Iron treatment normalizes cognitive functioning in young women[1–4]

Laura E Murray-Kolb and John L Beard

ABSTRACT

Background: Evidence suggests that brain iron deficiency at any time in life may disrupt metabolic processes and subsequently change cognitive and behavioral functioning. Women of reproductive age are among those most vulnerable to iron deficiency and may be at high risk for cognitive alterations due to iron deficiency.

Objective: We aimed to examine the relation between iron status and cognitive abilities in young women.

Design: A blinded, placebo-controlled, stratified intervention study was conducted in women aged 18–35 y of varied iron status who were randomly assigned to receive iron supplements or a placebo. Cognition was assessed by using 8 cognitive performance tasks (from Detterman's Cognitive Abilities Test) at baseline (n = 149) and after 16 wk of treatment (n = 113).

Results: At baseline, the iron-sufficient women (n = 42) performed better on cognitive tasks (P = 0.011) and completed them faster (P = 0.038) than did the women with iron deficiency anemia (n = 34). Factors representing performance accuracy and the time needed to complete the tasks by the iron-deficient but nonanemic women (n = 73) were intermediate between the 2 extremes of iron status. After treatment, a significant improvement in serum ferritin was associated with a 5–7-fold improvement in cognitive performance, whereas a significant improvement in hemoglobin was related to improved speed in completing the cognitive tasks.

Conclusions: Iron status is a significant factor in cognitive performance in women of reproductive age. Severity of anemia primarily affects processing speed, and severity of iron deficiency affects accuracy of cognitive function over a broad range of tasks. Thus, the effects of iron deficiency on cognition are not limited to the developing brain. *Am J Clin Nutr* 2007;85:778–87.

KEY WORDS Iron, women, cognition, attention, memory, learning, ferritin, hemoglobin

INTRODUCTION

Despite advances in the reduction of a number of nutrient deficiencies worldwide, iron deficiency (ID) remains the most prevalent single nutrient deficiency, and it affects those in both developing and developed countries. The World Health Organization (WHO) estimates that, worldwide, 2 billion people are anemic and twice as many are iron deficient (1). Because of their greater physiologic requirements, combined with increased losses and poor dietary intake, those at highest risk of developing ID and iron deficiency anemia (IDA) are infants, children, and women of reproductive age.

Nonhematologic manifestations of ID include reduced physical endurance, an impaired immune response, difficulty in regulating temperature, changes in energy metabolism, decreased cognitive performance, and behavioral disturbances (2–4). Over the past 30 y, a large effort has focused on understanding the relation between ID and development or behavior in infants and young children (4–8). As a result, we have strong evidence that IDA is associated with poorer performance on developmental ratings in infants and with lower scores on cognitive function tests and educational achievement tests in children. In adolescents, ID has been shown to impair cognitive abilities even in the absence of overt anemia (9).

Whereas data on the relation between iron status and cognition is mounting in both infants and children, a gap exists in our understanding of this same relation in adults. The large number of studies conducted in infants has led many to assume that ID disrupts brain functioning only during development (10). However, new evidence from animal models and in humans with restless leg syndrome (RLS) suggests that brain ID at any time in life is likely to disrupt metabolic processes and to be followed by changes in cognitive and behavioral functioning (4).

Reports have been published of cognitive improvement in adult renal dialysis patients who are receiving both erythropoietin and iron supplementation as part of treatment protocols (11–13), as well as in elderly whose iron nutritional status has improved (14). Recently, we reported a relation between iron status and cognitive abilities in poor South African mothers during the first postpartum year (15). Whereas these studies all point to a relation between iron status and cognition, the numerous confounding variables in each of them make the findings difficult to assess. Therefore, we undertook a more thorough investigation of the relation between iron status and cognition in women of reproductive age by conducting a blinded, placebo-controlled, intervention study. The overall aim of the study was to examine

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[2] Presented in part at the Experimental Biology Annual Meeting (FASEB), Washington, DC, April 2004.

[3] Supported by USDA NRICGP 99-35200-7610 and GCRC MO1RR10732.

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Received May 5, 2006.

Accepted for publication November 7, 2006.

the effects of ID and IDA on cognitive and emotional performance in young women. Here we report the results of the cognitive tasks. The primary outcomes of the study were the relation between iron status and cognition and that between changes in iron status and changes in cognition. We also wanted to examine whether different facets of cognition were differentially affected by iron status or a change in iron status. Given estimates that up to 50% of women in the world are iron deficient, a documented relation between iron status and cognitive abilities could provide a basis for interventions that would be relevant to the psychological functioning of a significant proportion of the world's population.

SUBJECTS AND METHODS

Subjects

This study was conducted on the University Park campus of The Pennsylvania State University in State College, PA between the fall of 1999 and the fall of 2002. Women aged 18–35 y were recruited via flyers as well as advertisements in the local newspaper. Interested women reported to the General Clinical Research Center (GCRC) for screening, which consisted of a venous blood draw and the completion of a health history questionnaire. The criteria for participation in the study included the following: female between 18 and 35 y of age, free from any chronic illness or serious health problems and where English is the primary language spoken in the home.

Written informed consent was obtained from each subject. All procedures used in this study were reviewed and approved by the Institutional Review Board at The Pennsylvania State University and were in accordance with the Helsinki Declaration of 1975 as revised in 1983.

Methods

The blood samples were used for the measurement of a complete blood count (including hemoglobin and hematocrit), serum ferritin (sFt) (Diagnostic Products Corporation, Los Angeles, CA), serum transferrin receptor (sTfR) (Ramco, Houston, TX), and plasma iron and total iron–binding capacity (TIBC) by standard methods (16). Transferrin saturation was then calculated as plasma iron/TIBC × 100, and body iron was calculated by using Cook's new algorithm (17). C-reactive protein was also measured with a qualitative kit (Wampole Laboratories, Princeton, NJ) to assess the presence of inflammation.

Women were classified as iron sufficient (control group; CN), nonanemic but with iron deficiency (ID group), or with iron deficiency anemia (IDA group). IDA was defined as a hemoglobin concentration between 105 and 119 g/L and ≥2 abnormal iron status values. ID was defined as above, except that hemoglobin concentrations had to be ≥120 g/L. Women with hemoglobin < 105 g/L were excluded from the study and referred to a physician for treatment. Once the women were stratified into the 3 iron status groups, they were randomly assigned (stratified randomization performed by using random permuted blocks) to receive either a slow-release iron supplement (160 mg ferrous sulfate containing 60 mg elemental iron) or a placebo. Only one of the researchers knew the allocation of the subjects, and the researchers responsible for testing the women were unaware of treatment allocation. The researcher who knew the treatment allocation was responsible for preparing the bottles of supplements to be given to the subjects. The bottles were labeled only with the subject's identification number and with the instruction to "take 1/d for the next 16 wk" (the women were also told to keep the pills away from children). A 4-mo intervention was chosen for 2 reasons. First, we wanted to ensure that the women given iron supplements would be truly iron replete at the end of the study. Second, evidence exists in animals that liver iron concentrations are replenished at a faster rate than are brain iron concentrations (18). Therefore, whereas studies have reported increases in hemoglobin and hematocrit concentrations after subjects have consumed iron supplements for 8–12 wk, we wanted to allow more time for brain iron concentrations to be replenished; thus, we chose a period of 16 wk. Our allocation of subjects to consume either iron or placebo resulted in 6 treatment groups: CN group taking placebo (CNPL), CN group taking iron (CNFe), ID group taking placebo (IDPL), ID group taking iron (IDFe), IDA group taking placebo (IDAPL), and IDA group women taking iron (IDAFe). The subjects were asked to refrain from taking any vitamin or mineral supplements during the trial.

Cognitive and emotional testing was conducted both at baseline and after 16 wk of treatment. Each woman first completed a questionnaire that obtained information about university grade-point average, level of physical activity, demographic variables [socioeconomic status (SES) was determined from the mother's and father's occupations and education level with the use of the Hollingshead 2 factor index; 19], oral contraceptive use, and menstrual cycle information. The subjects then completed the Shipley Institute of Living Scale (20), a self-administered test consisting of 2 subtests: a vocabulary subtest and an abstraction subtest. Scores from this scale were used to estimate intelligence quotient (IQ) for each woman through the use of a continuously adjusted age norms method (21).

Next, each woman completed 8 self-administered and automated computerized tasks of basic cognition using the Cognitive Abilities Test (CAT; 22). One of the advantages of the CAT is that it has been subject to the same psychometric rigors commonly used to develop intelligence tests. High reliabilities have been found for each of the tasks present in the CAT (23). These tasks were developed to measure the "modal model" of information processing that offers the opportunity to test specific aspects of cognition. This model is composed of 3 memory stores (very-short-term memory, short-term memory, and long-term memory), which are served by a stimulus encoding mechanism for input, a retrieval mechanism for output, and an output mechanism that executes responses. It also contains an executive functioning mechanism that oversees movement of information through the system.

Instructions appeared before each test and consisted of 3 parts—written instructions, clarification by the tester, and practice trials. The tests measure 3 domains—attention, memory, and learning—and correspond to levels of complexity as follows: attention: tachistoscopic threshold, reaction time, and stimulus discrimination; memory: probe recall, Sternberg memory search, and recognition memory; learning: and learning and progressive matrices.

The reaction time task includes both simple and choice reaction times in which several measures of speed and accuracy are obtained. The stimulus discrimination task is a modified match-to-sample test that yields measures of stimulus encoding and search processes. To establish a threshold, the tachistoscopic

threshold task measures the minimum amount of time needed for a subject to decide whether 2 stimuli are the same or different. These 3 measures of attention probe very-short-term memory as well as encoding and output mechanisms of information processing.

The probe recall task yields measures of memory, accuracy, and speed. The recognition memory task is a forced-choice recognition test used to measure memory, speed, and accuracy. The Sternberg memory search task is used to measure the amount of time it takes to search easy stimulus sets of various sizes. This test yields measures of memory, speed, and accuracy for 4 different set sizes. These 3 measures of memory probe short-term memory and the retrieval and executive functioning mechanisms of information processing.

The learning task requires subjects to encode increasingly larger sets of stimuli to measure learning rate, whereas the progressive matrices task is modeled after the progressive matrices type of intelligence tests and is the most complex of the tasks. These 2 measures of learning probe long-term memory and analytic reasoning.

After baseline testing, each subject was given either a placebo or a slow-release iron supplement (160 mg ferrous sulfate containing 60 mg elemental iron) and instructed to take 1 dose/d for the next 4 mo. After 16 wk, the women returned for a venous blood draw and repeated cognitive testing. Those women who were found to be anemic at the end of the study were provided with 30 days' worth of supplements and referred to their physician.

Statistical analysis

All data were analyzed with SAS for WINDOWS software (version 8e; SAS Institute, Cary, NC). Log transformation of ferritin as well as transferrin receptor variables was required for normalization. Factor analysis (principal axis with varimax rotation) was carried out for the cognitive and hematologic variables to reduce the number of variables and the probability of a type 1 error. The factors were then used for the analyses. Test scores were standardized by calculating z scores to facilitate the comparison of scores across the domains. Therefore, the scores have an SD of 1. Differences between groups (CN, ID, and IDA) at baseline were examined by using analysis of covariance (ANCOVA) with IQ as a covariate and Tukey's test as the post hoc test.

To examine change in the cognitive variables over time, repeated-measures analyses were employed after adjustment for IQ and baseline value on any particular task. Analyses were run with women classified as ferritin responders or nonresponders and hemoglobin responders or nonresponders. This step was taken because the current study was designed to determine the relation between changes in iron status and changes in cognition. Therefore, in keeping with our longitudinal hypothesis, which stated that an intervention that normalized the iron status of the young women would normalize their cognitive scores, data will be presented as comparing responders and nonresponders with respect to iron treatment, regardless of original group assignment. If we were to ignore our hypothesis and simply keep the group assignment as originally determined at baseline, a large potential would exist for erroneous conclusions because of the inclusion of women with no change in iron status in a group in which change may have been expected (or vice versa). Classifying the women as responder and nonresponders was done on an individual basis after determining whether the woman experienced a change in ferritin or hemoglobin greater or less than the known biological day-to-day variation (24) for each of these iron status variables. The analyses were then run by using repeated-measures ANCOVA with Tukey's test as the post hoc test.

RESULTS

The progress through the phases of this trial, which was carried out over a 3-y period, can be found in **Figure 1**. Of the 398 women who were screened, 152 were deemed eligible, stratified according to iron status, and randomly assigned to treatment. At follow-up, 113 women returned to complete the study. There were no differences between the groups at baseline with respect to mean age (21 $\pm$ 3 y), ethnic distribution, SES, birth control use, reported physical activity level, oral contraceptive use, GPA, or menstrual cycle characteristics (data not shown). Further exploration found no significant relation between these variables and iron status or cognition. Within each group, no significant hematologic differences were found between the women randomly assigned to placebo and those randomly assigned to iron at baseline.

Hematologic measurements are given in **Table 1**. As per the design of the study, at baseline, the iron status of the groups was significantly different. None of the women was found to have any indication of inflammation, as evidenced by negative results on the CRP test. At endpoint, those groups receiving iron had significantly improved their iron status. Significant increases occurred in the IDFe and IDAFe groups for ferritin ($P < 0.001$ and $P < 0.01$, respectively) and body iron ($P < 0.0001$ and $P < 0.01$, respectively). The IDAFe group also experienced a significant increase in hemoglobin ($P < 0.0001$), hematocrit ($P < 0.001$), and transferrin saturation ($P = 0.018$) and a nonsignificant decrease in transferrin receptor (TfR) concentrations. Groups consuming the placebo also experienced some hematologic changes over time. The CNPL group had a significant decrease in transferrin saturation (TSAT) ($P = 0.010$). However, for the IDPL and IDAPL groups, there was a regression to the mean with respect to ferritin (IDPL) and to hemoglobin and hematocrit concentrations (IDAPL), which rendered these groups no different at endpoint from their iron-receiving counterparts with respect to these variables. Of the women with the lowest hemoglobin values at baseline (<110 g/L), 67% experienced an increase in hemoglobin > 10 g/L, whereas only 10% of the women whose hemoglobin values were >120 g/L at baseline did so (**Figure 2**).

The factor analysis carried out on the hematologic variables found 4 factors, which we termed storage, transport, preanemia, and anemia (**Table 2**). Factor analysis of the cognitive variables within each cognitive domain (ie, attention, memory, and learning) found 2 factors for each domain tested. The factors were termed performance factor and time factor (**Table 3**). Factor analysis of all cognitive variables together found 2 overall factors, which were termed performance and time (Table 3). Data are represented by each of these factors (performance and time) as well as by a composite score that consists of the performance factor minus the time factor.

Cross-sectional baseline comparisons

Results of the overall scores (all cognitive domains considered together) are shown in **Figure 3**. The composite score shows that women in the CN group scored the highest, whereas women in the IDA group scored the lowest. Women in the ID group scored between women in the CN and IDA groups. Differences were

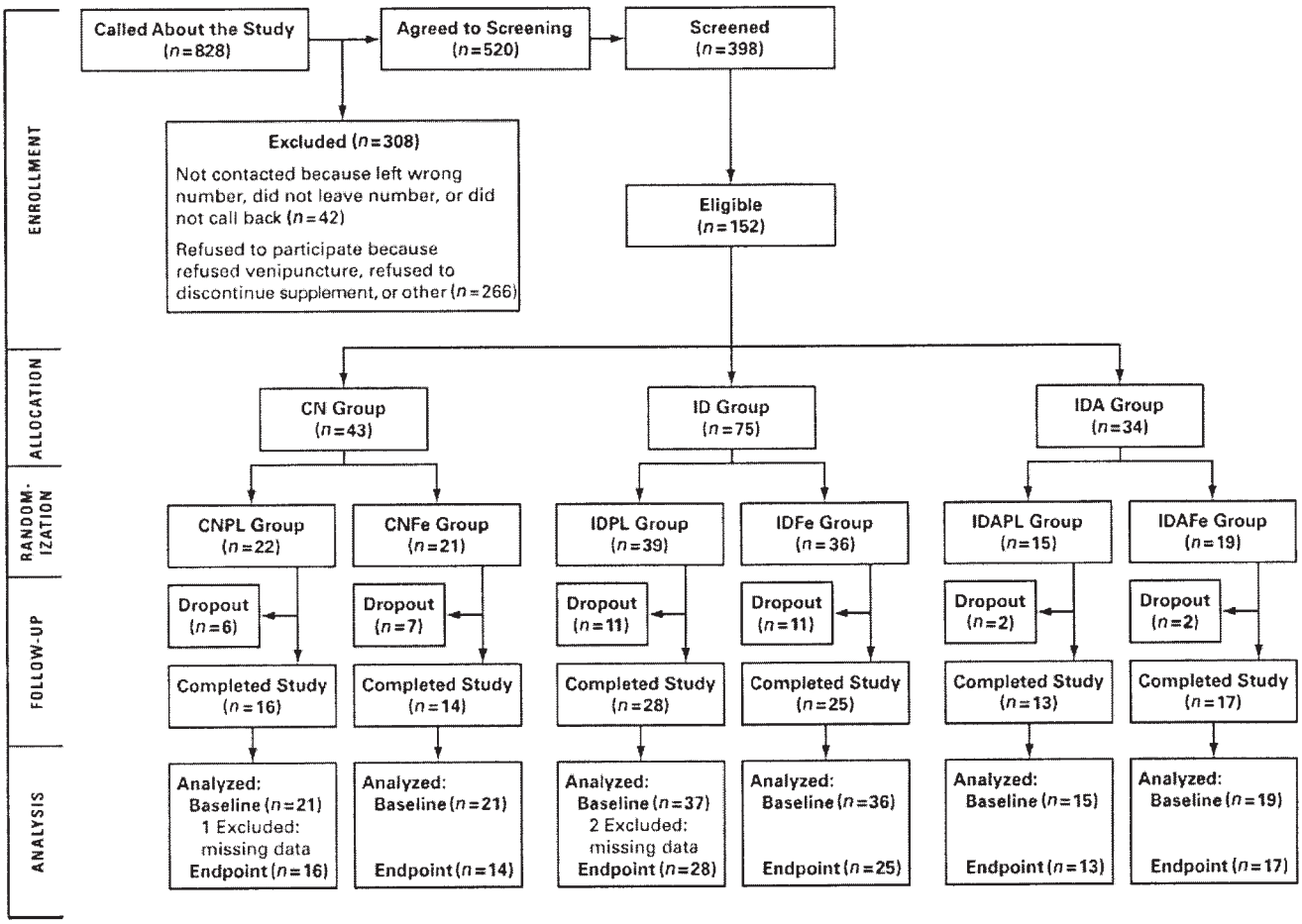

FIGURE 1. Profile for classification into iron status groups and randomization into iron versus placebo. CN, control group; ID, group with iron deficiency; IDA, group with iron deficiency anemia; CNPL, control group given placebo; CNFe, control group given iron; IDPL, ID group given placebo; IDFe, ID group given iron; IDAPL, IDA group given placebo; IDAFe, IDA group given iron.

found to be significant between the CN and IDA groups and between the ID and IDA groups ($P = 0.001$ for both). Scores on the performance factor followed this same pattern (CN>ID>IDA), and the differences were significant between the CN and IDA groups ($P = 0.011$) and between the ID and IDA groups ($P = 0.007$). The pattern for the score on the time factor was exactly the opposite (IDA>ID>CN), and the differences between the CN and IDA groups ($P = 0.038$) and the ID and IDA groups ($P = 0.036$) were significant. These scores indicate that not only was the CN group able to perform better on the cognitive tasks, but those women also were able to do so in a shorter amount of time.

Categorizing the women into the CN, ID, and IDA groups uses data that is continuous (iron status data) and places it into discrete categories (through the use of accepted cutoffs), thereby diminishing the power of the continuous data. To utilize the full power of our measures, data were sorted according to each hematologic factor (ie, storage, transport, preanemia, and anemia) and then divided into quintiles. ANCOVAs were then run on the data with the intention of specifically examining the extremes of the distribution. Analyses comparing the upper and lower quintiles for the storage factor found significantly better performance by those in the upper quintile (0.12 compared with −0.31; $P = 0.030$) but no difference in the time necessary to complete the tasks. The opposite was found when comparing the upper and lower quintiles for the anemia factor. That is, women in the highest quintile required significantly less time to complete the tasks than did those in the lower quintiles (−0.11 and 0.18, respectively; $P = 0.020$), but performance levels did not differ significantly between the quintiles.

To obtain a better understanding of exactly where the cognitive deficits lie, we then analyzed the cognitive tasks by cognitive domain (ie, attention, memory, and learning). Results from these analyses are shown in **Figure 4**. With respect to the attention domain (Figure 4A), the composite score did not differ between the CN and ID groups but was significantly lower in the IDA group than in the CN group ($P = 0.008$) or the ID group ($P = 0.003$). Performance in the attention domain did not differ between the CN and ID women but was significantly better than that of the IDA women ($P = 0.047$ and 0.008, respectively). However, women in the CN group completed the tasks quicker than did women in either the ID or IDA groups. Whereas these differences did not reach statistical significance, the difference between the CN and IDA groups trended toward significance ($P = 0.064$).

Composite scores on the memory domain (Figure 4B) show the CN and ID groups scoring equally well and significantly better than the IDA group ($P < 0.001$ for both). The performance

TABLE 1
Hematologic variables in subjects at baseline (n = 149) and endpoint (n = 113)[1]

	Control group		Iron deficiency group		Iron deficiency anemia group	
Hematologic variables	CNPL (n = 21, 16)[2]	CNFe (n = 21, 14)	IDPL (n = 37, 28)	IDFe (n = 36, 25)	IDAPL (n = 15, 13)	IDAFe (n = 19, 17)
Hb (g/L)						
Baseline	139 ± 9[a]	137 ± 8[a]	133 ± 6[b]	131 ± 6[b]	114 ± 5[c]	113 ± 5[c]
Endpoint	142 ± 11	134 ± 11	132 ± 10	131 ± 8	121 ± 9[3]	125 ± 9[3]
Hct (%)						
Baseline	42 ± 3[a]	42 ± 3[a]	41 ± 2[a,b]	40 ± 2[b]	35 ± 1[c]	35 ± 1[c]
Endpoint	44 ± 5	41 ± 4	41 ± 4	40 ± 3	37 ± 3[3]	38 ± 3[3]
MCV (fL)						
Baseline	90.4 ± 2.2[a]	90.8 ± 2.0[a]	88.2 ± 3.4[a]	88.0 ± 3.5[a]	79.7 ± 8.0[b]	78.6 ± 8.7[b]
Endpoint	90.2 ± 3.3	89.8 ± 3.9	87.5 ± 4.1	89.6 ± 4.1	81.8 ± 6.4	82.0 ± 7.0
RDW (%)						
Baseline	12.7 ± 0.5[a]	12.9 ± 0.5[a]	13.4 ± 1.0[b]	13.3 ± 1.0[b]	14.4 ± 1.1[c]	14.2 ± 1.4[c]
Endpoint	13.0 ± 0.7	13.0 ± 0.8	14.0 ± 1.0	13.0 ± 0.9	15.0 ± 1.0	15.0 ± 1.9
sFt (μg/L)						
Baseline	45.3 ± 20.1[a]	50.0 ± 19.7[a]	8.9 ± 3.4[b]	8.8 ± 4.0[b]	5.7 ± 4.5[b]	7.2 ± 5.7[b]
Endpoint	42.2 ± 20.7[a]	70.3 ± 44.4[3]	14.7 ± 14.2[c,d,3]	24.6 ± 22.4[d,3]	9.2 ± 7.7[d]	22.8 ± 17.7[d,3]
sTfR (mg/L)						
Baseline	4.6 ± 1.0[a]	4.6 ± 1.4[a]	6.5 ± 2.0[b,c]	5.9 ± 1.8[b]	9.1 ± 2.7[d]	8.2 ± 4.3[c,d]
Endpoint	5.3 ± 1.8	4.0 ± 1.5	6.4 ± 2.6	5.6 ± 2.3	7.9 ± 1.3	6.6 ± 2.5
TfSat (%)						
Baseline	33 ± 11[a]	33 ± 9[a]	24 ± 12[b]	22 ± 9[b]	19 ± 9[b]	18 ± 12[b]
Endpoint	23 ± 9[a,b,3]	25 ± 13[a,b]	24 ± 14[a,b]	29 ± 10[a]	16 ± 7[b]	32 ± 15[a,3]
Body iron (mg/kg)[4]						
Baseline	6.5 ± 1.5[a]	7.0 ± 1.7[a]	−0.4 ± 2.0[b]	−0.3 ± 2.3[b]	−4.0 ± 3.6[c]	−2.6 ± 4.6[c]
Endpoint	5.8 ± 2.2[a,c]	8.2 ± 3.6[a]	0.6 ± 3.9[b,d]	3.2 ± 3.3[c,d,3]	−1.9 ± 3.2[b]	2.0 ± 4.1[d,3]

[1] All values are $\bar{x}$ ± SD. Hb, hemoglobin; Hct, hematocrit; MCV, mean corpuscular volume; RDW, red blood cell distribution width; sFt, serum ferritin; sTfR, serum transferrin receptor; TfSat, transferrin saturation; CNPL, control group receiving placebo; CNFe, control group receiving iron; IDPL, iron deficiency group receiving placebo; IDFe, iron deficiency group receiving iron; IDAPL, iron deficiency anemia group receiving placebo; IDAFe, iron deficiency anemia group receiving iron. Values within a row with different superscript letters are significantly different, $P < 0.05$. ANOVA was used to compare groups at a given time point, and repeated-measures analyses were used after adjustment for baseline values to analyze change over time. Tukey's test was used to compare the groups at baseline and endpoint. The 3-way interaction (time × group × treatment) was significant ($P < 0.05$) for all variables except MCV and RDW. Two-way interaction (group × pills) was significant ($P < 0.01$) for sFt, TfSat, and body iron; 2-way interaction (group × time) was significant ($P < 0.05$) for all variables except MCV and RDW.

[2] n at baseline, endpoint (all such).

[3] Significantly different from baseline, $P < 0.05$.

[4] Calculated by method of Cook et al (17).

factor on the memory domain shows this same pattern, but the time factor shows the opposite. Differences with respect to the performance factor were significant between CN and IDA groups ($P = 0.002$) and between ID and IDA groups ($P < 0.001$).

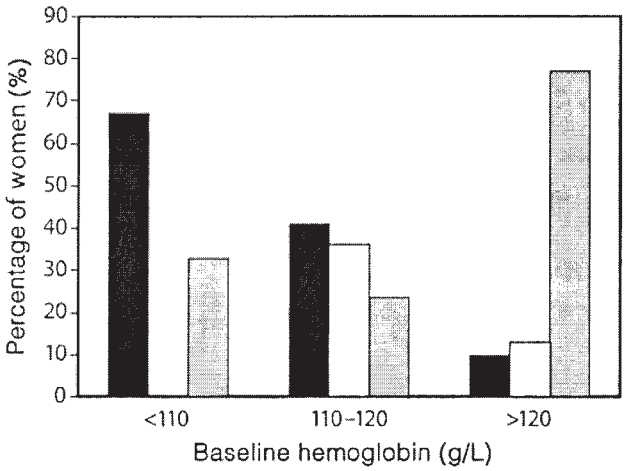

FIGURE 2. Change in hemoglobin concentration as a function of initial hemoglobin status ($P < 0.0001$, chi-square test). ■, Hemoglobin change >10 g/L; □, hemoglobin change 5–10 g/L; ▨, hemoglobin change <5 g/L.

The z scores for the time factor did not differ significantly between the CN and IDA groups ($P = 0.159$) but did differ significantly between the ID and IDA groups ($P = 0.038$).

Finally, the composite scores on the learning domain (Figure 4C) follow the pattern CN>ID>IDA with a significant difference between the CN and IDA groups ($P = 0.013$) and the ID and IDA groups ($P = 0.042$). The difference between the groups on

TABLE 2
Components of hematologic factors[1]

Factor name	Variables loaded
Storage	sFt, sTfR, TfRIX,[3] body iron
Transport	Iron, TfSat
Preanemia	MCV, MCH, MCHC, RDW
Anemia	Hb, Hct, RBC

[1] sFt, serum ferritin; sTfR, serum transferrin receptor; TfRIX, transferrin receptor index; TfSat, transferrin saturation; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; RDW, red cell distribution width; Hb, hemoglobin; Hct, hematocrit; RBC, red blood cells. Factor analysis (principal axis with varimax rotation) was used for statistical analysis.

[2] Calculated as log(sTfR/sFt).

TABLE 3
Components of cognitive factors[1]

Domain	Performance factor[2]	Time factor
Attention	RT, SD, TT: no. incorrect TT: no. attempted trials	RT, SD, TT: trial time RT, SD, TT: decision time RT, SD, TT: movement time
Memory	PR, RC: % correct ST: no. incorrect	PR, RC, ST: trial time RC, ST: decision time RC, ST: movement time PR, RC: reaction time
Learning	LR: no. attempted trials LR: no. blocks achieved LR: % correct PM: no. correct	LR: trial time LR, PM: reaction time
All domains	All of the variables listed above	All of the variables listed above

[1] RT, reaction time task; SD, stimulus discrimination task; TT, tachistoscopic threshold task; PR, probed recall task; RC, recognition memory task; ST, Sternberg memory search task; LR, learning task; PM, progressive matrices task. Factor analysis (principal axis with varimax rotation) was used for statistical analysis.

[2] For those tasks that measured "negative" performance, the absolute values of the scores were used; therefore, a higher score on the performance factor is always indicative of better performance.

the performance or time factor for the learning domain was not significant.

The use of the full power of our continuous iron status data by sorting according to our hematologic factors also showed a difference in performance that trended toward significance (for the attention and learning domains) when the upper and lower quintiles for storage factor were analyzed (attention: 0.01 compared with −0.26; $P = 0.091$; memory: 0.03 compared with −0.25; $P = 0.130$; learning: 0.31 compared with −0.43; $P = 0.061$), whereas no difference was found in the time necessary to complete the tasks for the attention and learning domains. However, the amount of time needed to complete the memory tasks differed significantly between the upper and lower quintiles (−0.09 compared with 0.22; $P = 0.031$). In contrast, when the data were compared between the upper and lower quintiles for the anemia factor, the women in the highest quintile completed the tasks in the attention domain significantly faster than did the women in the lowest quintile (−0.11 compared with 0.19, respectively; $P = 0.035$) but no differences were found for the memory and learning domains (memory: −0.12 compared with 0.09, respectively, $P = 0.151$; learning: −0.10 compared with 0.19, respectively, $P = 0.164$). In contrast, performance on the attention, memory, and learning domains did not differ significantly between the upper and lower quintiles for the anemia factor.

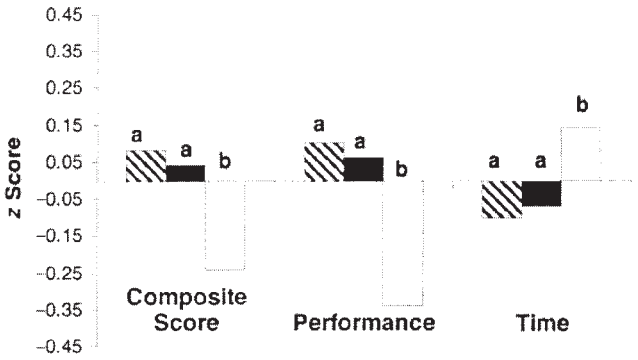

FIGURE 3. z Scores (SD = 1) for combined domains (attention, memory, learning) by group. ▨, Control group ($n = 42$); ■, iron deficiency group ($n = 73$); □, iron deficiency anemia group ($n = 34$). Bars within the same category (composite, performance, or time) with different superscript letters are significantly different, $P < 0.04$. P for trend: composite score ($P = 0.0002$); performance score ($P = 0.036$); time score ($P = 0.031$). Analyses were conducted with ANCOVA (Intelligence Quotient as covariate) with Tukey's test as the post hoc test.

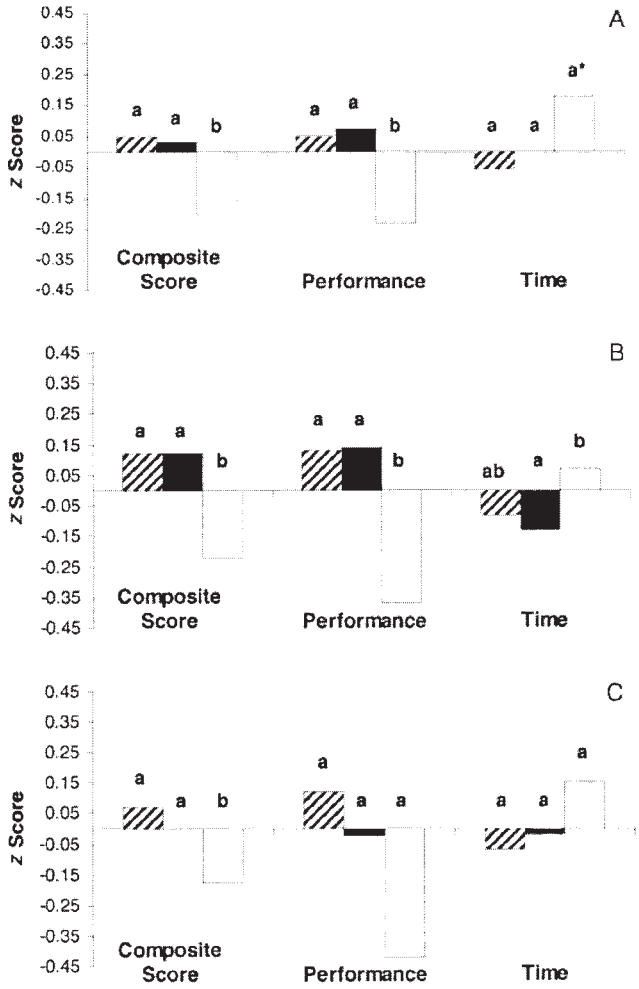

FIGURE 4. z Scores (SD = 1) for the attention (A), memory (B), and learning (C) domains by group. ▨, Control group ($n = 42$); ■, iron deficiency group ($n = 73$); □, iron deficiency anemia group ($n = 34$). A: Bars within the same category (composite, performance, or time) with different superscript letters are significantly different, $P < 0.04$. *Trend toward a significant difference when compared with the CN group ($P = 0.064$). B: Bars within the same category (composite, performance, or time) with different superscript letters are significantly different, $P < 0.04$. C: Bars within the same category (composite, performance, or time) with different superscript letters are significantly different, $P < 0.05$. For all domains, analyses were conducted with ANCOVA (Intelligence Quotient as covariate) with Tukey's test as the post hoc test.

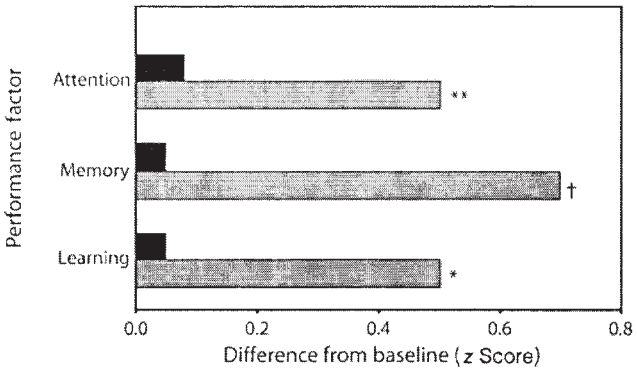

FIGURE 5. Change in performance (z scores with SD = 1) on attention, memory, and learning tasks for women classified as responders (▨; $n = 66$) and nonresponders (■; $n = 47$) with respect to ferritin change. Significantly different from baseline: $^{**}P < 0.01$, $^{\dagger}P < 0.07$, $^{*}P < 0.05$. Analyses conducted with repeated-measures analysis after adjustment for Intelligence Quotient as well as baseline value on any particular task.

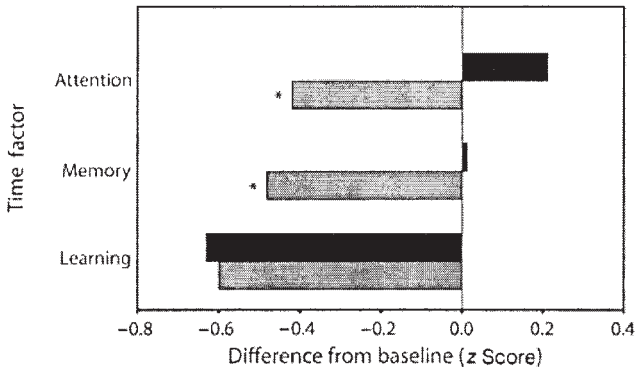

FIGURE 6. Change in time necessary to complete attention, memory, and learning tasks (z scores with SD = 1) for women classified as responders (▨; $n = 33$) and nonresponders (■; $n = 80$) with respect to hemoglobin change. *Significantly different from baseline, $P < 0.01$. Analyses were conducted with repeated-measures analysis after adjustment for Intelligence Quotient as well as baseline value on any particular task.

Longitudinal comparisons

Ferritin responders in our paradigm were defined as women who showed a significant rise in serum ferritin from baseline to the end of the study that was greater than the known biological within-subject day-to-day variability in ferritin (24). Hemoglobin responders were defined in the same way by using the known variability in hemoglobin (24). Those women who experienced a significant improvement in serum ferritin (ferritin responders, $n = 66$) also had improvements in the attention and learning domains and in the memory domain that were 5 and 7 times, respectively, the improvements in the women who did not experience a significant increase in ferritin (ferritin nonresponders, $n = 47$) (**Figure 5**). No significant relation between the size of the ferritin change and the size of the cognitive change was evident, although such a relation may be found with a larger sample size or a longer intervention duration. In contrast to the improvement in performance, the time necessary to complete the tasks did not differ significantly between the women classified as ferritin responders or nonresponders.

The opposite results were found for the hemoglobin responders. The women who showed a significant change in hemoglobin concentration (hemoglobin responders, $n = 33$) over the 16-wk study completed the attention and memory tasks significantly faster ($P < 0.001$ for both) than did the women who did not experience a change in hemoglobin (hemoglobin nonresponders, $n = 80$) (**Figure 6**). As with the ferritin responders, no detectable relation between the size of the hemoglobin change and the size of the cognitive change was evident, although such a relation may be found with a larger sample size or a longer intervention duration. With respect to the learning tasks, both the hemoglobin responders and nonresponders showed significantly faster completion times at the end of the study than at the beginning (Figure 6). This decrease in the time necessary to complete the tasks for the hemoglobin responders did not carry over into an improvement in the performance factor on any of the cognitive tasks.

DISCUSSION

This study is the first in this age group to systematically examine the effect of iron status and intervention on cognitive functioning. We specifically examined the effects of ID and IDA on 3 cognitive domains—attention, memory, and learning. Whereas 8 different tasks were administered to each woman, the current report focuses on the examination of the data at the domain level rather than at the task level. This approach has been proposed as a means of examining the relation between a nutrient deficiency and cognition (25). Penland (25) suggested, because of the many compensatory mechanisms that are available, seeking a consistent change in a class of measures that assesses a meaningful underlying function may be more useful than seeking changes on specific measures.

Examination of the data at the domain (ie, attention, memory, and learning) level as well as across all domains found greater inefficiency in information processing in those women who were iron deficient than in those with normal iron status. The 3 domains that we tested were affected in a similar way. That is, with increasing severity of ID, cognitive performance decreased, whereas the time needed to complete the tasks increased. We also showed that administration of iron sulfate and an improvement in iron status result in an improvement in both performance of and the time necessary to complete learning, memory, and attention tasks. The current report documents the more "global" relations, but future reports will present data on the dimensions of cognition that are specifically altered by dietary ID. It is important to note that many factors influence cognition. One major factor known to be related to cognition is SES, with those from a socially disadvantaged background generally having lower cognitive performance than those from a more advantaged background. Most of the women in the current study were from a middle to upper-middle SES, and no differences were found between the groups with respect to SES. Other variables that were considered as possible confounders in the current study but that had no effect on our findings were age, grade point average, and timing of menstrual cycle in relation to when the cognitive tasks were completed. Whereas it is impossible to measure every potential confounder, the groups in the current study did not differ significantly with respect to any of the confounders measured. Therefore, we have a high level of confidence that our findings of changes in cognition are in fact due to the changes in iron status experienced by these women.

The decreased performance in memory found in the current study is consistent with the observations of Bruner et al (9) of

alterations in memory in iron-deficient adolescent girls. However, their study did not have the capacity to differentiate between changes in anemia and changes in body iron stores because that was not part of the study design. In the current study, ID has a separate effect from anemia because these are 2 discrete factors in the statistical analyses, which are separated statistically from each another. The factor termed storage was related to the performance component of the cognitive tasks as opposed to the time component. This statistical factor contained the variables of sTfR, body iron, TfR index, and ferritin that are normal biomarkers for the variation in iron status preceding the emergence of IDA if there is a negative iron balance. When we focused on just one of these variables, ferritin, it became clear that the women who improved their ferritin status showed improvements in performance on attention, memory, and learning tasks, but that the time to complete the task was unaffected. This relation of changes in ferritin concentrations and in cognitive performance is a highly important relation because it shows that persons do not have to be anemic to have alterations in attention, memory, and learning.

The relation of variation in anemia factor and hemoglobin was of course also explored in both the cross-sectional and the longitudinal analyses. The anemia factor was significantly related to speed of processing in the cross-sectional analysis, and, more specifically, a change in hemoglobin was significantly related to improvement in the speed of processing for the attention and memory tasks in the longitudinal analysis. By utilizing known day-to-day variation in biology for hemoglobin and ferritin to classify women as responders and nonresponders, we were able to examine the strength of association between anemia (hemoglobin) or ID (ferritin) and changes in both the speed of processing and the performance. When examining change, it was not possible to use the factors (as was done in the cross-sectional analyses), because biologic variation in the factors is unknown. Nonetheless, the consistency of the relation between hemoglobin and speed and that between ferritin and performance in both the cross-sectional and longitudinal data analyses give reassurance that our findings are not spurious. These findings are evidence that ID without anemia affects "how well" we do simple and complex cognitive tasks, whereas anemia affects "how fast" we complete those tasks. The functional benefit of iron therapy for the hemoglobin responders compared with that for the ferritin responders differs, which implies different but possibly overlapping causalities. The animal studies that examine changes in brain iron and neurotransmitter metabolism provide strong support for the concept that brain regional responses to iron therapy exist (4).

Whereas a few existing reports document the relation between iron status and cognition in premenopausal women (26–32), almost all of these studies are confounded by other variables (eg, pregnancy, weight loss, and dialysis). Nevertheless, most of the findings are in agreement and provide us with data with which we can compare our results. With respect to observational studies, Kretsch et al (27) reported a correlation of both hemoglobin and transferrin saturation with sustained attention in obese dieting women, whereas Foley et al (28) found a correlation between iron concentrations (ie, zinc protoporphyrin) and spatial performance in male and female undergraduate students. Our observation of a significant difference in the attention domain between iron-sufficient and iron-deficient women is in agreement with these observations. Positive cognitive effects of iron treatment have also been reported in adults. Groner et al (26) observed an improvement in short-term memory with iron supplementation of young pregnant women, whereas several other investigators reported an improvement in quality of life and cognitive functioning as a result of the normalization of iron status via erythropoietin therapy (29–32). One study (32) reported significant improvements in IQ, concentration, speed of information processing, and memory after partial correction of anemia in patients treated with recombinant human erythropoietin. Whereas the abovementioned studies may provide a comparison, the many confounders associated with the treatment of those subjects precludes us from concluding that the improvement in hemoglobin or hematocrit was the sole cause of the improvements seen in cognition. Our data are significant in that they show that normalization of iron status affects mental functioning in otherwise healthy adult women. Thus, the effects of ID on mental functioning are not limited to the early stages of development.

Whereas categorizing persons as iron sufficient or as having ID or IDA is traditionally done through the use of set cutoffs, we felt that this method limited our data; we therefore decided to treat our iron status variables as continuous. This was done by using the hematologic factors, each of which represented a more robust measure than any individual iron status variable and were also the result of a data reduction strategy, thereby eliminating problems with redundant variables and decreasing the probability of type 1 errors. Consequently, sorting the data by the storage factor allowed us to parse out the relation between a deficit in storage iron (and not necessarily other indicators of iron status) and cognition, as compared with sorting the data by the anemia factor, which allowed for the understanding of the relation between those with the most severe anemia and cognition. Specifically, we were able to show that low concentrations of the storage factor were related to performance, whereas low concentrations of the anemia factor were related to the time necessary to complete the specific tasks. We conclude that treating the data as continuous allowed for the detection of subtle cognitive changes that were overlooked when iron status was treated as categorical. We have also shown that running the longitudinal analyses with women classified as responders or nonresponders is much more informative in efforts to detect cognitive changes than is simply running the analyses with women classified according to baseline groups.

The observed association between changes in ferritin and changes in performance strongly suggests that brain ID is causally related to these changes in cognitive performance. The neurologic underpinnings of these findings are uncertain, but it is possible to make a few educated guesses. Animal models show that ID in postweaning life alters brain iron content, biochemical functioning, and neurotransmitter metabolism (4). The aspects that are not dependent on age or development are brain iron and biochemistry, brain energy metabolism, and perhaps resultant cell functioning. Whereas researchers have documented altered transmission in auditory and visual systems in infants with ID (33, 34), similar measurements are lacking in adults. Adult patients with RLS do, however, have a decreased brain iron content, and strong evidence exists that striatum-based dopamine biology is altered (35). Indeed, these patients have dramatic alterations in periodic limb movements that are often responsive to either L-dopamine treatment, iron therapy, or both. The current study subjects did not participate in any biologic measurements of

brain functioning, although previous studies with adults did show asymmetry of electroencephalograms with ID (36, 37).

One neurotransmitter system, dopamine, has been heavily examined in relation to brain ID, and it is well established that dopamine is implicated in memory, learning, and attention as well as in motor control, hormonal regulation, stress responsivity, addiction, and emotional affect (38). Humans with attention deficit disorder or attention deficit hyperactivity disorder often show improvement in attentional performance when methyl phenadate is given (39). This is a multifunctional drug, but its primary target is the dopaminergic system. Studies of pharmacologic challenges in humans found that dopaminergic agonists facilitated cognitive performance (40, 41) and dopaminergic antagonists impaired cognitive performance (42). Studies in the literature also examined the relation between cognitive function deteriorations seen with advancing age and the declines in the nigrostriatal dopamine system across the adult life span. These reports document a strong relation between D2 receptor binding in the striatum and cognitive performance that persists after control for age (43). This is consistent with a change in D2 receptor expression resulting from dietary ID in rodents (44). Other evidence of biochemical abnormalities in iron-deficient adults include increased concentrations of catecholamines in plasma and urine and decreased concentrations of thyroid hormones; both of these abnormalities are normalized after iron therapy (45–47). Whereas plasma and urine measurements are quite distant from brain activity, they do show that IDA can alter neurotransmitter metabolism in adults. Other literature regarding brain ID points to effects on gamma-aminobutyric acid and serotonin metabolism as well as on fundamental cellular bioenergetics (48). It would be speculative for us to suggest any particular causal biochemical pathway at this time; suffice it to point out that numerous feasible possibilities do exist. Whereas most of the evidence to date has been collected in animals, it is hoped that recent advances in technology will serve to elucidate the mechanisms by which iron status affects cognition in humans.

It is important to place these highly artificial cognitive tasks within the framework of functioning in everyday "real life" tasks. We show alterations in memory, learning, and attention with ID. Throughout the day, most persons will be required to attend to various situations, to remember, and even to learn certain information. In some situations, multitasking is expected and may be required. If a person has a deficit in attention, memory, or learning accuracy because he or she have depletion of essential body iron pools, that person's ability to interact with the world at large will be lessened. This impairment may have negative consequences not only for the iron-deficient person but also for those around that person. An example is our recent demonstration that mother-child interactions are negatively affected when the mother is iron deficient (49, 50).

In conclusion, by using a conceptual model of adult intellectual abilities that encompasses a multistage approach, we showed a relation between iron status and information processing in adult women of reproductive age. Future studies should be conducted to replicate these findings and to expand the findings to an understanding of how these cognitive deficits affect everyday life. This study furthers our understanding of the consequences of ID in adults and challenges the traditionally held viewpoint that ID does not have functional consequences until it has reached the level of anemia. Given these findings, better iron status surveillance practices are encouraged to identify persons who may be at risk of cognitive deficits.

We thank the women who agreed to participate in this study; the medical and nursing staff at the Pennsylvania State General Clinical Research Center who performed the blood draws and reviewed each subject's medical history; Carrie Hegedus for her help with recruiting, analysis of the blood samples, and administration of the cognitive tasks.

Both authors were responsible for designing the experiment, analyzing the data, and writing the manuscript; LEM-K was responsible for collecting the data. Neither author had a personal or financial conflict of interest.

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