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## Factors affecting iron stores in infants 4-18 months of age

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**Objectives:** To determine the effects of dietary, physiological or environmental factors on body iron levels in infants aged 4-18 months.

**Design:** The daily iron intake of the infants was measured from a diet history obtained by interview using a standardised question sheet, previously validated against weighed intake (minimum 3 days) in an independent sample of 8 and 18 month old infants. Capillary blood samples were analyzed for haemoglobin, mean cell volume, haematocrit, zinc protoporphyrin and plasma ferritin concentration. Ferritin values were log-transformed prior to analysis to give a better approximation to the normal distribution and forward stepwise multiple linear regression was carried out using SPSS.

**Setting:** The city of Norwich, UK and some of its suburbs.

**Subjects:** One hundred and eighty-one healthy infants in age groups 4, 8, 12 and 18 months.

**Results:** Main determinants of iron stores in the 4 month old infants were birth weight (+ve ( $P < 0.001$ )) and body weight (-ve ( $P < 0.005$ )). In the 8 month old infants intake of cow's milk (-ve ( $P < 0.05$ )), belonging to a smoking household (-ve ( $P < 0.05$ )) and quantity of commercial babyfood consumed (+ve ( $P < 0.05$ )) were significant. In this age group there was a gender effect (girls > boys ( $P < 0.01$ )) and the gender effect remained at 12 months (girls > boys ( $P < 0.05$ )), but at 18 months only non-haem iron intake was a significant factor (-ve ( $P < 0.05$ )).

**Conclusions:** At 4 months of age birth weight and body weight exert the greatest influence on iron stores, whereas by 8 months components of the weaning diet have an effect (commercial babyfood (+ve), cow's milk (-ve)); there is also a gender effect (girls > boys), possibly reflecting the different growth rate between boys and girls. At 12 and 18 months the only significant factors are gender (girls > boys) and non-haem iron intake (-ve) respectively.

**Sponsorship:** Ministry of Agriculture, Fisheries and Food and the Biotechnology and Biological Sciences Research Council.

**Descriptors:** infants; iron stores; dietary iron

### Introduction

Infants have a higher relative requirement for iron than adults, mainly due to their rapid growth, and after the age of 4-6 months are wholly dependent upon dietary iron to meet their physiological needs. An insufficient iron supply will result in the depletion of body iron stores and finally lead to iron deficiency anaemia. Iron supplementation of infant formulas and weaning foods is one way of increasing the iron levels in the diet of growing infants, but without information on iron bioavailability, predictions about the effect of the added iron on body iron content are difficult to make. Ultimately, a better understanding of the components in the normal diet of growing infants that affect iron status is required to improve the iron nutrition of infants.

Many factors and interactions between them influence levels of iron in the body. These fall into three main categories: dietary, host-related and environmental factors. With regard to diet, the amount and type of iron ingested and the presence of inhibitors and enhancers of iron absorption determine the amount of iron that is available for absorption. Amongst the physiological factors, birth weight has an effect on the size of iron stores, and the rate

of growth can affect the depletion of those stores. Finally environmental factors such as socioeconomic background and birth order may indirectly affect neonatal iron endowment, the type of diet and the amount of food eaten.

### Methods

#### Subjects

A number of apparently healthy full-term infants aged 4, 8, 12 and 18 months, living in or near to the city of Norwich were selected for recruitment to the study. The sample of infants recruited were not intended to be representative of the local population, but was selected such that there were approximately equal numbers in each age group examined for sex and socioeconomic group, in order to give balanced groups for statistical analysis.

The recruitment of subjects was carried out through health visitor contact and mailshot. The response rate for the mailshot varied from month to month, but it was generally between 10 and 15%. Each infant was visited in the home on one occasion by the researcher and a research nurse. An information sheet with details of family structure, birth weight, parental occupation and environmental factors was completed by the researcher with the mother or father. Ethical approval for the study was given by the Norwich District and Institute of Food Research Human Research Ethics Committees.

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**Table 2** Summary of characteristics of infants

Age (months)	4	8	12	18
Number	43	54	40	44
Gender (%)				
Male	56	46	58	57
Female	44	54	42	43
Weight (kg) (s.e.m.)				
Male	7.3(0.03)	9.0(0.1)	10.2(0.2)	11.3(0.2)
Female	6.5(0.1)	8.9(0.2)	9.7(0.2)	10.9(0.1)
Socioeconomic class (%)				
Non-manual	56	52	45	50
Manual	44	48	55	50
Breastfed (%)	79	83	60	80
Duration of breastfeeding				
< 3 months	18	27	22	29
> 3 months	82	73	78	71
Smoking household (%)	30	28	40	30
First birth (%)	53	37	45	39

**Table 3** Mean (s.e.m.) daily iron intake and main sources of iron (mg) in infants aged 4-18 months

Age (months)	4	8	12	18
Males	24	25	23	25
Females	19	29	17	19
Iron intake (mg/d)				
Males	6.1(0.7)	7.8(1.1)	6.8(0.6)	6.7(0.4)
Females	4.8(0.8)	7.1(0.8)	7.1(0.6)	6.8(0.8)
Sources of iron				
Infant formula				
Males	3.0(0.6)	0.8(0.3)	0.4(0.2)	0.1(0.1)
Females	1.6(0.6)	1.6(0.3)	1.3(0.4)	0.2(0.2)
Babyfoods				
Males	2.6(0.6)	3.6(1.1)	0.3(0.2)	0.1(0.05)
Females	2.6(0.7)	2.7(0.8)	0.5(0.4)	0.1(0.1)
Non-babyfood cereals				
Males	0.0	1.5(0.2)	2.1(0.2)	3.3(0.3)
Females	0.0	1.2(0.2)	2.6(0.3)	2.7(0.4)
Non-milk animal protein				
Males	0.3(0.2)	1.2(0.3)	0.9(0.2)	0.7(0.2)
Females	0.1(0.1)	0.7(0.3)	0.9(0.2)	1.0(0.3)
Vegetables and fruit				
Males	0.2(0.1)	0.7(0.2)	1.6(0.3)	1.4(0.1)
Females	0.3(0.1)	0.9(0.1)	1.1(0.1)	1.5(0.2)

**Table 4** Comparison of mean energy and iron intakes as estimated by weighed intake and diet history

Age (m)	Number	Intake method	Energy intake (kJ/d)		Iron intake (mg/d)		Correlation for iron
			Mean ± s.e.m.		Mean ± s.e.m.		
8	20	Diet history	3792	140	7.7	0.9	0.93 (P < 0.001)
		Weighed intake	3592	113	7.1	0.7	
18	19	Diet history	4952	281	5.5	0.3	0.66 (P < 0.005)
		Weighed intake	4711	226	5.4	0.3	

**Table 5** Mean (s.d.) haemoglobin (Hb), mean cell volume (MCV), mean cell haemoglobin (MCH), plasma ferritin and zinc protoporphyrin (ZPP) in 4, 8, 12, and 18 month old children

	Age (months)							
	4		8		12		18	
	M	F	M	F	M	F	M	F
Number	24	19	25	29	23	17	25	19
Hb (g/L)	125 (9)	125 (11)	128 (11)	124 (9)	128 (12)	129 (9)	130 (8)	128 (9)
MCV (fl)	84 (5)	85 (4)	78 (4)	80 (4)	80 (6)	81 (6)	82 (5)	81 (4)
MCH (pg)	28.5 (1.9)	29.0 (1.9)	26.8 (2.0)	27.7 (1.9)	26.7 (3.3)	27.7 (1.3)	27.7 (2.4)	27.3 (2.5)
Ferritin (µg/L)	58 (48)	70 (45)	21 (16)	34 (28)	18 (14)	28 (15)	30 (39)	23 (20)
ZPP (µmol/L)	1.68 (0.44)	1.39 (0.36)	1.87 (0.48)	1.66 (0.52)	1.48 (0.49)	1.37 (0.38)	1.36 (0.44)	1.49 (0.35)

cient (*r*) for iron intake was 0.93 at 8 months ( $P < 0.001$ ) and 0.66 at 18 months ( $P < 0.005$ ).

The haematological results for boys and girls at 4, 8, 12 and 18 months of age are given in Table 5. There were no significant differences between the sexes for any of the measurements in all four groups. There was a low prevalence of iron deficiency (Hb < 110 g/L, International Nutritional Anemia Consultative Group, 1985) in the infants studied. The mean haemoglobin concentration of the 4 month old infants was significantly lower ( $P < 0.05$ ) than the 18 month old infants, otherwise there were no significant differences for haemoglobin concentration. The 4 month old infants also differed significantly from all other age groups ( $P < 0.001$ ) with respect to mean cell volume (higher) and ferritin (higher). The mean cell volume of the 8 month old infants was significantly different ( $P < 0.05$ ) to that of the 18 month old infants. Zinc protoporphyrin was only found to be significantly different in the 8 month old infants, with a mean value of 1.76 µmol/L compared with that of 1.56 µmol/L in the 4 month old infants ( $P < 0.05$ ) and 1.43 µmol/L and 1.42 µmol/L in the 12 and 18 month old infants respectively ( $P < 0.001$ ).

The factors used in the regression analysis are shown in Table 6. There were no significant differences in ferritin values between boys and girls, so the results were combined for the regression analysis. Of the original sample of 181 infants studied, 13 had white blood cell levels indicating the presence of infection and hence potentially elevated ferritin concentrations, thus these were excluded from the analysis. The multiple regression models for each of the four age groups are shown in Tables 7-10. In these stepwise regression models the criterion for inclusion was the 10% significance level, apart from the 8 month old infants where the 5% significance level is also given in Table 8 (see next paragraph for explanation).

At 4 months, birth weight ( $P < 0.001$ ) and body weight ( $P < 0.005$ ) had a positive effect. The ferritin concentrations in the 8 month old infants were affected by gender ( $P < 0.01$ ) in favor of the girls, the amount of babyfood consumed (+ve) and the effect of belonging to a smoking household (-ve) ( $P < 0.05$ ). The only other factor showing a significant effect on iron stores in this group was intake of cow's milk, which had a negative effect on plasma ferritin

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**Table 6** Factors used in regression analysis

Dietary factors (obtained from 24h dietary recall)	
Total iron (mg)	
Haem iron (mg)	
Non-haem iron (namely iron from all dietary sources except non-milk animal protein) (mg)	
Calcium (mg)	
Ascorbic acid (mg)	
Amount of formula milk (mls)	
Amount of cow's milk (mls)	
Amount of commercial babyfoods (g)	
Host-related factors	
Gender (M, F)	
Age (4, 8, 12, 18 months)	
Birth weight (kg)	
Body weight (kg)	
Environmental factors	
Socioeconomic group (non-manual/manual)	
Breastfeeding history (no breastfeeding/breastfed to 3 months/breastfed > 3 months)	
Birth order (only child/more than one child)	
Smoking in household (none/one or more smokers)	

**Table 7** Multiple regression analysis for plasma ferritin (logarithmically-transformed) for 4 month old infants

Variable	Coefficient <i>b</i>	Standard error SE ( <i>b</i> )	<i>t</i>	<i>P</i>
Constant	1.284	0.398	3.23	0.0027
Birth weight (g)	0.472	0.059	4.54	0.0001
Body weight (g)	-0.180	0.104	-3.06	0.0042
Commercial babyfood	-0.027	0.014	-0.26	0.0543

Residual standard deviation 0.244.  
Adjusted *R* squared 0.39.

**Table 8** Multiple regression analysis for plasma ferritin (logarithmically-transformed) for 8 month old infants

Variable	Coefficient <i>b</i>	Standard error SE ( <i>b</i> )	<i>t</i>	<i>P</i>
5% significance <sup>a,b</sup>				
Constant	1.346	0.059	22.89	0.0000
Cow's milk	-0.678	0.317	-2.14	0.0375
Commercial babyfood	0.018	0.009	2.08	0.0430
10% significance <sup>c,d</sup>				
Constant	1.209	0.081	17.25	0.0000
Gender	0.218	0.080	2.71	0.0094
Commercial babyfood	0.021	0.008	2.58	0.0131
Smoking	-0.208	0.088	-2.36	0.0227

<sup>a</sup>Residual standard deviation 0.295.

<sup>b</sup>Adjusted *R* squared 0.22.

<sup>c</sup>Residual standard deviation 0.279.

<sup>d</sup>Adjusted *R* squared 0.22.

**Table 9** Multiple regression analysis for plasma ferritin (logarithmically-transformed) for 12 month old infants

Variable	Coefficient <i>b</i>	Standard error SE ( <i>b</i> )	<i>t</i>	<i>P</i>
Constant	1.148	0.073	15.73	0.0000
Gender	0.240	0.115	2.10	0.0434

Residual standard deviation 0.342.  
Adjusted *R* squared 0.09.

**Table 10** Multiple regression analysis for plasma ferritin (logarithmically-transformed) for 18 month old infants

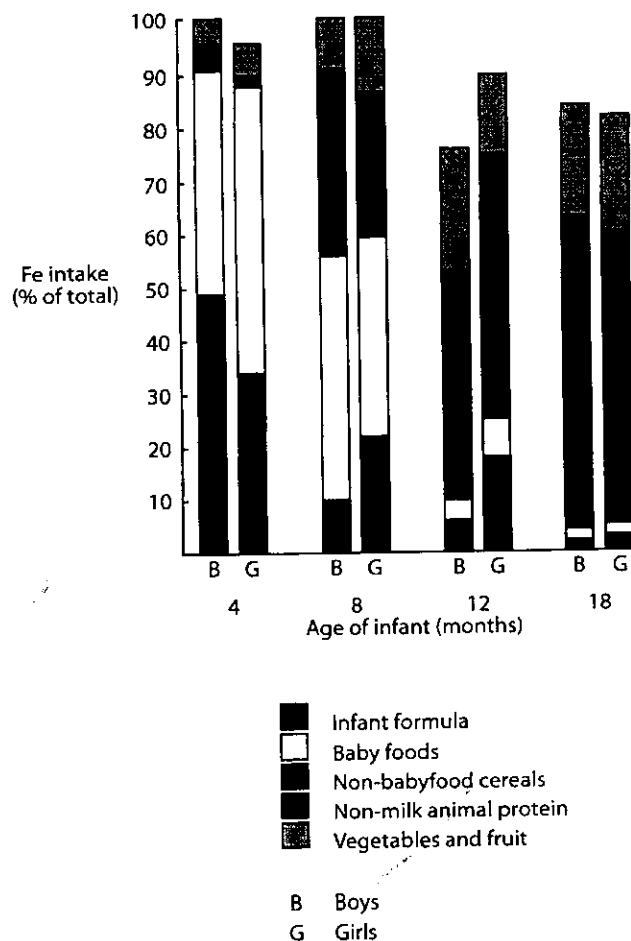
Variable	Coefficient <i>b</i>	Standard error SE ( <i>b</i> )	<i>t</i>	<i>P</i>
Constant	1.544	0.130	11.85	0.0000
Non-haem iron	-0.044	0.021	-2.04	0.0480

Residual standard deviation 0.286.  
Adjusted *R* squared 0.07.

concentrations ( $P < 0.05$ ), however as the amount of milk consumed correlated closely with gender, this effect was only shown at the 5% significance level, a not uncommon finding in stepwise regression. Gender was the only significant factor ( $P < 0.05$ ) at 12 months, with girls having higher iron stores than boys. At 18 months the only significant effect on iron stores was that of non-haem iron intake, namely iron from all sources except for non-milk animal protein foods), which had a negative effect ( $P < 0.05$ ).

### Discussion

There are limited data on the iron status of infants under the age of 18 months (Fairweather-Tait, 1996), but results from small individual studies suggest that iron deficiency is a very common problem amongst this age group, particularly in children from inner cities and ethnic minorities (Fairweather-Tait, 1992). Even in the recently published MAFF-



**Figure 1** Mean iron intake in 4, 8, 12 and 18 month old boys and girls: sources of iron and % contribution to total daily intake.

commissioned survey (Gregory *et al*, 1995) the youngest infants studied were aged 18–30 months, and although the NHANES II study (Pilch & Senti, 1984) carried out in the USA included some 6–12 month infants, they numbered only 22, so the data were considered statistically unrepresentative of the population. The recently published report 'Weaning and the weaning diet' (Department of Health, 1994) highlighted a large number of areas requiring further investigation with respect to iron and the weaning diet. Recommendations included the determination of levels of iron deficiency in UK infants and young children, and further investigation of relationships between iron status and dietary intakes, especially iron-fortified formula and foods.

There was a significant correlation between iron intake assessed from diet history and weighed intake. The agreement was better at 8 than 18 months, reflecting the fact that by 18 months a more varied diet has been introduced. It is generally accepted that weighed intakes and duplicate diet collections tend to underestimate habitual intake, whereas the 24 h recall overestimates habitual intake (Morgan *et al*, 1978). Diet histories fall somewhere in between, and therefore give more representative estimates of nutrient intake, particularly for groups consuming a restricted variety of foods, as is the case with infants.

The iron intake data collected by diet history in the present study indicated that the majority of infants were receiving sufficient iron in their diets to meet dietary recommendations (Department of Health, 1991). In a survey of UK infants (Mills & Tyler, 1992) the mean daily iron intake of 6–9 month old boys ( $n=130$ ) and girls ( $n=128$ ) was 9.6 and 9.0 mg, which the authors attributed to a high intake of fortified infant formulas and commercial infant food. The iron intake of 9–12 month old boys ( $n=96$ ) and girls ( $n=134$ ) was 7.2 and 6.4 mg respectively. A larger survey of UK pre-school children (Gregory *et al*, 1995) reported mean daily iron intakes of 4.9 mg in 576 children aged 1.5–2.5 y. Iron intakes of the 4 and 8 month old infants in the present study were much lower than those found by Mills & Tyler (1992), intakes of the 12 month old infants were in close agreement (6.8 mg in boys and 7.1 mg in girls), and intakes of the 18 month old children were higher than those found by Gregory *et al* (1995).

The infants recruited for this study had adequate body iron levels and there was a low prevalence of iron deficiency. This finding cannot, however, be taken as representative of the local population as a whole, as the sampling of infants for this study was not randomised. In particular, the prevalence and duration of breastfeeding was high, which suggests an above average motivation and health awareness of the mothers. This would be expected considering the type of recruitment procedure that was employed. Because of the low prevalence of iron deficiency anaemia plasma ferritin concentration was selected as the incidence of iron status to be used for the regression analysis to identify predictive factors for body iron content. Haemoglobin and mean cell volume are too insensitive, and transferrin saturation requires a larger blood sample than was available in the present study. Plasma ferritin reflects iron stores and a reduction in its level is the earliest sign of iron depletion in the body. Iron stores and thus ferritin levels are the last to rise, even when the iron depletion is reversed, but the main disadvantage of using plasma ferritin is that it can be falsely elevated in the presence of infection. The appropriate cut-off value that is indicative of iron

deficiency is still under discussion and it has been shown (Bergstrom *et al*, 1995) that the choice of value can greatly change the numbers of individuals categorised as iron deficient. To overcome this problem individual values for plasma ferritin were used as opposed to high or low groups.

ZPP is also known to be elevated in the presence of infection and inflammatory disease, and also in lead poisoning. Although the infants examined were mainly urban dwellers, it is unlikely that the levels of lead in Norwich were high enough to produce such a marked elevation of values as observed in the present cohort of infants. It is also unlikely that the incidence of infection in this group of infants should have resulted in approximately 50% of the 4–8 month and 45% of the 18 month old infants to have levels of ZPP above the commonly-used cut-off values for iron deficiency. Blood samples that were checked by measuring on a second machine gave similar results after correction for low blood control values. In a recent survey (unpublished), Leeds City Council Environmental Health Department measured ZPP in 6234 children aged 0.5–5 y old to examine levels of lead poisoning and iron deficiency. They commented that a high number of the infants studied exceeded the recognised cut-off values for iron deficiency (personal communication), and reported an inconsistency in results from samples measured on different machines, which casts doubt upon the robustness of this measure of iron status. The use of different units in reporting ZPP results also makes comparison across studies difficult.

By 4 months of age the iron stores endowed at birth are depleted and diet is the main supply of iron. At this age, physiological variables have a strong influence on body iron stores, as demonstrated by the results of this study; at 4 months the two factors that had a significant effect on plasma ferritin concentration were birth weight and body weight. Since the effect of birth weight on iron stores was strongly positive at four months ( $P < 0.001$ ), this suggests that higher birth weight babies have larger iron stores. This is consistent with the results of the work carried out by Widdowson and Spray (1951) who found a linear relationship between body weight at birth and total body iron. Conversely, the highly significantly negative effect ( $P < 0.005$ ) of body weight at 4 months on plasma ferritin concentration suggests that heavier babies have lower iron stores, presumably due to their more rapid growth.

At 8 months it appears that physiological factors are no longer important and dietary factors play a role in determining body iron stores. The amount of commercial baby-food consumed (+ve) and the amount of cow's milk ingested (–ve) influence iron stores. Weaning foods are usually supplemented with iron and ascorbic acid, but levels of absorption from these have been shown to be relatively low (Fairweather-Tait *et al*, 1995). The question of the suitability of cow's milk as a food/drink for infants has been vigorously debated (Zlotkin, 1993) due to its low iron content and an association with intestinal bleeding. The results of this study appear to support the recommendation for the avoidance of cow's milk at an early age (Department of Health, 1994). The only socioeconomic factor that was significant in this age group was smoking in the household. In the present study there was no significant difference between birth weights of babies from non-smoking and smoking households, thus we cannot explain this observation. The gender of the infant was a significant factor at 8 months, but since there was a significant association between gender and quantity of cow's milk consumed, with boys drinking more cow's milk than girls,

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(correlation coefficient  $r = -0.394$  ( $P < 0.01$ )), this may partially explain the gender effect seen in these infants.

At 12 months, gender was the only factor that showed a significant effect ( $P < 0.05$ ). Girls had higher plasma ferritin concentration than boys. This effect however disappeared by 18 months, where only non-haem iron intake had an effect on iron stores ( $P < 0.05$ ). Surprisingly, this effect was a negative one, possibly reflecting the low bioavailability of non-haem iron and the adverse effect of inhibitory factors associated with foods higher in non-haem iron.

### Conclusions

Different categories of factors (dietary, physiological and environment) affected iron stores at ages 4, 8, 12 and 18 months. In the very young (4 months old) infants birth weight and body weight had greatest influence. At 8 months dietary factors associated with the weaning diet (commercial babyfood and cow's milk intake) had the most significant effect on iron stores, although the sex of the infant also appeared to play a part. The effect of gender continued to exert an influence on iron stores at 12 months but by 18 months the diet of the infant, specifically its non-haem content, was the only significant determinant of iron stores.

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