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Iron Deficiency and Cognitive Test Performance in Preschool Children

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This study demonstrates reversible alterations in cognitive function in mildly iron-deficient 3- to 6-yr-old children. Behavorial tests were administered to 15 iron-deficient and 15 matched controls before and after the former group changed from a deficient to a normal iron status level. In the first evaluation, in comparison to the controls, the sideropenic children took more trials to reach a learning criterion in three discrimination learning tasks, and made more errors in the simplest of the memory tests. The differences in learning disappeared in the second evaluation.

Key words: **iron deficiency, anemia, cognitive function**

INTRODUCTION

In addition to its widely recognized role in systemic oxygen transport, iron is also a structural component of, or a cofactor for, enzymes critical in oxidative metabolism, DNA synthesis, and neurotransmitter synthesis and catabolism [Dallman et al., 1979]. Until recently, it was believed that these iron-containing (or -dependent) enzymes were not affected until very late in the development of iron-deficiency anemia. However, the availability of increasingly sophisticated measures of systemic iron status has revealed that certain iron-related enzyme derangements may precede the development of anemia and respond more rapidly than hemoglobin concentration to systemic iron administration [Beutler, 1957; Dagg et al., 1966; MacDougall et al., 1975; Finch et al., 1976].

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The original study design was to allow for a comparison of treated and untreated children with mild iron deficiency but no anemia. The final number of subjects in this category was not sufficient to allow this comparison.

There is an extensive, and largely anecdotal literature describing the behavioral correlates of iron-deficiency anemia [Pollitt and Leibel, 1976]. Lassitude, shortened attention span, irritability, and pica have been noted frequently in adults and children with iron-deficiency anemia. Also commented upon has been the rapid resolution of these symptoms following the onset of iron therapy, substantially before any significant increment in hemoglobin concentration has occurred [Harris and Kellermeyer, 1970].

Recent efforts by Oski et al. [1978] and Lozoff et al. [1982] to quantify the apparent psychoaffective correlates of systemic iron deficiency in infants have tended to buttress the clinical impression that disorders of behavior and cognition may attend the iron-deficient state. These prospective studies suggest that iron deficiency may affect the behavior of infants. The dependent measure used in both of these studies was the Bayley Scale of Infant Development, a global developmental test that assesses cognition, attention, reactivity, and motor development. Since the group differences obtained in these studies could reflect deficits in any of these processes, the specific process affected by iron in infancy remains unknown.

The present study demonstrates reversible alterations in attentional deficits in mildly iron-deficient 3- to 6-yr-old children. The study design included a prospective intervention and behavioral assessment both prior to and following iron repletion. The behavioral test battery was based on an information-processing theory [Fisher and Zeaman, 1973] that provided for the interpretation of normal–iron-deficient group differences in two important respects. First, specific effects of iron deficiency on learning, attention, and memory could be identified. Second, the theory allowed for the determination of whether differences on any of these cognitive processes were due to irreversible structural or reversible state-dependent changes in the iron-deficient child.

SUBJECTS AND METHODS

The 30 children who are the subjects of this report were selected from a pool of 110 subjects enrolled in Cambridge, Massachusetts (1975–1977) in a study of iron deficiency and behavior. Criteria for entry into the study included: (1) birth weight > 2500 g, (2) benign past medical history, (3) anthropometry within 2 SD of mean for age, (4) normal physical and neurological examination. Children meeting these criteria had approximately 700 μl of capillary blood withdrawn by finger stick (between 10:00 a.m. and 2:00 p.m.) into appropriate metal-free containers and standard Coulter S diluents. Duplicate determinations of hemoglobin (Hb) and mean cell volume (MCV) (Coulter Model S), whole blood lead [Barthel et al., 1973], free erythrocyte protoporphyrin [Blumberg et al., 1977], iron [Olsen et al., 1973], and total iron-binding capacity (TIBC) [Yeh and Zee, 1974] were performed on each sample. The percentage of transferrin saturation was calculated as serum iron/TIBC × 100.

Any child with a blood lead > 40 μg% was eliminated from further study. In order to maximize the number of potential subjects with very mild iron deficiency, iron deficiency was *initially defined* as a percentage transferrin saturation less than 20% with or without attendant hematologic changes such as reduced Hb or MCV. Iron-deficiency anemia was defined as a Hb less than 11 g% accompanied by a transferrin saturation less than 17%. In all instances of iron deficiency and/or anemia, confirmatory hematologic and iron determinations were done on samples obtained by venipuncture. When indicated, further studies were used to eliminate subjects with anemias not due solely to iron deficiency. These rather arbitrary definitions of iron deficiency and iron-deficiency anemia were used only to allow a clinical decision regarding eligibility for iron therapy. As described below an operational definition of initial iron status, based on change in iron status during the months of observation, was ultimately used to select the iron-deficient (index) cases.

Following the performance of the psychometrics described below (T_1), all children with iron-deficiency anemia (Hb $<$ 11 g%, transferrin saturation $<$ 17%) and half of the children with iron deficiency but no anemia (transferrin saturation $<$ 20%; Hb $\geqq$ 11 g%) were treated with an oral iron preparation (Fer-in-Sol, Mead Johnson, Evansville, IN.) at a dosage of 4–5 mg elemental iron/kg of body weight/day for 4–6 mo. This schedule was calculated to deliver sufficient iron to rectify completely any systemic iron deficit. The iron was provided at no charge, and compliance was monitored by weekly telephone calls and monthly home visits to collect and replace empty medication bottles. From 4 to 6 mo after T_1, all subjects had repeat physical examination, psychometrics (T_2), and blood letting. In those children receiving iron, the blood studies (by venipuncture) were done after at least 2 wk of not receiving iron medication.

There is known to be considerable overlap of normal and mildly abnormal values for serum iron and transferrin saturation [Koerper and Dallman, 1977; Dallman and Siimes, 1979], rendering an operational definition of iron deficiency—such as change in iron biochemical or hematologic status with iron therapy—the most convincing means of assessing the existence of mild iron deficiency [Dallman et al., 1981; Leibel et al., 1982]. In order to generate such an operational definition of iron deficiency, we computed a "normal" range for change in transferrin saturation from T_1 to T_2 and used deviation from this norm to identify index cases. For this purpose, the change in transferrin saturation (Δ sat) from T_1 to T_2 for all children with a T_1 transferrin saturation $>$ 20% (N=72) was calculated. The mean delta was -1.51 ± 9.0. The cutoff T_1 transferrin saturation ($>$ 20%) was chosen as being well within the age-normal range and as reflecting a somatic iron status unlikely to change over a 4- to 6-mo period due to a change in iron intake.

The 15 experimental children (8 males, 7 females) described below represent all of the original 110 subjects meeting the two following criteria: (1) transferrin saturation at $T_1 < \bar{X} - 1$ SD for the entire study group (N=110) at T_1 (23 $\pm$ 9; range 7–46%); (2) a change in transferrin saturation (Δ saturation) from $T_1 - T_2 > \bar{X} + 1.5$ SD for the Δ saturation of those 72 subjects with a T_1 transferrin saturation $>$ 20%. Use of these criteria resulted in the inclusion as index cases of some children (N=4) other than those actually treated with iron. Parents of untreated children with apparent mild hypoferremia but no anemia were told that the child might be mildly iron deficient and that repeat blood studies would be done at T_2. We believe that several of these parents took matters into their own hands and, by dietary and/or medicinal manipulation, succeeded in elevating their child's serum iron level. Some treated children (N=9) were excluded from the index sample because of a lack of a significant increment in transferrin saturation from T_1 to T_2. This implied that they were not, in fact, iron deficient at T_1 despite suggestive biochemistries. An alternative hypothesis for this lack of response was failure to absorb or ingest the prescribed iron. However. since iron status remained unchanged from T_1 to T_2 in these children, they were not suitable for inclusion as index cases in any event.

Fifteen controls, selected from the children with a T_1 transferrin saturation > 20% and a Hb > 11%, were matched to the index children for age, sex, race, and height age. Table 1 presents the relevant anthropometric, hematologic, and iron-biochemical data on both groups at T_1 and T_2. As expected, there is a small but statistically significant difference in Hb at T_1 between index and control children, which is absent at T_2 due to iron repletion of index cases. The small difference in T_1 Hb between index and control children, the presence in the index group of a T_1 mean Hb well above the level generally associated with physiologic alterations due to impaired O_2 carriage [Varat et al., 1972], and the fact that MCV and serum FEP levels were not significantly different in these two groups at T_1 and T_2 suggest that the relative hypoferremia of the index group is the physiologically more significant difference from the control group.

The mean serum iron levels and transferrin saturations of our control patients at T_1 (71.8 μg/dl and 26.1%, respectively) are comparable to median values recorded for 2- to 12-yr-old children (70 μg/dl and 24%) without any laboratory evidence of iron deficiency (normal serum ferritin; free erythrocyte protoporphyrin, hemoglobin, and mean cell volume) [Koerper and Dallman, 1977]. In this latter group, the lower limits of the 95% range were serum iron 20 μg/dl and transferrin saturation 7%. Many children with iron biochemistries this low will be found to have other chemical and hematologic evidence of iron deficiency. The index cases in the present study had T_1 values (serum iron 28.5 μg/dl and transferrin saturation 11%) very close to the lower limits of this 95% range.

PSYCHOLOGICAL TESTING

The behavioral test battery for this study was derived from an information processing model originally designed to study attention, learning, and memory processes in retarded children [Fisher and Zeaman, 1973]. In addition to assessing these processes independently, the theory also provides for a distinction between permanent capacity (structural feature) aspects such as the amount of information to which one can simultaneously attend (breadth of attention), and state-dependent behavior (control processes) such as a strategy for use of attention when there is both relevant and irrelevant information available (direction of attention). Details of the test battery are given below.

Discrimination Learning Tasks

Two-choice discrimination learning with three-dimensional objects. The child is presented two three-dimensional objects: (toy car, toy whistle, for example) mounted on 3 × 3-in.^{2} wooden bases. A yellow "happy face" is pasted on the bottom of only one of the bases. The child's task is to discover which stimulus "hides" the "happy face" underneath it. After each trial the stimuli are rearranged (for possible position preferences) out of the child's view, and the procedure continues until a criterion of seven correct responses in a row is met. The reverse problem (the previous incorrect stimulus is now correct) is then administered to the same criterion.

Two-choice discrimination learning with two-dimensional pictures. The procedure is identical to the three-dimensional problem except that the two stimuli

TABLE 1. Experimental and Control Children: Mean Age, Height, and Weight Age, Hemoglobin, Serum Iron, and Transferrin Saturation

	Evaluation time					
	T_1			T_2		
	(Mean)	(SD)	(P)	(Mean)	(SD)	(P)
Age (months)						
Experimental	43.1	(7.8)	NS	50.4	(9.2)	NS
Control	44.1	(7.0)		48.5	(7.4)	
Height age (mo)						
Experimental	42.9	(10.0)	NS	50.3	(12.2)	NS
Control	43.1	(10.3)		49.3	(11.9)	
Hemoglobin (g/dl)						
Experimental	11.2	(0.80)	< .05	12.4	(0.64)	NS
Control	12.1	(0.50)		12.3	(0.50)	
Serum iron (μg/dl)						
Experimental	29.5	(7.2)	< .0001	81.0	(20.7)	< .05
Control	71.8	(22.2)		67.13	(20.4)	
Transferrin saturation (%)						
Experimental	11.0	(2.0)	< .0001	33.3	(10.0)	< .01
Control	26.1	(7.6)		25.6	(6.5)	

are two-dimensional pictures cut from children's books, pasted on black poster-board with a "happy face" attached to the back of the appropriate stimulus.

Two-choice discrimination learning with two-dimensional color-form pictures. Identical to the two-dimensional colored pictures. The same two colors and two forms are randomly paired on each trial (trial 1: blue X, red O; trial 2: red X, blue O) but only the form (always X or always O) is consistently correct.

Short-term Memory

Memory test adopted from the paradigm of Peterson et al [1962]. A large number of two-choice visual discrimination learning problems consisting of two-dimensional pictures are presented concurrently for a total of four trials each. Trials 1 and 2 are massed (consecutive). Trials 2 and 3 have either 0, 4, or 8 interpolated items separating them. Trial 4 occurs 24 h later. A "happy face" is posted on the back of the correct stimulus.

Oddity Learning

Three stimuli (two-dimensional pictures), two of which are identical, are presented simultaneously on a 7 × 18-in.^{2} black poster-board. The child must point to the correct picture, the one different from the other two (oddity). The only instructions given are "to find the winner." In the first series of problems, new stimuli are used on every trial. In the remaining series, the same stimuli are repeated every trial in an AAB, ABB manner. In this task, the specific character of a stimulus does not determine its correctness; correctness is determined by the relationship of a character to other stimuli in the array.

IQ Test

All children were individually administered the Stanford-Binet Intelligence Scale.

RESULTS

The mean values for the first part of each of the three discrimination learning tasks are presented in Figure 1a,b,c. Two-way analysis of variance with repeated measures to test for main effects of iron status and time of evaluation were used for all the statistical comparisons reported.

In the first discrimination task (three-dimensional objects) there were no statistically significant time effects but there were iron status effects on trials to learning criterion ($F = 4.24$; $P < 0.05$). Paired comparisons showed that the only statistically meaningful bivariate comparisons were restricted to those that included the experimental group at T_1. That is, the experimental children took more trials to reach the learning criterion at T_1 than at T_2. Also, their performance was poorer at T_1, but not at T_2, than that of the control children. In the discrimination reversal shift in both T_1 and T_2 there were no differences between groups.

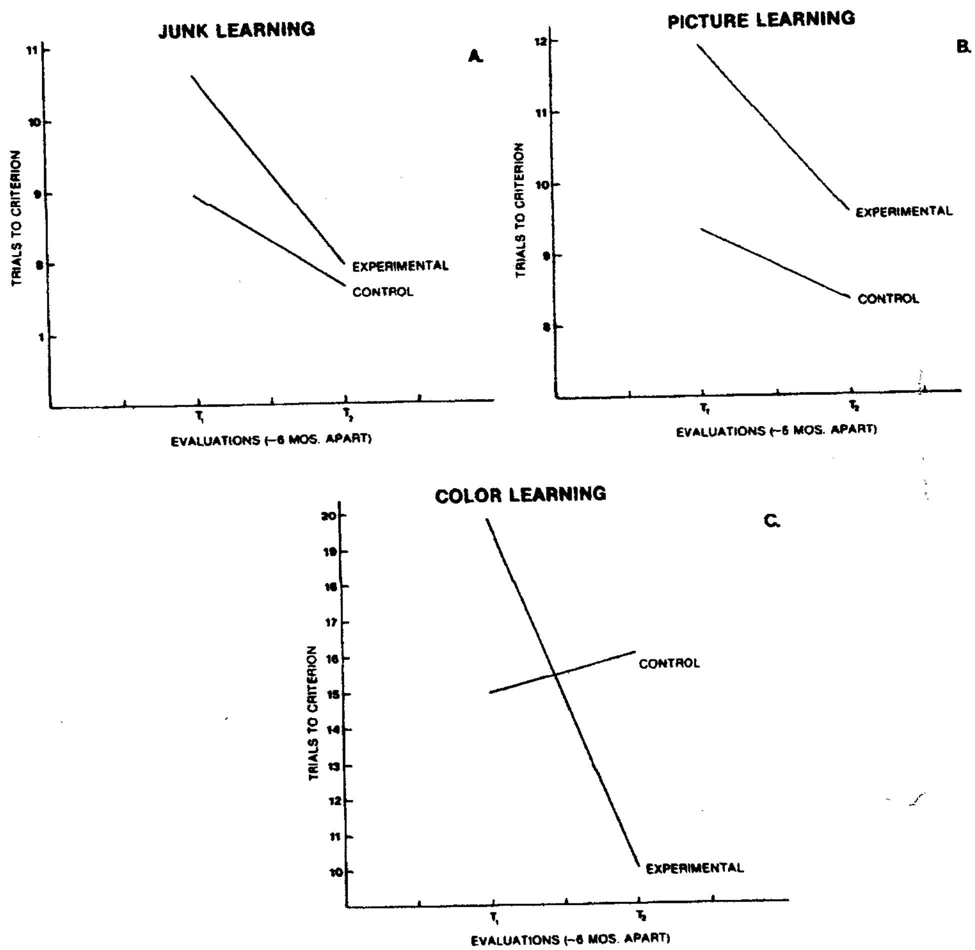

Fig. 1. Mean scores of experimental and control children in three discrimination learning tasks at T_1 and T_2.

In the second discrimination learning task (two-dimensional pictures) there were again no time effects, but there was a significant iron-status effect on performance ($F = 4.49$; $P < .05$). Thus, as compared to the controls, the experimental children took more trials to reach the learning criterion. Paired comparisons showed that as in the first discrimination learning test, the experimental children at T_1 took more trials than at T_2, and more than the controls at both time points. In the reversal shift conditions all differences again disappeared.

The third discrimination learning test (colors) consisted of geometric figures of different colors. The children had to learn to choose the correct color, ignoring the geometric form. Here there were no main effects on trials to criterion. The time by iron-status interaction did reach the .08 probability level. A paired comparison showed that at T_1 the iron-deficient children took more trials ($P < .05$) to reach criterion than the controls; but at T_2 the direction of this difference was reversed ($P < .05$). As in the two previous discrimination tests, no statistically significant differences were found in the analysis of the reversal shift.

Tests to assess short- and long-term memory were also administered to all children at both time points. These tasks consisted of a number of two-choice discrimination problems that were presented concurrently, varying the number of different intervening items between trials. Conditions 0, 4, and 8 refer to the number of intervening items between the second and third trial of a single problem. Thus, for example, condition 0 problems had consecutive trials (a measure of attention), whereas a condition 8 problem had 8 intervening items between trials 2 and 3 (a measure of memory interference). All problems were repeated a day later to test long-term memory differences.

Comparisons for the 4 and 8 interval situations on both testing days showed no statistically significant findings at T_1 and T_2. In the 0-interference conditions for both first and second day testing, both at T_1 and T_2, there were significant iron-status effects. Thus, in contrast to the discrimination learning tasks the difference of the experimental children—as compared with that of the controls—was poorer at both T_1 and T_2 in the memory test.

There were no statistically significant differences between groups at either T_1 or T_2 in the mean number of correct responses in the oddity learning tasks. Likewise, there were no statistically significant differences between the IQs of these two groups of children. For T_1 and T_2 the IQs of the experimental children were 107 and 111, while that of the controls were 103 and 107, respectively.

DISCUSSION

Bellar and Howell [1972] and Howell [1971] were among the first to call attention to the possible existence of cognitive disorders in iron-deficient children. Subsequently, Webb and Oski [1973] described the association of iron-deficiency anemia with impaired scholastic performance and behavior in adolescents of low socioeconomic class. In more recent studies, Oski and Honig [1978] demonstrated cognitive disturbances in iron-deficient children under 1 yr of age which were reversed within several days following the administration of parenteral iron. Similar deficits were demonstrated by Lozoff et al. [1982], but these were not reversed following short-term oral iron therapy. Deinard et al. [1981] found no effect of

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