Randomised study of cognitive effects of iron supplementation in non-anaemic iron-deficient adolescent girls

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Summary
Background Up to 25% of adolescent girls in the USA are iron deficient. This double-blind, placebo-controlled clinical trial assessed the effects of iron supplementation on cognitive function in adolescent girls with non-anaemic iron deficiency.

Methods 716 girls who enrolled at four Baltimore high schools were screened for non-anaemic iron deficiency (serum ferritin $<12$ $\mu$g/L with normal haemoglobin). 98 (13.7%) girls had non-anaemic iron deficiency of whom 81 were enrolled in the trial. Participants were randomly assigned oral ferrous sulphate (650 mg twice daily) or placebo for 8 weeks. The effect of iron treatment was assessed by questionnaires and haematological and cognitive tests, which were done before treatment started and repeated after the intervention. We used four tests of attention and memory to measure cognitive functioning. Intention-to-treat and per-protocol analyses were done.

Findings Of the 81 enrolled girls with non-anaemic iron deficiency, 78 (96%) completed the study (39 in each group). Five girls (three control, two treatment) developed anaemia during the intervention and were excluded from the analyses. Thus, 73 girls were included in the per-protocol analysis. Ethnic distribution, mean age, serum ferritin concentrations, haemoglobin concentrations, and cognitive test scores of the groups did not differ significantly at baseline. Postintervention haematological measures of iron status were significantly improved in the treatment group (serum ferritin 27.3 vs 12.1 $\mu$g/L, $p<0.001$). Regression analysis showed that girls who received iron performed better on a test of verbal learning and memory than girls in the control group ($p<0.02$).

Interpretation In this urban population of non-anaemic iron-deficient adolescent girls, iron supplementation improved verbal learning and memory.

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lower scores on a standardised scholastic achievement test than non-anaemic students. Thus, a better understanding of the possible cognitive effects of iron deficiency in the absence of anaemia is particularly important because of the high rate of this condition in adolescent girls.

The main aim of this randomised, double-blind, placebo-controlled trial was to examine the effects of iron supplementation on attention, memory, and learning in non-anaemic iron-deficient adolescent girls.

Methods
The study was based at two public (ie, state-supported) high schools and two private Catholic high schools in Baltimore, Maryland, USA. During August and September, 1993, girls who enrolled at the four schools (grades 9–12 [ages 13–18]) were asked to take part in a voluntary screening for iron deficiency, which consisted of a complete blood count and measurement of serum ferritin concentration. An explanatory letter and consent form were sent to all girls who attended the schools, and distributed at school open-houses and in science and health classes. Written consent from a parent or guardian was required for participation in the screening.

Students completed a brief demographic and medical history questionnaire before blood samples were taken at the school by venepuncture. Haemoglobin concentrations were obtained from a complete blood count, and serum ferritin was measured by chemilumino-metric immunoassay (CIBA Corning Diagnostic Corporation, Norwood, Massachusetts, USA). All laboratory tests were done by SmithKline Beecham Clinical Laboratories.

We used established race-adjusted and age-adjusted values for haemoglobin and serum ferritin. Girls were classified as normal (non-iron deficient), non-anaemic iron-deficient, or anaemic. Anaemia was defined as a haemoglobin concentration of less than 11·5 g/dL for African American girls or less than 12·0 g/dL for white girls. Non-anaemic iron deficiency was defined as a serum ferritin concentration of less than 12·0 µg/L with a normal haemoglobin concentration. Only girls with non-anaemic iron deficiency were eligible for enrolment in the trial.

After we obtained written informed consent from the student and a parent or guardian, eligible participants were randomly assigned to a treatment or control group. Assignment was done by computer-generated random number lists, in blocks of four with stratification by school. Participants and investigators were unaware of group assignment. Participants were asked not to take any vitamins or iron supplements during the trial.

After randomisation, baseline cognitive functioning was assessed. Standard cognitive tests were administered by trained research assistants at the participants’ schools; three measures of attention and one multicomponent test of verbal learning and memory were used. The Brief Test of Attention (BTA) is a measure of auditory divided attention, in which participants listen to a tape of letters and numbers (eg, 4-A-8-G-3-2) and are asked to report how many numbers or letters they hear. The Symbol Digit Modalities Test (SDMT) is a timed measure of visual attention, motor speed, and rapid coding, in which participants print the number that corresponds to a written symbol listed at the top of the test page and the task is then repeated with the participant saying the digits. The Visual Search and Attention Test (VSAT) is a timed test of visual scanning, target detection, and cancellation, in which participants locate and cross out letters or symbols that look like the target. The Hopkins Verbal Learning Test (HVLT) is a 12-item, semantically categorised word-list learning test with three free recall trials, a delayed recall trial, and yes/no recognition; participants are read the same list of words three times and each time are asked to repeat as many words as they can recall, 20 min later they are asked to say which words they remember, and are read 24 words which include the original 12 words plus 12 semantically related and unrelated words.

Participants were randomly allocated a non-prescription ferrous sulphate preparation (Feosol, SmithKline Beecham) or placebo for 8 weeks. Two 325 mg tablets of ferrous sulphate were taken twice daily; the daily dose of 1300 mg was equivalent to 260 mg elemental iron daily. Iron and placebo (SmithKline Beecham) tablets were identical in appearance and dose regimen. Research assistants were unaware of treatment allocation throughout the study; treatment received was not disclosed until all subjects had completed postintervention testing.

We ensured optimum compliance in several ways. Research assistants administered one dose of treatment or placebo every school day; on weekends and holidays girls were contacted at home and reminded to take their tablets. In addition, researchers worked closely with girls and their families to encourage continuing participation, monitor compliance, and ask about side-effects. Every week girls received their supply of tablets and were asked to report any doses that they had not taken. Finally, participants were given small prizes at school, which they could receive only at the time scheduled for that day’s regimen.

After treatment had stopped, haematological and cognitive tests were repeated and participants completed a brief questionnaire about their group assignment (iron, placebo, don’t know), any side-effects (nausea, stomach ache, headache, diarrhoea, change in stool colour, constipation, other); and behavioural and cognitive changes in energy, attention, memory, mood, and sleep pattern (more/better, the same, less/worse).

We estimated that 70 participants (35 in each group) were required to detect a 0·5 SD change in cognitive test scores by a one-tailed Students’ t test (α = 0·05, β = 0·1). The initial intention-to-treat analyses were done by χ². Student’s t test, and linear regression; a per-protocol analysis was then done.

The study was approved by the Department of Research and Evaluation of the Baltimore City Public Schools, the Division of Schools of the Archdiocese of Baltimore, and the Joint Committee on Clinical Investigation of the Johns Hopkins Medical Institutions.

Results
Signed consent forms for the screening blood tests were obtained from 803 of about 2000 female students who enrolled at the four schools (figure 1). 716 girls were
than the control group (18·2 [SD 12·6] g/L, p<0·001; table 2). Similarly, girls who took iron had a significantly higher mean haemoglobin concentration (g/dL) at the start of treatment. After the 8-week intervention, the treatment group had a similar haemoglobin concentration in both groups at the start of treatment. After the 8-week intervention, the treatment group had a significantly higher mean serum ferritin concentration than the control group (18·2 [SD 12·6] g/L, p<0·001; table 2). Similarly, girls who took iron had a significantly higher mean haemoglobin concentration than the control group (18·2 [SD 12·6] g/L, p<0·001).

Factor analysis of the cognitive test scores showed four distinct factors; therefore, each test was analysed separately. We used multiple-linear regression analysis to assess the effect of iron treatment on postintervention cognitive test scores, after adjustment for baseline scores (table 3). Iron treatment had no significant effect on postintervention BTA, SDMT, or VSAT scores (the three measures of attention). However, on the total recall score of the HVLT (sum of trials 1–3), girls who took iron showed significant improvement over baseline and end of treatment compared with the control group (p<0·02). Baseline performance on the HVLT accounted for 93% of the variability in postintervention scores, whereas treatment condition accounted for the remainder. However, there were no significant differences between groups in other components of the HVLT (delayed recall, yes/no recognition). With the exception of one outlier, analysis of residuals showed no deviations from linear regression assumptions of linearity, constant variance, and normal distribution of standardised residuals. After adjustment for baseline scores, the correlation between the change in serum ferritin and postintervention score on the HVLT was 0·21 (p=0·04).

We compared preintervention and postintervention scores on each trial of the HVLT to assess differences detected between groups in total HVLT scores. The sum of the three learning trials of the HVLT is the HVLT total score (maximum score 36). We used ANOVA to analyse the HVLT learning curve by group assignment (treatment or control), session (baseline or postintervention), and trial (three free recall trials per test; figure 2). At baseline, both groups had similar learning curves. All girls recalled more words at each successive trial than girls in the control group. However, the effect of group assignment on postintervention cognitive test scores was not significant (F_{2,142}=2·67, p=0·06). The three-way interaction of group by session by trial was not significant (F_{2,142}=p=0·85).
Although all features of cognition in whom iron deficiency had a negative effect on language girls accord with previous research on infants and toddlers intention-to-treat analyses. Our findings in adolescent memory was shown in both the per-protocol and iron supplementation improves some aspects of cognitive The findings suggest that, even in the absence of anaemia, iron supplementation improves some aspects of cognitive performance among adolescent girls. More than 6% of girls developed iron-deficiency anaemia during the study. By definition, none of these girls was anaemic at the start of the intervention. However, in the treatment group, iron therapy increased mean haemoglobin concentrations and mean cell volume, whereas in the control group there was a mean decrease in haemoglobin concentration, which suggests that some girls did have a degree of biochemical iron deficiency.

The methodological limitations of our study need to be considered. Ferritin was the only measure of iron status in the study. Primary statistical analysis was by group assignment rather than changes in serum ferritin, because serum ferritin concentrations can increase secondary to infection or inflammation. A linear correlation was also seen between iron status and cognitive function. By contrast, Fordy and Benton measured ferritin concentrations in young men and women and found no association between low iron status and cognitive functioning. One possible explanation for these conflicting findings is that the effect of iron deficiency on cognitive function may be subtle. We did not assess whether there were any baseline differences in cognitive functioning between normal and iron-deficient adolescents.

Iron treatment had no effect on the three measures of attention. Previous studies have shown that iron deficiency has a detrimental effect on attention, but it is not clear why our study did not detect this effect. Perhaps the measures we used were not sensitive enough to detect changes in attention. For example, mean baseline scores on the BTA were at or near 100% of the possible test score in both groups; this ceiling effect may have limited the ability of the BTA to detect any changes in attention between the groups. We assessed the effects of iron supplementation only on standardised cognitive tests; further research is needed to assess whether such cognitive effects are limited to neuropsychological measures or are also evident in academic performance.

Although iron fortification of formulas and cereals has improved the iron status of infants and toddlers, young women remain at high risk of iron deficiency and anaemia—the prevalence of iron deficiency in this population was more than 15%. This study suggests that, even in the absence of anaemia, iron supplementation improves verbal learning and memory among adolescent...
girls, which suggests that further investigations of the non-haematological effects of iron deficiency are warranted.

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