

## Haemoglobin, ferritin, and iron intakes in British children aged 12–14 years: a preliminary investigation

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The purpose of the study was to assess the prevalence of Fe deficiency and Fe-deficiency anaemia in a group of apparently healthy adolescents, and to assess the value of a food frequency and amount questionnaire as a screening tool to identify children at risk of Fe deficiency. White schoolchildren (399) aged 12–14 years living in a Southwest London suburb completed a food frequency and amount questionnaire to assess usual Fe and vitamin C intake, and provided a thumb-prick blood sample for analysis of haemoglobin (Hb), packed cell volume (PCV), and serum ferritin (SF). Children were classified as 'anaemic' if Hb was below the Dallman 3rd percentile (girls: < 120 g/l; boys: < 122, < 124 and < 126 g/l at ages 12, 13 and 14 years respectively); and 'low' or 'borderline' in Fe stores if SF was < 12 µg/l, or between 12 and 20 µg/l respectively. Of the boys and girls 3.5 and 10.5% respectively were anaemic; 1% of boys and 4% of girls had low ferritin values, and 14% of boys and 16% of girls were borderline. Fe intakes were significantly higher in boys than in girls (12.3 v. 9.6 mg/d,  $P < 0.001$ ). Prevalence of anaemia was 14.5% in the group with both low Fe intakes (< lower reference nutrient intake) and low vitamin C intakes (< median), compared with 2.3% in the group with both high Fe intakes (> reference nutrient intake) and high vitamin C intakes (> median). Anaemia was three times more common in vegetarians than omnivores (25 v. 9%), and in girls who had tried to lose weight in the last year compared with those who had not (23 v. 7%). The questionnaire did not prove satisfactory as a screening tool for risk of Fe deficiency. The higher-than-expected prevalence of Fe deficiency in apparently healthy white girls suggests that other groups should also be investigated.

### Haemoglobin: Ferritin: Iron: Adolescence

Fe intakes in British adolescents have been declining over the last two decades (Durnin *et al.* 1974; Darke *et al.* 1980; Department of Health, 1989; Nelson *et al.* 1990). Two-thirds of British adolescents now have reported intakes below the recommended daily amounts (RDA; 12 mg/d; Department of Health and Social Security, 1979), and an even higher proportion of girls have intakes below the new reference nutrient intake (RNI 14.8 mg/d; Department of Health, 1991). Some of these children, particularly girls with heavy menstrual losses, will probably be failing to meet their individual requirements for Fe, and will be at increased risk of Fe deficiency (ID) and Fe-deficiency anaemia (IDA).

There are no recent studies on the prevalence of ID amongst UK adolescents. Goel *et al.* (1978) showed that in young adolescents living in Glasgow, low haemoglobin (Hb) levels (Hb < 120 g/l) were more common in children of non-caucasian origin (20%) than those of caucasian origin (16%). More recently, Armstrong (1989) reported that 13% of boys and 7% of girls aged 14–18 years living in County Sligo, Republic of Ireland, had Hb levels below 130 and 120 g/l respectively, and that 75% had serum ferritin (SF) levels below 20 µg/l, and 40% had values below 10 µg/l. In neither study was Fe intake assessed, but in the absence of high levels of infection or hookworm infestation, these indices are likely to be indicative of ID.

ID and IDA in schoolchildren are known to be associated with less than optimal behaviour and poorer performance in intelligence tests (Pollitt *et al.* 1989; Pollitt, 1990), and, in adolescents in particular, with poorer school performance (Webb & Oski, 1973) and disruptive behaviour (Webb & Oski, 1974). Recent, well-designed studies in Indonesia (Soemantri *et al.* 1985; Soemantri, 1989), Thailand (Pollitt *et al.* 1989) and India (Seshadri & Gopaldas, 1989) all indicate that anaemia in adolescence is associated with poor performance in academic tests, and that Fe supplementation of anaemic children over 3 months results in significant improvements in performance.

Godfrey *et al.* (1991) have shown that low Hb in pregnancy is associated with a raised placental weight:birth weight ratio, which in turn is a predictor of high blood pressure in adult life (Barker *et al.* 1990). If anaemia in adolescent girls persists into their reproductive years, this has important implications for the long-term health of their offspring. It is, therefore, important to discover the likely extent of ID and IDA in UK schoolchildren.

This preliminary study was designed to assess the prevalence of ID and IDA in a group of apparently healthy white schoolchildren living in a predominantly middle-class area near London, and to investigate the use of a short food frequency and amount questionnaire (FAQ) to screen children for dietary ID, using biochemical measures of Fe status as an external marker of the FAQ validity. If the prevalence of ID and IDA were substantially greater than expected in an apparently healthy population of white children (about 3%, using the 3rd percentiles for Hb and SF as cut-off points), then further studies would be warranted to investigate the origins and potential consequences of such deficiency, and to assess the prevalence of ID and IDA in other groups of children in the population who were potentially at risk, such as vegetarians or children from low-income families.

#### METHODS

In June and July, 1990, all 8th and 9th year pupils aged 12–14 years attending two comprehensive schools in Epsom, Surrey were asked to take part in the study. Of 622 pupils, 399 (64%) (202 males and 197 females) agreed to participate in the study and had parents who gave written consent.

During the morning registration period at school, subjects were asked to complete a twenty-three item frequency and amount questionnaire on sources of Fe and vitamin C in the diet. The items in the questionnaire were selected on the basis of an analysis of the diets of a similar group of children from North London (unpublished results). The twenty-three items included foods which together contributed over 90% of dietary Fe and vitamin C. The questionnaire asked the children to describe their diets over the previous month.

Pupils were then seen individually at 10 min intervals throughout the day by J.W. and C.R., who checked the responses on the questionnaire and filled in any missing information. At the end of the interview a thumb-prick blood sample of approximately 250  $\mu$ l was obtained from each subject using an Autolet DL. Hb was measured in duplicate on fresh 20  $\mu$ l blood samples collected in heparinized capillary tubes, using the cyanomethemoglobin method (Boehringer-Mannheim; International Committee for Standardization in Haematology, 1967) and a Pye Unicam SP600 spectrophotometer. Packed cell volume (PCV) was measured on 10  $\mu$ l samples collected in heparinized capillary tubes, using a Compur micro-haematocrit or Fisons Haematocrit Minor. Blood samples (200  $\mu$ l) were collected using a Gilson pipette, transferred to plain 1.5 ml microcentrifuge tubes, left to stand at room temperature for 10 min, and spun at 3000 rev./min for 10 min. Serum was collected by pasteur pipette, transferred to fresh microcentrifuge vials, and stored at  $-70^{\circ}$ . SF was determined in duplicate using the ELISA (immunosorbent assay) technique (Boehringer-Mannheim).

'Anaemia' was defined as Hb below the 3rd centile (Dallman & Siimes, 1979): in girls, less than 120 g/l, and in boys, less than 122, 124 and 126 g/l at ages 12, 13 and 14 years respectively. Anaemia was defined as 'microcytic' if mean corpuscular Hb concentration (MCHC; Hb/PCV) was less than 300 g/l. Children were deemed to have low Fe stores if SF was less than 12  $\mu\text{g/l}$ , and borderline stores if SF was between 12 and 20  $\mu\text{g/l}$ .

Fe and vitamin C intakes were estimated from the questionnaire responses using the FOODTABS computer program (T. A. B. Sanders, unpublished results) based on the McCance and Widdowson food composition tables (Paul & Southgate, 1978). Children were classified according to their Fe intakes into low, intermediate, or high groups, using cut-offs based on the Department of Health dietary reference values (Department of Health, 1991). For boys, 'low' was less than 6.1 mg/d (lower RNI; LRNI), 'intermediate' between 6.1 and 11.3 mg/d (RNI), and 'high' greater than or equal to 11.3 mg/d. For girls, 'low' was less than 8.0 mg/d (LRNI), 'intermediate' between 8.0 and 14.8 mg/d (RNI), and 'high' greater than or equal to 14.8 mg/d. They were classified according to vitamin C intake as being above or below the median vitamin C intake, assessed separately by sex (66.5 mg/d in boys, 67 mg/d in girls). The validity of the questionnaire for correctly classifying children into groups with low, medium or high Fe intakes was assessed in relation to their classification for anaemia (based on Hb) and low or borderline Fe stores (based on SF), taking vitamin C intakes into account.

Results were analysed using the *Statistical Package for the Social Sciences* (SPSS, 1988). Statistical significance of differences in mean values between sexes was assessed using unpaired *t* tests; significance of association between Fe or vitamin C intake and Hb or ferritin status, and between dietary practices (vegetarianism or dieting) and Hb status, was assessed using the chi-squared test.

## RESULTS

All 399 children recruited completed the questionnaire, and provided a blood sample. Mean values for age, Hb, PCV, MCHC, SF, and Fe and vitamin C intakes are given in Table 1. There were statistically significant differences between boys and girls for Hb, PCV, MCHC, SF and Fe intake.

Seven (3.5%) of the boys and twenty-one (10.5%) of the girls were classified as anaemic, the values for Hb in the anaemic boys ranging from 112 to 125 g/l, and in the anaemic girls from 91 to 119 g/l.

Because of problems with the equipment, PCV was measured in only a subset of the children. Six of 178 boys (3.4%) and ten of 101 girls (9.9%) had MCHC < 300 g/l. Of the six boys and twelve girls who were anaemic and in whom PCV was also measured, microcytic anaemia was present in two of the boys and six of the girls.

Sufficient serum was collected for ferritin assays from 140 boys and 156 girls. One boy (0.7%) and six girls (3.8%) had SF levels less than 12  $\mu\text{g/l}$ . A further twenty boys (14.3%) and twenty-five girls (16.0%) had SF levels between 12 and 20  $\mu\text{g/l}$ . The boy with low Fe stores was not anaemic, but two of the twenty boys with borderline ferritin values were anaemic; of the six girls with low Fe stores, two were anaemic, and of the twenty-five with borderline ferritin values, four were anaemic.

Table 2 shows the relationships between anaemia, low ferritin, and low MCHC in the 204 children (128 boys, 76 girls) in whom all three variables were measured. Two boys (1.6%) and eight girls (10.5%) satisfied two or more criteria for ID (low Hb, low MCHC, low or borderline SF).

Mean (with SE) Fe intake was significantly greater in boys (12.3 (SE 0.34) mg/d) than in girls (9.6 (SE 0.25) mg/d; unpaired *t* test,  $P < 0.001$ ). When children were classified

Table 1. Mean values for age, iron and vitamin C intakes, haemoglobin (Hb), packed cell volume (PCV), mean corpuscular Hb concentration (MCHC), and serum ferritin in 399 British adolescents†

(Mean values and standard deviations)

	Boys			Girls		
	Mean	SD	n	Mean	SD	n
Age (years)	13.3	0.61	202	13.2	0.67	197
Hb (g/l)	143***	109	202	133	102	197
PCV (%)	40.9***	2.8	178	39.0	3.0	101
MCHC (g/l)	34.9*	2.7	178	34.1	3.5	101
Serum ferritin‡ (µg/l)	31.4	1.5	140	30.4	1.6	156
Fe (mg/d)	12.3***	4.8	202	9.6	3.6	197
Vitamin C (mg/d)	70.7	33.5	202	70.6	31.4	197

Mean values were significantly different from those for girls (unpaired *t* test): \*  $P < 0.05$ , \*\*\*  $P < 0.001$ .

† For details of subjects and procedures, see pp. 148–149.

‡ Means and SD based on log-transformed values.

Table 2. Classification of 204 12–14-year-old British boys and girls according to levels of haemoglobin, serum ferritin, and mean corpuscular haemoglobin concentration (MCHC)\*

Serum ferritin (µg/l)	MCHC (g/l)	Boys (n 128)		Girls (n 76)	
		Anaemic (n 5)	Non-anaemic (n 123)	Anaemic (n 11)	Non-anaemic (n 65)
< 12	< 300	0	0	1	0
	> 300	0	1	1	2
12–20	< 300	1	0	1	0
	> 300	0	17	1	12
≥ 20	< 300	1	3	4	3
	> 300	3	102	3	48

\* For details of subjects and procedures, see pp. 148–149.

according to level of Fe intake, there were 5, 41 and 54% of boys in the low, intermediate and high group respectively; and 35, 57, and 8% of girls respectively (Fig. 1).

Table 3 shows the mean Fe and vitamin C intakes according to Hb and ferritin levels. There were no statistically significant differences in either mean Fe or mean vitamin C intakes between anaemic and non-anaemic boys and girls, or between boys and girls with low or normal Fe stores, although Fe and vitamin C intakes were consistently lower in the anaemic groups, and Fe intakes were lower in the low-ferritin groups. There were no significant differences in Fe or vitamin C intakes between children with MCHC above or below 300 g/l.

Children were asked if they had tried to lose weight in the last year, or were vegetarian. There were no relationships between Fe status and these dietary practices amongst boys. Of the forty-eight girls attempting to lose weight, eleven (23%) were anaemic, compared with 7% of the girls not trying to lose weight ( $\chi^2$  10.011,  $P = 0.002$ ). Of the anaemic girls 52% had attempted to lose weight. Four of the sixteen vegetarians (25%) were anaemic, compared with 9% of the non-vegetarians. Average Fe intakes amongst those attempting

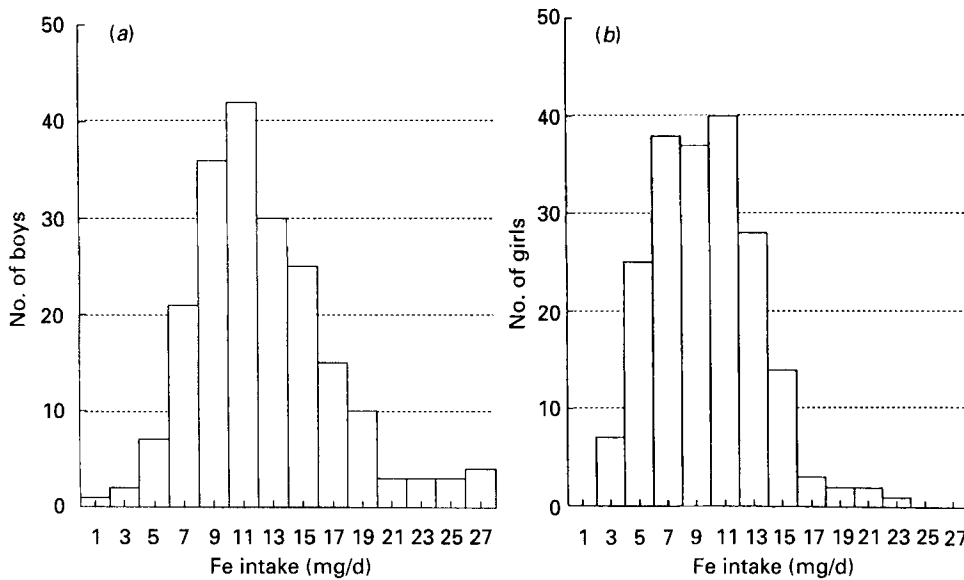


Fig. 1. Iron intake (mg/d) obtained from frequency and amount questionnaire from British schoolchildren aged 12–14 years (a) boys (*n* 202); (b) girls (*n* 197). For details of subjects and procedures, see pp. 148–149.

Table 3. Mean iron and vitamin C intakes (mg/d) of 12–14-year-old British boys and girls according to sex, haemoglobin and serum ferritin status\*

(Mean values with their standard errors)

	<i>n</i>	Iron		Vitamin C	
		Mean	SE	Mean	SE
<b>Haemoglobin</b>					
<b>Boys</b>					
A	7	9.5	1.9	60.7	17.0
NA	195	12.4	0.3	71.1	2.4
<b>Girls</b>					
A	21	9.2	0.8	61.8	6.8
NA	176	9.7	0.3	71.7	2.4
<b>Serum ferritin (µg/l)</b>					
<b>Boys</b>					
< 12	1	10.5	0	116	0
≥ 12	139	12.1	0.4	69	2.7
<b>Girls</b>					
< 12	6	8.6	1.0	69	9.3
≥ 12	150	9.6	0.3	69	2.6

A, anaemic; NA, non-anaemic.

\* For details of subjects and procedures, see pp. 148–149.

to lose weight was lower than those amongst the remainder (8.5 v. 10.0 mg/d, *P* = 0.015, unpaired *t* test), and were lower amongst the vegetarian girls compared with the omnivores (8.1 v. 9.7 mg/d, *P* = 0.07, unpaired *t* test).

Table 4 shows, for all subjects, the relationships between low, intermediate or high Fe intakes, vitamin C intakes above or below the median (controlling for sex), and Hb and

Table 4. Relationship between iron intake (low, intermediate or high), vitamin C intake (above or below median intake) and Fe status, showing the percentage anaemic or with serum ferritin (SF) < 20 µg/l in 12-14-year-old British schoolchildren

(No. of schoolchildren who were anaemic or have SF < 20 µg/l shown in parentheses)

Fe intake group...	Low	Intermediate	High	All
<b>Haemoglobin</b>				
Vitamin C < median				
<i>n</i>	55	108	36	199
Percentage anaemic	14.5 (8)	9.3 (10)	2.8 (1)	9.6 (19)
Vitamin C > median				
<i>n</i>	26	86	88	200
Percentage anaemic	7.7 (2)	5.8 (5)	2.3 (2)	4.5 (9)
All children:				
<i>n</i>	81	194	124	399
Percentage anaemic	12.3 (10)	7.7 (15)	2.4 (3)	
<b>Serum ferritin</b>				
Vitamin C < median				
<i>n</i>	44	84	27	155
Percentage < 20 µg/l	22.7 (10)	17.9 (15)	25.9 (7)	20.6 (32)
Vitamin C > median				
<i>n</i>	17	63	61	141
Percentage < 20 µg/l	17.7 (3)	12.7 (8)	14.8 (9)	14.2 (20)
All children:				
<i>n</i>	61	147	88	296
Percentage < 20 µg/l	21.3 (13)	15.6 (23)	18.2 (16)	

Relationship between presence of anaemia and Fe intake ( $\chi^2$  7.69,  $P = 0.02$ ); and vitamin C intake ( $\chi^2$  3.90,  $P = 0.049$ ).

ferritin status. There was a statistically significant relationship between the presence of anaemia and low Fe intake ( $\chi^2$  7.69,  $P = 0.02$ ) and vitamin C intake below the median ( $\chi^2$  3.90,  $P = 0.049$ ). The prevalence of anaemia in the low-Fe, low-vitamin C group (14.5%) was more than six times that in the high-Fe, high-vitamin C group (2.3%). The same pattern was observed when data were analysed separately by sex, but the relationships failed to reach statistical significance with the smaller numbers. No statistically significant relationships were observed between Fe or vitamin C intakes and risk of low SF levels, but the trends were in the expected direction.

The validity of the FAQ for use as a screening device to detect poor Fe status was assessed by calculating the sensitivity (ability of the questionnaire to identify those who were anaemic, i.e. true positives), specificity (ability to identify those who were not anaemic, i.e. true negatives), and predictive value (the proportion of those with a positive questionnaire response who were anaemic). The sensitivity of the questionnaire was good (86%), but the specificity was poor (34%) and the predictive value low (13%).

#### DISCUSSION

The purpose of the present study was twofold: to assess the prevalence of anaemia in a group of apparently healthy white adolescents from a typical suburban background in Southeast England in order to consider whether further investigation of the problem in other groups was warranted, and to assess the value of a brief dietary questionnaire to identify those children who might be at risk of anaemia.

A response rate of 64% was regarded as acceptable. Although it was not possible to gather information on the characteristics of the non-responders, discussions with the Headteachers led us to believe that the non-responders did not differ from the responders with regard to social class or general health (based on absenteeism). The 204 children for whom all three blood measures of Fe status were obtained did not differ on average from those of the other children in the study with regard to age, height, weight, or Hb. The results presented are believed, therefore, to be representative of the children from this area. Moreover, Table 1 shows that the children are typical of others in this age-group: the mean values reported here agree well with previous reported values (Dallman *et al.* 1980; Department of Health, 1989; Nelson *et al.* 1990).

The data on multiple criteria (Table 2) are not wholly consistent with expectations. One would expect the majority of children with low Hb levels to have low Fe stores also, and for this to be reflected in low ferritin values (Pollitt, 1990). The present results may indicate, therefore, confounding by infection, which has the temporary effect of reducing Hb and increasing SF (Beaton *et al.* 1989; information on infection was not collected). Alternatively, some of the samples may have been haemolysed during collection, which would release ferritin from erythrocytes into the serum and give false high values. While the value of a pin-prick sample for whole-blood analyses is well established (Hinchliffe & Lilleyman, 1987), the same may not be true for serum analyses. Also, some misclassification error may have been due to the use of a single blood sample (Borel *et al.* 1991). Nevertheless, the presence of anaemia and low or borderline ferritin values were consistent with the dietary findings (Tables 3 and 4). Although some of the low estimates of Fe intake based on the FAQ probably understate true intake (a far higher proportion of children with intakes truly below the LRNI would be expected to be anaemic), there is an internal consistency to the findings which support the interpretation that Fe and vitamin C intakes are inadequate to prevent anaemia in a substantial number of children.

The risk of anaemia amongst the girls was strongly associated with attempting to lose weight and vegetarianism, which were associated with lower levels of dietary Fe intake. Whilst these practices in themselves are not necessarily harmful, the present results, particularly in relation to attempting to lose weight, indicate that nutrition education is necessary to prevent unwanted consequences, and that there may be a need to monitor Fe status in these children.

There are, of course, other possible reasons for the appearance of anaemia, such as low vitamin B<sub>12</sub> or folic acid intakes, or haemoglobinopathies. Existing dietary findings on children in this age-group suggest that intakes of these nutrients are likely to be adequate, and the schools were chosen deliberately to include a predominantly white population, in whom haemoglobinopathies are rare.

The validity of the FAQ for screening purposes was assessed against the biochemical findings. While the mean Fe intakes in the present study were virtually the same as those reported by the Department of Health (1989) in 14–15-year-old schoolchildren and similar to the values reported by Nelson *et al.* (1990) in 11–12-year-old schoolchildren (both based on 7 d weighed records), the spread of values for Fe intake (as measured by standard deviation) was greater in the present study. This suggests that the FAQ may have exaggerated intakes in some individuals and underestimated it in others, resulting in an overestimate of the proportion of children whose intakes fall below the LRNI. While the questionnaire successfully identified the majority of those with true low intakes (based on their biochemical results), it is clear that a number of subjects with reported low intakes were misclassified. The lack of specificity and predictive value indicate that the questionnaire in its present form is not satisfactory as a screening tool for identifying children at risk of being anaemic.

The levels of Hb reported here correspond to a diagnosis of 'mild' anaemia. There is no information from the UK to show whether borderline IDA of the type reported here is associated in adolescents with poorer physical health, behaviour, or academic performance. Evidence from both cross-sectional and intervention studies in Third World countries (in which the level of ID is much greater) strongly supports an association in adolescents between Fe status and academic performance (Pollitt, 1990). Although the mechanisms of the influence of Fe are not fully understood (Tucker *et al.* 1984; Hallberg, 1989), particularly in cases of less severe ID, the benefits of Fe supplementation have been clear in relation to academic and behavioural tests. The implications of the work by Godfrey *et al.* (1991) also need careful consideration with regard to adolescent girls who may enter their reproductive phase with low Fe stores.

The present results suggest that ID may be common in apparently healthy middle-class white children. If that is so, the prevalence can be expected to be higher in other groups in the population in whom Fe or vitamin C intakes may be lower, such as children from low-income families, Asian vegetarian children, or children living in areas with lower vitamin C intake (such as Scotland). In the face of apparently declining Fe intakes in the UK, it is important to assess the prevalence of ID in these groups, and to examine the possible consequences of low Fe status.

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