6). No association between lower pretreatment test scores and lesser degrees of iron deficiency has been documented (4–6,8). After 1 week, neither intramuscular nor oral iron treatments differed from placebo treatment in effects on scores (4,5,9; M.E.K. Moffatt, personal communication, 1987). After 2 to 6 months of treatment, one study reported an overall improvement in mental and motor test scores (6), two studies observed continued lower test scores in the majority of anemic infants, with improved scores
in a minority who showed the best response to iron treatment (4,10), and two found no improvement at all, regardless of hematologic response (5,7). Thus, the ability of iron treatment to correct the lower test scores and the conditions under which iron therapy can affect test score improvements remain open questions.

I go over our own studies in more detail to illustrate some issues about environmental influences. The overall lesson is that most of us clinical investigators are naive and simplistic in our approach to environmental factors. Someone tried to teach me this lesson in my initial study in Guatemala years ago, but the issue continues to be challenging in every single subsequent study. In that first study (1), conducted in Guatemala in 1978 in collaboration with the Institute of Nutrition of Central America and Panama (INCAP), the sample was drawn from a socioeconomically homogeneous settlement constructed after the 1976 earthquake. Thus, housing, sanitation, and health care facilities were the same for everyone. In this study, as in all the subsequent ones, babies were candidates for participation only if they had been born at term, were not of low birth weight, and were free of acute or chronic illness. We compared 28 infants with iron deficiency anemia with a nonanemic group of 40 infants and assessed the effects of short-term oral iron and placebo treatment. The mean mental development test score of the infants with iron deficiency anemia (Hb ≤ 105 g/liter) was 87, compared to the mean score of 100 among the nonanemic infants (Hb ≥ 120 g/liter). A 9-point pretreatment difference in motor scores was also observed. There were no significant differences between anemic and nonanemic groups in birth history, socioeconomic factors, or general nutritional status that might otherwise explain the lower developmental test scores. Mental test score deficits were especially marked in older anemic infants (19- to 24-month-olds), and a substantial correlation between the degree of iron deficiency and mental test scores (r = 0.73) was observed in this age group (11). No changes from short-term oral iron treatment were noted. Iron-treated anemic infants did not show significantly greater increases in their Bayley mental or motor test scores than either placebo-treated anemic babies or infants in the iron or placebo-treated nonanemic groups; all groups increased by 4 to 6 points.

When, as a new investigator, I presented these data at the Society for Research and Child Development meetings in 1981, I made a big point of the similarity in all the background factors, arguing this meant that the pretreatment differences really could be attributed to iron deficiency anemia. A member of the audience, neurologist Marcel Kinsbourne, came up to me afterwards and gently pointed out that the usual indicators of socioeconomic status, such as parental education and occupation, might not be nearly as revealing in research in developing countries as in highly industrialized settings. Education might be a good proxy for parental intelligence and socioeconomic status in a country such as the United States but not in a developing country where educational opportunities were limited. At the time, I thought the lesson of the Guatemala study was that ways of assessing environmental influences might need to be different depending on whether the research was conducted in industrialized societies or a developing country. But it really was just the beginning of recognizing that addressing environmental influences adequately is a complicated and sophisticated undertaking.
The next study, started in Costa Rica in 1981 in conjunction with the Hospital Nacional de Niños, was designed to answer some questions posed by the results of studies available at that time. None of the previous studies assessed whether there was a particular degree of iron deficiency at which infant behavior and development was altered. Furthermore, the apparent discrepancies regarding treatment (1,8,9) suggested that oral and intramuscular iron might differ in short-term effects on developmental test scores. We tried to address these issues by enrolling a relatively large number of infants with varied iron status in a single study, including double-blind randomized controlled comparisons of short-term oral iron, intramuscular iron, and placebo treatment, and long-term oral iron or placebo treatment (with placebo only for iron-sufficient children) (4). The sample consisted of 191 12- to 23-month-old infants divided into groups ranging from most to least iron-deficient as follows: (a) iron-deficient anemic \( n = 52 \); (b) intermediate in hemoglobin level and iron-deficient \( n = 45 \); (c) nonanemic iron-deficient \( n = 21 \); (d) nonanemic iron-depleted \( n = 38 \); and (e) nonanemic iron-sufficient \( n = 35 \). The data from the anemic infants were further analyzed with respect to actual Hb level because lower Hb levels indicate more severe iron deficiency once anemia is present. Iron-deficient and iron-depleted conditions were subsequently confirmed by hematologic response to iron treatment.

Infants with moderate iron deficiency anemia (Hb \( \leq 100 \) g/liter) were found to have lower mental and motor test scores than appropriate controls; infants with mild anemia (Hb 101 to 105 g/liter) received lower motor scores but not mental scores; and infants with lesser degrees of iron deficiency did not have impairments in developmental test performance (Figs. 1 and 2). The mean mental test score of the moderately anemic infants was 8 points below that of infants with higher Hb levels (\( >100 \) g/liter), and the mean motor score of the entire anemic group was 10 points below that of infants with Hb \( >105 \) g/liter. Mental test scores decreased with age in all groups; the differential decrease observed among older anemic infants in the Guatemalan study (11) was not found. However, anemic infants in all age groups seemed to have trouble with particular motor functions involving balance and coordination.

After 1 week of treatment, the increases in Bayley test scores and hematologic variables among iron-deficient infants receiving intramuscular iron did not differ from those of iron-deficient infants receiving oral iron. There was no benefit of iron therapy over placebo on developmental test scores.

More important than short-term outcome, this study was designed to examine the effects of a course of treatment commonly used in practice (3 months of oral iron treatment). All iron-deficient and iron-depleted children were treated with iron, and the iron-sufficient group was randomly assigned to oral iron or placebo treatment. After 3 months, the anemia of all iron-deficient anemic infants was corrected, but the majority (64%) still had biochemical evidence of iron deficiency, suggesting greater severity or chronicity. On average, previously anemic infants continued to have lower test scores in mental and motor development (Figs. 1 and 2). However, in the minority of infants who became completely iron-sufficient after 3 months of
treatment, there was some indication that iron treatment benefited test scores. These infants, who probably had less severe or less chronic iron deficiency anemia, showed a 10-point increase in motor test scores and did not show the decline in mental scores observed in the rest of the sample.

Despite the encouraging response in this subset of infants, it was worrying that lower mental and motor test scores persisted among the majority of initially anemic infants. We were again faced with a clinical trial that did not show a test score benefit of iron treatment for most babies. The whole issue of environmental factors becomes crucial in this situation, because establishing a causal relationship between a risk factor and poorer development then depends heavily on eliminating other

FIG. 1. Mental development index from the Bayley Scales of Infant Development before and after treatment (4), reproduced with permission.
FIG. 2. Psychomotor development index from the Bayley Scales of Infant Development before and after treatment (4), reproduced with permission.

Factors that might account for the developmental test score differences. In the Costa Rica study (4), the population was generally lower middle class, highly literate, with excellent health care, and the babies were free of undernutrition, increased blood lead, hemoglobinopathies, and parasitic disease. However, Kinsbourne’s comments had led us to include a much more comprehensive assessment of the environment. We administered full-scale IQ tests to the mothers (and to fathers where possible), determined parental weight and height, made home visits to complete the HOME
Inventory (12) (a measure of the stimulation a child receives at home), and collected dietary information. On these more detailed measures, infants with iron deficiency anemia had several environmental disadvantages. They were less likely to have been breast-fed; if breast-fed, they were weaned earlier; they consumed more unmodified cow's milk; their mothers had lower IQ scores; and their home environments were less stimulating (4). Nonetheless, mental and motor test score differences were statistically significant after control for these differences. We also did a stepwise multiple regression entering all of the background variables first and found that iron deficiency anemia contributed significantly to the variance even after control for family background variables.

Initially, I thought that the problem of differences in family background had been handled satisfactorily, and there was reason to conclude that iron deficiency anemia accounted for the lower test scores. Ultimately, however, the lesson from this study was that the coincidence of nutrient deficiencies and other disadvantages must be taken even more seriously (13). As with several other risk conditions, such as low birth weight, raised lead levels, and generalized undernutrition, iron deficiency is associated with environmental disadvantages (14–19). Such disadvantageous conditions might include poverty, limited knowledge of optimal ways to feed and care for infants, lack of stimulation in the home, and so on. These factors are known to have adverse effects on infant development and could account both for nutritional deficiency and for poorer developmental outcome. For instance, parents who are more limited because of restriction of intellectual capacity, stress, or depression might make worse decisions about feeding their children and at the same time provide less stimulating environments, both physically and intellectually. Thus, the association between the nutrient disorder and poorer development might not be a causal one; instead, each might be caused independently by family limitations.

In the clinical trials of iron deficiency anemia and iron treatment, a consistent pattern seemed to be emerging from studies of a routine course of treatment. Not only had the majority of iron-deficient anemic infants continued to receive lower developmental test scores despite 2 to 3 months of treatment and excellent hematologic response to iron in the Costa Rica study, but similar results were obtained in studies in the United Kingdom (10) and Chile (5). However, a recent study by Idradinata and Pollitt in Indonesia (6) has reopened the question of effective reversal of test score deficits with iron treatment. In a double-blind randomized trial, 12- to 18-month-old infants (50 iron-deficient anemic, 29 nonanemic iron-deficient, and 47 iron-sufficient babies) were assigned within iron status group to oral iron or placebo treatment for 4 months. Before treatment the mean mental and motor scores of the iron-deficient infants were 12 to 15 points lower than those of nonanemic iron-deficient and iron-sufficient groups. Iron-treated anemic infants dramatically improved their mental and motor test scores (+19 points and +23 points, respectively) compared to no change in placebo-treated anemic infants. There was no evidence of lower test scores in nonanemic iron-deficient infants and no effect of iron treatment on test scores in either the nonanemic iron-deficient or iron-sufficient groups. However, the marked improvement in test scores of iron-deficient anemic
infants treated with iron provided convincing evidence that iron deficiency anemia in infancy can cause lower developmental test scores.

One possible explanation for improvement in the Indonesia study and lack of clear-cut effects in previous studies was the longer course of iron treatment (4 months). In a new study in Costa Rica in collaboration with the Hospital Nacional de Niños, we assessed the effects of 6 months of oral iron treatment (7). This community study of 12- to 23-month-old infants compared 32 babies with moderate iron deficiency anemia (Hb ≤ 100 g/liter) with 54 nonanemic controls (Hb ≥ 125 g/liter). The iron-deficient anemic group averaged 6 points lower in mental test scores than the nonanemic group. No differences in motor scores were observed. All anemic infants were treated with oral iron for 6 months. Nonanemic infants were randomly assigned to oral iron or placebo treatment, but there was no difference between the two conditions with respect to test score change; all nonanemic infants could therefore constitute a single comparison group. Anemic infants continued to receive lower mental test scores after 3 and 6 months despite an excellent hematologic response with correction of anemia in all infants and iron sufficiency in 71% at both 3 and 6 months.

The seriousness of the challenges presented by the confluence of nutritional and environmental disadvantage is further illustrated by this study. Once again there were differences in family background—in this case lower levels of maternal education, lower HOME scores, and less breast-feeding (7). However, in contrast to the previous study in Costa Rica, developmental test score differences were no longer statistically significant after control for the background factors. In trying to understand the differing results, I became very aware of the issue of power with respect to environmental factors. Power analysis indicated that the sample size was adequate (90% power) to detect a simple main effect but could only have detected a considerably bigger difference (11 points) between anemic and nonanemic groups after control for background variables. Thus, one lesson about environmental factors from this new study is that the sample size necessary to have sufficient power to determine a simple main effect is much smaller than the sample size necessary to be able to identify a main effect after control for a range of environmental factors. I certainly have not considered that problem in the power calculations I have generally used in designing studies.

Given the uncertainty about the reversibility of test score differences with iron treatment, an important related question is whether preventing iron deficiency will prevent poorer developmental test performance. Two preventive trials have been published, and a third large trial is near completion in Chile. The first, by Heywood et al. in Papua New Guinea (20), compared 1-year-olds, half of whom received intramuscular iron at 2 months and half a placebo injection. Although the design of the study was strong, the results are difficult to interpret because malaria was endemic, all groups were anemic at 12 months, and iron status measures did not clearly indicate iron deficiency. However, it seemed that iron-treated infants who were malaria-negative showed better attentional abilities. The other preventive trial, recently published by Moffatt et al. (21), included 283 Native American infants in
Canada, half of whom received iron-fortified formula and half received unfortified formula from birth, with follow-up until 15 months. The groups were initially comparable in iron status, development, and family background but diverged in hemoglobin, iron status, and psychomotor test scores at 9 and 12 months, with poorer outcome in the unfortified group. Because of its design, this study also provides convincing evidence that iron deficiency causes lower test scores in infancy. Two findings qualify this conclusion, however: (a) no differences in mental scores were noted, even though they have been consistently found in virtually all case-control studies; (b) the differences in motor scores resolved spontaneously by 15 months. These observations raise the possibility that some other factor explains the differences in mental and motor development in iron-deficient anemic infants.

The third preventive trial, almost completed in Chile, was designed to address the question of a causal relationship between iron deficiency anemia and poorer developmental outcome. It is a double-blind randomized study of preventing iron deficiency through the use of iron supplementation, starting at 6 months of age. There are several reasons for this study design: (a) by the process of random allocation, children at most environmental risk would be equally represented in the supplemented and unsupplemented groups; (b) by starting the trial at an early age and excluding infants with iron deficiency anemia at the outset, infants would not yet be iron-deficient, and the confounding effects of nutrient deficiency and environmental disadvantage would not yet have occurred. This study has involved 1185 children. (The sample size had to be very large because only 20% to 25% of the unsupplemented children were expected to develop iron deficiency anemia. Because they are the only ones likely to show lower scores, the overall group difference between supplemented and unsupplemented children would be quite small and hence require a large sample to be detected with confidence.)

What we are finding is surprising (22). The supplemented and unsupplemented children are very different in iron status but do not differ in developmental test scores. These results highlight two issues in particular. Once again, the study raises the possibility that any differences in previous studies are not caused by iron deficiency itself but by some other closely associated factor, or by iron deficiency only under certain conditions. For instance, results of a second component of the study, showing evidence of altered neurophysiological variables in 6-month-olds with iron deficiency anemia (23), suggest that factors such as the timing and duration of iron deficiency anemia may be critical. The results of the preventive trial also raise the possibility that when iron status is experimentally manipulated rather than being a product of family decisions, the relationship to developmental test performance is different.

Out of these experiences, I have become increasingly convinced that the issue of environmental disadvantage is a major dilemma for studies of nutritional deficiencies. Figure 3 is a conceptual model of my current thinking about the interrelationship among environmental disadvantage, nutrient deficiency, and poorer developmental outcome in infants (24,25). On the left side of the figure are the more biological explanations that focus on the baby. There is a reduction
in brain iron when iron deficiency anemia occurs early in development (26),
with concomitant alterations in myelination and neurotransmitter function contrib-
uting to stress vulnerability and delayed neuromaturation (23). These combine
to produce an altered behavior pattern—increased wariness and proximity seeking
and decreased activity (24). On the right side of the figure are the environmental/
familial influences. Environmental disadvantage might contribute to poor feeding
practices, such as early weaning and use of unmodified cow’s milk. Other effects
of environmental disadvantage might be mediated through parenting behavior
(perhaps through maternal stress and depression). One would expect transactional
relationships between the child’s behavior pattern and limitations in parenting
behavior, such that there would be less support for the child’s development,
contributing to decreased learning experiences. Again, one would expect transac-
tional relationships between functional isolation as a result of the child’s behavior
(27,28) and the decreased support of the child’s development because of environ-
mental disadvantage. Collectively, these influences would lead to poorer outcome
for iron-deficient anemic infants. Such a model provides a useful framework for
thinking about how a nutritional deficiency could fit together with environmental
disadvantage to produce poorer developmental outcome in affected infants.
I have touched on only a few of the issues of environmental factors in research on nutrient deficiencies and infant development. There are many other concerns. For instance, even though having a model helps guide the choice of factors one should try to assess, measuring the relevant environmental influences is truly challenging. Deciding the right things to measure is also of critical importance in assessing whether differential attrition might affect results, whether the groups in a clinical trial are really comparable, and so on. Furthermore, an intervention might be effective only under certain environmental conditions (for example, the most or the least advantaged environments) or only in infants with certain characteristics (for example, relatively younger or older; at biological risk because of birth weight or health problems; or completely free of health concerns). Finally, there are many methodologic and statistical issues related to confounding and mediating variables. Several recent papers provide thoughtful discussions of these subjects (29–33). In any case, the issues related to environmental factors require expert methodologic advice at all stages, but especially in relation to study design, selection of measures, data analysis, and interpretation.

In sum, researchers in infant nutrition should expect that nutrient deficiencies will go along with environmental disadvantage. Even if we use statistical control for these environmental differences, I am convinced that the families are in fact different in ways that will influence their children’s behavioral and developmental outcome. Rather than seeing this as a methodologic annoyance, I have come to think about this concurrence of environmental and nutritional risk as the reality in which children live. The challenge for researchers is to describe this reality in trying to understand what places children at risk and what components are most amenable to intervention.

Acknowledgments

This work was supported in parts by grants from the National Institutes of Health (HD14122 and 31606). I also appreciate the thoughtful ideas of Sandra Jacobson, Ph.D., Department of Psychology, Wayne State University, about the general issues of environmental factors in research on risk factors and infant development.

REFERENCES


DISCUSSION

Dr. Hamburger: Would you go so far as to state that the anemia would make no difference if you really could remove all of the environmental defects?

Dr. Lozoff: I have not reached that conclusion. I would like to make several points about the absence of developmental test score differences in the preventive trial I described (1):

1. Other developmental measures, such as visual attention and motor milestones, have yet to be analyzed;
2. Previous studies in Guatemala and Costa Rica did not find Bayley test score differences at 12 months (the endpoint in the preventive trial) but showed lower scores in older anemic infants;
3. The entrance criteria were different in the preventive trial in Chile: the birth weight cutoff was 3 kg rather than 2.5 kg as in earlier studies, and babies with iron deficiency anemia at 6 months were excluded. Thus, it is possible that the results of previous studies were caused by the inclusion of children whose iron deficiency started earlier and lasted longer. Also, in Chile, all babies were initially breast-fed, whereas cow milk feeding was an important factor contributing to iron deficiency anemia in other samples;
4. Finally, and most importantly, another part of the study in Chile clearly indicates central nervous system effects of early iron deficiency anemia (2). We screened the children at 6 months for anemia and allowed only those with normal hemoglobin levels to enter the trial. The small proportion of children who met criteria for iron deficiency anemia at 6 months and a comparison group who had normal hemoglobin levels went into the neurophysiology part of the study. We found that infants with iron deficiency anemia showed altered nerve conduction in auditory brainstem responses, reduced vagal tone, immature cardiorespiratory control, and so on. So for the very first time in all my years of research, I think we have gotten close to effects on the brain, and the results really do fit with what is coming from the animal model.

Dr. Hamburger: Could it be that the early group were not really severely iron deficient? I noticed you used a 10 g/dl cutoff for hemoglobin. We begin to see problems when they get down around 6 to 8 g/dl, so I wonder if, perhaps, the iron deficiency wasn’t severe enough to bring out these differences early on.

Dr. Lozoff: These were community studies in which we decided in advance that any child with a hemoglobin below 6 g/dl would be treated immediately; we wouldn’t allow even a week’s wait. But we never saw a child with a hemoglobin that low. In fact, the lowest hemoglobin in all of the hundreds of children that we looked at was 7.8 g/dl. However, the average hemoglobin level of anemic infants in the Chile study was very similar to that in our previous studies in Guatemala and Costa Rica, where we did see test score differences.

Dr. Haschke: Is it possible that you found no difference because you eliminated all the severely anemic children from the study?

Dr. Lozoff: In the big NIH study that we are talking about, out of several thousand we screened, only about 2% had iron deficiency anemia at 6 months. Physiologically, it should be uncommon, and it was in fact uncommon. But the problem was still there, and there is still the question whether early iron deficiency could have influenced some of the results in other studies. Chronicity and severity are factors that we haven’t had adequate control over in the past. In the preventive trial that I have described here, we know the maximum duration. It can’t ever have been more than 6 months because we had hematologic screening at 6-month intervals.
Unidentified participant: The question from the audience is whether the effects are caused by anemia or iron deficiency. Would it be possible to design a matched-pair study comparing thalassemia minor patients with iron-deficient patients based on age, body weight, and hemoglobin? Has anybody done this or has such an approach been considered?

Dr. Lozoff: People have tried to look at this. There are studies looking at children with sickle cell anemia or sickle-C disease (summarized in ref. 3), but they are not particularly good, partly because those who are anemic to the degree we are talking about often have some other problems. The sicklers do have poorer school performance, but no particular cognitive deficits have been identified. I have not seen a study of thalassemia.

Dr. Lucas: We have just started the analysis of a randomized preventive trial on about 450 subjects, which we started at 9 months of age. These were all subjects whose mothers had decided to put them on cow’s milk, and we randomly assigned them to stay on cow’s milk and to be put on a formula with iron or a formula without iron until 18 months. Iron screening is not routine in Britain, so we were allowed to do this without prior screening because that would have been normal practice. So all the subjects were included regardless of their initial iron status, and presumably there would be quite a few anemic children at 9 months. And again, like Dr. Lozoff, we found absolutely no difference in cognitive scores or in growth between the randomized groups at 18 months. We are at a preliminary stage of analysis, and we haven’t got subgroup analyses and so forth, but what is interesting here is that now, we have two randomized studies with 1600 or 1700 children that have gone right in the face of a large amount of existing philosophy on the importance of iron in infant nutrition.

Dr. Hamburger: What level of iron deficiency are you talking about?

Dr. Lucas: We are in the middle of documenting that, but we know from the big national survey that we have done that the incidence of iron deficiency anemia in Britain is about 10% to 15%, and the incidence of low ferritin is around 30% to 40%. So we assume that we are going to be in that range.

Dr. Lozoff: I only learned of this study by coming to this meeting, so this has been very exciting. I thought our study in Chile (1) had produced results that were so at variance with perceived wisdom. The fact that Dr. Lucas has got very similar results in an industrialized society and has also extended the developmental range to 18 months makes the results all the stronger.

Dr. Lucas: Dr. Aggett’s department has been looking at the iron status of these children. The preliminary results, and these are very preliminary, suggest—as you’ve shown—that iron supplementation does make a difference to iron status, so there is an effect on iron status, but it has no corresponding effect on growth and neurodevelopment. This is the power of randomized studies as opposed to epidemiologic ones in confounded research.

Dr. Lozoff: What was the birth weight cutoff in your study?

Dr. Lucas: They were normal term infants.

Dr. Rey: I was always very surprised by the notion of checking mental or motor development after only 8 days of iron supplementation. In your conceptual model, you spoke about the turnover of iron in rat brain being very slow, so if iron deficiency were to have an effect on mental performance, we would expect a long time interval before we saw anything. And in fact, you say now that you observed absolutely no difference. But I recall you published a paper in the New England Journal of Medicine a few years ago in which you said that there were some differences between the two groups (4). I am surprised at this. Can you comment?

Dr. Lozoff: First about the short-term studies. Oski argued that if we wanted to detect effects on the brain, we should be looking before there was a change in hemoglobin, at a
time when you would be seeing an effect on CNS enzymes. That was the rationale. In addition, he had done two studies showing very rapid changes. Having myself been involved with two studies trying to replicate those results, both of which were negative, I am no longer concerned with that question. Instead, we have focused on the clinical question of what happens if you treat iron deficiency anemia with a full course of iron. With regard to brain iron, it isn’t only a question of turnover, it’s a question of timing. Barbara Felt looked at this issue in the rat model as part of a behavioral pediatrics fellowship. Her work was published last year in the Journal of Nutrition (5). She pointed out that earlier studies showing lower brain iron in iron deficiency anemia in the rat had involved postnatal iron deficiency. Her question was, suppose that the period of iron deficiency and its treatment were earlier in development—might there be a time when brain iron deficit could be reversed? She studied four groups—early gestation, late gestation, early lactation, and late lactation—and there was a deficit in brain iron in all groups, even in the early gestation group, where iron deficiency had been corrected during gestation. We are talking about a 27% to 33% deficit in brain iron here, and the evidence we have so far is that it cannot be corrected by treatment.

With regard to your question about the New England Journal of Medicine paper, we are talking about two different studies. The New England Journal of Medicine paper was a follow-up of the Costa Rican children, comparing formerly anemic children with the rest of the sample. That was a study where there were several environmental differences, which we controlled statistically. The conclusion to that paper, which I still hold by, was that iron deficiency anemia identifies children at risk for long lasting developmental disadvantage. But did iron deficiency anemia cause the developmental differences? That particular study can never say that the developmental differences were caused solely by iron deficiency anemia. The new study was a preventive trial in which environmental factors were randomly allocated. That could tell us about causality, if differences are found.

Dr. Uary: In your study, did you identify other home factors or social factors that affected development, and was there interaction with iron status in any way, despite the fact that it was randomized to iron treatment?

Dr. Lozoff: Where we have done the background analyses to the point of satisfaction is in the Costa Rican study. For the Chilean randomized trial, we are waiting the analyses. For the Costa Rica study, yes, there was a substantial correlation between the HOME score and later development, of the order of 0.3 or 0.4, which is what the home environment typically does, and of course, we have a relation between lower HOME scores and iron deficiency anemia. So this is an interconnected set. I expect to see such relationships in Chile as well.

Dr. Perman: Did you ever look directly at the route of administration? I realize that the iron must be getting absorbed because you were correcting the iron deficiency, but did you study this?

Dr. Lozoff: In this same Costa Rica study, the iron-deficient children with low hemoglobin levels were randomly allocated to placebo, oral iron, or intramuscular iron in the short term. There was no difference between oral and intramuscular iron. After 1 week, the children on placebo were also treated with oral iron. For the 3-month follow-up, we had children who were just on intramuscular iron and children who were just on oral iron. The only difference was that the iron status of children on oral iron was better than that in those on intramuscular iron. We gave the IM group enough iron to bring the hemoglobin level up to 12.5, while the oral iron group ended the study with hemoglobin levels higher than that.

Dr. Uary: Very briefly, what is the policy implication of your finding?

Dr. Lozoff: It is hard to argue that having iron deficiency anemia is a good thing for children. On those grounds, I continue to be very comfortable with the idea that prevention
is the safest course. But if you are tackling the question of scarce resources and public health priorities, then I would have to say, at present, that I do not think we have the scientific evidence to inform public policy.

**Dr. Hamburger:** I don’t think there is anything radical about the notion that a bad environment contributes to a bad outcome, whether or not you have confounding variables. I think we are looking at this through the narrow vision of people who have to do clinical studies and therefore see poverty, ignorance, filth, and so on. I really don’t think that we should be all that surprised that they feed into each other and help to produce that kind of result.

**Dr. Lozoff:** Nevertheless, around the world, Health Departments are making decisions about iron fortification and iron supplementation. Those decisions are going to affect resources that might go toward relieving some conditions underlying poverty and disadvantage. So, there are real public policy decisions that depend on these trials.

**Dr. Sorensen:** You mentioned that all these children were healthy, but at 6 to 12 months old, children are rarely entirely healthy, especially in Santiago! They are likely to have had a lot of respiratory infections at the very least, and I wonder if you factored those things in. Such factors are likely to set some children back in their development.

**Dr. Lozoff:** In both studies, the Costa Rican and the Chilean, we did home monitoring of infections in a prospective fashion. In Costa Rica, we had daily records, and in Chile, we had weekly records. I haven’t yet looked at the Chilean data. However, the mean developmental scores of the Chilean children were absolutely average for the standard sample used in the Bayley test. So we do not have any evidence, either for Costa Rica or Chile, that the children were developmentally behind U.S. children.

**Dr. Whitehead:** This will be the chapter I remember because I think it contains some really salutary results. I would also like to congratulate the organizers of the meeting for deciding to put this paper in at this point. I think there would have been a danger of us going away a bit self-satisfied. We have got to accept that there are a large number of confounding variables, things we don’t know anything about, things that perhaps science doesn’t yet know anything about, which we clearly can’t standardize for. I think that is the main message that you are putting across. I would like to add one more thing that is going to be increasingly important in community studies, and that is gene polymorphism. Once we can really get to terms with that subject, I think it is going to revolutionize our approach, both at national and international level.

**Dr. Lucas:** I want to explore this message further. What we have discovered today is that environmental factors seriously confound epidemiologic studies in nutrition. We have discussed the number of them—we have discussed the way they confound iron studies, general malnutrition studies, the comparison of breast- and bottle-fed babies, Barker’s data, and so forth. The question is whether you feel that your randomized trial, which should equalize out all these factors, actually does take care of the environmental confounders and factors them out. Do you actually feel that the environmental entanglements you showed in your complex diagram have been taken out of the equation to a large extent by randomization, or do you think they are still there?

**Dr. Lozoff:** I wanted to make the point that all of the studies I showed you were randomized clinical trials. The last one was a preventive trial. In the other ones, they were treatment trials. Had we shown a treatment effect, we could have argued that environmental factors had been taken care of, because children were randomly allocated to treatment or placebo. But there are lots of treatment studies in which, even though they begin out as randomized controlled trials, you don’t see a treatment effect, and then, all the environmental issues come up again. In the preventive trial, we certainly saw an effect of iron supplementation on hematologic
status, but we didn’t see any effect on development. We then went into the data again and looked at just the anemic children compared to the nonanemic children in the no-iron group—that would have been the equivalent of the eight earlier studies that made such comparisons. Children with iron deficiency anemia from the no-iron arm of the preventive trial did not have lower developmental test scores than the nonanemic children. So there is something different about this study. I am not satisfied that we can simply say that environmental factors are irrelevant; within this trial, the environmental factors were randomly allocated, but that doesn’t mean environmental influences are unimportant.

REFERENCES