ORIGINAL ARTICLE

Infant feeding in the second 6 months of life related to iron status: an observational study

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Objective: To investigate the relationship between iron status in infancy and type of milk and weaning solids consumed.
Design: An observational cohort study.
Setting: 928 term infants from the Avon Longitudinal Study of Parents and Children in 1993–94.
Methods: Haemoglobin and ferritin concentrations at 8 and 12 months were assessed in relation to type and quantity of milk intake at 8 months.
Results: By WHO criteria, 22.7% of the infants were anaemic at 8 months and 18.1% at 12 months. More breast- than formula-fed infants were anaemic at 8 and 12 months. Cows' milk as the main drink was associated with increased anaemia at 12 months and low ferritin at 8 and 12 months. No association was found between any nutrients and haemoglobin concentrations. Protein and non-haem iron intakes were positively associated with ferritin concentrations and calcium intake negatively. This effect was more marked in infants being fed cows' milk. More than 25% of infants in the breast milk and cows' milk groups and 41% of infants having >6 breast feeds per day had iron intakes below the lower reference nutrient intake. Feeding cows' milk or formula above 600 ml or >6 breast feeds per day was associated with lower intakes of solids.
Conclusions: Both breast and cows' milk feeding were associated with higher levels of anaemia. Satisfactory iron intake from solids in later infancy is more likely if formula intake is <600 ml per day and breast feeds are limited to <6 feeds per day. Cows' milk should be strongly discouraged as a main drink before 12 months.

A WHO review on the length of exclusive breast feeding has recommended an extension to 6 months.[1] The adoption of this recommendation by the UK government has highlighted a need for suitable weaning advice to ensure transition to an adequate diet. Previous guidance documents have recommended exclusive breast or formula feeding for the first 4 months of life[2] and there is good evidence for adopting such feeding practices.[3–6] Some benefits, in terms of development of atopic disease, have been shown with later introduction of weaning solids (>4 months) in pre-term infants[7] but not in term infants.[8] Some have questioned the feasibility of adhering to the 6-month recommendation[9] and highlighted slower rates of growth in those exclusively breast fed for 6 months[10] and the fact that a proportion may later fail to thrive.[11]

Iron deficiency anaemia is the most common nutrient deficiency in the developed world, particularly among preschool children,[12] and there is evidence of an association with delayed mental development.[13 14] There is some evidence that breastfed infants may be at greater risk if they are exclusively breast fed beyond 6 months.[15] A recent review by Griffin and Abrams[16] has pointed out that there remain areas of uncertainty regarding the optimal age of introduction of iron-fortified weaning foods. The development of iron deficiency in infancy has been linked to poor feeding practices, including the use of cows' milk.[17 18] The Department of Health recommends that whole cows' milk should not be used as the main milk drink until 1 year of age[2] and that 500–600 ml of either breast milk or infant formula should be given each day between the ages of 6 and 12 months.[2] Data from the Avon Longitudinal Study of Parents and Children (ALSPAC) have shown that ferritin levels were negatively associated with intake of cows' milk and calcium and positively associated with iron, vitamin C and non-starch polysaccharide (NSP) intakes at 18 months.[19]

In this study we investigated the relationship between the type of milk and quality of weaning solids and measures of iron status in infants at 8 and 12 months of age.

METHODS

Over 14 000 pregnant women resident in a defined area of south-west England who had an expected date of delivery between April 1991 and December 1992 were recruited.[19] From this population cohort, a 10% sample of term infants were randomly selected from the final 6 months of the cohort and invited to research clinics at 8 and 12 months of age in 1993–94.

Blood samples obtained at these clinics, after signed parental consent, were analysed for haemoglobin (Hb) and ferritin.[21] Previous work in this cohort showed that the fifth centile for Hb was 97 g/l at 8 months[21] and 100 g/l at 12 months.[22] For this study, the ALSPAC cut-off for anaemia in infancy of 100 g/l was compared with the WHO criteria of 110 g/l and low ferritin was defined as <16 µg/l (the fifth centile).[22]

Dietary information was obtained when the infants were 8 months old, using a structured 3-day unweighed dietary record (available on www.alspac.bristol.ac.uk) completed by the infant's main carer. The carer attended a clinic with the child at which the diet diary was checked and expanded in a short interview with SN who then interpreted and analysed the results. Food weights and codes were allocated and combined with British food tables to calculate mean daily food/drink and nutrient intakes. Breast milk volume was assessed from duration of feed allowing 10 ml per minute up to a maximum

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Abbreviations: ALSPAC, the Avon Longitudinal Study of Parents and Children; BM, breast milk group; CM, cows' milk group; FM, formula milk group; Hb, haemoglobin; LRNI, lower reference nutrient intake; NSP, non-starch polysaccharide; OR, odds ratio of 100 ml.[23] Six breast feeds/day were taken to equate to an intake of 600 ml. Noble *et al*[24] give a description of the dietary method used. Infants were categorised according to the type of milk drunk:

- breast milk group (BM): breast-milk with or without some cows' milk but no formula,
- formula milk group (FM): formula with or without some breast and/or cows' milk,
- cows' milk group (CM): cows' milk but no formula or breast milk.

These groups were subdivided by number of breast feeds or volume of milk taken. The breast milk group was divided into <6 (low BM) and ⩾6 breast feeds per day (high BM). The formula and cows' milk groups were divided by volume taken into <600 ml (low FM/CM) and ⩾600 ml (high FM/CM).

Nutrients examined were chosen in the light of previous work showing their association with iron status. These were haem iron,[25] non-haem iron,[25] protein,[26] vitamin C,[25] calcium[26] and fibre/phytate[27] (measured as NSP). Iron-containing foods were grouped according to type: breakfast cereals, meat/fish family foods, vegetables (including legumes and baked beans) and commercial infant foods.

Parity,[19] vegetarian status of the child[19] (the status of the mother was used as a proxy), use of iron supplements during pregnancy, educational level of mother,[18] gender,[18 19 29] birth weight,[19 22] current weight,[22] growth from birth[22 30] and a positive response from the mother regarding recent infection[18] were included as confounders since previous work had shown their potential influence on iron status. These data were obtained from questionnaires completed during pregnancy, medical records or assessments at the clinics.

Statistical analysis

SPSS version 12.0.1 and Stata version 8 were used for statistical calculations. Ferritin levels were transformed to natural log values to account for their skewed distribution. Two-tailed Fisher's exact tests were used to assess differences in the prevalence of anaemia and low ferritin. This test was used in preference to Pearson's χ^2 test due to the low expected cell count, particularly for ferritin. Each nutrient was standardised to account for their different scales of measurement, thus allowing easier comparisons of their effect on iron levels and iron status. Multiple regression was used to assess the independent contribution of nutrients in explaining haemoglobin and ferritin concentrations after adjusting for confounding variables and total energy intake. In the case of anaemia or low ferritin outcomes, analyses utilised logistic regression. Student's t tests were used to assess differences in unadjusted mean values of energy and nutrients from weaning foods for high and low milk intake within each milk group.

Ethical approval for the study was obtained from the ALSPAC Law and Ethics Committee and the three Local Research Ethics Committees.

RESULTS

At 8 months of age, 1338 term infants and their carers were invited to the clinic and 1250 (93%) attended. One or both parents of 2.5% of these infants were of non-white ethnic background. Dietary diaries were received for 1124 infants (90% of attendees) and blood samples were obtained from 1079 (86%). At 12 months of age, 1182 (90% of invitees) attended and blood samples were obtained from 906. Infections were present in 27% and 23% of the children giving blood at 8 and 12 months of age, respectively. Full data for both diet at 8 months and haemoglobin concentrations at 8 months (928 infants) and 12 months (782) were available. Equivalent data for diet and ferritin were available for 650 and 645 infants. At 8 months, the percentage taking the different milks was: BM 12%, FM 74% and CM 14%. Follow-on formula was being taken by 25% in the low FM group and 14% in the high FM group. All infants were established on solids by 6 months of age.[30]

Incidence of anaemia

At 8 months of age, 7.1% of the study population were anaemic according to the ALSPAC cut-off level and 22.7% by the WHO criteria, reducing to 4% and 18%, respectively, by 12 months (table 1). Within this population a higher proportion of infants in the breast milk and cows' milk groups were anaemic. The cows' milk group also had a higher proportion of infants with low ferritin.

Confounding variables

All confounders were significantly associated with at least one iron status outcome and were subsequently used in all regression analyses investigating the effect of diet.

Effect of nutrient intake on iron status and iron levels

Multivariable analysis of iron status in the whole study population at 8 months showed no association between intake of any of the nutrients and low haemoglobin. At 8 months, NSP intake was the only dietary variable associated with low ferritin. The odds ratio (OR) of 2.28 ($p = 0.007$) indicates that children were twice as likely to have low ferritin for each standard deviation increase in NSP (about 2 g/day). At 12 months, low ferritin was associated with calcium intake ($OR = 4.16$, $p = 0.003$) and NSP ($OR = 2.69$, $p = 0.005$).

Table 2 shows the relationship between nutrient intakes at 8 months in each milk group and haemoglobin and ferritin concentrations after controlling for the confounders and each of the other nutrients. Protein and non-haem iron intake generally had a positive influence on markers of iron status, while calcium had a negative effect. These influences were most

Table 1 Incidence of anaemia and low ferritin concentration in infants at 8 and 12 months according to the type of milk taken at 8 months of age

		Dietary group			p	
	All (n/n)	**Breast milk (n/n)**	**Formula (n/n)**	**Cows' (n/n)**	**FM vs BM**	**FM vs CM**
8 months						
% Anaemic (<100 g/dl)	7 (66/926)	9 (10/113)	7 (45/687)	9 (11/126)	0.4	0.3
% Anaemic (<110 g/dl)	23 (210/926)	32 (36/113)	20 (139/687)	28 (35/126)	0.009	0.08
% Low ferritin (<16 μg/l)	3 (20/649)	5 (4/85)	2 (10/481)	7 (6/83)	0.2	0.02
12 months						
% Anaemic (<100 g/dl)	4 (31/781)	11 (11/102)	3 (15/574)	5 (5/105)	0.001	0.2
% Anaemic (<110 g/dl)	18 (141/781)	27 (27/102)	15 (86/574)	27 (28/105)	0.006	0.006
% Low ferritin (<16 μg/l)	4 (27/644)	5 (4/83)	3 (14/477)	11 (9/84)	0.3	0.003

marked in the cows' milk group. The negative association between calcium intake and ferritin concentrations was observed in all three groups at 8 months. Overall analyses suggested that for every standard deviation increase (about 250 mg) in calcium content of the diet there was a drop in mean ferritin concentration of about 20%.

Table 3 shows energy and nutrient intakes from the whole diet and from ''solids'' (foods and drinks other than the main milk drink). The estimated mean total energy intakes of the low and high BM groups were very similar but high BM obtained 31% less energy from solids. High BM had lower mean intakes of iron, protein, calcium and fibre than low BM. Both the low and the high BM groups had a high proportion of infants with iron intakes below the lower reference nutrient intake (LRNI; 4.2 mg/day) at 27% and 41%, respectively.[2] Energy intakes in the other four ''milk'' groups were between 5% and 24% higher than in the BM groups and in both FM and CM groups greater ''milk'' volume led to higher energy intake. High FM obtained 23% less energy from solids than low FM. Mean intakes of all nutrients except NSP were significantly higher in high FM compared to low FM. Energy intake from solids in high CM was 17% lower than in low CM. Mean intakes of protein and calcium but not iron, vitamin C and NSP were higher in high CM than in low CM. More than a quarter of infants in the CM groups had iron intakes below the LRNI. In the high FM and CM groups, energy intakes were similar to each other ($p = 0.2$). The same was true of the low FM and CM groups ($p = 0.2$) and the two BM groups ($p = 0.5$). Furthermore, high FM and high CM had greater mean energy intakes than the other four feeding groups ($p<0.001$).

Iron intake from foods consumed at 8 months

The largest contributor to iron intake was commercial baby food (eaten by 90.7%) with fortified breakfast cereals (71.6%) being the next most important (table 4). Family foods such as meat/fish (64.6%) and vegetables (76.0%) also contributed. Iron intake from breakfast cereals in high BM was half that of low BM. Iron intake from vegetables in high CM was half that in low CM ($p = 0.003$).

DISCUSSION

We have shown associations between haemoglobin concentrations and iron stores at 8 and 12 months and the type of milk fed at 8 months. Both breast milk and cows' milk feeding were associated with lower indices of ferritin and a higher incidence of anaemia than formula feeding. In all three milk groups, there was evidence of a positive association of iron status with protein intake and a negative association with calcium. Infants having ⩾6 breast feeds per day were obtaining less energy from solid foods than the other ''milk'' groups and their mean iron intake was very low, with a small contribution from breakfast cereals and meat/fish. Non-haem iron intakes were fairly consistently associated with better iron status in these infants. The negative association of haem iron with ferritin at 8 months might be accounted for by very low intakes of haem iron in the diet at this age, with other factors being more important in determining levels. Both the cows' milk and breast milk groups had a high proportion of infants with iron intakes below the LRNI. The feeding of cows' milk as a main drink is particularly detrimental due to its low iron and high calcium content. Breastfed infants having ⩾6 breast feeds had an average iron intake from meat and fortified cereals that was half that of those having ⩽5 breast feeds. Our study suggests that milk feeding of any kind in excess of 600 ml/day or 6 breast feeds has a significant detrimental effect on energy and iron intake from solids.

The main limitation is that the dietary data were derived from untrained parental reports of infant food/drink intake over 3 days. This method is prone to inaccuracy in recording and interpretation by researchers. However, in this study the parents were questioned about the record within a few days of making it, to increase completeness and understanding. A 3-day recording of food/drink should be sufficient to characterise diet in an 8-month-old infant at least with regard to energy

Table 2 Associations between nutrient intakes at 8 months and concentrations of haemoglobin (Hb) and ferritin at 8 and 12 months in the three milk groups*

	Hb at 8 months		Log ferritin at 8 months		Hb at 12 months		Log ferritin at 12 months	
Nutrient intake	β† (95% CI)	p	β† (95% CI)	p	β† (95% CI)	p	β† (95% CI)	p
Breast milk								
Haem iron	−0.05 (−0.49 to 0.38)	0.8	−0.14 (−0.38 to 0.09)	0.2	0.23 (−0.21 to 0.66)	0.3	−0.18 (−0.41 to 0.04)	0.1
Non-haem iron	0.15 (−0.23 to 0.52)	0.4	−0.07 (−0.28 to 0.15)	0.5	0.05 (−0.33 to 0.44)	0.8	−0.11 (−0.32 to 0.10)	0.3
Protein	−0.38 (−1.00 to 0.23)	0.2	0.44 (0.10 to 0.78)	0.01	0.15 (−0.54 to 0.84)	0.7	0.44 (0.10 to 0.79)	0.01
Calcium	0.04 (−0.52 to 0.60)	0.9	−0.32 (−0.65 to 0.02)	0.06	0.08 (−0.55 to 0.71)	0.8	−0.14 (−0.45 to 0.17)	0.4
Vitamin C	−0.01 (−0.30 to 0.28)	0.96	−0.05 (−0.24 to 0.13)	0.6	−0.13 (−0.40 to 0.14)	0.3	−0.01 (−0.18 to 0.15)	0.9
NSP	0.03 (−0.26 to 0.33)	0.8	−0.04 (−0.22 to 0.13)	0.6	−0.01 (−0.29 to 0.27)	0.9	−0.04 (−0.18 to 0.10)	0.5
n	101		75		92		74	
Formula								
Haem iron	0.04 (−0.09 to 0.18)	0.5	−0.06 (−0.12 to 0.01)	0.09	−0.05 (−0.18 to 0.08)	0.5	−0.04 (−0.10 to 0.03)	0.3
Non-haem iron	−0.05 (−0.17 to 0.09)	0.5	0.09 (0.02 to 0.15)	0.01	−0.02 (−0.14 to 0.10)	0.7	0.02 (−0.04 to 0.09)	0.5
Protein	−0.07 (−0.33 to 0.18)	0.6	0.08 (−0.04 to 0.20)	0.2	0.01 (−0.22 to 0.25)	0.9	0.13 (0.01 to 0.26)	0.03
Calcium	0.09 (−0.09 to 0.28)	0.3	−0.18 (−0.28 to −0.09)	<0.001	0.05 (−0.13 to 0.22)	0.6	−0.03 (−0.12 to 0.06)	0.5
Vitamin C	−0.03 (−0.14 to 0.08)	0.6	0.04 (−0.01 to 0.10)	0.1	−0.01 (−0.11 to 0.10)	0.9	0.02 (−0.04 to 0.07)	0.5
NSP	−0.01 (−0.14 to 0.12)	0.8	−0.05 (−0.11 to 0.01)	0.1	−0.04 (−0.16 to 0.09)	0.6	−0.03 (−0.10 to 0.03)	0.3
n	598		414		517		431	
Cows' milk								
Haem iron	0.13 (−0.09 to 0.34)	0.3	−0.19 (−0.32 to −0.05)	0.008	−0.05 (−0.25 to 0.16)	0.7	0.00 (−0.11 to 0.10)	0.9
Non-haem iron	0.47 (0.11 to 0.83)	0.01	0.26 (0.11 to 0.42)	0.001	0.47 (0.09 to 0.85)	0.02	0.10 (−0.11 to 0.30)	0.3
Protein	−0.03 (−0.62 to 0.56)	0.9	0.66 (0.36 to 0.96)	<0.001	0.59 (−0.05 to 1.20)	0.07	0.19 (−0.14 to 0.52)	0.3
Calcium	0.15 (−0.31 to 0.61)	0.5	−0.36 (−0.56 to −0.16)	<0.001	−0.55 (−1.00 to −0.08)	0.02	−0.25 (−0.50 to −0.01)	0.05
Vitamin C	0.18 (−0.14 to 0.49)	0.3	0.00 (−0.14 to 0.14)	0.99	0.03 (−0.26 to 0.31)	0.8	0.05 (−0.10 to 0.19)	0.5
NSP	−0.13 (−0.49 to 0.22)	0.4	−0.15 (−0.29 to −0.01)	0.04	−0.18 (−0.54 to 0.18)	0.3	−0.10 (−0.29 to 0.10)	0.3
n	108		73		93		74	

*All analyses were adjusted for nine confounders: gender, birth weight, age, weight, recent infections as assessed at the 8-month or 12-month clinic, parity, maternal education, vegetarian status and use of iron supplements during pregnancy.

†Results reported are the effect on Hb or log ferritin of a one standard deviation change in nutrient after adjusting for confounders, all other nutrients and total energy intake.

Table 3 Mean energy and nutrient intakes within groups of 8-month-old infants identified by the type and volume of milk consumed

Nutrient intake per day	<6 Breast feeds/day (n = 101)	≥6 Breast feeds/day (n = 41)	p
Total energy (kJ)	3111	3193	0.5
Energy from solids (kJ)	2198	1516	<0.001
Total protein (g)	24	20	<0.001
Total iron (mg)	6.2	5	0.04
Total calcium (mg)	501	438	0.03
Total vitamin C (mg)	79	74	0.6
Total NSP (g)	4.6	3.7	0.02
Iron <4.2 mg (LRNI) (%)	26.7	41.5	0.09
	<600 ml formula/day (n = 602)	≥600 ml formula/day (n = 230)	
Total energy (kJ)	3298	3750	<0.001
Energy from solids (kJ)	2217	1702	<0.001
Total protein (g)	27	28	0.04
Total iron (mg)	9	10.7	<0.001
Total calcium (mg)	617	715	<0.001
Total vitamin C (mg)	95	104	0.02
Total NSP (g)	4.3	3.7	<0.001
Iron <4.2 mg (LRNI) (%)	2.8	0	<0.001
	<600 ml cows' milk/day (n = 79)	≥600 ml cows' milk/day (n = 69)	
Total energy (kJ)	3388	3897	<0.001
Energy from solids (kJ)	2190	1812	<0.001
Total protein (g)	33.8	40	<0.001
Total iron (mg)	6.3	6.2	0.8
Total calcium (mg)	799	1117	<0.001
Total vitamin C (mg)	57	56	0.8
Total NSP (g)	4.7	3.8	0.01
Iron <4.2 mg (LRNI) (%)	29.1	26.1	0.7

Energy from solids includes that from all foods/drinks other than the specified milk in each group.
LRNI, lower reference nutrient intake below which intake is inadequate for 97.5% of a given population.

intake[32] since infants tend to have a limited range of food. Of necessity breast milk intake was estimated from duration of feed, which provides only a crude estimate of amount consumed. However, the method of assessing intake of other drinks and solids was exactly the same in all the milk groups and should not have led to bias. Although the data were collected in 1993–94, the most recent British infant feeding survey in 2000[33] shows 16% of infants being breast fed and 8% having cows' milk at 8 months. Our findings therefore indicate that a high proportion of these infants may be at risk of having iron intakes below the LRNI.

This study has provided insight into an under-researched area relating to the weaning process. Its strength is that the data were prospectively collected from a large representative population-based sample. To our knowledge this is the first study to collect detailed dietary data on all foods and drinks taken by infants and link them to longitudinal measures of iron status.

A study in Pakistan showed that late weaning was the most important predictor of iron deficiency anaemia at 1–2 years of age.[34] In our study, the nutrient intake of the formula-fed group remained satisfactory, but there was a significant impact on nutrient intake in the breast and cows' milk groups. Attempts at using health education to reduce the incidence of iron deficiency anaemia in vulnerable population groups have met with disappointing results.[35] The addition of iron fortified "sprinkles" to homemade weaning foods may be an effective strategy in reducing iron deficiency in a developing country.[36] Iron-fortified formula is useful in combating pre-existing iron deficiency anaemia and may have the added benefit over medicinal iron of improving mean corpuscular volume with less gastrointestinal side effects[37] assuming that a clean water supply is available. In our study there was less anaemia at 8 and 12 months in those infants taking any formula, which made a significant contribution to iron intake.

Table 4 Mean iron intake (mg) from weaning foods within groups of 8-month-old infants identified by the type and volume of milk consumed

Breast milk group	<6 feeds/day (n = 101)	≥6 feeds/day (n = 41)	p
Baby foods	3.8	3.1	0.3
Fortified breakfast cereals	0.7	0.4	0.006
Meat/fish	0.2	0.1	0.07
Vegetables	0.4	0.3	0.1
Formula group	<600 ml/day (n = 602)	≥600 ml/day (n = 230)	
Baby foods	4.0	4.1	0.9
Fortified breakfast cereals	0.9	0.8	0.01
Meat/fish	0.3	0.3	0.3
Vegetables	0.3	0.3	0.09
Cows' milk group	<600 ml/day (n = 79)	≥600 ml/day (n = 69)	
Baby foods	3.0	3.5	0.4
Fortified breakfast cereals	1.3	1.1	0.3
Meat/fish	0.5	0.3	0.1
Vegetables	0.4	0.2	0.003

Suggestions, arising from this research, likely to reduce the incidence of iron deficiency anaemia in the developed world

Once weaning is established, the amount of milk offered to infants should be reduced to <6 breast feeds or 600 ml of formula a day unless the child is unwell. To increase iron intake in breastfed infants from 6 months onwards, supplementation using up to 200 ml per day of iron-fortified formula may be beneficial. This may be done by using formula with fortified breakfast cereals, or by offering a drink of formula from a "feeder beaker" or cup so as not to have an adverse effect on the mechanics of breast feeding.[38] It may be beneficial for some tokens in the government's Healthy Start programme to be

What is already known on this topic

- Exclusive breast feeding up to 4 months is beneficial.
- Iron deficiency is common in infancy and early childhood.
- Cows' milk is not suitable as a main drink before 1 year of age.

What this study adds

- Infants fed breast milk or cows' milk, without formula, in later infancy had a high risk of anaemia.
- Feeding high volumes of milk reduced energy and iron intake from solid foods.

"ring fenced" to provide foods high in iron and iron-fortified formula to try to achieve a balanced diet in early childhood. The use of cows' milk as a main milk drink before 12 months of age should be strongly discouraged with warnings in weaning leaflets about the likely problems.

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