The determinants of iron status in early pregnancy

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In pregnancy, the additional demands for Fe are thought to be met principally through increased maternal dietary Fe absorption and by mobilization of maternal Fe stores. In a general population sample of 576 women we examined the maternal and dietary characteristics which influenced Fe stores (assessed by serum ferritin concentration) in early pregnancy. The effects of these characteristics on two measures of functional Fe status (mean cell volume and haemoglobin concentration) were also considered. Serum ferritin concentrations were lower in multiparous women ($P < 0{\cdot}0001$) and in those with a lower BMI ($P = 0{\cdot}01$), and rose with increasing alcohol intake ($P < 0{\cdot}0001$). Ferritin concentrations fell with increasing Ca intake ($P < 0{\cdot}0001$); the proportion of women with serum ferritin values ≤ 12 μg/l rose from 14 % of the women in the lowest quarter of Ca intake to 29 % of the women in the highest quarter. Mean cell volume and haemoglobin concentration were not related to Ca intake in early pregnancy. Although Ca added to test-meals reduces Fe absorption, long-term Ca supplementation has not been shown to lower plasma ferritin concentration, suggesting that high habitual Ca intakes would be unlikely to influence Fe status in non-pregnant individuals. Our findings show that in early pregnancy there is an association between high dietary Ca intake and lower Fe stores. This effect of Ca on one aspect of Fe status may result from its influence on Fe bioavailability.

Iron: Ferritin: Pregnancy

Fe requirements are increased in pregnancy. In theory they are met through the combined actions of mobilization of maternal Fe stores (Taylor *et al.* 1982), increased dietary Fe absorption (Barrett *et al.* 1994) and from savings made in basal Fe losses during the period of amenorrhoea. For women who start pregnancy with adequate Fe reserves these adaptations should allow the additional Fe requirements to be met without any need to increase dietary Fe intake (Department of Health, 1991).

Although the ideal level of storage Fe at the start of pregnancy is not known (British Nutrition Foundation, 1995), studies in the UK show low Fe stores in considerable numbers of women of child-bearing age (Gregory *et al.* 1990). In addition, whilst large increases in Fe absorption from test-meals have been measured in pregnant women (Barrett *et al.* 1994), it is not clear how these changes would be influenced by the 'bioavailability' of Fe in the diet. This describes the proportion of Fe intake available for absorption and utilization from the diet and depends on the balance of factors present which inhibit and promote Fe absorption (Department of Health, 1991).

In a recent dietary survey of a general population of pregnant women (Robinson *et al.* 1996), nearly half the group reported that their consumption of milk was increased in early pregnancy relative to their non-pregnant intake. Since Ca is a potent inhibitor of Fe absorption from test meals (Hallberg *et al.* 1992), we investigated whether there was a relationship between Fe status in early pregnancy and Ca intake. As indices of Fe status we considered serum ferritin concentration as a measure of Fe stores, and mean red cell volume (MCV) and haemoglobin (Hb) concentration as measures of functional Fe status (British Nutrition Foundation, 1995). We also considered the influences of maternal characteristics which affect Fe status, as well as other dietary factors which influence Fe bioavailability.

Subjects and methods

Subjects and study design

The study population consisted of all white Caucasian women aged 16 years or older with singleton pregnancies who registered under two consultants over 1 year at the Princess Anne Hospital in Southampton. Of the 643 eligible women, 603 (94 %) agreed to take part in the study. In the

Abbreviations: FFQ, food-frequency questionnaire; Hb, haemoglobin; MCV, mean cell volume.

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present paper we report data for 576 women (90%) for whom we have dietary information, serum ferritin, MCV and Hb values in early pregnancy.

At a home visit a research nurse administered a food-frequency questionnaire (FFQ) which assessed the average frequency of consumption of 100 foods or food groups during the preceding 3 months. This period roughly corresponded with the first trimester of pregnancy since the median gestation for the visits was 15·1 weeks (5th and 95th percentiles, 12·1 and 18·3 weeks respectively). Each woman reported on her use of dietary supplements during the 3-month period and gave an assessment of how her intake of specific foods (including milk) had changed in pregnancy in relation to non-pregnant levels. Additional questions were asked about the woman's social and medical background, health in pregnancy, smoking habits and alcohol consumption. Alcoholic drinks were recorded in pub measures (glasses or pints) and converted to units of alcohol before analysis. Details of the study have been reported previously (Robinson *et al.* 1996).

Age, height, first recorded weight in pregnancy, MCV and Hb values were abstracted from hospital notes. Gestational age was assessed using the date of the last menstrual period or, if uncertain, from ultrasound scan data (Howe *et al.* 1995). Social class was determined from the woman's current or last occupation (Office of Population Censuses and Surveys, 1980). Approval for the study was given by the local ethical committee.

Blood analyses

Blood samples taken at the first hospital visit were used for the determination of a full blood count and for serum ferritin estimation. In cases where a sample had previously been taken by the woman's general practitioner for a blood count, a second sample was taken at the hospital visit for ferritin estimation. Samples were taken for serum ferritin determination at a median of 101 d (5th and 95th percentiles, 81 and 120 d respectively), and at a median of 99 d (5th and 95th percentiles, 63 and 118 d respectively) for Hb and MCV determination. All measurements were carried out in the hospital haematology department which subscribes to the UK National External Quality Assessment Scheme (NEQAS). Serum ferritin concentration was measured by radioimmunoassay (Beckton Dickinson UK Ltd, Oxford, Oxon., UK) which incorporated both low- and high-end reference standards. An automated counter (Coulter STKR; Coulter Electronics Ltd, Luton, Beds., UK) was used for the determination of blood counts. The between-batch CV were 9% for serum ferritin concentration, and less than 0·8% for Hb and MCV measurements (Howe *et al.* 1995).

Data analysis

Nutrient intake was calculated by multiplying the nutrient content of a portion of each food (Holland *et al.* 1988, 1989, 1991*a*,*b*) by its reported frequency of consumption over 3 months. Nutrient intakes from dietary supplements taken during the period of assessment were added to food nutrient intakes assessed by the FFQ to give an average daily intake. Nutrient intakes assessed by this FFQ have previously been validated against estimates from 4 d food diaries kept in early pregnancy (Robinson *et al.* 1996). Nutrient intakes were either log or square-root transformed to approximate normality before analysis.

Hb and MCV values did not need transformation, but serum ferritin values were positively skewed and were square-root transformed before analysis. Serum ferritin and Hb concentrations fell with increasing duration of gestation (by 0·03 $\sqrt{(\mu g/l)}$ per d (95% CI 0·04, 0·01) and 0·11 g/l per d (95% CI 0·16, 0·07) respectively). MCV was not related to duration of gestation. Using the slopes of these regression lines, serum ferritin and Hb values were adjusted to a standard gestation of 98 d before analysis, and gestation-adjusted values are reported. The back-transformed serum ferritin values are given in the text and tables.

Data were analysed to examine the influences of maternal and dietary factors on the three measures of Fe status. Smoking status and number of blood donations were treated as dichotomous variables, and their effects analysed using two-sample *t* tests. The effects of ordered categorical variables (social class, tea and alcohol intakes) were assessed using tests for linear trend by ANOVA. All other factors were treated as continuous variables and their effects analysed using linear regression. Multiple linear regression analysis was used to assess the independent effects of separate variables when considered simultaneously.

Results

Table 1 shows the characteristics of the women together with their intakes of selected nutrients and tea. Primiparous women constituted 53%, and there were similar numbers of women in social classes I and II (26·4%) as in classes IV and V (21·1%). Of the women 25% were smokers at the time of interview. At 14 weeks gestation, 24% of the women had low serum ferritin values ($\leq$12 μg/l), 4% had low MCV values ($\leq$80 fl), and 6% had low Hb values (<110 g/l).

Iron status and maternal characteristics

Table 2 shows mean serum ferritin, MCV and Hb concentrations according to the women's characteristics. Serum ferritin concentrations fell with decreasing maternal BMI, were lower in multiparous women and tended to be lower in women of lower social class. Allowing for the effects of parity reduced the association between serum ferritin concentration and social class ($P=0·30$), but did reveal independent associations with maternal height, age and the number of blood donations over the past year. In a simultaneous analysis, lower serum ferritin levels were seen in multiparous women ($P<0·001$) and in those who had given blood in the past year ($P=0·01$), and levels fell with increasing maternal height ($P=0·04$), lower age ($P=0·001$) and lower BMI ($P=0·001$). Smoking status was not related to serum ferritin level.

Table 1. Characteristics and dietary intakes of the 576 women

	Median	5th, 95th percentile
Age (years)	26·4	18·2, 34·4
Height (m)	1·63	1·53, 1·74
BMI* (kg/m^2)	23·1	18·9, 32·1
Serum ferritin† (μg/l)	25	3, 79
Mean cell volume (fl)	87	81, 93
Haemoglobin† (g/l)	124	108, 138
Fe intake (mg/d)	15·5	8·9, 40·4
Ca intake (mg/d)	1219	642, 2046
Vitamin C intake (mg/d)	125	48, 346
Folic acid intake (μg/d)	317	188, 602
Meat protein intake (g/d)	28	8, 53
Tea intake (cups/d)	2	0, 8
Alcohol intake (units/week)	0·2	0, 3·5

* Calculated from first recorded weight in pregnancy.
† Values adjusted for length of gestation at time of blood sample.

MCV was lower in multiparous women and higher in those who smoked. After allowing for the effects of parity and smoking, independent associations were demonstrated between MCV and maternal height and social class. In a simultaneous analysis, MCV fell with increasing maternal height ($P = 0·04$), and was lower in multiparous women ($P = 0·04$), in non-smokers ($P < 0·0001$) and in women of lower social class ($P = 0·003$).

Hb concentration was positively related to maternal BMI but showed no association with any other maternal characteristic. Allowing for the effect of maternal BMI did not change the relationship between Hb concentration and the other maternal factors.

Iron status and dietary intakes

Mean serum ferritin, MCV and Hb concentrations are shown in Table 3 according to the intakes of selected nutrients and tea. Lower serum ferritin concentrations were associated with higher Ca intakes and lower alcohol intakes. In a simultaneous analysis which included maternal parity, BMI, height, age and blood donation status, Ca and alcohol intakes remained strong independent predictors of serum ferritin concentration in early pregnancy. Mean serum ferritin concentration adjusted for these maternal factors fell from 31 μg/l (95% CI 27, 35) in the lowest quarter of Ca intake to 22 μg/l (95% CI 19, 25) in the highest ($P = 0·0003$), and rose from 24 μg/l (95% CI 21, 26) in individuals consuming no alcohol to 37 μg/l (95% CI 30, 45) in women drinking more than 2 units/week ($P < 0·0001$). After allowing both for the effects of Ca and alcohol intakes as well as the maternal factors in a simultaneous analysis, a weak positive

Table 2. Mean serum ferritin concentration, mean cell volume (MCV) and haemoglobin (Hb) concentration according to categories of maternal characteristics

		n	Serum ferritin† (μg/l)	MCV† (fl)	Hb† (g/l)
Parity	Primiparous	306	32	87·5	124
	Multiparous	270	20	86·7	124
	P value		<0·0001	0·01	0·97
Age (years)	−20	58	25	86·8	122
	−25	161	24	86·9	124
	−30	225	30	87·1	125
	>30	132	25	87·3	124
	P value		0·53	0·35	0·39
Height (m)	−1·59	157	29	87·5	124
	−1·63	155	24	87·0	124
	−1·67	136	27	86·8	124
	>1·67	128	24	87·1	123
	P value		0·13	0·13	0·14
BMI (kg/m^2)	−20	71	23	86·5	121
	−25	332	26	87·4	123
	−30	116	25	86·9	125
	>30	57	33	86·3	127
	P value		0·01	0·31	<0·0001
Social class of woman*	I and II	148	28	87·5	124
	IIIN	245	28	87·1	124
	IIIM	49	23	86·4	124
	IV and V	118	24	87·0	124
	P value		0·06	0·18	0·83
Number of blood donations in past year	0	540	27	87·1	124
	≥1	36	22	86·7	125
	P value		0·20	0·54	0·41
Smoking status at 15 weeks gestation	Non-smoker	431	26	86·7	124
	Smoker	145	28	88·4	124
	P value		0·39	<0·0001	0·67

* Unknown for sixteen women.
† Mean values (−1 SD, +1 SD) for whole group; ferritin 26 (9, 53) μg/l; MCV 87·1 (83·2, 91·0) fl; Hb 124 (115, 133) g/l.

Table 3. Mean serum ferritin concentration, mean cell volume (MCV) and haemoglobin (Hb) concentration according to maternal dietary intakes

Dietary intake		*n*	Serum ferritin (μg/l)*	MCV (fl)*	Hb (g/l)*
Fe (mg/d)	−12·6	145	28	87·2	125
	−15·5	143	26	87·0	123
	−21·1	144	28	86·9	124
	>21·1	144	23	87·3	123
	P value		0·15	0·54	0·007
Ca (mg/d)	−945	144	32	87·2	125
	−1219	144	26	87·3	123
	−1518	144	26	86·9	124
	>1518	144	21	87·0	123
	P value		<0·0001	0·57	0·13
Vitamin C (mg/d)	−87	144	26	86·9	124
	−125	144	27	87·0	124
	−174	144	27	87·1	123
	>174	144	25	87·4	124
	P value		0·88	0·08	0·88
Folic acid (μg/d)	−255	144	29	87·1	125
	−317	144	26	86·7	123
	−397	144	27	87·0	124
	>397	144	24	87·5	123
	P value		0·20	0·21	0·06
Meat protein (g/d)	−19·2	144	27	87·2	122
	−28·2	145	24	86·7	125
	−37·5	143	30	87·6	125
	>37·5	144	24	86·9	124
	P value		0·47	0·82	0·21
Tea (cups/d)	0	94	25	86·7	125
	−2	233	28	87·3	124
	−5	165	26	86·8	123
	>5	84	23	87·3	123
	P value		0·31	0·72	0·02
Alcohol (units/week)	0	250	24	87·0	125
	−1	212	26	86·8	123
	−2	62	32	87·9	124
	>2	52	37	87·9	123
	P value		<0·0001	0·06	0·17

* Mean values (−1 SD, +1 SD) for whole group; ferritin 26 (9, 53) μg/l; MCV 87·1 (83·2, 91·0) fl; Hb 124 (115, 133) g/l.

association was seen between serum ferritin concentration and vitamin C intake ($P=0·09$), but there was no association with any of the other dietary factors listed.

Table 4 shows the proportion of women with a serum ferritin concentration ≤12 μg/l according to parity and quarter of the Ca intake distribution. As expected, at each level of Ca intake, the proportion of multiparous women with a low ferritin level was greater than that of primiparous women, but within each parity group there was a twofold increase in the proportion of women with a low serum ferritin concentration between the lowest and highest quarters of Ca intake. Supplementary Ca contributed less than 1 % of the group's Ca intake, and exclusion of supplementary Ca had little influence on the relationship between Ca intake and serum ferritin concentration. The inverse association observed between Ca intake and serum ferritin concentration was not restricted to women with low Fe intakes, and was independent of the woman's experience of nausea and her reported changes in food intake in early pregnancy.

Table 4. Proportion of women with low serum ferritin values in early pregnancy according to parity and level of calcium intake

	Primiparous		Multiparous	
Ca intake (mg/d)	*n*	Women with ferritin values ≤12 μg/l (%)	*n*	Women with ferritin values ≤12 μg/l (%)
−945	76	9	68	19
−1219	77	14	67	33
−1518	76	15	68	44
>1518	77	17	67	43

There were weak trends for MCV to increase as alcohol and vitamin C intakes rose (Table 3), but these influences were only seen among women who smoked ($P=0·08$ and $P=0·006$ respectively). The effects were independent of the influences of maternal parity, height and social class.

Hb concentration was related to Fe, folic acid and tea intakes (Table 3). Inclusion of maternal BMI in a multiple linear regression did not affect the inverse association between tea intake and Hb concentration ($P=0·04$), but weakened the relationships between Hb concentration and both folic acid intake ($P=0·28$) and Fe intake ($P=0·08$). The inverse association between Fe intake and Hb

concentration was largely dependent on Fe intake from diet supplements and was not seen among unsupplemented women.

Discussion

We have described associations between maternal and dietary factors, and three measures of Fe status in a general population of women in early pregnancy. Serum ferritin concentration was included as an indicator of the level of stored Fe, and Hb concentration and MCV as measures of functional Fe status. Since we have data for 90% of the study population, and the social class distribution of the group is similar to that of women in the UK, the women should be representative of pregnant women in the general population. Values of the three measures of Fe status were in the normal range, although lower than those observed in non-pregnant women (Gregory *et al.* 1990; Fogelholm *et al.* 1993).

Our main findings relate to serum ferritin concentration in early pregnancy. Lower serum ferritin concentrations were seen in multiparous, taller, thinner and younger women, and in those who had given blood over the year before becoming pregnant. The two most important maternal characteristics were the woman's parity and her BMI, both of which have been shown to relate to serum ferritin level in non-pregnant women (Fricker *et al.* 1990; Milman *et al.* 1992; White *et al.* 1993).

When we considered maternal dietary intakes, we found strong influences of alcohol and Ca intake on serum ferritin concentrations which were independent of the maternal influences described. Even though reported alcohol intakes were low, serum ferritin concentration was higher in the women who drank alcohol in early pregnancy. This effect appeared to be dose-related, the highest ferritin levels being seen in women who reported consumption of more than 2 units/week. A positive association between alcohol intake and ferritin level has been described in non-pregnant women (Gregory *et al.* 1990; White *et al.* 1993), although it was seen at higher levels of intake, and we were surprised to find an influence of alcohol in women whose reported intakes were so low. Whilst the influence of alcohol on ferritin level is thought to represent a real effect on Fe stores (Strain & Thompson, 1991) it is not clear whether alcohol affects Fe absorption. Experimental studies of the effect of alcohol vary in their findings, and, depending in part on the nature of the alcoholic drink given, both positive and negative influences on Fe absorption have been described (Cook *et al.* 1995).

Ca intake was inversely related to serum ferritin concentration. This association was not restricted to women who reported increasing milk consumption in pregnancy or to those with low Fe intakes. While Ca supplementation of non-pregnant individuals has not resulted in lower plasma ferritin levels (Sokoll & Dawson-Hughes, 1992; Yan *et al.* 1996), an inverse relationship between the intake of dairy products and serum ferritin has been reported in non-pregnant women (Soustre *et al.* 1986) and Ca added to test-meals has been shown to reduce Fe absorption (Hallberg *et al.* 1992; Gleerup *et al.* 1995). Our findings suggest that higher intakes of Ca in early pregnancy affect the bioavailability of dietary Fe and result, at least in the short term, in lower Fe stores.

We assessed Ca intake using an administered FFQ (Robinson *et al.* 1996). Whilst such questionnaires are thought to give useful information on the ranking of individuals within the distribution of nutrient intake, they may not give good information regarding absolute nutrient intakes, and can be subject to bias (McKeigue, 1995). However, when Ca intakes determined by the present questionnaire were compared with those assessed using 4 d food diaries, the correlation coefficient was 0·41 (Robinson *et al.* 1996), and for 564 women in the present study who kept food diaries, diary-assessed Ca intake was also inversely related to serum ferritin concentration (results not shown).

Since the influences of Ca and alcohol on ferritin concentration were seen at intakes within the normal range, it raises the possibility that in early pregnancy the level of Fe absorption is strongly influenced by dietary Fe bioavailability. If true, this may have implications for the projected increases in Fe absorption believed to occur during pregnancy. In non-pregnant women, Hulthén *et al.* (1995) have shown independent effects of Fe status and dietary Fe bioavailability in determining the level of Fe absorption. Whilst large increases in Fe absorption have been measured in pregnant women consuming test diets (Barrett *et al.* 1994) it is not known how the bioavailability of Fe in a woman's free-living diet could influence these changes. Our findings suggest that Fe bioavailability may have important effects on Fe absorption in pregnancy.

There were modest trends for MCV to be reduced in multiparous women, taller women and in those of lower social class. There was a strong influence of smoking habits on MCV, higher levels being seen amongst women who were smokers at the time of their first interview. This association has been described in other non-pregnant populations and is attributed to the chronic hypoxic effects of smoking (Strain *et al.* 1990). Within women who smoked, there were positive effects of increasing vitamin C intakes on MCV.

In early pregnancy Hb concentration was related to maternal BMI. A positive association of rising Hb level with increasing maternal fatness has also been described in non-pregnant women (Micozzi *et al.* 1989; Fricker *et al.* 1990). There was a modest inverse independent association between Hb concentration and tea consumption. The inverse association with Fe intake and Hb was dependent on the inclusion of women taking supplementary Fe and is likely to be a result of women with low Hb values having been prescribed Fe supplements.

Overall, we found modest influences of diet on the two measures of functional Fe status in early pregnancy, whilst there was an effect of both Ca and alcohol intakes on the level of Fe stores. Our findings rely on the use of serum ferritin concentration as a marker of the amount of Fe stored. Although this correlates well with the level of Fe stores in non-pregnant individuals (British Nutrition Foundation, 1995), interpretation of ferritin values in pregnant individuals is complicated by the effects of

haemodilution occurring in early pregnancy (Whittaker & Lind, 1993). Additional problems in the interpretation of ferritin values arise from its normal day-to-day variability (Cooper & Zlotkin, 1996) and from its role in acute-phase reactions to inflammatory conditions. However, unless there are specific effects of Ca and alcohol intakes on plasma volume expansion or on the level of acute-phase reactions experienced in our population, these factors are unlikely to explain the associations observed.

Although high Ca intakes appear to lower Fe stores in early pregnancy, we do not see this as a reason to limit Ca intake. We found no association between Ca intake in early pregnancy and the two measures of functional Fe status, and, whilst serum ferritin concentration relates to placental volume at 18 weeks gestation (Howe *et al.* 1995), it is not related to fetal or placental size at term (K Godfrey, unpublished results). The importance of our findings is that they suggest that dietary effects on Fe bioavailability are of significance in pregnancy and this may have implications for the definition of dietary Fe requirements.

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