HOW CAN WE IDENTIFY SCHOOLGIRLS AT RISK OF LOW IRON STATUS AND WHAT DIETARY ADVICE SHOULD WE BE GIVING?

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EXECUTIVE SUMMARY

Project N08017: How can we identify schoolgirls at risk of low iron status and what dietary advice should we be giving?

Introduction

Iron is arguably the most marginal nutrient in the diet of girls and young women in Britain. The risk of poor iron status is increased in puberty and adolescence due to a combination of accelerated growth and the onset of menarche, exacerbated by low intakes of iron in some groups. The NDNS of young people found that 27% of the oldest girls (15-18 years) had low iron stores (serum ferritin < 15 μg/l) while 9% were anaemic (Hb below 12g/dl).

Among children of school age, low iron status has been associated with poorer IQ scores, maths scores and educational attainment (Halterman, Kaczorowski et al. 2001). One study of iron supplementation has shown benefits on verbal learning and memory in girls who were iron deficient but not anaemic (Bruner, Joffe et al. 1996), but other smaller studies have not (Pollitt, Soemantri et al. 1985) (Pollitt, Hathirat et al. 1989). A major recent review has concluded that the evidence from randomised controlled trials is "reasonably convincing but not conclusive" (Grantham-McGregor and Ani 2001). In the light of evidence that a significant proportion of girls and young women in Britain have undiagnosed mild iron deficiency, there may however be grounds to support a public health nutrition approach to improving iron status in this population. The Scientific Advisory Committee on Nutrition (Working Group on Iron) is currently considering the issue of iron status in vulnerable groups.

Methods

Using data from the NDNS of young people (Gregory and Lowe 2000) our objectives were:

- to identify the principal dietary and physiological predictors of low iron status in girls aged 7-18 years (n=443),
- to devise a scoring tool or checklist to identify individuals at risk of low iron status
- to suggest appropriate messages that might improve iron status in this vulnerable group.

The approach recognises that iron intake *per se* is poorly correlated with iron status, and seeks to identify messages that reflect the importance of iron bioavailability and the presence of enhancers and inhibitors of iron absorption.

The NDNS survey of young people aged 4-18 years assessed diet via a 7–day weighed dietary record of all food and drink consumed. Blood samples (mostly fasting) were obtained from consenting children shortly after completion of the dietary records. Of the sample of girls aged 7 years and over who had completed a dietary record (n=665), two thirds (n=443) had data on iron status in the form of haemoglobin concentration, (Hb), serum ferritin (SF) or transferrin saturation (TS). We classified children as having **low**

iron status if *any* of their iron status measurements was below certain thresholds, based on the definitions used in the NDNS report (i.e. Hb<11.5g/dl for girls under 13 years, or < 12g/dl for girls 13 and over; SF<15µg/dl; TS<15%). Differences in background variables, iron and food intakes were compared between groups. Multiple logistic regression was used to identify the main predictors of low iron status, and separately, of *good* iron status, using different criteria (Hb>12g/dl; SF>25µg/l; TS>25%). The results of the best models were used to derive a simple scoring tool based on five questions, using weightings based on the relative values (beta coefficients) of the predictor variables.

Results

Background characteristics were very similar in the low and adequate iron status groups. Girls with low iron status were on average one year older, with an attendant difference in height, weight and BMI but there was no difference in age-adjusted BMI, or in energy intake. Consistent with their older age, girls with low iron status were more likely to have reached menarche (62% vs. 46%; P=0.002). Total iron intakes were identical, but the group with low iron status had lower intakes of *haem* iron (0.32mg *vs*. 0.38mg/d; P=0.016). This reflected the higher prevalence of vegetarianism in this group (12.8% vs. 4.5%; P<0.002) but also a lower consumption of *red meat and meat products* among the non-vegetarians. Only 10 girls were taking iron supplements (2%) and those who were taking useful quantities were not those at greatest risk.

In logistic regression, the best model predicting low iron status was based on one physiological variable *(menarche)* and three dietary variables *(red meat, fruit or fruit juice, and tea).* Girls who had reached menarche had a 50% increased odds of low iron status (OR =1.5; 95% CI= 1.0 to 2.4). Eating no red meat at all during the survey week was associated with more than treble the odds of low iron status (95% CI =1.8 to 8.0), while eating some red meat (but less than 7 portions a week, or 90g/d) carried a slightly elevated risk compared with a high meat eater (>90g/day). Sixty girls consumed neither fruit nor fruit juice during the survey week and this doubled their odds of having low iron status. Drinking more than one cup of tea (125ml) per day was associated with almost double the odds of low iron status, while lesser amounts were not associated with significant excess risk. Predictors of *good* status included eating raw vegetables (salads), but the most consistent factor in all models was consumption of red meat.

The scoring tool, or questionnaire was developed using the 5 main predictor variables *(menarche, red meat, fruit/fruit juice, tea and salads).* These were entered in a new logistic regression, in order to derive weightings for each response in the scoring tool (see table).

Scoring tool

	Question	Response	Points	Your score
1	How much red meat (or meat products) do you eat?	7 portions a week 1-6 portions a week Less than once a week or never	0 1 3	
2	How often do you eat fruit or drink fruit juice?	At least once a week Less often	0 1	
3	How often do you eat salads or raw vegetables?	At least once a week Less often	0 1	
4	How much tea do you drink?	One a cup a day (or more) Less than a cup a day	1 0	
5	Have you started having periods yet? *	No Yes	0 1	
		Total score		

* Question 5 could be replaced by "are you over 13 years of age" with similar results.

The scoring tool had an overall accuracy of about 65%. It is probably too insensitive to be used as a diagnostic tool in predicting a dichotomous outcome (low vs. normal iron status), but is best seen as a rough self-assessment, giving a score on a scale of 0 to 7.

An interpretation of relative risk might be as follows:

A score of 1 or less indicates below-average risk (approx 1 in 5)…but doesn't guarantee good iron status
A score of 2 indicates average risk (approx 1 in 3)
A score of 3 or more indicates above-average risk (approximately 1 in 2)… but doesn't guarantee poor iron status.

Among girls in this study, 63% of those achieving a score of 4+ would have low iron status, and 7/19 would be false positives. It might be worth referring girls with such high scores to a doctor for blood test if they had symptoms such as pallor and tiredness. Girls with lower scores might be encouraged to look at the dietary messages designed to improve iron status.

Dietary messages

The main dietary factors identified in our models and scoring tool are consistent with the results of other studies and illustrate the importance of bioavailability of iron in the diet, rather than mere iron intake. However, iron absorption is also modulated by the iron status of the individual, being enhanced where iron stores are low. This enhanced absorption may not be sufficient to compensate for iron losses in some girls after menarche and our models found post-menarcheal status (or age above 13 years as a surrogate) to be a predictor of iron status. The results of our models were combined with other data on the sources and bioavailability of iron in order to design the dietary messages suitable for dissemination to girls of school age. These are given below.

Dietary messages
Red meat (beef, lamb, pork, offal) is rich in iron that is easily absorbed (haem iron). The darker the meat the more iron it contains
Poultry contains some haem iron, and leg meat contains more iron than breast meat.
Fish contains some haem iron too, especially oily fish and molluscs (mussels etc)

If you are vegetarian eating no meat or fish, the following messages are even more important for you :

Fresh fruit or fruit juice with meals greatly helps to improve iron absorption
Salad vegetables (including tomatoes) contain vitamin C to help iron absorption.
Green leafy vegetables contain some iron (e.g. watercress, kale)
Avoid drinking tea with meals (or within 30 minutes of a meal) as this greatly reduces iron absorption
If you have heavy periods you may need extra iron. See your doctor if you think you may be anaemic

Conclusions

The models and scoring tool scoring tool gave a fair prediction of low iron status based on 5 key variables (~65%), whereas a prediction based on low iron intake (<LRNI) was no better than chance. However, the tool developed from these data should ideally be validated in an independent sample of girls or young women. The new NDNS data on 19-34 year old women could be analysed for comparative purposes. Dietary messages such as those suggested could be used by the Agency to help provide guidance for young girls who may be at risk of low iron status.

Abbreviations used in the report

NDNS	National Diet and Nutrition Survey
Fe	iron
Hb	haemoglobin
SF	serum ferritin
TS	transferrin saturation
SE	Standard Error
SD	Standard Deviation
B	Beta coefficient (in logistic regression)
OR	Odds Ratio
CI	Confidence interval
BMI	Body mass index
BMR	Basal metabolic rate
LRNI	Lower Reference Nutrient Intake
NHANES	National Health and Nutrition Examination Survey (US)
WHO	World Health Organisation

Scientific Report

1. INTRODUCTION

1.1 Aims and objectives of the project

Using data from the NDNS of young people (Gregory and Lowe 2000) our objectives were to identify the principal dietary and physiological predictors of low iron status in girls aged 7-18 years, to devise a scoring tool or checklist based on these, and to use these data to suggest appropriate messages to improve iron status in this vulnerable group. The approach recognises that iron intake *per se* is poorly correlated with iron status, and seeks to identify messages that reflect the importance of iron bioavailability and the presence of enhancers and inhibitors of iron absorption.

1.2. Importance of low iron status to public health

Iron is arguably the most marginal nutrient in the diet of girls and young women in Britain. The risk of poor iron status may be increased in puberty and adolescence due to a combination of accelerated growth and the onset of menarche, exacerbated by low intakes of iron in some groups. The NDNS of young people found that over 45% of girls aged 11-18 had iron intakes below the LRNI. Furthermore, 27% of the oldest girls (15-18 years) had low iron stores (serum ferritin < 15 μg/l) while 9% had haemoglobin levels below 12g/dl, the WHO limit defining anaemia for adult women.

The impact of poor iron status in terms of child health and development has been extensively researched, although in many studies the possibility of confounding effects of socioeconomic background prevent causal inferences from being made. Among school-children, low iron status has been associated with poorer IQ scores (Otero, Aguirre et al. 1999) and lower standardized math scores (Halterman, Kaczorowski et al. 2001). One supplementation study of iron-deficient but non-anaemic girls aged 13-18 years showed an improvement in (free call) verbal learning (Bruner, Joffe et al. 1996), while a larger English Study of 12-16 year olds showed some improvement in visual reasoning among the low status group, but inconsistent effects of treatment in the other groups (Lynn and Harland 1998). Overall, the evidence from randomised controlled trials (RCT) has been described as “reasonably convincing but not conclusive” (Grantham-McGregor and Ani 2001). However, given that a significant proportion of girls and young women in Britain have undiagnosed mild iron deficiency, there may be grounds to support a public health approach to improving iron status in this population. The Scientific Advisory Committee on Nutrition (Working Group on Iron) is currently considering the issue of iron status in vulnerable groups.

2. METHODS

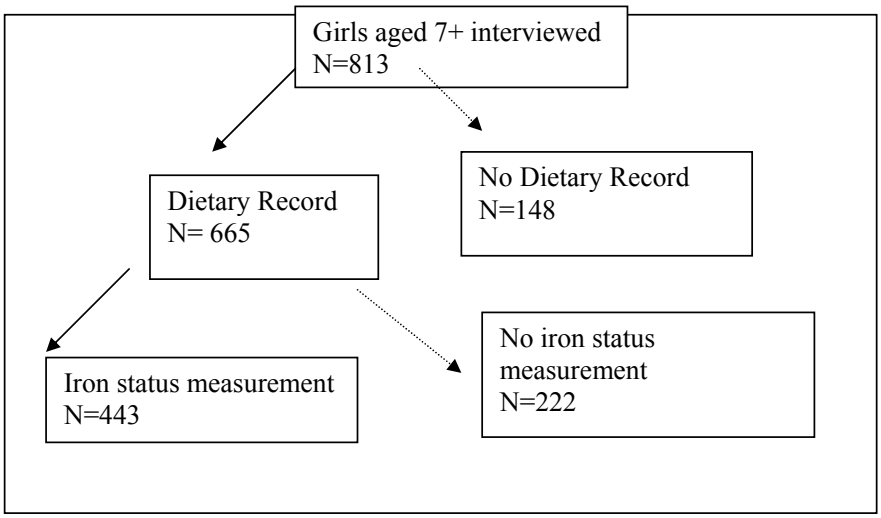
2.1. Data collected

The NDNS survey of young people aged 4-18 years assessed diet via a 7–day weighed dietary record of all food and drink consumed. Participants were required to give detailed descriptions of foods, including brands, and a database of more than 5000 foods and recipes was used to calculate nutrient intakes from the dietary data. Blood samples (mostly fasting) were obtained from consenting children shortly after completion of the dietary records, and these were subjected to biochemical and haematological analyses, including haemoglobin (Hb), serum ferritin (SF); percentage plasma transferrin saturation (%TS); total iron-binding capacity (TIBC); mean corpuscular volume (MCV); and mean cell haemoglobin (MCH). Analytical procedures are detailed in the published documentation (Gregory and Lowe 2000).

2.2. Data manipulation and sample selection

For the present project, NDNS data files and documentation were obtained from the University of Essex Data Archive and analysed using SPSS (v.11). Of the nationally representative sample of 2127 children initially interviewed in the survey, approximately (80%) completed a 7-day weighed dietary record. Of the sample of girls aged 7 years and over who had completed a dietary record (n=665), two thirds (n=443) had data on iron status measured by haemoglobin (Hb), serum ferritin (SF) or transferrin saturation (TS). These 443 girls formed our sample for analysis (Fig 1). Further restricting the sample to include only the 308 girls who had all three measurements of iron status was considered, but this would have resulted in the loss of a further 135 children and compromised the power of the analyses.

Fig 1. Numbers of girls at each stage of the NDNS sample



2.3. Criteria and cut-offs for low iron status

Iron status can be assessed through several laboratory tests, each assessing a different aspect of iron metabolism, or a different stage of iron deficiency. Initially, in iron *depletion*, the amount of stored iron (as measured by **serum ferritin** concentration) is reduced but the amount of functional iron may not be affected. In *iron-deficient erythropoiesis*, stored iron is depleted and transport iron (as measured by **transferrin saturation**) is reduced. In this stage, the amount of iron absorbed is not sufficient to replace the amount lost or to provide for red blood cell production. In *iron-deficiency anaemia* the shortage of iron leads to underproduction of iron-containing functional compounds, including **haemoglobin**.

Since no single test is accepted for diagnosing iron deficiency, a combined approach is often adopted in epidemiological studies (Cohen and Haas 1999). However, it has been suggested that use of multiple criteria to diagnose iron deficiency falsely reduces prevalence figures for iron deficiency (Hallberg, Bengtsson et al. 1993). We therefore classified children as having low iron status if *any* of their iron status measurements was below certain thresholds. These are defined below, as Hb <11.5g/dl or 12g/dl (for under or over 13 years, respectively); SF<15µg/l; TS<15% (Table 1). The chosen thresholds were based on the definitions used in the NDNS report itself, to ensure consistency, but alternatives were also examined.

Although all cut-offs are essentially arbitrary, the validity of the cut-off for SF of <15 µg/l has been established by several studies. In adult women SF<15ug/l used alone had the best predictive value to establish a diagnosis of iron deficiency (absent iron stores and a compromised supply of iron to the erythron) (Hallberg, Bengtsson et al. 1993). Furthermore a SF<15µg/l coincided with a drastic decrease in Hb concentration and disappearance of stainable iron in bone marrow smears (Hulten, Gramatkovski et al. 1995). Although no such comprehensive studies have been conducted in teenagers, signs of an iron deficient erythropoiesis appear at the same SF level as in adults, suggesting the same cut-off should be used in this age group (Hallberg, Hulthen et al. 1993) (Hulthen and Hallberg 1996). For TS a cut-off of <16% is often quoted as indicating an inadequate iron supply (Bainton and Finch 1964), although others have suggested higher values (25% or more) should be used (Hallberg and Hulthen 1996). However, this cut-off would have implicated half the sample of schoolgirls as having low iron status, and the less sensitive cut-off of <15% was therefore used.

Table 1.
Criteria for low iron status (from NDNS report)

	Girls under 13 years	Girls 13 years and over	References
Low Haemoglobin	<11.5g/dl	< 12g/dl	Great Ormond Street Hospital (Gregory and Lowe 2000)
Low Serum Ferritin	<15µg/l	<15µg/l	(Dacie and Lewis 1995)
Low Transferrin saturation	<15%	<15%	(Bates, Thurnham et al. 1997)

2.4. Statistical approach

Variables were examined statistically and visually, using histograms (for continuous data) and cross tabulations (for categorical data). Nutrient and food intakes were compared between iron status groups using T-Test or a non-parametric test (Mann Whitney) as appropriate. Associations between continuous variables were examined using Pearson and Spearman correlations. Background characteristics of the groups were compared using Chi-square tests. Although the conventional level of $P<0.05$ has been taken as indicative of statistical significance, in most instances actual P values are quoted and their relevance discussed in the text.

Logistic regression was then used to explore the impact of variables in predicting low iron status, defined as any one of three indices (Hb or SF or TS) below the thresholds specified in Table 1. The forward stepwise method of selection was used, adding significant variables sequentially (i.e. with smallest P value first) until no more reached the criterion for inclusion ($P<0.05$). The process also removed variables that became non-significant ($P>0.1$). The final model was attained when no further improvement was possible (as measured by the change in the –2 log-likelihood).

For initial model exploration, background variables (such as age, menarcheal status and ethnic group) and dietary intake data (grams per day from the seven day weighed food records) were used as independent variables in the logistic model. Details of food groups covered by the survey are given in Table 8. Foods that were weakly significant at the 10% level ($P<0.1$) were then categorised into two or three levels of consumption appropriate for questionnaire use, using portion sizes/frequency as a guide (Table 9). Various categorisations were evaluated for their significance in models and the most powerful were then used to build the main models (for example; for red meat the 3 group classification: (none *vs*. 1-90g *vs*. >90g/d) gave the best prediction, while for tea, a two group classification (above or below 125g/d) was most significant in the regression.
The main model was designed to predict low iron status, but supplementary models were also explored to predict good iron status, using separate criteria, (Hb>12g/dl, SF>25µg/l,

TS>25%). Model statistics (Nagelkerke R-square) ROC curves and classification tables (sensitivity and specificity) were used to evaluate the explanatory power of models.

Finally a simplified scoring tool, for identifying level of risk of low iron status, was devised using the relative importance (Beta coefficients) of the main predictor variables. The accuracy of this tool was evaluated using ROC curves and indices based on sensitivity and specificity such as Youden's J and Cohen's kappa.

3. RESULTS

3.1. Comparison of girls with and without iron status measurement

(n=443 vs. n=222)

It was important to compare girls that were excluded with those in the analysis to identify any potential bias that might affect the generalisability of the conclusions. As expected, girls who provided a blood sample for iron status measurement tended to be slightly older than those who did not provide a blood sample (mean age 13.1 vs. 12.4 years), but there were no differences in energy intake, or iron intake, whether from diet or from all sources. Neither were there any significant differences in body mass index (weight in kg/ (height in m)^2, or in the ratio of energy intake to BMR (EI:BMR), which is used as an indicator of low energy reporting or dieting.

Table 2
Characteristics of girls with blood measurement of iron status vs. those without (i.e. excluded from analysis)

		iron status measurement	no iron status measurement	
	N	443	222	P value
Age (years)	Mean	13.1	12.4	0.009
	Std Deviation	3.3	3.4	
energy (kJ)	Mean	6941	6811	0.30
	Std Deviation	1540	1449	
Dietary Iron intake (mg/d)	Mean	8.7	8.8	0.70
	Std Deviation	2.8	2.9	
Total iron (mg/d)	Mean	8.9	8.9	0.85
	Std Deviation	3.2	3.1	
Body Mass Index (kg/m^{-2})	Mean	20.3	20.0	0.40
	Std Deviation	3.9	4.3	
EI:BMR ratio	Mean	1.28	1.30	0.48
	Std Deviation	.30	.30	

3.2. Prevalence of low iron status in girls aged 7-18 years

Thirty percent of girls (n=133/443) had one or more low iron status values (Hb, SF or TS). A small subset of 34 girls (7.7% of the total sample) had two or more low status values, but this number was insufficient for meaningful separate analysis.

Table 3
Number and percentage of girls with low iron status measurements, by age group

Number of low Fe status measurements		Age group at blood sample			Total
		7-10	11-14	15-18	
0	Count	101	120	89	310
	%	77%	76%	58%	70%
1	Count	30	29	40	99
	%	23%	18%	26%	22%
2+	Count	1	9	24	34
	%	1%	6%	16%	8%
Total	Count	132	158	153	443
	%	100%	100%	100%	100%

3.2.1. Prevalence of low iron status according to different indices

Table 4
Prevalence of low iron status on three indices

	Age group				**Chi sq**
	7-10	11-14	15-18	Total	P
Low Hb <11.5 or < 12g/dl	N=131 5 4%	N=155 4 3%	N=153 14 9%	N=439 23 5%	0.024
Low SF (<15µg/l)	N=91 4 4%	N=121 18 15%	N=124 35 28%	N=336 57 17%	0.0001
Low TS (<15%)	N=109 23 21%	N=142 27 19%	N=143 44 31%	N=394 94 24%	0.049

Approximately 5% of the sample was anaemic on the criteria used in the NDNS report, with the highest prevalence among older girls aged 15-18 years (9%) (Table 4).
One in six girls (17%) had evidence of low iron stores (SF <15μg/l). The prevalence rose steadily with increasing age (P<0.0001) such that 28% of the oldest group of girls had evidence of depleted iron stores compared with only 4% of 7-10 year olds.
Almost one in four girls had evidence of low levels of transport iron (TS<15%), again with a higher prevalence among the older group of girls (31%).

3.3. Background differences according to iron status

As mentioned previously, we classified girls into two groups, normal or low iron status (see Table 1). Background characteristics were very similar in the two groups. There was no significant difference in region, household composition (lone parent, other children) or in any of the socio-economic factors investigated (social class, income, receipt of benefits). Low iron status was most prevalent among Asian girls (50%) but due to the low numbers (n 16), this did not approach statistical significance.

Table 5
Background characteristics of girls according to iron status

		Iron Status		
	N	Normal	Low	P value
Ethnic group				0.29
White	404	71%	29%	
Asian	16	50%	50%	
Black	16	63%	38%	
other/mixed race	7	71%	29%	
Social class				0.47
I and II	148	69%	31%	
III non-manual	53	79%	21%	
III manual	131	66%	34%	
IV and V	64	72%	28%	
Unclassified	47	72%	28%	
Region				0.62
Scotland	30	70%	30%	
Northern	115	65%	35%	
C, SW & Wales	159	71%	29%	
London & SE	139	73%	27%	
Mothers education				
Degree	45	82%	18%	0.17
Other qualification/exam	287	68%	32%	
No formal qualifications	89	70%	30%	
Parenting				0.78
Living with both parents	350	70%	30%	
Living with lone parent	93	69%	31%	
Other children in household				0.36
Yes	303	71%	29%	
No	133	67%	33%	
Receiving benefit				0.68
Yes	109	72%	28%	
No	334	69%	31%	

3.4. Anthropometric and physiological differences according to iron status

Girls with low iron status were on average one year older, with an attendant difference in height (+4 cm), weight (+5 kg) and BMI (+0.9). However, there was no significant difference in standardised (age-adjusted) BMI nor in energy intake (EI) nor the ratio of EI to basal metabolic rate (EI: BMR), an indicator of low energy reporting.
Girls with low iron status were more likely to have reached menarche (62% vs. 46%; P=0.002), consistent with their older age. One in every four girls over 15 was taking an oral contraceptive pill, but this was not associated with iron status, either positively or negatively.

Table 6. Anthropometric and physiological differences according to iron status

			Iron status			
			Normal	**Low**	**Total**	**P value**
	N=		310	133	443	
Age (years)	443	Mean	12.8	13.9	13.2	0.002
		SD	3.3	3.4	3.3	
Energy (kJ)	443	Mean	6890	7059	6941	0.29
		SD	1569	1469	1540	
EI: BMR	440	Mean	1.3	1.3	1.3	0.39
		SD	.3	.2	.3	
Body Mass Index (kg/m^2)	441	Mean	20.0	20.9	20.3	0.02
		SD	3.8	4.0	3.9	
Standardised BMI (z-score)	441	Mean	.4	.5	.4	0.40
		SD	1.1	1.1	1.1	
Height (cm)	441	Mean	150	154	151	0.005
		SD	14	14	14	
Weight (kg)	442	Mean	46	51	48	0.002
		SD	15	15	15	
Waist (cm)	306	Mean	70	72	71	0.08
		SD	9	8	9	
Hip (cm)	306	Mean	93	95	93	0.04
		SD	10	10	10	
% Who had reached menarche			46%	62%	51%	0.002

3.5. Differences in iron intake according to iron status

Girls with low iron status had identical total iron intakes, but lower intakes of *haem* iron (0.32 vs. 0.38mg/d; P=0.016) compared with girls with normal status (Table 7). Haem iron is calculated in the NDNS as 40% of the iron in meat and fish and proportional amounts in meat and fish dishes, (Monsen, Hallberg et al. 1978). The lower intake of haem iron in the low iron status group was mainly attributable to the higher prevalence of vegetarianism (12.8% vs. 4.5%; P=0.002), coupled with a tendency for the non-vegetarians in that group to eat less red meat than their counterparts who had normal iron status (mean red meat consumption 67g vs. 80g/d; P=0.01).

The contribution from iron supplements was not significantly different between the groups because so few were taking them (10 girls, or 2% in each group). The five girls receiving 12mg Fe per day or more from iron supplements all had normal iron status, although it is not possible to tell whether they were taking supplements on the advice of a doctor, for anaemia, or whether they were self-medicated.

Table 7.
Iron intake and sources of iron according to iron status

		Iron status			
		Normal N=310	Low N=133	Total N=443	P value
Total iron (mg/d) incl. supplements	Mean	8.9	8.9	8.9	0.98
	SD	3.5	2.6	3.2	
	Median	8.3	8.5	8.4	
	Percentile 25	6.6	7.2	6.6	
	Percentile 75	10.3	10.5	10.4	
Dietary iron (mg/d)	Mean	8.6	8.8	8.7	0.44
	SD	2.8	2.6	2.8	
	Median	8.2	8.5	8.3	
	Percentile 25	6.5	7.1	6.6	
	Percentile 75	10.2	10.4	10.2	
Dietary Haem iron (mg/d) #	Mean	.38	.32	.36	0.016
	SD	.25	.22	.24	
	Median	.34	.28	.33	
	Percentile 25	.20	.18	.20	
	Percentile 75	.50	.44	.47	
Dietary Non-haem iron (mg/d)	Mean	8.2	8.5	8.3	0.32
	SD	2.8	2.6	2.7	
	Median	7.8	8.3	7.9	
	Percentile 25	6.1	6.7	6.3	
	Percentile 75	9.8	10.0	9.9	
Iron (mg/d) from supplements	Mean	.27	.04	.20	0.98*
	SD	2.06	.32	1.74	
	Median	.00	.00	.00	
	Percentile 25	.00	.00	.00	
	Percentile 75	.00	.00	.00	

* non-parametric test (Mann Whitney)

3.6. Food intakes according to iron status

Food intake data from the dietary records were aggregated into 31 broader food groups for comparison of food intakes between girls with low and normal iron status. Table 8 shows values for median and quartiles of consumption (g/d).

The most statistically significant result was the lower consumption of *red meat and meat products* among girls with low iron status (median 57g vs. 70g; P=0.001). This was not counteracted by a higher consumption of other sources of haem iron (poultry and fish). More than half the girls who ate no meat had low iron status (53%) compared with 28% of those who ate meat (Table 9). Non-consumption of meat was broadly consistent with (but not identical to) the reported prevalence of vegetarianism, which was nearly three times as common in those with low iron status (12.8% vs. 4.5%; P<0.002; Table 10).

Tea consumption was higher in the low iron status group (P=0.01) a quarter of whom consumed over 200g/day). Consuming more than about one cup per day (125 ml) was associated with a higher risk of low iron status (41% vs. 26%) (Table 9). Tea consumption rose with age but was not associated with vegetarianism (see 3.6.1. and Table 11). The association of tea consumption with low iron status is in accordance with our understanding of the adverse effect of polyphenols on non-haem iron absorption. Coffee, by contrast, was consumed by very few children and did not appear to be associated with low iron status.

Vegetables may contain both enhancers such as vitamin C and inhibitors of iron absorption such as phytate (in beans) and polyphenols. Cooked vegetables appeared to be associated with low iron status but this association is confounded by age. For raw vegetables (mainly salads) there was no association in univariate tables, although in some logistic models salad vegetables subsequently showed up as a weak predictor of good iron status (see 4.2).

Girls with low iron status tended to have a slightly lower intake of fruit overall (median 30g vs. 40g/d; P=0.08) and 29% consumed no fruit during the survey week, compared with 19% of girls with normal iron status (data not shown). There was no significant difference in the consumption of fruit juice between low status and normal status groups, but consuming *neither* fruit nor fruit juice was subsequently found to be a significant predictor of low iron status in the logistic models (see 4.1.3).

Soft drink consumption was weakly associated with good iron status (P=0.013). Whilst many fruit squashes contain ascorbic and citric acid, which could plausibly enhance iron absorption, the association is also confounded by age. Younger children, who have better iron status, tended to consume more soft drinks, and these were not significantly associated with iron status in the logistic models.

Consumption of milk, eggs and cheese was similar between the iron status groups. There was also no difference for cereal consumed, even when high fibre and non-high fibre varieties were considered separately (it was not possible to distinguish fortified and non-

fortified cereals in this analysis). Neither was there any association of iron status with the amount or type of bread eaten.

Table 8
Food intake differences by iron status group

Food group	Iron status						Mann Whitney
	Normal			Low			P value
	Median	Percentile 25	Percentile 75	Median	Percentile 25	Percentile 75	
High fibre breakfast cereal	0	0	12	0	0	12	.32
Low fibre breakfast cereal	4	0	22	6	0	20	.52
Breakfast cereal	18	0	36	16	3	33	.89
Biscuits, cakes, pies	34	14	54	31	13	55	.82
Bread	61	44	88	61	38	90	.68
Wholemeal bread	0	0	0	0	0	8	.20
Other bread	55	34	79	53	29	81	.52
Pasta and rice	32	13	68	39	16	68	.38
Pizza	0	0	18	0	0	20	.75
Milk	135	54	220	138	67	233	.41
Yogurt, desserts, ice cream	25	2	49	20	0	50	.44
Puddings	0	0	25	0	0	31	.93
Cheese	8	0	17	6	0	17	.39
Eggs and dishes	0	0	16	4	0	17	.37
Fats and oils	8	4	13	7	4	14	.95
Fish	9	0	22	8	0	26	.59
Red meat, liver, and meat products	70	40	104	57	28	91	.001
Poultry and prods	25	10	55	27	9	56	.74
Chips and fried potatoes	47	23	83	48	23	76	.84
Other potatoes	34	15	58	34	18	65	.31
Salad vegetables	10	0	23	6	0	25	.29
Cooked vegetables Inc baked beans	49	24	75	58	33	93	.01
Fruit	40	11	86	30	0	75	.08
Fruit juice	6	0	85	0	0	78	.68
Savoury snacks	14	6	23	15	6	26	.64
Chocolate and sweets	20	7	40	21	10	38	.70
Soft drinks	505	290	805	394	197	668	.013
Table sugar	1	0	7	2	0	8	.34
Jam and other sugar spreads	0	0	3	0	0	4	.47
Tea and herbal tea	6	0	96	29	0	201	.010
Coffee	0	0	0	0	0	0	.73

Table 9
Prevalence of low iron status according to consumption of selected foods

Food	Consumption in week	N in consumption category	% with low iron status	Chi square P value
Breakfast cereal	No Yes	111 332	27.0% 31.0%	0.43
High fibre breakfast cereal	No Yes	253 190	31.6% 27.9%	0.40
Bread/cereals	>70g <70g	275 168	29.8% 30.4%	0.90
Fruit (in week)	Yes No	346 97	27.2% 40.2%	0.013
Fruit juice (in week)	Yes No	221 222	29.4% 30.6%	0.78
Fruit or fruit juice (in week)	Yes No	383 60	27.9% 43.3%	0.016
Milk (g/d)	<125g <250g >250g	211 146 86	29.9% 30.8% 29.1%	0.96
Cooked vegetables (in week)	None <50g/d >50g/d	26 192 225	26.9% 25.0% 34.7%	0.09
Poultry (in week)	No Yes	74 369	35.1% 29.0%	0.29
Red meat and products	Yes No	409 34	28.1% 52.9%	0.002
Red meat and products	>90g/d 1-90g/d None	142 267 34	23.9% 30.3% 52.9%	0.003
Raw vegetables (salad)	Yes No	302 141	27.8% 34.8%	0.14
Tea	None <125g/d >125g/d	211 118 114	26.5% 25.4% 41.2%	0.01
Tea	<125g/d >125g/d	329 114	26.1% 41.2%	0.002
Age over13	No Yes	220 223	22.3% 37.7%	0.0001
Age over 15	No Yes	290 153	23.8% 41.8%	0.0001
Menarche reached	No Yes	219 224	23.3% 36.6%	0.002

3.6.1. Vegetarianism, meat and tea consumption

The association between reported vegetarianism and consumption of meat (from the one week diet records) was investigated further to identify which might be more appropriate to use in the checklist or screening tool. Vegetarianism means different things to different people and the correspondence between claiming to be vegetarian and not eating meat was not as close as expected (Table 10). One in four vegetarians (8/31) ate red meat during the survey week, whilst a few non-vegetarians had a meat-free week. Furthermore, the non-vegetarians with low iron status tended to eat less red meat than the non-vegetarians who had normal iron status (67g/d vs. 80g/d, P=0.01). Given the ambiguity in the term vegetarianism, a score based on usual consumption of red meat is likely to reflect haem iron intake more closely.

Tea consumption rose with age (data not shown) but was virtually identical among vegetarians and non-vegetarians (Table 11). Approximately half the girls consumed some tea in the week, but only a quarter drank one cup or more daily.

Table 10
Numbers of children who reported being vegetarian, according to consumption of red meat and poultry during survey week

	Vegetarian (n=31)	Non-vegetarian (n=412)	All girls aged 7-18 (n=443)
No red meat	23	11	34
No red meat or poultry	22	2	24
Consumers of red meat			
<=90g/d red meat	6	261	267
>90g/d red meat	2	140	142

Table 11
Tea consumption among vegetarians and non-vegetarians

		Vegetarian		Non-vegetarian	
		N	%	N	%
Tea consumption	none	15	48%	196	48%
	1-125g/d	7	23%	111	27%
	>125g/d	9	29%	105	25%
Total		31	100%	412	100%

4. LOGISTIC MODELS OF LOW IRON STATUS AND GOOD IRON STATUS

Logistic regression was used to identify the dietary and non-dietary predictors of low iron status (defined as one or more low status measurements). The methods employed for the logistic regression have been described in section 2.4. Details of the best models from these procedures are given in Appendix 1. In the first step, the dietary variables were "tested" in continuous format, in a conditional stepwise regression procedure, together with the main background variables (Appendix 1: model 1). Factors significant in these early models were then explored further and simplified with a view to producing a model based on categorical variables (defined in Table 9) that might be suitable for use as a questionnaire (Appendix 1: models 2 and 3). Summaries of the final model are given below.

4.1. Predictors of low iron status

The best model predicting low iron status (one or more low status measurements) was based on one physiological variable *(menarche)* and three dietary variables *(red meat, fruit or fruit juice, and tea).* Model statistics are given in Table 12a and 12b. The sensitivity and specificity of the model at a cut point of 0.3 (based on the prevalence of low iron status in the sample) is given in 12c: (overall accuracy 66%; sensitivity 54%, specificity 71%).

4.1.1. Menarche

Girls who had reached menarche had a 50% increased odds of low iron status (OR=1.5; 95% CI= 1.0 to 2.4). Using age group (above or below 13 years of age, the median age of menarche) gave similar results, but menarche was chosen as the more intuitive risk factor. There was no significant residual effect of age after adjusting for menarcheal status.

4.1.2. Red meat

Eating no red meat at all during the survey week (8% of girls; Table 10) was associated with more than treble the odds of low iron status (OR 3.6; 95% CI=1.6 to 8.0), while eating some red meat (but less than a portion a day <90g) carried a slightly elevated risk compared with high meat eater (>90g a day). A simpler dichotomous classification (no red meat vs. any red meat) could be substituted for the three-level red meat variable (OR 2.7; 95% CI 1.3 to 5.6) but this model had poorer explanatory power (Nagelkerke R-square 0.083).

Being a vegetarian could also substitute for red meat consumption as a risk factor, but was considered less suitable for risk assessment since it can be variously interpreted

(Table 10). Poultry had a negligible impact in predicting iron status, possibly because it contains less iron (especially breast meat).

4.1.3. Fruit juice and fruit

Approximately one in seven girls (n=60) consumed neither fruit nor fruit juice during the survey week. According to the model this doubled their odds of having low iron status. As individual variables non-consumption of fruit and non-consumption of fruit juice were less significant predictors of iron status than when combined. Vitamin C is a powerful enhancer of non-haem iron absorption, but interestingly, total dietary vitamin C intake was a weaker predictor of iron status than fruit and fruit juice consumption, although highly correlated with these (Spearman's rho =0.71, $P<0.0001$). This raises the possibility that other constituents of fruit and fruit juice may also have an impact. In a previous analysis of this survey, Thane found that girls aged 11-18 years who consumed fruit juice were less likely to have low Hb (Thane, Bates et al. 2003) .

4.1.4. Tea

Tea is a potential inhibitor of iron absorption, when consumed with or shortly after meals (Disler, Lynch et al. 1975). We found that drinking more than one cup of tea (125ml) per day was associated with almost double the odds of low iron status, while lesser amounts were not associated with significant excess risk. There were no interactions between tea consumption and other dietary variables such as milk or cereals. It was not possible to assess whether tea was consumed with or after meals in the present study, although timing on consumption may be crucial to the inhibitory effect of tea on iron absorption.

4.1.5. Other variables not included in the model

Not eating any raw vegetables (salads) was weakly associated with low iron status and this is consistent with the enhancing effect of vitamin C in many salad vegetables as well as the iron content of some (e.g. watercress). Breakfast cereals were not associated with lower risk, despite their positive contribution to iron intakes. Other studies have failed to find a positive association of breakfast cereals with iron status, (Gibson 1999) (Kato, Dnistrian et al. 2000). This may be a result of low bioavailability of cereal iron, although the possibility of confounders (e.g. an inverse association with meat consumption) could not be excluded. In our study, two-way interactions were explored between combinations of food variables where enhancers and inhibitors could have different effects (e.g. cereals and milk, meat, tea, fruit/fruit juice), but none were significant in any model.
No background factors such as ethnic group, income, dieting, low energy reporting or illness were significant predictors of low iron status.

Table 12a
Model variables for low iron status on any criterion

	B	S.E.	Sig. P=	Odds ratio	95.0% C.I for ORs	
					lower	upper
Menarche reached	.44	.22	.048	1.56	1.00	2.42
Tea (more than 125ml tea/day vs. none)	.60	.24	.011	1.83	1.15	2.91
No red meat	1.27	.41	.002	3.58	1.61	7.96
Some red meat but less than 7 portions a week	.42	.25	.088	1.52	.94	2.46
No fruit or fruit juice	.69	.29	.020	1.99	1.12	3.55
Constant	-1.73	.25	.000	.18		

Table 12b
Model Statistics for low iron status model

Step		-2 Log likelihood	Nagelkerke R Square
1	Menarche	531.976	.030
2	+Tea	525.964	.049
3	+Red meat	517.043	.076
4	+Fruit or fruit juice	511.728	.092

Table 12c
Classification Table for low iron status model

Observed			Predicted		
			Iron status		Percentage Correct
			Normal	Low	
Final model	Iron status	Normal	219	91	70.6
		Low	61	72	54.1
	Overall Percentage				65.7

(Overall accuracy 66%; sensitivity approx 54%, specificity approx 71%)

4.2. Predictors of good iron status

Separate logistic regressions were conducted to identify predictors of *good* iron status. To differentiate these from the model for low iron status in 4.1, we explored the predictors of each of the three indices of iron status, SF, Hb and TS. Good status was defined as SF>25 µg/l for the model based on iron stores; Hb>12g/dl for the model based on functional iron and TS>25% for the model based on transport iron. The proportion of girls with good status on these criteria was 55%, 90%, and 33% respectively and cut points for classification were set according to these prevalences. Model results are summarized below with further details given in Appendix 1 (models 4-6).

4.2.1. Model for serum ferritin >25µg/l

The best model predicting good iron stores (SF>25 µg/l) was based on just two variables: menarche and red meat consumption (Table 13a). Having attained menarche halved the odds of having SF>25µg/l (OR 0.45; CI 0.28 to 0.70). Iron losses in menstruation would be expected first to deplete iron stores (of which serum ferritin is a sensitive indicator) and only later and if severe to affect functional iron. Eating >90g red meat per day (equivalent to eating red meat most days) more than trebled the odds of good SF, while eating less than 90g/d was associated with around double the odds of good iron stores (compared with eating none). The confidence interval for this latter estimate was wide and included zero, but the results are consistent with a graded improvement in iron stores with increasing red meat consumption. The SF model correctly classified 59% of girls at a cut point of 0.55 (Table 13b): sensitivity 70%, specificity 46%.

Table 13a
Model variables for good iron stores (SF>25µg/l)

	B	S.E.	df	Sig.	Odds ratio	95.0% C.I.for Odds Ratio	
						Lower	Upper
Menarche reached	-.809	.233	1	.001	0.45	0.28	0.70
Red meat intake:			2	.012			
1<90g/day	.692	.456	1	.129	2.00	0.82	4.88
>90g/d	1.240	.473	1	.009	3.45	1.37	8.73
Constant	-.190	.467	1	.683	.83		

Table 13b
Model statistics for good iron stores (SF>25µg/l)

Step		-2 Log likelihood	Nagelkerke R Square
1	Menarche	448.799	.056
2	+Red meat	439.523	.091

Table 13b
Classification Table for good iron stores (SF >25µg/l)

Serum Ferritin		Predicted		
Observed		Low	High	Percentage Correct
	Low	70	83	45.8
	High	54	129	70.5
Overall Percentage				59.2

Cut value 0.55
(Overall model accuracy approx 59%; Sensitivity approx 70%, specificity 46%)

4.2.2. Model for haemoglobin>12g/dl

The best Hb model was based on three dietary variables. Eating fruit/fruit juice in the week and eating salad each doubled the odds of having a normal Hb level, while being non-vegetarian increased the odds by a factor of 3.8. This model correctly classified approx 60% of girls at a cut point of 0.9 (sensitivity 59%, specificity 66%). Eating red meat could be substituted for non-vegetarianism to give a model with very similar classification but slightly lower R-squared statistic (0.078) (see Appendix 1 model 5b).

Table 14a
Model variables for haemoglobin (Hb>12 g/dl)

	B	S.E.	Wald	df	Sig.	Odds ratio	95.0% C.I.for OR	
							Lower	Upper
Non-vegetarian	1.35	.51	6.89	1	.009	3.85	1.41	10.54
Salad veg.	.88	.35	6.21	1	.013	2.40	1.21	4.79
Fruit/juice	.98	.39	6.26	1	.012	2.67	1.24	5.76
Constant	-.25	.62	.16	1	.686	.78		

Table 14b
Model statistics for haemoglobin (Hb>12g/dl)

Step		-2 Log likelihood	Nagelkerke R Square
1	Fruit/ juice	264.844	.037
2	+Non-vegetarian	260.540	.058
3	+Salad veg	254.381	.087

Table 14c
Classification Table for haemoglobin (Hb >12g/dl)

Haemoglobin (g/dl)		Predicted		
Observed	Hb	<12	>12	Percentage Correct
	<12	27	14	65.9
	>12	164	234	58.8
Overall Percentage				59.5

cut value 0.9
(Overall model accuracy approx 60%; Sensitivity approx 59%, specificity 66%)

4.2.3. Model for iron saturation (TS>25%)

One in three girls had TS levels over 25%. The best TS model was based on two dietary variables: eating red meat and eating salad, both associated with a better likelihood of having a normal Hb level. According to this model, red meat eaters had approximately 5 times the odds of having a transferrin saturation level of over 25%. The model, however, was less powerful than those for SF and Hb and gave a prediction only just over 50% (sensitivity 74%, specificity 42%)

Table 15a
Model variables for TS>25%

	B	S.E.	Wald	df	Sig.	Odds ratio	95.0% C.I. for OR	
							Lower	Upper
Salad veg.	.58	.25	5.51	1	.019	1.78	1.10	2.88
Red meat	1.64	.62	6.94	1	.008	5.15	1.52	17.44
Constant	-2.67	.65	16.88	1	.000	.07		

Table 15b
Model statistics for TS>25%

Step	-2 Log likelihood	Nagelkerke R Square
Red Meat	490.991	.030
Salad veg	485.252	.050

Table 15c
Classification Table for TS>25%

Transferrin saturation Observed		Predicted <25%	Predicted >25%	Percentage Correct
Transferrin saturation	<25%	112	152	42.4
	>25%	34	96	73.8
Overall Percentage				52.8

a The cut value is .33
(Overall accuracy 53%: Sensitivity 74%, specificity 42%)

Model based on all three targets (n=308)

Out of 308 girls with measurements for all three indices, sixty-one had good status on all three criteria. However, no model could be created to predict these individuals from dietary and background variables, since none were significant.

4.2.5. Conclusions from good status models

The good status models provided some reassurance of the validity of the variables identified by the low status model and particularly of the strong association with red meat. They also identified salad vegetables as a dietary predictor of good status, and this was subsequently included in the screening tool and associated dietary messages.

5. DEVELOPMENT OF SCORING TOOL FOR LOW IRON STATUS

5.1. Model for scoring tool

The scoring tool was based on the best low status model (menarche, red meat, fruit/juice, and tea (Table 12a), but with the addition of *no salad vegetables*, which appeared in two of the good status models but was not correlated with avoiding fruit/juice. These 5 variables were then used to generate the model shown below (Table 16) to provide the basis for weightings for the scoring tool.

Deleted:

Table 16
Model for scoring tool

	B	S.E.	Df	Sig.	Odds Ratio i.e. Exp(B)	95% Confidence interval for Odds Ratio	
						Lower	Upper
Red meat:			2	.005			
Some red meat (but less than 90g/d)*	.43	.25	1	.079	1.54	.95	2.50
No red meat*	1.32	.41	1	.001	3.73	1.67	8.35
No fruit/fruit juice *	.59	.30	1	.053	1.80	.99	3.25
>125 ml tea/day	.60	.24	1	.011	1.83	1.15	2.92
Menarche reached	.49	.23	1	.031	1.63	1.05	2.55
No salad vegetables*	.38	.24	1	.110	1.46	.92	2.31
Constant	-1.87	.27	1	.000	.15		

* over one week

5.2. Weighting for scoring tool

In designing the weightings for the scoring tool based on this model, we could have exploited either the estimated B coefficients of the five-variable model, or their derived odds ratios. Although odds ratios are widely understood, the use of B coefficients is mathematically more correct where an additive self-assessment tool is required. We therefore explored the performance of the scoring tool based on the B coefficients of the above model (5.2.1.) and then subsequently devised a simpler version based on rounded weightings that would be easier to use (5.2.2).

5.2.1 Scoring tool based on unrounded B coefficients

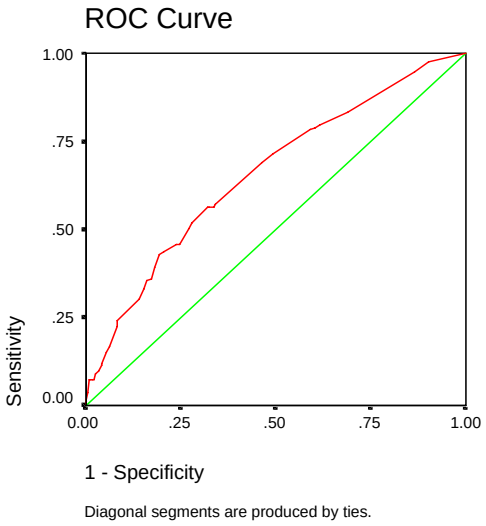
Based on the model in Table 16, the algorithm for this score is given below:

*Score = (no red meat*1.32) + (less than 90g/d red meat *0.43) + (no fruit or fruit juice*0.59) + (>125ml/d tea *0.60) +(menarche reached *0.49)+(no salad*0.38).*

The formula gave a score for each individual ranging from 0 to 3.38 (although in this sample no girl had the maximum score).

The overall accuracy of this model can be illustrated by the ROC curve (Fig A1), which shows the trade off between sensitivity and specificity (axis reversed). The area under the curve measures accuracy, according to ROC analysis; this is compared with the area of 0.5 lying under the diagonal line, which represents a worthless diagnostic test. The area for this model was 0.66 which, although significantly better than 0.5 ($p<0.001$, 95%CI = 0.6-0.72), is best considered as "fair". It is therefore of some potential value as the basis of a checklist, though not a substitute for a clinical diagnostic tool. More details of the performance statistics are given in the Appendix to this report.

Fig 2. ROC Curve for logistic model and scoring tool based on unrounded coefficients



5.2.2. Scoring tool based on integers (Questionnaire)

Rather than use the complex series of unrounded scores as above, a much simpler weighting scheme can be derived using simple integers based on rounded B coefficients, which has exactly the same ordering. In this scheme, the scoring tool, in the form of a

questionnaire (see Table 17) gives a weight of 3 for "no red meat" and a weight of 1 for all other factors. Possible scores range from 0 (suggesting good iron status) to a maximum of 7 (which indicates a high likelihood of poor iron status).

Table 17
Questionnaire: Are you at risk of having a low iron status?

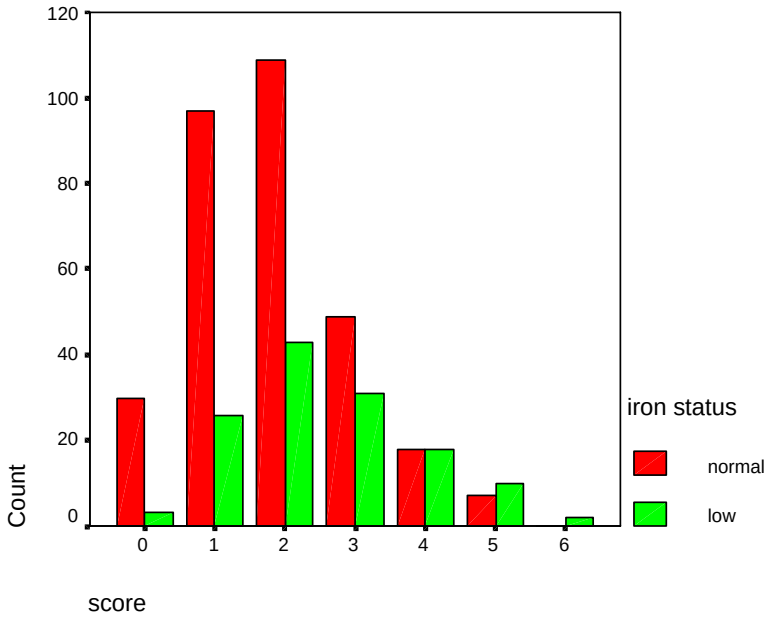
	Question	Response	Points	Your score
1	How much red meat do you eat? (or meat products)	7 portions a week	0	
		1-6 portions a week	1	
		Less than once a week or never	3	
2	How often do you eat fruit or drink fruit juice?	At least once a week	0	
		Less often	1	
3	How often do you eat salads or raw vegetables?	At least once a week	0	
		Less often	1	
4	How much tea do you drink?	One a cup a day (or more)	1	
		Less than a cup a day	0	
5	Have you started having periods yet? *	No	0	
		Yes	1	
		Total score		

* This question could be replaced by "are you over 13 years of age" with minor loss of accuracy.

5.3. Performance of the simple scoring tool

The simpler scoring tool (questionnaire) provided reasonable discrimination when tabulated against actual iron status of the 443 girls in the sample (Figure 3; Table 18). Substitution of the question on menarche by the alternative question ("are you more than 13 years old?") gave similar prediction (data not shown).

Fig 3. Score by iron status



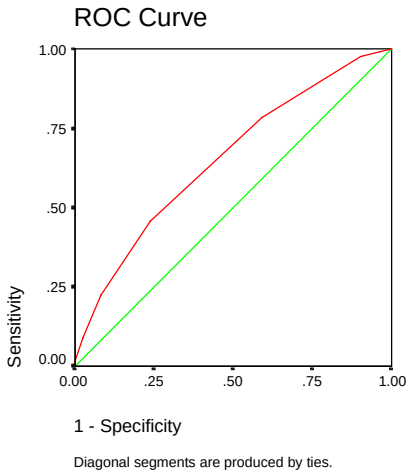
Performance measures are given in Figure 4 (ROC Curve) and Table 19. These indicate a predictive power almost identical to that of the model using unrounded coefficients (i.e. Area under curve 0.65 vs. 0.66). By contrast, the prediction obtained by using low iron intake (<LRNI) is no better than chance (50%).

If the intention were to define a single cut-off point for classifying girls as "high risk" *vs.* "low risk" of low iron status, Youden's J index [#] and Cohen's kappa[Ψ] were maximal (0.22) at a cut-off of ≥3 (Table 19) at which point agreement between the prediction of low status and actual low status in these girls could be regarded as "fair" (accuracy ~ 67%). However, we would suggest that this tool be used to grade risk on a continuous scale, rather than to predict a dichotomous outcome (at risk vs. not at risk).

Fig 4. Sensitivity and specificity of scoring tool

Youden's J index = %sensitivity + %specificity-100

Ψ Cohen's Kappa is a measure of agreement corrected against chance. A value of 0.24 or 24% indicates fair agreement



Area Under the Curve

Area	Std. Error(a)	Asymptotic Sig.(b)	Asymptotic 95% Confidence Interval	
			Lower Bound	Upper Bound
.653	.028	.000	.598	.709

a Under the nonparametric assumption
b Null hypothesis: true area = 0.5

Table 18. Crosstabulation of Questionnaire score vs. Actual iron status

		Iron status		
Score		Normal	Low	Total
0	Count	30	3	33
	%	91%	9%	100%
1	Count	97	26	123
	%	79%	21%	100%
2	Count	109	43	152
	%	72%	28%	100%
3	Count	49	31	80
	%	61%	39%	100%
4	Count	18	18	36
	%	50%	50%	100%
5	Count	7	10	17
	%	41%	59%	100%
6	Count		2	2
	%		100%	100%
	Count	310	133	443

(max score achieved was 6 out of possible score of 7)

Table 19. Performance indicators for scoring tool

	Iron status	Performance indicators at each cut-off point *

Score>=	Normal	Low	Total	Sensitivity %	Specificity %	Positive predictive value (%)	Negative predictive value (%)	True positives (n)	True negatives (n)	False positives (n)	False negatives (n)	Efficiency%	Cohens k	Youden J
0	30	3	33	100	0	30	100	133	0	310	0	30	0	0
1	97	26	123	98	10	32	91	130	30	280	3	36	5	7
2	109	43	152	78	41	36	81	104	127	183	29	52	14	19
3	49	31	80	46	76	45	77	61	236	74	72	67	22	22
4	18	18	36	23	92	55	73	30	285	25	103	71	17	14
5	7	10	17	9	98	63	71	12	303	7	121	71	9	7
6	0	2	2	2	100	100	70	2	310	0	131	70	2	2
	310	133	443	0	100	100	70	0	310	0	133	70	0	0

If cut-offs are used to assess risk, Table 19 can be used to indicate misclassification, although in practice using these data on other populations is likely to result in poorer agreement than in those on whom the tool was developed. Given these data, 63% of girls achieving a score of 4+, would actually have low iron status, and 7/19 would be false positives. It might be worth referring girls with such high scores to a doctor for blood test if have symptoms such as pallor and tiredness. Girls with lower scores might be encouraged to look at the dietary messages designed to improve iron status (section 7) and for these an interpretation of relative risk might be as follows:

A score of 1 or less indicates below-average risk (approx 1 in 5)…but doesn't guarantee good iron status
A score of 2 indicates average risk (approx 1 in 3)
A score of 3 or more indicates above-average risk (approximately 1 in 2)… but doesn't guarantee poor iron status.

5.4. Conclusion of logistic models and scoring tool

The main dietary factors identified in our models and scoring tool illustrate the importance of bioavailability of iron in the diet, rather than mere iron intake. However, iron absorption is also modulated by the iron status of the individual, being enhanced where iron stores are low. This enhanced absorption may not be sufficient to compensate for iron losses in some girls after menarche and our models found post-menarcheal status (or age above 13 years as a surrogate) to be a predictor of iron status. The results of our models were combined with other data on the sources and bioavailability of iron (section 6) in order to design the dietary messages suitable for dissemination to girls of school age (section 7).

6. DISCUSSION

6.1. Validity of approach and limitations

This study is based on data from the NDNS of young people aged 4-18 years, and in particular on the sample of 443 girls aged 7-18 years with a blood measurement of iron status. The challenge was to identify whether certain dietary habits could provide a better prediction of iron status than iron intake alone, given the known poor correlation between intake and status. The scoring tool devised is of similar power to a model recently published to help doctors gauge which patients would respond to withdrawal of treatment for high blood pressure (Nelson, Reid et al. 2002). In our case, the questionnaire is envisaged not as a diagnostic tool with a dichotomous outcome, (yes/no for low iron status) but as an educational tool for potential use with teenage girls.

The model is only applicable to this population (girls of school age) and should not be extrapolated to other age/sex groups. In addition, it would be desirable to validate the scoring tool against an independent sample of young girls, because the scoring tool is likely to be less efficient than in the sample from which it was generated. Ideally, our sample size would have permitted a true cross-validation (model-building on random 50% sample; model testing on remaining 50%), but the numbers were too small. Numbers were also too small to generate meaningful models for different age groups, although associations in subgroups (<>13 yrs) were explored. Simplification of the 7-d weighed dietary record data into two or three categories of consumption, to simulate a questionnaire, slightly reduced the power of the analyses, but the models were consistent with the preliminary analyses using continuous dietary data. Moreover, the significant associations found in the study are plausible and in broad agreement with other epidemiological and experimental studies (see 6.2. to 6.5).

6.2. Scientific background to the main predictive variables in models

6.2.1. Physiological variables

The physiological variables investigated in this study included age, and menarcheal status, body weight and height, which are closely correlated with age. There was no association between iron status and BMI adjusted for age. Menarche occurs at about the age of 13 and age, if dichotomised (over 13 vs. under 13yrs), could be substituted for menarche in models. However, menarche was chosen for the final model and scoring tool because it is the more biologically plausible.

Menarche

It is well recognised that high blood, and therefore iron, losses in menstruation can lead to poor iron status (Department of Health 1991). A recent, yet unpublished UK study (Harvey 2002) has generated data from direct measurements of menstrual blood loss and compared these to iron status. These investigators studied 90 women aged between 18 and 45y and found a mean blood loss of 31ml (range 1-129ml). Menstrual iron loss was

found to be inversely correlated with iron status, with higher menstrual iron losses resulting in a poorer iron status.

Our study could only ascertain whether or not the girls had reached menarche or not, rather than quantify size of menstrual losses, but this still proved to be strongly predictive factor for iron status.

6.2.2. Dietary variables

Several dietary factors have been shown to have a strong influence on iron absorption. The main promoters have been traditionally recognised as organic acids (ascorbic, citric and oxalic acids), animal tissue and alcohol. The main inhibitors are phytate, polyphenols, calcium, and phoshoproteins (British Nutrition Foundation Task Force 1995). Recently it has been reported that vitamin A and ß-carotene are strong promoters (Garcia-Casal MN, Layrisse M et al. 1997). Several other factors may also have an effect but have not been investigated as extensively. These include phosphate, zinc, copper, cobalt, manganese and fermented foods. It is the combination of these factors in a meal, which (in part) determines non-haem iron absorption and therefore (in part) determines iron status.

Three of these dietary variables were predictive in our model for schoolgirls and they will be discussed in detail here.

Red meat

There are two main reasons why eating red meat can improve iron status (British Nutrition Foundation Task Force 1995). Meat is a rich source of haem iron (around 45-60% of the iron in meat is haem) and this has a higher bioavailability than non-haem iron. In general 20-30% of haem iron is absorbed, in contrast to only 5-15% of non- haem iron (Monsen 1988) although this is highly variable due to the effect of enhancers and inhibitors of absorption (British Nutrition Foundation 1999). One enhancer is an unidentified factor associated with red meat. Suggestions for the 'meat factor' include the formation of iron complexes by amino acids such as cysteine or by peptides in meat, which counteract the luminal factors inhibiting iron absorption.

In our analysis of schoolgirls, eating no red meat at all during the survey week was associated with more than treble the odds of low iron status while eating a little red meat (but less than a portion a day <90g) carried a slightly elevated risk compared with being high meat eater (>90g a day). We have recently reported an association between iron status and consumption of red meat and meat products in an analysis of the 1986/7 survey of British adults (Gibson and Ashwell 2003). Two smaller studies among women have also shown an association between iron status and meat (Ortega, Lopez-Sobaler et al. 1998), or red meat in particular (Worthington-Roberts, Breskin et al. 1988).

Fruit juice and fruit

A number of schoolgirls in our study consumed neither fruit nor fruit juice during the survey week and this doubled their odds of having low iron status. In a separate analysis of young people from the same survey, Thane et al also found that girls aged 11-18 years who consumed fruit juice were less likely to have low Hb (Thane, Bates et al. 2003).

Fruits and their juices contain organic acids, mainly ascorbic and citric acid, and these are all strong promoters of non-haem iron absorption (Cook and Monsen 1977; Hallberg, Brune et al. 1986; Hallberg, Brune et al. 1989; Cook and Reddy 2001). These reducing agents change the valency of iron from Fe III to Fe II; this increases absorption because Fe II is more soluble at acid pH values found in the duodenum and small intestine. Vitamin C intake may have a greater impact on Hb than on SF (Thane, Bates et al. 2003) (Nelson, White et al. 1993) and one supplementation study has suggested vitamin C supplementation may be better than iron salts among vegetarians (Sharma and Mathur 1995).

Salad vegetables

Salad or raw vegetables were included in our scoring tool because weekly consumption was associated with double the odds of good iron status (P=0.003) and (non-consumption) of salad vegetables was a (weak) predictor of low iron status (at P>0.1). Salad vegetables contain vitamin C and other constituents such as carotenoids that may have a role in enhancing iron absorption. Salad vegetables are also a minor source of non-haem iron.

Tea

Tea is a potential inhibitor of iron absorption, when consumed with (or shortly after) meals. Tea contains polyphenols, which have an adverse effect on iron absorption because they chelate iron, forming large polymers which are not easily absorbed (Disler, Lynch et al. 1975; Disler, Lynch et al. 1975). Alkyl (galloyl) groups with their 3 adjacent hydroxyl groups were found to be the main common structure in polyphenols binding iron(Brune, Rossander et al. 1989). Coffee is also thought to inhibit iron absorption (Hallberg and Rossander 1982; Brune, Rossander et al. 1989) but no association was found with iron status among the schoolgirls in our study.

We found that drinking more than one cup of tea (125ml) per day was associated with almost double the odds of low iron status, while lesser amounts were not associated with significant excess risk. In contrast, the analysis by Thane et al. dichotomised consumption (into consumers vs. non-consumers) and found no difference in prevalence of low Hb (Thane, Bates et al. 2003). The low prevalence of anaemia in this study may have weakened the ability to detect a significant difference between consumers and non-consumers in Thane's analysis. However, there were also other differences in the definition of low iron status and in the children included in each analysis.

In other population groups, such as toddlers (Gibson 1999), menstruating women (Razagui, Barlow et al. 1991) and older people (Doyle, Crawley et al. 1999) drinking tea has also been shown to be associated with low iron status.

6.3 Variables not in our main model but known to influence iron status

Iron

Total iron intake did not figure as a predictor in our main model. This is not surprising when viewed in the light of the poor correlation generally found between total iron intake and iron status (Miles, Collins et al. 1984; Southon, Wright et al. 1992) (Spodaryk 1999). It is generally recognised that intakes of enhancers and inhibitors of iron absorption are more important than the actual intake of iron.

Phytates and cereal based foods

There is evidence to show that phytates (inositol phosphates with 6 phosphate groups) and other inositol phosphates are potent binders of iron and therefore inhibitors of non-haem iron absorption (Hallberg, Brune et al. 1989; Brune, Rossander-Hulten et al. 1992; Hurrell, Juillerat et al. 1992). On the other hand, the major foods containing phytate (breakfast cereals and bread) also contribute substantial amounts of iron to the diet. Breakfast cereals, for example, contributed over 20% of total iron of girls in the NDNS.

There was no association of iron status with bread consumption, whether wholegrain or other varieties, in any of our models. Neither were breakfast cereals significant in the models. We examined other ways of classifying breakfast cereal consumption (as high fibre vs. low fibre, by amount, and by iron contribution) but this did not increase the predictive power. We also assessed the impact of including breakfast cereal consumption as a dichotomous variable in the scoring tool but this did not improve the discrimination of low status. On these grounds, the suggested dietary messages do not make reference to any benefits of fortified breakfast cereals in relation to iron status.

Calcium and dairy foods

A strong dose-effect relation between the amount of calcium in a meal and the reduction in non-haem and haem iron absorption has often been observed in short term studies (Hallberg, Rossander-Hulten et al. 1992) but it is uncertain whether this has long lasting effects. Other studies are conflicting: one cross-sectional study in 6 European countries found a linear association between serum ferritin and calcium intake, after adjustment for other factors including iron intake, protein, tea, vitamin C and menarche, (van de Vijver, Kardinaal et al. 1999). On the other hand, calcium supplements do not appear to reduce iron stores (Fairweather-Tait 1995; Yan, Prentice et al. 1996; Minihane and Fairweather-Tait 1998). It was therefore not too surprising that calcium and dairy foods were not predictors in our main model of iron status in schoolgirls.

Eggs

Some evidence exists for an inhibitory effect of eggs, which is probably due to binding substances, notably phoshoproteins and albumin (Callender, Marney et al. 1970) (Hallberg and Hulthen 2000). Egg consumption by our schoolgirls was low, however, (with half consuming no eggs in a week and only 25% eating more than 2 eggs a week) and there was no association with iron status.

Alcohol

Alcohol has been shown to increase the absorption of Fe III but not Fe II iron, probably due to an enhancement of gastric acid secretion, lowering the pH of the proximal small intestine and increasing solubility of iron (Derman, Bothwell et al. 1980),(Derman, Bothwell et al. 1980) (Hallberg and Rossander 1982). However, reported alcohol consumption was extremely low among the girls in the study and no associations were apparent with iron status.

Other factors

Other known inhibitors of non-haem iron absorption such as soy protein (Hurrell, Juillerat et al. 1992) (Hallberg and Rossander 1982), nuts (Macfarlane, Bezwoda et al. 1988) would not have been present in sufficient amounts in the diets of schoolgirls to have any noticeable effect on the main predictive factors in our model.

6.4. Other models and algorithms

Several authors have attempted to predict non-haem iron absorption from diets on the basis of the foods or nutrients present in the diet.

Our study differed in that iron *status,* rather than iron absorption, was the outcome measure. However, if the dietary factors we identified were reasonably consistent with those from other studies, this would give increased confidence to promote these as dietary messages.

The results of some of the more recent studies are shown in Table 20. Most have used isotopically-labelled Fe to measure non-haem iron absorption in complex meals. In one of these Reddy fed 25 meals to 86 subjects and found that only the amount of animal tissue, phytic acid and ascorbic acid were significant predictors of non-haem iron absorption, collectively accounting for 16.4% of the variation in absorption (Reddy, Hurrell et al. 2000). Hallberg and Hulthen used a different approach. Their method involved calculating eleven separate absorption ratios, each taking into account only one or two dietary factors, and then combining the results of these ratios to produce a final estimate of non-haem iron absorption Their algorithm is based on the results of several studies published by the group over the past 30 years. Using a food (a wheat roll) with no known enhancing or inhibiting factors they evaluated the effect of different factors added in different amounts. In addition, the iron status of the subjects was taken into account.

Predictive factors included uncooked meat, Vitamin C, Phytate phosphorus, polyphenols, Calcium, Soya, protein, Eggs and Alcohol (Hallberg and Hulthen 2000).

Very recently, Conway and Geissler) performed a meta-analysis of 48 studies to estimate the quantitative impact of the principal enhancers and inhibitors of non-haem Fe absorption (Conway and Geisler 2002) (FSA project N05017). Studies were only included where it was possible to adjust for the Fe status of the individual or group. The resulting prediction equation included two promoters (animal tissue and high vitamin C fruit/juice) and eight inhibitors (beans/lentils, wholegrain cereals, tea, dairy foods, cheese, eggs, soya and nuts) (R^2=0.218, P<0.001).

Table 20
Predictors of non-haem iron absorption in other studies

Reference	Predictors of iron absorption	
	Enhancers	Inhibitors
(Tseng, Chakraborty et al. 1997)	Cooked meat Vitamin C	Phytate Tea
(Du, Zhai et al. 2000)	Animal tissue Vitamin C Fruit & vegetables	Dry tea Rice Beans
(Hallberg and Hulthen 2000)	Uncooked meat Vitamin C Alcohol	Phytate phosphorus Polyphenols Calcium Soya protein Eggs
(Reddy, Hurrell et al. 2000)	Animal tissue Vitamin C	Phytic acid
(Conway and Geisler 2002)	Animal tissue Vitamin C fruit/juice	Beans/lentils, Wholegrain cereals, Tea, Dairy foods, cheese Eggs Soya and nuts

6.5. Conclusion of comparison with other studies

The main dietary factors in our models and scoring tool (i.e. red meat, fruit/fruit juice, salads and tea) are consistent with the results of other studies and illustrate the importance of bioavailability, rather than total intake, of iron. However, iron absorption is

also modulated by the iron status of the individual, being enhanced where iron stores are low (Hallberg, Hulthen et al. 1997) (Hallberg, Hulthen et al. 2000). This enhanced absorption may not be sufficient to compensate for iron losses in some girls after menarche (Hallberg 1992) and our models found menarcheal status (or age) to be a predictor of low iron status. The results of these models were combined with other evidence to design the dietary messages suitable for dissemination to girls of school age.

7. DESIGN OF MESSAGES TO OPTIMISE IRON STATUS

The final phase of this project was the identification of evidence-based messages to optimise iron status in schoolgirls. In contrast to the conventional approach of general messages directed at the whole population, this approach is more focused. The scoring tool provides individuals with a rough, non-invasive guide to likely risk so that they can assess their need and link this to appropriate dietary messages. The messages are based on the predictors identified in models, augmented by additional information based on current scientific understanding of the determinants of iron status.

Table 21. Suggested dietary messages

Red meat (beef, lamb, pork, offal) is rich in iron that is easily absorbed (haem iron). The darker the meat the more iron it contains
Poultry contains some haem iron, and leg meat contains more iron than breast meat.
Fish contains some haem iron too, especially oily fish and molluscs (mussels etc)

If you are vegetarian eating no meat or fish, the following messages are even more important for you :

Fresh fruit or fruit juice with meals greatly helps to improve iron absorption
Salad vegetables (including tomatoes) contain vitamin C to help iron absorption.
Green leafy vegetables contain some iron (e.g. watercress, kale)
Avoid drinking tea with meals (or within 30 minutes of a meal) as this greatly reduces iron absorption
If you have heavy periods you may need extra iron. See your doctor if you think you may be anaemic

Such messages could be disseminated by the Agency via its website, providing a cost-effective means of delivering evidence-based nutrition advice to young girls.

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