Relationships among Blood Lead Levels, Iron Deficiency, and Cognitive Development in Two-Year-Old Children

Holly A. Ruff,[1] Morri E. Markowitz,[1,2] Polly E. Bijur,[1] and John F. Rosen[1,2]

[1]Department of Pediatrics, Albert Einstein College of Medicine, Bronx, NY 10461 USA; [2]Montefiore Medical Center, Bronx, NY 10467 USA

The goals of this study were to explore the relationship of declining blood lead levels and cognitive development in 42 moderately lead-poisoned children around 2 years of age and to investigate the potential interaction between iron and lead levels in the course of development. The cognitive functioning of children was assessed upon enrollment into a comprehensive intervention and 6 months later. The intervention consisted of chelation treatment, if appropriate, iron supplementation, if needed, and steps to eliminate the source of lead in the home environment. The children were referred because of blood lead levels between 25 and 55 µg/dl; they were also selected on the basis of age between 18 and 30 months. The outcome measures were the global score on a standardized test of cognitive development and subscale scores for perceptual-motor and language functioning. Cognitive change over 6 months was related to an interaction between change in blood lead and initial iron status. Specifically, the change in standardized score (particularly change in perceptual-motor performance) was strongly related to change in blood lead in children who were iron sufficient at the outset: there was an increase of 1.2 points for every 1 µg/dl decrease in blood lead. There was no such relationship in iron-deficient children. Secondary analyses suggested that 1) the change in cognitive functioning of iron-deficient children was related to change in hemoglobin, and 2) the decline in blood lead was less in iron-deficient than in iron-sufficient children. Thus, when iron is sufficient, changes in blood lead and changes in cognition are inversely related. When iron is deficient, other processes affect the outcome. *Key words*: blood lead, cognitive development, intervention, iron deficiency, lead poisoning, perceptual-motor functioning. *Environ Health Perspect* 104:180–185 (1996)

Several recent prospective studies relating blood lead to behavior in children (*1–9*) have implicated lead as having a negative influence on young children's cognitive development. Although in some of these studies early relationships with prenatal exposure disappear with time, several studies suggest that postnatal exposure is related to later outcomes (*1–5,8,9*). Bellinger et al. (*2,3*) reported that blood lead level at 2 years of age is negatively related to cognitive outcome at 4.5 and 10 years. Australian investigators (*1,5*) reported that integrated blood lead levels are negatively related to performance at 4 and 7 years of age, and blood lead levels at 2 and 3 years contributed most to the integrated blood lead concentration. Wasserman and colleagues (*9*) found that blood lead levels after 18 months of age were more strongly related to cognitive development at 4 years of age than blood lead levels before 18 months. In all of these studies, the strongest relationships at 4 years were between blood lead and perceptual-motor performance on the McCarthy scales. Although the results of the Cincinnati study (*10*) have often been nonsignificant when appropriate covariates were controlled for, Dietrich et al. (*4*) found that decrements in performance on several subscales on a test of fine motor proficiency at 6 years were associated with both current and earlier blood lead levels. These results suggest that blood lead levels around 2 or 3 years of age contribute to cognitive development, particularly perceptual-motor skills. The general timing of this contribution coincides with the natural course of blood lead in children for whom there is no systematic intervention; blood lead levels peak between 18 and 30 months and decline slowly but steadily thereafter (*1,4*).

The age of 2 years may be especially important in neurobiological development as synaptic connections within the brain reach a peak and are then selectively reduced (*11,12*). Goldstein (*11*) suggests that lead may augment the background level of neurotransmitter activity and thereby diminish the efficiency with which these connections are pruned. In this way, the cognitive development of children around 2 years of age may be particularly vulnerable to the effects of lead. Another factor is that children around this age are more likely than older children to be iron deficient. In our sample (*13*), for example, 52% of children under 3 years had ferritin concentrations of less than 16 ng/ml. Wasserman et al. (*8*) found that both lead and iron contributed to cognitive performance in a group of 2-year-old children. Because iron levels may influence children's neurological and cognitive development (*14*) and also affect absorption and excretion of lead (*15,16*), they may interact with lead in influencing development and the outcomes of intervention.

We recently reported (*13*) an inverse relationship between declining blood lead levels and improvements in cognitive performance in 1- to 7-year-old children who participated in a comprehensive intervention program to reduce blood lead levels. The average trend in this group was significant, but there was a great deal of variation. The variability stemmed in part from the necessary use of different tests at different ages and from variations in iron levels and treatment patterns. We thus decided to examine in more detail the data of a subgroup of these children who were close to 2 years old at enrollment and who were administered only the Bayley Scale of Mental Development during the study. The use of a single test and the narrowness of the age range meant that many test items were common across the group and permitted an examination of particular types of items.

In summary, the two questions motivating the analyses were related specifically to the age of the children. First, if 2 year olds are particularly vulnerable to the effects of lead, will they also be particularly responsive to intervention to reduce blood lead levels? Second, given the prevalence of iron deficiency at this age, are iron-deficient children less responsive than iron-sufficient children to such intervention?

Methods

The goal of the analyses reported here was to explore the relationships between cognitive performance and both iron status and changing lead levels in a sample of children between 18 and 30 months of age. The chil-

Address correspondence to H. A. Ruff, Room 222, Kennedy Center, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, NY 10461 USA.

This research was supported by grants ES04039 from the National Institute of Environmental Health Sciences; 86109286 from the W. T. Grant Foundation; and a Research Scientist Development Award, MH00652, from the National Institute of Mental Health. The research was also facilitated by grant HD07199 from the National Institute of Child Health and Human Development to the Rose F. Kennedy Center for Research in Mental Retardation and Human Development at Albert Einstein College of Medicine.

Received 17 July 1995; accepted 27 October 1995.

dren were evaluated at three time points in the course of a 6-month intervention period. The intervention included abatement procedures in the home to reduce exposure to lead, chelation treatment if indicated by the results of the lead mobilization test (LMT), and iron supplementation for those children who were iron deficient as determined by serum ferritin levels (*13*).

The study was approved by the local Institutional Review Boards, and consent was obtained from the parents or guardians of all participating children. Children participated in the study if they had not been treated previously for lead poisoning, their blood lead (BPb) levels at the time of enrollment were between 25 and 54 µg/dl, and their levels of erythrocyte protoporphyrin (EP) were >34 µg/dl. The children's clinical management throughout the study followed standard procedures. If the child's LMT was positive, the child was hospitalized for treatment with $CaNa_2EDTA$. If the child was iron deficient (ferritin <16 ng/ml), oral iron was prescribed (6 mg/kg/day). Depending on the diagnostic outcomes, the enrolled children could be given both forms of treatment, neither form, or either one of them alone. For all children, there were sequential inspections of the home and attempts to eliminate exposure to lead paint through existing housing codes. That these efforts were at least partially successful is demonstrated by a significant decline in exposure as measured during home visits (unpublished observations).

The children's cognitive development and behavior were evaluated at three time points, but we report here the data from the first and last visits: 1) time 1: approximately 1 week after enrollment and before the treatment course was determined, and 2) time 3: approximately 6 months after the first visit. The dependent variable was the child's performance on the Bayley Scale of Mental Development (*17*). Assessors were blind to the treatment status of children and to their BPbs.

Sample and Procedures

We selected 42 children (26 males, 16 females) from the 154 children who constituted the sample in our recent report (*13*). The selection criteria were as follows: 1) age at enrollment of at least 18 months but no more than 30 months; 2) complete data for BPb, EP, and cognitive functioning at both time points and for all background factors except mother's IQ; and 3) use of the Bayley Scale at both visits. The subsample was 69% Hispanic and 31% black.

Early in the week after enrollment, each child was evaluated with the Bayley Scales of Mental Development. Within 3 days of this evaluation, each child underwent an LMT; the LMT consists of the intramuscular administration of a single dose of $CaNa_2EDTA$ followed by an 8-hr urine collection for determination of lead. A new blood sample was obtained on the same day and analyzed for BPb, EP, and ferritin. Based on the results of the LMT, the child was either hospitalized for a 5-day course of $CaNa_2EDTA$ treatment (1 g/m^2/day) or not. Oral iron (6 mg/kg/day) was prescribed if necessary, and a housing inspection was conducted. Except for the LMT, which depended on current BPb, this entire process was repeated approximately 6 months later whether or not the child had been hospitalized for EDTA chelation treatment. The cognitive and biochemical measures are therefore closely linked in time.

Measures

The dependent measure of cognitive functioning was the mental development index (MDI) from the Bayley Scales of Infant Development (*17*). The MDI is a standardized score with a mean of 100 and a standard deviation of 16. In addition to the global score, we analyzed the cognitive and language subscales according to the Kent scoring system. These scores are age equivalents in months. Despite the label, the cognitive subscale consists almost exclusively of perceptual-motor items such as performance on pegboards and formboards, imitating crayon strokes, and stacking blocks. The language subscale includes both comprehension and production of single words, simple combinations, and comprehension of simple commands.

There were several biochemical measures used in the analyses as independent or moderator variables: BPb, EP, ferritin, and several hematologic indices. BPb (95% confidence limit for the reliability of duplicate samples, ± 1.0 µg/dl) was measured by graphite furnace atomic absorption spectroscopy. The EP level was determined by the extraction method of Piomelli et al. (*18*). Our laboratory participates successfully in the proficiency testing programs of New York City, New York State, and The Centers for Disease Control.

Ferritin was measured by radioimmunoassay. The intra-assay and interassay coefficients of variation were 3% and 4%, respectively. Ferritin levels were missing at the last time point for five children, for whom data were otherwise complete. These missing data points were replaced by regressing ferritin on age in the entire sample (*13*) and using the resulting values to estimate missing ferritin values; this process resulted in replacement values of ferritin >20, as mean replacement would have. Complete blood counts were also conducted by the hospital laboratory, which provided information about hematologic variables. To form groups for use as a categorical variable, the children were separated into those with low iron and those with adequate iron. There were 20 (48%) children whose ferritin was <16 ng/ml at time 1; 14 of them had average ferritin levels over the course of the study of <16 ng/ml. This cut-off, which determined whether oral iron was prescribed, was based on a large population-based study of healthy, nonanemic children (*19*) and has been used successfully by us and others to indicate the need for intervention (*13,20*). Of the 22 children whose ferritin was >16 ng/ml at time 1, 1 child with a ferritin level of 16 had a hemoglobin (Hb) level of 10.5 g/dl, indicative of mild anemia, and was moved to the iron-deficient group. Ten children in the iron-deficient group fit the criteria for mild to moderate anemia [Hb ≤11 g/dl (*21*)]; 11 were judged to be deficient or depleted (ferritin <16 ng/ml, Hb >11 g/dl).

The distributions of all variables were checked for this sample, and any outlying values, as determined by the statistical program, were pulled in to 1 point above (or below) the next largest (or smallest) value, a process known as winsorizing (*22*). One or two values changed on three time 1 variables (BPb, mean corpuscular volume, and urinary Pb), four change variables (MDI, perceptual-motor score, ferritin, Hb), and one background variable (socioeconomic status; SES); these represent 27% of distributions tested. These changes increased the precision, but not the direction or general magnitude, of the results.

A number of background variables were considered; these included SES, score on the Caldwell HOME scale as a percentage of items scored positively (*23*), maternal IQ (replaced with the mean in five cases of missing data), child's birth order, number of people in the household, number of prenatal and perinatal complications, sex, language of test administration (Spanish/English), race, and age. We controlled those variables that were associated with both dependent and independent variables with a probability of <0.15 (*24*). We also included background variables related to the dependent variable if their inclusion increased the precision of the particular model being tested (*25*). SES, Caldwell HOME score, and language of test administration were used in analyses of change in MDI; covariates added to other models will be noted as relevant.

Analyses

For the main analyses, we examined regressed change in MDI or subtest scores

in relation to regressed change in BPb or other biochemical or hematologic variables during the study period (*26*). For example, in assessing the relationship between change in MDI and change in BPb, we controlled for initial levels of BPb and MDI. Potential interactions between change in BPb and the covariates and background variables were tested; none was significant. Because of our interest in iron status as a potential modifier or confounder of the relationship between change in BPb and change in MDI, iron status at time 1 was entered into the regression equation as a categorical variable. Finally, the term representing the interaction between iron status and change in BPb was entered to determine whether the interaction accounted for a significant increase in R^2. This same procedure was followed for most of the analyses involving the entire group of children. All significant relationships were tested for the effects of individuals with undue influence. Analyses were conducted with the SYSTAT computer program.

Results

Table 1 presents descriptive data for iron-deficient and iron-sufficient subgroups at time 1. The groups differed mainly on the iron-related measures, as expected. The iron-deficient children were, by definition, lower in ferritin [t (40) = 8.2, $p < 0.001$]; they were also significantly lower in mean ferritin over 6 months [t (40 df) = 6.1, p <0.001], Hb [t (40 df) = 2.8, p = 0.008], MCV [t (40 df) = 3.1, p = 0.003], and mean corpuscular hemoglobin [MCH; t (40 df) = 3.0, p = 0.005]. There was a marginally larger proportion of girls in the iron-deficient group [t (40 df) = 1.9, p = 0.06]. Although iron-deficient children were less likely to receive chelation treatment, only 33% (7/21) of the iron-sufficient children underwent chelation therapy during the course of the 6-month study. The two groups did not differ on any other variables, including their initial cognitive scores and their initial BPb levels.

Relationships between Cognitive and Biochemical Measures

The analyses did not reveal any relationships at time 1 between the Bayley MDI or separate subscale scores and BPb, EP, or ferritin levels in the whole group or within either the iron-deficient or iron-sufficient groups. In the iron-deficient group, MDI was positively related to Hb level (r = 0.51, p = 0.02) and hematocrit (Hct; r = 0.56, p = 0.02). Neither of these was significant for the iron-sufficient children. With age controlled, the perceptual-motor and language subscales for the iron-deficient children were related to Hb (r_s = 0.48 and 0.55) and Hct (r_s = 0.51 and 0.54) at time 1, and the language subscale was also related to MCV (r = 0.51).

Relationship between Cognitive Change and BPb Change

In the analysis of the relationship between changes in MDI over 6 months and the concurrent changes in BPb, the regression coefficient (β) for BPb change was -0.344, not too different from the -0.310 reported for the age range 1–7 years (13). The result was not significant, and iron status alone

Table 1. Comparison of iron-deficient and iron-sufficient children at time 1

Variable	Mean (SD) Iron deficient (n = 21)	Iron sufficient (n = 21)
Iron-related		
Ferritin	10.0 (3.2)	26.5 (8.7)
Mean ferritin[a]	14.3 (5.3)	23.4 (4.4)
Hemoglobin	11.2 (1.0)	12.0 (0.8)
Hematocrit	35.2 (2.9)	36.8 (2.8)
MCV	71.0 (6.6)	76.4 (4.5)
MCH	22.6 (3.0)	24.9 (2.0)
Background		
Age	23.8 (3.5)	23.6 (3.6)
SES	19.2 (6.9)	16.6 (6.2)
Mother's IQ	94.9 (9.3)	91.3 (10.1)
HOME[b]	0.66 (.13)	0.62 (.11)
Birth order	2.4 (1.5)	2.8 (1.8)
No. in household	4.8 (1.7)	4.8 (1.3)
No. of complications[c]	2.0 (1.6)	2.2 (1.9)
% Female	52	24
% Hispanic	67	71
% Tests in Spanish	52	52
Lead and cognitive		
MDI	78.8 (10.9)	77.1 (12.3)
P-M score[d]	20.5 (3.4)	19.8 (2.1)
Language score[d]	19.3 (3.0)	18.2 (3.5)
BPb	28.3 (6.0)	30.4 (6.1)
EP	76.2 (36.5)	85.2 (35.8)
% Receiving chelation	11	33

Abbreviations: MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; SES, socioeconomic status; EP, erythrocyte protoporphyrin; MDI, mental development index from Bayley Scale; P-M, perceptual-motor subscale; BPb, blood lead level.
[a]Mean ferritin level over 6 months.
[b]HOME = Caldwell score.
[c]Number of prenatal and perinatal complications.
[d]Age equivalent in months.

Table 2. Hierarchical regression models and increases in R^2 for variables used to predict change in mental development index (MDI)[a]

	R	Increase in R^2	p-value
MDI change = Constant + MDI at Time 1 (MDI_1)	0.31	0.095	0.05
MDI change = Constant + MDI_1 + Background Variables[b]	0.57	0.22	0.02
MDI change = Constant + MDI_1 + Background + Blood Lead at Time 1 (BPb_1) + BPb change	0.61	0.05	NS
MDI change = Constant + MDI_1 + Background + BPb_1 + BPb Change + Iron Status (Fe)	0.62	0.01	NS
MDI change = Constant + MDI_1 + Background + BPb_1 + BPb Change + Fe + Fe•BPb_1	0.63	0.01	NS
MDI change = Constant + MDI_1 + Background + BPb_1 + BPb Change + Fe + Fe•BPb_1 + Fe•BPb Change	0.72	0.12	0.007

[a]Each step in this table represents the addition of the underlined variable or set of variables to show the consequent increase in the amount of variance accounted for in change in MDI over 6 months.
[b]The set of background variables was socioeconomic status, score on HOME, and language of test administration.

Table 3. Unadjusted and adjusted means for BPb and MDI at times 1 and 3 for subgroups based on degree of change in BPb

BPb change groups	BPb_1 (μg/dl)	Change in BPb (μg/dl) Unadjusted	Adjusted[a]	MDI_1	Change in MDI (standardized points) Unadjusted	Adjusted[b]	$P\text{-}M_1$	Change in P-M score (Age equivalent, months) Unadjusted	Adjusted[c]
Large declines (n = 6)	34.2	-14.5	-13.4	80.0	+ 5.3	+ 7.9	19.8	+ 5.6	+ 6.1
Medium declines (n = 8)	30.1	-9.6	-9.7	73.1	+0.8	+1.4	19.6	+ 4.6	+4.5
Small declines/increases (n = 7)	27.4	-2.1	-3.0	79.1	- 4.3	- 7.2	20.1	+ 3.5	+ 3.1

Abbreviations: BPb, blood lead; MDI, mental development index of Bayley Scale; P-M, perceptual-motor subscale.
[a]Adjusted for BPb at time 1.
[b]Adjusted for BPb and MDI at time 1.
[c]Adjusted for BPb and P-M score at time 1.

did not increase the R^2, nor did the interaction between iron status and initial BPb level. However, when the interaction between iron status and change in BPb was entered into the equation, the R^2 increased by 12% (p = 0.007; Table 2), suggesting that the relationship between changes in MDI and BPb was different for the two iron groups.

To test this interaction further, we analyzed only those children who remained iron deficient throughout the 6 months (n = 10) and those who remained sufficient throughout (n = 17). Though the numbers are smaller, the results were of the same magnitude and still significant (increase in R^2 = 15.2%, p = 0.03). We also conducted the same analyses using raw scores from the Bayley rather than the standardized scores. Some MDI scores had to be extrapolated from the norms (*13*) for children who became too old for the norms at some point during the 6 months. Using raw scores bypasses any potential problems with the extrapolation; even so, the analysis of the raw scores reveals the same significant interaction (increase in R^2 = 7.3, p = 0.03). We are confident, therefore, about the interaction between change in BPb and iron status.

The relationship between changes in MDI and changes in BPb was then assessed separately for the two groups. The iron-deficient group showed no relationship between cognitive and BPb changes (β = 0.038, p = 0.91). The iron-sufficient group, in contrast, showed a large and significant relationship (β = -1.22, p = 0.009). The change in BPb contributed 33% to the R^2 and thus explained considerable variance in MDI change beyond that accounted for by initial levels of both MDI and BPb and the background covariates of SES, HOME score, and language of test administration. The regression coefficient can be interpreted as showing an increase of 1.2 points in MDI for every decrease of 1 µg/dl of BPb. There were no bivariate outliers with undue influence.

For descriptive purposes, the 21 iron-sufficient children were categorized into three subgroups according to changes in BPb: large decreases in BPb (-22 to -11 µg/dl), moderate decreases in BPb (-11 to -6 µg/dl), and modest decreases or actual increases (-6 to +2 µg/dl). Table 3 presents the unadjusted and adjusted means for MDI change and BPb change for these three groups along with the mean initial levels. As can be seen in the table, the changes in MDI ranged from large increases to large declines over time; thus, conceptually, both smaller decrements as well as increases could be viewed as improvement. In other words, declining BPb seemed to be related to both a halting of decline in MDI and to facilitation of development over the 6 months.

Age equivalents on the perceptual-motor and language subscales of the Bayley as scored by the Kent system (unpublished manuscript) were examined next. There was an interaction between change in BPb and iron status observed with the perceptual-motor score (β = -0.22, p = 0.055). When the two iron groups were analyzed separately, there was, again, no relationship for the iron-deficient children (β = 0.01, p = 0.93), but a sizeable and significant relationship in the iron-sufficient children (β = -0.27, p = 0.007). For every decrease of 1 µg/dl in BPb, iron-sufficient children gained about 0.25 months on the perceptual-motor subscale. Table 3 shows adjusted and unadjusted means for the BPb-change subgroups. Note that a score in age equivalents almost always increases because children usually pass at least as many items on a second testing; the MDI, in contrast, may decrease because a child passes fewer items than expected for an average rate of development. There was no relationship between BPb changes and changes on the language subscale in either the iron-sufficient or iron-deficient groups. Thus, relationships between changes in cognitive functioning and changes in BPb are seen only in the iron-sufficient children, and then only in the perceptual-motor subscale.

Changes in the Iron-Deficient Group

The absence of a systematic relationship between BPb changes and changes in MDI in the iron-deficient group may result from other factors that obscure the relationship. These other factors may affect changes in MDI over time, or they may influence changes in BPb over time. To explore these possibilities, changes in the MDIs of the iron-deficient group were tested against changes in the iron and hematologic indices. There was no relationship with changes in ferritin, but changes in Hb and MCV were both significantly related to changes in MDI (β = 5.07 and 1.29, p < 0.05). After entering the initial levels of MDI and Hb, SES, HOME score, and language of test administration as covariates and potential confounders, the addition of change in Hb accounted for an increase of 11% in the R^2 (p = 0.08). The change in MCV also accounted for 10% of the variance (p = 0.08). These two indices are related to one another (r = 0.57, p = 0.007) at time 1, and here we pursue Hb only. One child had undue influence in the opposite direction; when that child's data are omitted, the relationship between MDI change and Hb change is even stronger, with change in Hb accounting for 19% increase in the R^2 over the initial values and the background variables (p = 0.02). When the data for all sample children with Hb values at time 3 were entered into an analysis, the interaction between change in Hb and iron status was significant (p = 0.05); that is, only for iron-deficient children was the relationship significant.

The iron-deficient children were then divided for descriptive purposes into three equal groups according to degree of change in Hb: substantial increases in Hb (+0.8 to 2.3 g/dl); modest increases (+0.2 to +0.7 g/dl); and very small increases or decreases (+0.1 to -1.0 g/dl). Adjusted means for both change in MDI and change in Hb were calculated with control for the initial levels as well as the background variables and are presented in Table 4. The largest increases in hemoglobin were related to increases in MDI, but moderate to small increases or decreases in Hb were associated with declines in MDI. Unadjusted scores showed the same pattern. The distribution of children with anemia at time 1 was 5, 3, and 2 for the three groups, respectively.

When we examined change in BPb in the two groups, the iron-deficient group had about half the reduction in BPb of the iron-sufficient group (-4.8 µg/dl vs. -8.5 µg/dl, $F(1,40)$ = 4.3, p <0.05). The results were virtually the same with initial BPb level and sex controlled (-4.9 vs. -8.4 µg/dl, p = 0.07). Although the iron-deficient and iron-sufficient children were not significantly different in their BPbs at time 1, more iron-deficient children were below

Table 4. Unadjusted and adjusted means for changes in Hb and MDI over 6 months for subgroups based on degree of change in Hb

		Change in Hb (g/dl)			Change in MDI (standardized points)	
Hb change groups	Hb_1	Unadjusted	Adjusted[a]	MDI_1	Unadjusted	Adjusted[b]
Large increases (n = 7)	10.7	+1.47	+1.44	76.3	+2.40	+2.90
Medium increases (n = 7)	11.4	+0.40	+0.43	77.7	-2.40	-2.14
Small increases/declines (n = 7)	11.6	-0.36	-0.35	82.3	-6.00	-6.19

Abbreviations: Hb, hemoglobin; MDI, mental development index of Bayley Scale.
[a]Adjusted for Hb at time 1 (Hb_1).
[b]Adjusted for Hb and MDI at time 1 (MDI_1).

the median of 29 μg/dl (13 vs. 7), raising the possibility that the iron-deficient children may have had somewhat less room for change in BPb. However, when we compared only children who were above the median on BPb at time 1, the iron-deficient children still had significantly lower reductions in BPb [F (1,18) = 7.2, p = 0.015]. These results suggest that iron-deficient children with moderately elevated BPb may have more difficulty in correcting elevated blood leads than children who are sufficient in iron.

Discussion and Conclusions

There were several interesting results of this study. The first was the striking relationship between changes in BPb and changes in cognitive performance over a 6-month period for iron-sufficient children who participated in a comprehensive intervention program. Because of the observational nature of the study, it is not possible to make definitive causal statements; however, degree of improvement in cognitive performance was related to the degree of change in BPb in a linear fashion, suggesting that cognitive development may be directly related to declining BPb levels. Given the comprehensive nature of the intervention, we cannot attribute the decline in BPb to any particular component.

Follow-up analyses indicated that the relationship was strongest between change in BPb and change in perceptual-motor performance, a finding that is consistent with the results of some of the recent prospective studies (*2,4,5,9*). One potential problem in interpretation stems from the lack of a concurrent relationship between initial BPb level and performance on the cognitive test at time 1. To some extent, this may reflect a restriction in range of the initial values; the range in initial BPb levels was, by design, restricted to the moderately elevated level; the observed standard deviation in MDI scores was less than the normative standard deviation (11.5 vs. 16). The change scores, on the other hand, had considerably higher coefficients of variation than the initial scores (37% vs. 21% in the case of BPb; 48% vs. 15% in the case of MDI).

Another explanation is more theoretical. A delay in the effects of lead on cognition may result in children's performance being more related to earlier levels than concurrent levels of BPb. A survey of the literature from the prospective studies suggests such a possibility. Although there are occasional reports of concurrent relationships between BPb and cognitive functioning (*8,9*), it is more common for cognitive functioning to be related to earlier BPb levels. For example, Bellinger et al. (*27*) found that MDI scores in the first 2 years of life were related to umbilical cord BPb, but not to concurrent blood lead levels. Wigg et al. (*28*) found that MDI scores at 2 years were related to BPb at 6 months but not to concurrent BPb levels. Bellinger et al. (*2,3*) found a relationship of BPb at 2 years with later cognitive functioning but not with concurrent cognitive functioning. In the Australian prospective study, McMichael et al. (*5*) reported relationships between cognitive functioning at 4 years and BPb values at 2 and 3 years, but no correlation with BPb at 4 years, when appropriate covariates were controlled. If such a delay were operating here, then the initial cognitive performance of the children in our sample would have been determined more by their history with exposure to lead than by their current BPb levels. The 6-month course of the study was presumably long enough to detect a relationship between the changes in lead levels and changes in cognition without those changes having to be strictly contemporaneous. Further investigation would be needed to clarify this possibility.

It is not clear how low iron levels interfere with the observed relationship between declining BPb and cognitive functioning. Low levels of iron may slow down the rate at which lead can be excreted and increase the rate at which lead is absorbed (*15,16*), leading to less predictable changes in lead levels. The significantly lower reduction in BPb over the course of 6 months in the iron-deficient group would support such a possibility. Another factor is that iron levels are associated with cognitive performance, either directly or indirectly, thus complicating the relationship between lead and cognition. This possibility is supported by our findings that the performance of the iron-deficient children seems to change along with changes in Hb and MCV. In this group, there was also an association between Hb and Bayley MDI at time 1. In other words, the lower the Hb at time 1, the lower the MDI; over time, the more Hb increased, the more MDI increased. We cannot entirely rule out some third variable that is related to both increases in Hb and improvements in performance. For example, compliance with the therapeutic regimen of oral iron might be related to some background factor that was also related to cognitive development. However, no background variable, including maternal IQ, quality of home environment, or SES, eliminated the significant association between Hb and MDI when entered into the equation.

Another hypothetical possibility is that the anemic children, who showed the largest increases in Hb, as expected, were initially more shy and fearful than children with higher initial Hb and smaller increases. The MDIs of the anemic children may then have increased more from time 1 to time 3 because they were significantly more comfortable with the test situation at time 3 (*29*). We do not have the measures to determine whether, in fact, the anemic children were more fearful initially. However, because MDI change was related to change in Hb in a linear fashion, noncognitive factors like fearfulness would more likely be mediators, rather than confounders, of the relationship between cognitive and hematologic changes.

If the association is not a spurious one, the iron-deficient children's performance might conceivably have been influenced directly by oxygenation of neural tissue or indirectly by increasing energy and thus attention and motivation. The findings were not limited to the anemic children, a fact that is consistent with some previous literature and not others. Walter et al. (*30*) and Oski et al. (*31*) report improvements in the cognitive scores of iron-deficient, nonanemic children after short-term iron therapy, while Lozoff et al. (*32*) found changes only in the anemic children. Only a few investigators, however, have reported changes in hematologic indices as continuous variables (*33,34*), a potentially powerful way to detect associations between biological and behavioral variables.

In summary, there seem to be at least two biochemical changes associated with changes in the children's cognitive performance in this study; one is improvement in hematologic indices in iron-deficient children and the other is decline in BPb in iron-sufficient children. The number of subjects is relatively small, but the results, if replicated, would have important implications for our understanding of the way multiple biological factors interact to influence the behavior and functioning of young children.

References

1. Baghurst PA, McMichael AJ, Wigg NR, Vimpani GV, Robertson EF, Roberts RJ, Tong S. Environmental exposure to lead and children's intelligence at the age of seven years. N Engl J Med 327:1279–1284 (1992).
2. Bellinger D, Sloman J, Leviton A, Rabinowitz M, Needleman HL, Waternaux C. Low-level lead exposure and children's cognitive function in the preschool years. Pediatrics 87:219–227 (1991).
3. Bellinger DC, Stiles KM, Needleman HL. Low-level lead exposure, intelligence and academic achievement: a long-term follow-up study. Pediatrics 90:855–861 (1992).
4. Dietrich KN, Berger OG, Succop PA. Lead

exposure and the motor developmental status of urban six-year-old children in the Cincinnati prospective study. Pediatrics 91:301–307 (1993).
5. McMichael AJ, Baghurst PA, Wigg NR, Vimpani GV, Robertson EF, Roberts RJ. Port pirie cohort study: environmental exposure to lead and children's abilities at the age of four years. N Engl J Med 319:468–475 (1988).
6. Ernhart CB, Morrow-Tlucak M, Marler MR, Wolf AW. Low level lead exposure in the prenatal and early preschool periods: early preschool development. Neurotoxicol Teratol 9:259–270 (1987).
7. McBride WG, Carter CJ, Bratel JR, Cooney G, Bell A. The Sydney study of health effects of lead in urban children. In: Lead exposure and child development: an international assessment (Smith MA, Grant LD, Sors AI, eds). Dordrecht, the Netherlands:Kluwer, 1988; 255–259.
8. Wasserman G, Graziano JH, Factor-Litvak P, Popovac D, Morina N, Musabegovic A, Vrenezi N, Capuni-Paracka S, Lekic V, Preteni-Redjepi E, Hadzialijevic S, Slavkovich V, Kline J, Shrout P, Stein Z. Independent effects of lead exposure and iron deficiency anemia on developmental outcome at age 2 years. J Pediatr 121:695–703 (1992).
9. Wasserman GA, Graziano JH, Factor-Litvak P, Popovac D, Morina N, Musabegovic A, Vrenezi N, Capuni-Paracka S, Lekic V, Preteni-Redjepi E, Hadzialijevic S, Slavkovich V, Kline J, Shrout P, Stein Z. Consequences of lead exposure and iron supplementation on childhood development at age 4 years. Neurotoxicol Teratol 16:233–240 (1994).
10. Dietrich KN, Succop PA, Berger OG, Hammond PB, Bornschein RL. Lead exposure and the cognitive development of urban preschool children: the Cincinnati lead study cohort at age 4 years. Neurotoxicol Teratol 13:203–211 (1991).
11. Goldstein W. Neurologic concepts of lead poisoning in children. Pediatr Ann 21:384–388 (1992).
12. Bellinger D. Lead and neuropsychologic function in children: progress and problems in establishing brain-behavior relationships. Adv Child Neuropsychol 3:12–47 (1995).
13. Ruff HA, Bijur PE, Markowitz M, Ma Y, Rosen JF. Declining blood lead levels and cognitive changes in moderately lead-poisoned children. J Am Med Assoc 269:1641–1646 (1993).
14. Lozoff B. Iron and learning potential in childhood. Bull NY Acad Med 65:1050–1066 (1989).
15. Watson WS, Hume R, Moore MR. Oral absorption of lead and iron. Lancet 2:236–237 (1980).
16. Markowitz ME, Rosen JF, Bijur PE. Effects of iron deficiency on lead excretion in children with moderate lead intoxication. J Pediatr 116: 360–364 (1990).
17. Bayley N. Bayley scales of infant development. New York:The Psychological Corporation, 1969.
18. Piomelli S, Seaman C, Zullow D, Curran A, Davidow B. Threshold for lead damage to heme synthesis in urban children. Proc Natl Acad Sci USA 79:3335–3339 (1982).
19. Yip R, Schwartz S, Deinard AS. Screening for iron deficiency with erythrocyte protoporphyrin test. Pediatrics 72:214–219 (1983).
20. Yip R, Norris TN, Anderson AS. Iron status of children with elevated blood lead concentrations. J Pediatrics 98: 922–925 (1981).
21. Oski FA. Iron deficiency in infancy and childhood. N Engl J Med 329: 190–193 (1993).
22. Winer BJ. Statistical principles in experimental design, 2nd ed. New York:McGraw-Hill, 1971.
23. Bradley RH, Caldwell BM, Rock SL, Ramey CT, Barnard KE, Gray C, Hammond MA, Mitchell S, Gottfried AW, Siegel L, Johnson DL. Home environment and cognitive development in the first 3 years of life: a collaborative study involving six sites and three ethnic groups in North America. Dev Psychol 25:217–235 (1989).
24. Dales LG, Ury HK. An improper use of statistical significance testing in studying covariables. Int J Epidemiol 7:373–375 (1978).
25. Kleinbaum DG, Kupper LL, Muller KE. Applied regression analysis and other mutivariable methods, 2nd ed. Boston, PWS-Kent, 1988.
26. Cohen J, Cohen P. Applied multiple regression/correlation analysis for the behavioral sciences, 2nd ed. Hillsdale, NJ:Erlbaum, 1983.
27. Bellinger D, Leviton A, Waternaux C, Needleman H, Rabinowitz M. Longitudinal analyses of prenatal and postnatal lead exposure and early cognitive development. N Engl J Med 316:1037–1043 (1987).
28. Wigg NR, Vimpani GV, McMichael AJ, Baghurst PA, Robertson EF, Roberts RJ. Port pirie cohort study: childhood blood lead and neuropsychological development at age two years. J Epidemiol Commun Health 42:213–219 (1988).
29. Lozoff B, Brittenham GM. Behavioral aspects of iron deficiency. Prog Hematol 14:23–53 (1986).
30. Walter T, Kovalskys J, Stekel A. Effect of mild iron deficiency on infant mental development scores. J Pediatr 102:519–522 (1983).
31. Oski FA, Honig AS, Helu B, Howanitz P. Effect of iron therapy on behavior performance in nonanemic, iron-deficient infants. Pediatrics 71:877–880 (1983).
32. Lozoff B, Brittenham GM, Wolf AW, McClish DK, Kuhnert PM, Jimenez E, Jimenez R, Mora LA, Gomez I, Krauskoph D. Iron deficiency anemia and iron therapy effects on infant developmental test performance. Pediatrics 79:981–995 (1987).
33. Aukett MA, Parks YA, Scott PH, Wharton BA. Treatment with iron increases weight gain and psychomotor development. Arch Dis Child 61:849–857 (1986).
34. Lozoff B, Brittenham GM, Viteri FE, Wolf AW, Urrutia JJ. The effects of short-term oral iron therapy on developmental deficits in iron deficient anemic infants. J Pediatr 100: 351–357 (1982).