Early reports

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

Ann B Bruner, Alain Joffe, Anne K Duggan, James F Casella, Jason Brandt

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 μg/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 μg/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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Division of General Pediatrics, Departments of Pediatrics (A B Bruner MD, A Joffe MD, A K Duggan ScD) **and Hematology** (J F Casella MD), **and Division of Medical Psychology, Department of Psychiatry and Behavioural Sciences** (J Brandt PhD), **Johns Hopkins University School of Medicine, Baltimore, Maryland 21287-2530, USA**

Correspondence to: Dr Ann B Bruner

Introduction

Iron deficiency is the most common nutritional disorder, which affects about 20% of the world population. This disorder is not limited to developing countries, and is the main cause of anaemia in the USA.[1] Iron deficiency is a systemic condition, which has many non-haematological consequences: it impairs physical endurance, work capacity, infant growth and development, and depresses immune function.[2,3]

Since the initial studies by Oski and Honig,[4] most research on the cognitive effects of iron deficiency has focused on infants and very young children (toddlers). Several studies have shown that iron deficiency causes changes in behaviour and lowers development test scores in infancy.[5,6] Animal models have revealed several mechanisms by which iron deficiency may affect cognition; these include changes in brain iron content and distribution, and in neurotransmitter function. Body iron stores, such as central nervous system iron, decrease before red blood cell production is affected by iron deficiency. Changes in cognition and behaviour may be independent of haemoglobin concentrations; for example, pica is a well-known manifestation of iron deficiency from which individuals recover soon after the start of iron therapy.[7,8] In addition, people who receive iron for iron-deficiency anaemia commonly report improved memory, attention, mood, and energy before any improvement in haemoglobin indices.[9] Decreased brain iron stores may impair the activity of iron-dependent enzymes necessary for the synthesis, function, and degradation of neurotransmitters, such as dopamine, serotonin, and noradrenaline.[10] During the past 10 years, the increased use of iron-fortified formulas and cereals has improved the iron status of children and reduced the prevalence of iron-deficiency anaemia.[11] However, adolescent girls and young women are still at high risk of developing iron deficiency because of increased iron demands during puberty, menstrual losses, and limited dietary iron intake. Prevalence estimates of iron deficiency in adolescent girls range from 9% to 40%, depending on the population studied and the criteria used to define iron deficiency.[12] The Second National Health and Nutrition Examination Survey (1976–80) found that 14·2% of adolescent girls in the USA were iron deficient.[13]

The non-haematological sequelae of iron deficiency in adolescents have not been well defined. A study by Groner et al[14] of pregnant young women found better performance on psychometric tests in women treated with iron than in controls. Ballin and colleagues[15] found that iron-treated adolescent girls reported decreased lassitude and improved mood and ability to concentrate. Many studies of the systemic effects of iron deficiency have focused on individuals with iron-deficiency anaemia. Webb and Oski[16] observed that anaemic students had

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 chemiluminometric 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.[17] Non-anaemic iron deficiency was defined as a serum ferritin concentration of less than 12·0 µg/L with a normal haemoglobin concentration.[18,19] 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)[20] 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)[21] 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)[22] 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)[23] 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 schoolday; 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 questionnnaire 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 χ^2, Student's t test, and linear regression; a per-protocol analysis was then done.[24]

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

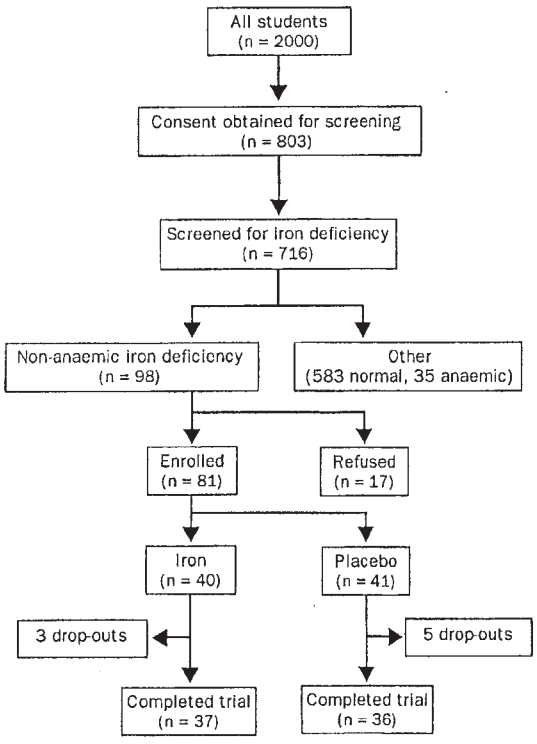

Figure 1: **Profile for randomisation into iron versus placebo**

	Treatment (n=37)	Control (n=36)	p*
Age (years)	16·2 (1·1)	157 (1·2)	0·06
Ethnic origin			
African American	16 (43%)	20 (56%)	
White	21 (57%)	16 (44%)	0·29
School			
Public	14 (38%)	16 (44%)	
Private	23 (62%)	20 (56%)	0·56
Haematological measurements			
Haemoglobin concentration (g/dL)	13·1 (0·7)	13·0 (0·7)	0·49
Mean cell volume (fL)	86·1 (3·8)	84·4 (4·8)	0·10
Red cell distribution width (%)	13·2 (0·9)	13·5 (1·1)	0·16
Serum ferritin concentration (μg/L)	9·1 (2·2)	8·5 (2·6)	0·28
Cognitive functioning test scores			
SDMT	118·1 (19·3)	115·1 (14·9)	0·46
VSAT	125·4 (22·5)	130·0 (22·9)	0·39
BTA	16·1 (2·6)	16·1 (2·7)	0·96
HVLT	25·2 (4·3)	24·9 (4·1)	0·72

Data are mean (SD) unless otherwise shown. *Two-tailed Student's *t* test or χ^2. SDMT=Symbol Digit Modalities Test; VSAT=Visual Search and Attention Test; BTA=Brief Test of Attention; HVLT=Hopkins Verbal Learning Test.

Table 1: **Characteristics of patients at start of treatment**

subsequently tested for iron deficiency (87 were absent or refused to take part). Of these 716 girls, 112 (16%) were iron deficient—14 had iron-deficiency anaemia and 98 were not anaemic and therefore eligible to enrol in the trial. 17 of the 98 non-anaemic iron-deficient girls did not want to take part in the trial. Of the 81 girls who enrolled, 78 (96%) completed the intervention (two girls transferred to other schools, one withdrew on the first day of the intervention) and were included in the initial intention-to-treat analyses. Five girls (three control, two treatment) developed anaemia during the intervention and were, therefore, excluded. 73 girls were therefore included in the final per-protocol analyses.

Both groups were well matched in terms of ethnic distribution, mean age, and school attended (table 1). Baseline haematological and cognitive measures were similar 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] *vs* 3·5 [6·6] μg/L, p<0·001; table 2). Similarly, girls who took iron had a significantly higher mean haemoglobin concentration than the control group (13·5 [0·8] *vs* 12·7 [0·7] g/dL, p<0·001).

Factor analysis of the cognitive tests 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

Cognitive test	R^2	R^2 change attributable to iron therapy	Baseline score	p
Attention				
SDMT	0·49	N/A*	−0·43	0·90
VSAT	0·41	N/A*	−1·39	0·75
BTA	0·21	N/A*	−0·23	0·64
Learning				
HVLT	0·25	0·07	1·79	<0·02

Independent variables: group assignment and baseline score; dependent variable: postintervention score. *Not applicable because independent variable did not reach significance for entry into equation. SDMT=Symbol Digit Modalities Test; VSAT=Visual Search and Attention Test; BTA=Brief Test of Attention; HVLT=Hopkins Verbal Learning Test.

Table 3: **Summary of stepwise linear-regression analysis of group assignment on postintervention cognitive test scores**

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 ($F_{2,142}$=273·70, p<0·001) with no significant differences between groups. Although both groups did better after the intervention ($F_{1,71}$=6·30, p<0·02), girls who took iron recalled more words at each successive trial than girls in the control group. However, the effect of group assignment on session did not achieve significance ($F_{1,71}$=2·67, p=0·106). The three-way interaction of group by session by trial was not significant ($F_{2,142}$ p=0·85).

65 of 73 subjects were available to complete the postintervention questionnaire on side-effects, subjective behaviour, and cognitive changes (table 4). Blinding to group assignment was successful. There was no significant difference between groups when girls were

	Treatment (n=37)	Control (n=36)	p*
Haemoglobin concentration (g/dL)			
Mean	13·5 (0·8)	12·7 (0·7)	<0·001
Change since start of intervention	0·4 (0·8)	−0·3 (0·5)	<0·001
Mean cell volume (fL)	88·5 (3·6)	85·1 (4·8)	0·001
Red cell dustribution width (%)	13·3 (1·2)	13·6 (1·0)	0·41
Serum ferritin concentration (μg/L)			
Mean	27·3 (13·2)	12·1 (7·6)	<0·001
Change since start of intervention	18·2 (12·6)	3·5 (6·6)	<0·001

*Two-tailed Student's *t* test. Data are mean (SD).

Table 2: **Haematological measurements at end of treatment**

	Treatment (n=34)	Control (n=31)	p*
Correctly guessed treatment	21 (61·8%)	14 (45·2%)	0·18
Side-effects			
Constipation	2 (5·9%)	1 (3·2%)	0·61
Change in stool colour	22 (64·7%)	3 (9·7%)	<0·001
Reported improvements			
Energy	12 (35·3%)	7 (22·6%)	0·26
Mood	5 (15·2%)	6 (16·7%)	0·87
Concentration	8 (25·8%)	6 (20·0%)	0·59
Memory	7 (21·2%)	3 (9·7%)	0·20

*χ^2.

Table 4: **Postintervention questionnaire**

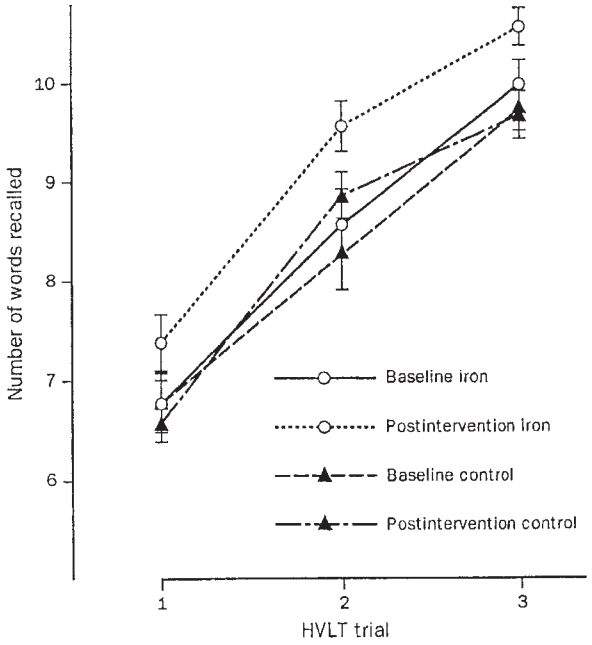

Figure 2: **HVLT learning curves**
Mean (SE) by group, session (baseline or postintervention), and trial (three free recall trials per test) at baseline and postintervention.

asked to guess whether they had taken iron or placebo: 21 (62%) girls in the treatment group and 14 (45%) in the control group correctly guessed their group assignment (p=0·18). Regression analysis did not show any association between cognitive performance and which intervention girls thought they had received. Overall, both groups reported the same frequency of side-effects: abdominal pain (26%), constipation (4%), diarrhoea (6%), headache (20%), nausea (19%). However, significantly more iron-treated girls reported changes in stool colour than girls in the control group (22 [65%] *vs* 3 [10%], p<0·001). Data on subjective behaviour changes were divided into two groups (more/better or same/less/worse) for analytical purposes because of the small number of participants. We found no significant differences in energy, mood, or attention; a few more girls who took iron reported slightly more improvement in memory than girls in the control group (7 [21%] *vs* 3 [10%], p=0·20).

Discussion

The findings suggest that, even in the absence of anaemia, iron supplementation improves some aspects of cognitive functioning in iron-deficient adolescent girls. The positive effect of iron supplementation on verbal learning and memory was shown in both the per-protocol and intention-to-treat analyses. Our findings in adolescent girls accord with previous research on infants and toddlers in whom iron deficiency had a negative effect on language development.[25] Although all features of cognition are important, verbal learning and memory are particularly important in terms of academic performance.

The effect of iron therapy on learning, shown by the increased HVLT scores of the treatment group, raises questions about the overall effects of iron deficiency on cognition. Although there was a significant difference between groups in total HVLT scores, the difference across trials did not achieve significance. This finding may have been due to the small number of participants, because our study was designed to detect significant changes on total cognitive test scores rather than subtest or trial scores. Although causal mechanisms by which iron deficiency may alter brain function have not been thoroughly defined, various theories have been proposed. Animal models show that iron deficiency is associated with changes in: neurotransmitter synthesis, uptake, and degradation; mitochondrial function; brain iron deposition; protein synthesis; and oxidation-reduction and electron transport.[26] Little clinical research to validate such animal models has been done. However, one study[27] showed an association between ferritin concentrations and electroencephalographic asymmetry, and another[28] observed changes in urinary catecholamines in iron deficient infants, which returned to normal after iron therapy. Clearly, the association between iron status and cognition is complex, and further research is needed.

Our study focused on the effects of iron deficiency in the absence of anaemia in a cohort of 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 serrum 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[29] measured ferritin concentrations in young men and women and found no association between low iron status and psychological 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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References

1 Cook J, Finch C. Assessing iron status of a population. *Am J Clin Nutr* 1979; **32**: 2115–19.
2 Dallman PR. Manifestations of iron deficiency. *Semin Hematol* 1982; **19**: 19–30.
3 Oski FA. The nonhematologic manifestations of iron deficiency. *Am J Dis Child* 1979; **133**: 315–22.
4 Oski F, Honig A. The effects of therapy on the development scores of iron deficient infants. *J Pediatr* 1978; **92**: 21–25.
5 Oski F, Honig A, Helu B, et al. Effect of iron therapy on behavior performance in nonanaemic, iron deficient infants. *Pediatrics* 1983; **71**: 877–80.
6 Lozoff B, Brittenham G, Wolf A, et al. Iron deficiency anemia and iron therapy effects on infant developmental test performance. *Pediatrics* 1987; **79**: 981–95.
7 Fairbanks V, Beutler E. Iron deficiency. In: Beutler E, ed. Williams Hematology, 5th edn. New York: McGraw-Hill, 1995: 495.
8 Pollitt E, Leibel RL. Iron deficiency and behavior. *J Pediatr* 1976; **88**: 372–81.
9 Yehuda S, Youdim MBH. Brain iron: a lesson for animal models. *Am J Clin Nutr* 1989; **50**: 618–29.
10 Dallman PR. Biochemical basis for the manifestation of iron deficiency. *Annu Rev Nutr* 1986; **6**: 13–40.
11 Hallberg L, Hulten L, Lindstedt G, et al. Prevalence of iron deficiency in Swedish adolescents. *Pediatr Res* 1993; **34**: 680–87.
12 Scott D, Pritchard JA. Iron deficiency in healthy young college women. *JAMA* 1967; **199**: 897–900.
13 Expert Scientific Working Group. Summary of a report on assessment of the iron status of the United States populations. *Am J Clin Nutr* 1985; **42**: 1318–30.
14 Groner JA, Holtzman NA, Charney E, et al. A randomized trial of oral iron on tests of short-term memory and attention span in young pregnant women. *J Adolesc Health Care* 1986; **7**: 44–48.
15 Ballin A, Berar M, Rubinstein U, et al. Iron state in female adolescents. *Am J Dis Child* 1992; **146**: 803–05.
16 Webb TE, Oski FA. Iron deficiency anemia and scholastic achievement in young adolescents. *J Pediatr* 1973; **82**: 827–30.
17 Williams WJ, Morris MW, Nelson DA. Examination of the blood. In: Beutler E, ed. Williams hematology, 5th edn. New York: McGraw-Hill, 1995: 8–15.
18 Dallman PR, Siimes MA, Stekel A. Iron deficiency in infancy and childhood. *Am J Clin Nutr* 1980; **33**: 86–118.
19 Cook JD, Skikne BS. Iron deficiency: definition and diagnosis. *J Int Med* 1989; **226**: 349–55.
20 Schretlen D, Bobholz J, Brandt J. Development and psychometric properties of the brief test of attention. *Clin Neuropsychol* 1996; **10**: 80–89.
21 Smith A. Symbol digit modalities test, manual. Los Angeles: Western Psychological Services, 1973.
22 Psychological Assessment Resources. Visual search and attention test: professional manual. Odessa, Florida: Psychological Assessment Resource Inc, 1990.
23 Brandt J. The Hopkins verbal learning test: development of a new memory test with six equivalent forms. *Clin Neuropsychol* 1991; **5**: 125–42.
24 Newell DJ. Intention-to-treat analysis: implications for quantitative and qualitative research. *Int J Epidemiol* 1992; **21**: 837–41.
25 Walter T. Effects of iron-deficiency anaemia on cognitive skills in infancy and childhood. *Baillieres Clin Haematol* 1994; **7**: 815–27.
26 Beard JL, Connor JD, Jopnes BC. Brain iron: location and function. *Prog Food Nutr Sci* 1993; **17**: 183–221.
27 Tucker DM, Sandstead HH, Swenson RA, et al. Longitudinal study of brain function and depletion of iron stores in individual subjects. *Physiol Behav* 1982; **29**: 737–40.
28 Voorhess ML, Stuart MJ, Stockman JA, et al. Iron deficiency anemia and increased urinary norepinephrine excretion. *J Pediatr* 1975; **86**: 542–47.
29 Fordy J, Benton D. Does low iron influence psychological functioning? *J Hum Nutr Diet* 1994; **7**: 127–33.

Can iron supplementation improve cognitive functioning?

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Bruner and colleagues have examined the effects of iron supplementation on cognitive function in adolescent girls with iron deficiency. The idea that intellectual performance can be affected adversely by nutritional deficiencies, which can then be remedied by taking nutritional supplements, is not new. Those who remember the vitamins and intelligence quotient story will have a sense of *déjà vu*.[1] However, iron deficiency is widespread, so Bruner and colleagues' study is potentially important.

It is unclear whether the primary objective for the study is to further understanding of the effects of iron deficiency on cognitive function in adolescent girls through rigorous experimental design (an explanatory approach), or whether it is to investigate the case for prescribing regular iron supplementation in adolescent girls with iron deficiency (a pragmatic approach). In practice the trial is likely to contribute to both debates, but the most appropriate study design and analysis differ for the two objectives. Murray[2] gives a clear discussion of the differences, and on balance the study falls into the explanatory category. The subjects chosen for the study were iron-deficient but not anaemic, with subsequent exclusion of those becoming anaemic. Treatment was given for only 8 weeks, many strategies were used to ensure compliance, and other vitamins or iron supplements were prohibited. The outcome measures were the standardised cognitive measures of attention and verbal learning and memory, rather than more clinically relevant measures, such as school performance. The conduct of the study was fairly intensive, with both haematological and cognitive testing at baseline and the end of the study. Analysis was per protocol, which includes only non-anaemic girls completing treatment, rather than by intention-to-treat, which includes all patients as randomised.

The trial looked at four endpoints: improvement from baseline in three measures of attention, and one multicomponent test of verbal learning and memory. Only the last of these showed any statistically significant difference between the treatment and control groups. And even that was only in one component of the test: other aspects did not show a significant difference between groups. This finding is difficult to interpret. It could just be a chance result, it may be that the trial was inadequately powered for the other endpoints, or it may be that there is some subtle effect on one aspect but not the others. Had the trial been pragmatic it might have been important to specify which was the primary endpoint, or to make some adjustment for multiple-hypothesis testing, which would reduce the statistical significance level of the verbal learning and memory results. For an explanatory trial this is not necessary, but it would be useful to see confidence intervals presented for between-group comparisons for all of the endpoints. This would help clarify the potential magnitude of differences on these endpoints, and provide important information for anybody planning further trials, or wishing to review this trial alongside other trials.

Bruner and colleagues recommend further investigations. One should be explicit about the purpose of further studies. Logically the scientific question comes first: to demonstrate whether iron deficiency has measurable effects on attention or verbal learning and memory. How such an effect should be remedied is a much more difficult question. Dietary change would be one strategy, but is a delicate issue in teenage girls, among whom eating disorders are prevalent. The alternative, of long-term supplementation, raises complex issues. Trials set up for this purpose would need to follow a pragmatic model, to mirror the situation likely to occur in clinical practice, to include long-term follow-up of clinically relevant endpoints using an intention-to-treat approach, and to consider long-term safety very carefully.

The authors conclude that iron supplementation improved verbal learning and memory in iron-deficient adolescent girls. This is a small explanatory study designed to explore potential mechanisms, which found a difference in only one of four endpoints studied. It should not be mistaken for a pragmatic trial designed to confirm whether any potential benefit could be realised in practice. Until the results of such studies are available, to improve cognitive functioning, a balanced diet and a good night's sleep are unlikely to go amiss.

Deborah Ashby
Departments of Mathematical Sciences and Public Health, University of Liverpool, Liverpool L69 3BX, UK

1 Benton D, Roberts G. Effects of vitamin and mineral supplementation on intelligence of a sample of school-children. *Lancet* 1988; i: 140–43.
2 Murray GD. Statistical aspects of research methodology. *Br J Surg* 1991; 78: 777–81.
3 Lewis JA, Machin D. Intention to treat—who should use ITT. *Br J Cancer* 1993; 68: 647–50.

Interferon therapy for hepatitis C

Hepatitis C virus (HCV) affects at least 200 million people worldwide, and although infection can result in progressive liver disease, there may be several decades between exposure to the virus and development of complications.[1] In one study, cirrhosis was found at presentation in 50% of patients, and life-threatening complications occurred in 15% in the subsequent 4 years.[1] This study suggests that infection causes rapid progression to serious disease in a substantial proportion of patients. However, in a population-based study of patients with post-transfusion hepatitis followed up for 18 years after exposure no difference in overall mortality and only a minor increase in liver-related mortality was observed in study patients compared with control patients.[2] Both these studies show that HCV infection can cause progressive liver disease, yet neither study addresses adequately the risk of progression in the individual. Factors that may influence natural history include mode of acquisition, viral load, viral genotype, and concomitant alcohol use. Of these, the data are strongest for genotype 1b and alcohol.

Interferon is the only drug approved for HCV infection in the USA and Europe, at a dose of 3 MU three times a week for 24 and 48 weeks, respectively. Candidates for treatment have persistently raised serum alanine aminotransferase (ALT) concentrations and hepatic inflammation on biopsy. Treatment is inadvisable in those with hepatic decompensation or contraindications such as thrombocytopenia, severe psychiatric illness, and significant non-hepatic disease. Although initial responses are acceptable at 50%, sustained off-treatment response

reminded of a 51-year-old woman who received octreotide because of daily losses of 6 L of fluid through her jejunostomy. After the first dose of 100 μg she developed upper abdominal pain and serumamylase rose to 18 μkat/L. After stopping concomitant codeine treatment the patient tolerated further injections of octreotide well. The duration of pancreatitis, lasting for days, in two patients after the single dose of octreotide is consistent with retention of activated pancreatic enzymes due to outflow obstruction.[3]

Octreotide and natural somatostatin inhibit secretion of pancreatic juice and release of hormones relaxing the sphincter of Oddi. However, while somatostatin has a relaxing effect on the sphincter of Oddi, octreotide seems not to.[3] After octreotides spasm can be induced in susceptible patients.[3] Pancreatitis occur, more often after octreotide than placebo when administered before ERCP[4] and the papilla can be more difficult to cannulate.[5]

Our third patient did not develop pancreatitis during treatment with codeine before nor with octreotide after her single attack of pancreatitis. The tendency of codeine to contract the sphincter of Oddi may have made her susceptible to the contractile effect of octreotide. Thus, the concomitant treatment with both drugs probably induced spasm of the sphincter of Oddi and pancreatitis.

We suggest that this side-effect of octreotide should be part of the drug information for octreotide, both available to the authorities and when the drug is promoted to doctors.

*Göran Bodemar, Henrik Hjortswang
Department of Medico-Surgical Gastroenterology and Internal Medicine, University Hospital, S-581 85 Linköping, Sweden

1 Paran H, Neufeld D, Mayo A, et al. Preliminary report of a prospective randomised study of octreotide in the treatment of severe acute pancreatitis. *J Am Coll Surg* 1995; 181 (2): 121–24.
2 Buchler M, Friess M, Klempa I, et al. Role of octreotide in the prevention of postoperative complications following pancreatic resection. *Am J Surg* 1992; 163: 125–31.
3 Jenkins SA, Berein A. Review article: the relative effectiveness of somatostatin and octreotide in pancreatic disease. *Aliment Pharmacol Ther* 1995; 9 (4): 349–61.
4 Sternlieb JM, Aronchick CA, Retig JN, et al. A multicentre randomised controlled study trial to evaluate the effects of prophylactic octreotide on ERCP-induced acute pancreatitis. *Am J Gastroenterol* 1992; 87: 1561–66.
5 Binmoeller KT, Harris AG, Dumas R, Grimaldi C, Delmont JP. Does the somatostatin analogue octreotide protect against ERCP induced pancreatitis. *Gut* 1992; 33:1129–33.

Iron supplementation and cognitive function

SIR—Ashby (Oct 12, p 973),[1] commenting on a study of the effects of an iron supplement given to iron-deficient adolescent girls,[2] in which benefit was evident in only one of the four end-points investigated, emphasises the need for trials to ascertain whether any benefit can be realised in actual practice, such as in school performance. This need is especially apposite in the African setting, where the huge mass of the people are impoverished, and moreover, where most have non-dietary as well as dietary disadvantages, including iron-deficiency anaemia. Among children, we may wonder whether a benefit from iron supplementation would be manifest, say, in school examination results. Lozoff,[3] has called for long-term studies to evaluate factors such as poor school achievement, vocational versus academic career tracking, absenteeism, school drop-out tendencies, and behavioural problems.

We believe that the benefits of supplementation should be scrutinised in all instances of nutritional shortfalls. Among black Africans, there is the intriguing situation of very low calcium intake and the apparent absence of deficiency stigmata.[4] Daily intake of calcium is usually half or less of the recommended dietary allowance. The intake of elderly black African women is very much less than the 1000–1500 mg now recommended for the avoidance of hip fracture. To comply with the dietary guidelines, all such Africans would require supplements, but this is not really practical. Besides, do Africans really need the intake of calcium recommended? Breast milk has a satisfactory composition, as shown in extensive studies in the Gambia;[5] calcium-deficient rickets is very rare; rural African children have excellent teeth; and in South Africa, the frequency of hip fracture in elderly black African women is still about one tenth of that in white women. In the particular context described, it is not unreasonable to question the merit of including calcium in the dietary recommendations for this population.

While the developed countries are reducing their public funding of health services, in developing states the effects of funding restrictions are even more acute, as frequently described in *The Lancet*. In the context of poverty, we need to know what the minimum intakes of nutrients consistent with reasonable health performance and wellbeing really are. In situations in which specific supplementation is called for, the benefits to be gained must be clear, and—to secure funding—the proposed supplementation must be able to compete, in terms of cost-benefits, with other interventions, dietary and non-dietary, in the quest for disease avoidance and the maintenance of health.

*A R P Walker, B F Walker
Human Biochemistry Research Unit, Department of Tropical Diseases, School of Pathology of the University of the Witwatersrand, and the South African Institute for Medical Research, Johannesburg, South Africa, 2000

1 Ashby D. Can iron supplementation improve cognitive functioning? *Lancet* 1996; 348: 973.
2 Bruner AB, Jaffe A, Duggan AK, Casella JF, Brandt J. Randomised study of cognitive effects of iron supplementation in non-anaemic iron-deficient adolescent girls. *Lancet* 1996; 348: 992–96.
3 Lozoff B. Iron deficiency and infant development. *Pediatr* 1994; 125: 577–78.
4 Walker ARP, Walker BF. Nutrition-related diseases in Southern Africa: with special reference to urban populations in transition. *Nutr Res* 1995; 15: 1053–94.
5 Prentice A, Jarjou LMA, Cole TJ, Stirling DM, Dibba B, Fairweather-Tait S. Calcium requirements of lactating Gambian mothers: effects of a calcium supplement on breast-milk calcium concentration, maternal bone mineral content, and urinary calcium excretion. *Am J Clin Nutr* 1995; 62: 58–67.

SIR—Ashby's[1] commentary on Bruner and colleagues'[2] report is important. Her warning that long-term follow-up of clinically relevant endpoints should be included and long-term safety should be carefully considered in all intention-to-treat approaches needs to be applied to the recommendations for iron supplementation in healthy and patient populations. There is a need of public health efforts to detect hereditary haemochromatosis and to prevent the consequences of this common but under-recognised genetic disorder.

Feder and colleagues[3] reported two easily detectable mutations in the novel HLA-H gene; one of these was homozygous in 83% of 178 patients with hereditary haemochromatosis. Their findings offer the possibility for screening carriers of the defective gene and preventing the development of severe disease which often has a fatal outcome. Little[4] points out some doubts about the candidacy of HLA-H to be the hereditary haemochromatosis gene, and underlines the importance of investigating recombinants by familial analyses. Barton and colleagues and Little[1] explain the extraordinarily high

frequency of this disease by the selective advantage of heterozygous child-bearing women to impart greater quantities of iron to their offspring. This argument is based on an as yet unproven hypothesis, and unfortunately reinforces the widespread and routine use of unselective iron medication for pregnant women and infants.

In 1984 a joint symposium of the International Society of Haematology, International Society of Blood Transfusion, and WHO initiated investigations to determine adequate iron stores in periods of increased cell proliferation, and launched a campaign against indiscriminate iron supplementation that would interfere with physiological adaptations at such times. Many investigations and clinical trials have corroborated the validity and the public health importance of these aims, but have not changed the prevailing clinical practice in most countries.

Iron supplementation during pregnancy will increase the circulating red-cell volume, leading to reduced blood flow, enhanced platelet-vessel wall interaction, and platelet aggregation. These changes would raise the risk of thromboembolic complications in the physiologically hypercoagulable state of pregnancy, combined with a rise in femoral venous pressure as a result of compression of the vena cava by the enlarging uterus. Additionally, iron-catalysed release of free radicals is involved in mediating immunodeficiency and increased mutagenesis in the highly proliferative tissues of the fetus, especially in the presence of viral infections or any other oncogenic factors. Since malignant diseases arise as a result of multistep mutations the consequences of interuterine damage may be manifested after several decades of latency. It seems probable that hypoferraemia during pregnancy is an important physiological adaptation for the prevention of these risks to mother and fetus.[5]

Susan Hollán
National Institute of Haematology, Budapest, PO Box 44, H-1518, Hungary

1 Ashby D. Can iron supplementation improve cognitive functioning? *Lancet* 1996; 348: 973.
2 Bruner AB, Joffe A, Duggan AK, Casella JF, Brandt J. Randomised study of cognitive effects of iron supplementation in non-anaemic iron-deficient adolescent girls. *Lancet* 1996; 348: 992–96.
3 Feder JN, Gurke A, Thomas W, et al. *Nat Genet* 1996; 13: 399–408.
4 Little P. *Nature* 1996; 382: 494–95.
5 Hollán S, Staehr-Johansen K. *Haematologia* 1993; 25: 69–84.

The superficial femoral vein: a cause of therapeutic error

SIR—There is general agreement about the need to prescribe anticoagulants to patients with deep vein thrombosis (DVT), in order to prevent pulmonary embolism and ameliorate post-thrombotic syndrome. In addition, the rapid achievement of adequate anticoagulation with heparin has an important prognostic influence.[1] However, we have recently seen two patients admitted to hospital with suspected pulmonary embolism, who were not anticoagulated urgently despite an ultrasound scan of the lower extremities reporting "signs of thrombosis of the superficial femoral vein". We asked the junior doctors who first attended those patients about the reasons for not prescribing immediate anticoagulation. They explained that they knew the necessity of anticoagulation in DVT, but they thought that the superficial femoral vein was indeed a superficial vein, not a deep one.

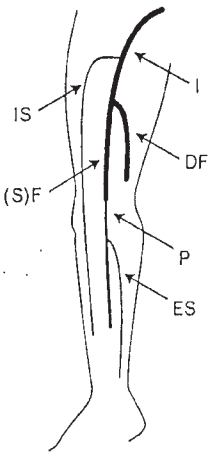

Figure: **Schematic diagram of main veins of leg**
Superficial veins (thin lines): IS, internal saphenous; ES, external saphenous. Deep veins (thick lines): P, popliteal; (S)F, femoral, also known as superficial femoral; DF, deep femoral; I, iliac. The segment of the femoral vein proximal to the confluence of the deep femoral is sometimes called the common femoral vein.

To find out if this was a common misinterpretation, we passed a questionnaire to 34 junior doctors in family medicine (8), general internal medicine (7), or internal medicine subspecialities (19). Only 9 (26%) considered anticoagulation indicated for thrombosis of the superficial femoral vein. However, all adequately answered that anticoagulants should be prescribed for thrombosis of the iliac, femoral, common femoral, or deep femoral veins. All but one (97%) would also anticoagulate popliteal vein thrombosis, whereas only two (6%) would anticoagulate external saphenous vein thrombosis.

These results clearly indicate that the failure to anticoagulate patients with thrombosis of the superficial femoral vein was not due to ignorance about proper therapy of DVT, but to a misinterpretation of the term superficial applied to the femoral vein. In fact this seems to be a common worldwide mistake.[2] The denomination superficial femoral for the vein segment joining the popliteal and iliac veins is frequent in ultrasound publications and patient reports (figure). However, since it seems to be a common cause of clinicians' confusion, it should be abandoned. The more easily interpreted term femoral (which is also more adequate from an anatomical point of view) should be used instead of the potentially dangerous superficial femoral.

**José A Riancho, Ana Ontañón*
Department of Internal Medicine, Hospital M Valdecilla, University of Cantabria, Santander 39008, Spain

1 Pineo GF, Hull RD. Classical anticoagulant therapy for venous thromboembolism. *Prog Cardiovasc Res*, 1994; 37: 59–70.
2 Bundens WP, Bergan JJ, Halasz NA, Murray J, Drehobl M. The superficial femoral vein. *JAMA* 1995; 274: 1296–98.

DEPARTMENT OF ERROR

An air stewardess with puzzling diarrhoea—In this Case Report by Greaves and colleagues (Nov 30, 1996), the second author should be R L Bown, not R L Brown.

CHD prevention in clinical practice—A line was lost from panel 1 in Professor Pyörälä's contribution to the supplement on Coronary Heart Disease (*Lancet* 1996; 348 (suppl 1): s26–s28): the middle section (modifiable biochemical or physiological characteristics) should have included raised plasma triglycerides.