Archives of Disease in Childhood 1995; **73**: 216–220

The effect of iron in formula milk after 6 months of age

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Abstract

Ninety two normal birthweight infants aged 6 months entered a double blind controlled trial which compared a follow on formula milk with no added iron against the same formula milk containing 1·2 mg of iron per 100 ml. There was no significant difference in the social class or demographic characteristics of the two treatment groups or in the proportion of each group completing the trial. There was no difference between the two groups in the quantity of milk taken but the amounts taken lessened between 6 and 18 months of age. There was no difference between the two groups with respect to mean haemoglobin and median serum ferritin at 6, 9, 12, 15, and 18 months of age. Very few infants developed iron deficiency anaemia in either group but there was a tendency for serum ferritin levels to fall between 6 and 18 months of age in both groups. The results suggest that iron added to follow on milk was not an important source of dietary iron in the infants studied.

(*Arch Dis Child* 1995; **73**: 216–220)

Keywords: iron deficiency, infancy, iron supplementation of formula.

Evidence that iron deficiency can impair the developmental progress of young children[1 2] has drawn attention to the importance of introducing nutritional campaigns to prevent iron deficiency in infancy. Iron fortified cereals can be an effective source of iron[3] but the intake of solid foods varies greatly in the first year of life. Formula milks are widely used in infancy and are a potentially useful source of iron. However, only a small proportion of iron in formula milk is absorbed.[4 5] Follow on milks contain higher concentrations of iron than standard formula milks and this extra iron might be expected to compensate for poor absorption and provide an important source of iron for infants. However, studies have shown that increasing the iron level in formula milk above 0·3–0·7 mg/100 ml has very little additional effect in the prevention of iron deficiency anaemia.[6 7] To investigate this further we compared the effect of follow on milk fortified with iron against the same milk without added iron. The study took the form of a randomised double blind controlled trial on infants aged six to 18 months of age and compared a follow on milk containing 1·2 mg of iron per 100 ml with exactly the same follow on milk without any iron added during manufacture.

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Accepted 18 May 1995

Methods

SUBJECTS

Infants were eligible for the trial if they were born in Gloucester Maternity Hospital, had an address in Gloucester City, were of normal birth weight, not breast fed, not ill, had no major congenital malformation, had not been given any iron therapy, and if parental permission was obtained. Infants entered the trial at 6 months of age. After entry to the trial infants were excluded if they became ill (they were not excluded for upper respiratory infections), were given iron medication, were fed with milk other than the trial milk for more than 25% of the time between each examination, developed iron deficiency anaemia (haemoglobin <110 g/l and serum ferritin <10 μg/l), moderate anaemia (haemoglobin <100g/l) for any reason, or if they missed more than one follow up appointment. Infants who developed mild anaemia (haemoglobin between 100 and 109 g/l) were not excluded if their serum ferritin levels were above 10 μg/l and infants with serum ferritin levels below 10 μg/l were not excluded if they were not anaemic (haemoglobin <110 g/l).

MILK

The fortified follow on milk contained 1·2 mg of iron per 100 ml, in the form of ferrous sulphate. The unfortified milk was identical except that no iron had been added during manufacture and it was therefore assumed to contain the same concentration of iron as in unmodified cows' milk (approximately 0·1 mg of iron per 100 ml). The milks were identical in all other respects and contained 2·6 g of fat, 1·9 g of protein (40% casein, 60% whey protein), 115 mg calcium, 94 mg phosphorus, and 10 mg of vitamin C per 100 ml. The milk was supplied in cans which were numbered but did not reveal the name of the manufacturer. Infants who entered the trial were randomly allocated to either fortified or unfortified follow on milk. The mothers and the personnel involved in the trial were not aware of which milk the infants consumed until the end of the trial when the code was broken and the results analysed.

The mothers were asked to replace the formula milk which their infants were receiving with the trial milk (no infants were taking unmodified cows' milk as their main source of milk at entry into the trial), to continue feeding their infants with their normal diet, and to avoid all other forms of milk. No other dietary advice was offered or given. The trial milk was supplied free of charge and delivered to the home every three months or more frequently if more milk was used.

EXAMINATIONS

The children were examined at entry to the trial (6 months of age) and at 9, 12, 15, and 18 months of age. Inquiry was made about illnesses and about whether the milk was tolerated. A dietary history was taken by a paediatric dietitian. At each examination free flowing blood was taken by heel stab for full blood count and serum ferritin. Haemoglobin electrophoresis, including HbA_2 estimation, was performed on blood from non-Caucasian infants.

DIETARY METHODS

Dietary data were collected at three monthly intervals using a structured questionnaire giving information on the type, frequency, and quantity of meals, snack foods, and fluids, including non-milk drinks. At the onset of the trial, reliable quantitative estimates of milk intake and iron containing cereals could be obtained and an estimate of the iron intake from fortified foods (milk+iron fortified cereals) was made, but as the diet became more complex it became increasingly difficult to obtain accurate estimates of the quantity of iron in the diet from sources other than milk. Dietary iron was classified at 18 months on a qualitative graded scale from 1 (poor) to 5 (excellent), based on haem iron sources, non-haem iron sources, fruit, vegetables, cereals, snack foods, and non-milk drinks. The effect of iron in milk was excluded in this assessment. An estimate of the amount of milk ingested was obtained by dietary history and by counting the number of cans of milk supplied for that infant. These two estimates did not correlate well and on further inquiry we found that mothers were sometimes using formula milk for other members of the household or spilt or lost cans of milk. We have therefore quoted volumes of milk as estimated from the dietary history.

LABORATORY METHODS

Standard haematological methods were used, and serum ferritin was estimated with an ELISA (enzyme linked immunosorbent assay) technique.[8]

SATISTICS

Students *t* test, the Mann-Whitney U test, and χ^2 tests were used when appropriate.

Results

Four hundred and fifty seven randomly selected infants born at the Gloucester Maternity Hospital fulfilled the initial entry criteria, and parental permission was obtained for 105 of these, of whom 92 attended for the first blood test and were included in the trial. Infants whose parents gave permission and who were included in the trial were from less affluent groups, with a significantly greater proportion from social classes IV and V and fewer from social classes I and II compared to those whose parents did not wish their infants to enter the trial ($\chi^2=23{\cdot}7$, df=4, $p<0{\cdot}001$). There were only two non-Caucasian infants in the trial and 15 from the group whose parents did not give permission (NS). Comparison of the infants randomised to fortified milk and unfortified milk showed no significant difference with respect to social class, sex, mother's age, and number of siblings.

Six children were withdrawn from the trial because they developed iron deficiency anaemia (haemoglobin <110 g/l and serum ferritin <10 μg/l); four were receiving fortified milk and two unfortified milk (NS). One infant was withdrawn from each group because a milk other than the trial milk was taken for more than 25% of the time between two examinations. The other children were withdrawn because of failure to attend follow up. The parents were not pressurised to continue with the trial and the mothers were not closely questioned as to the reasons for failure to attend for follow up. It was not always possible to take enough blood for both a full blood count and serum ferritin, which explains the minor differences in the numbers of these estimations at each age.

There was no significant difference in the proportion in each treatment group remaining in the trial at any age, nor was there a difference in the number of siblings, the mother's age, or the social class distribution between those withdrawn and those remaining in the trial in each treatment group at each age; and the reasons given for leaving the trial were similar. Infants who were withdrawn from the trial did not differ from those not withdrawn with respect to mean haemoglobin or median serum ferritin at entry into the trial. Power was assessed on the basis of an estimated clinically significant difference of 7·5 g/l for haemoglobin and 20 μg/l for serum ferritin. The power was assessed at each examination and remained more than 80% at the 5% significant level for mean haemoglobin and median serum ferritin estimations throughout the trial.

AGE AT EXAMINATIONS

There was no difference between the two treatment groups with regard to the median age in days at each examination throughout the trial.

DIET

There was no difference in the volume of milk (ml/d or ml/kg/d) between the two treatment groups throughout the period of the trial, nor was there a difference in the mean age at weaning and the amount of iron taken in fortified

Table 1 Mean dietary iron (mg/d) from fortified infants foods+formula milk and mean age of weaning (weeks) at entry into the trial (6 months)

	Fortified milk			*Unfortified milk*		
	No	*Mean*	*SD*	*No*	*Mean*	*SD*
Iron (mg/d) from fortified foods (milk+cereals)	43	4·9	2·59	44	4·38	2·3
Age of weaning (weeks)	43	13·2	4·14	44	12·7	3·81

Comparison of the two treatment groups showed no significant difference with respect to mean dietary iron and mean age of weaning.

Table 2 Mean haemoglobin (Hb) levels and number of anaemic infants (Hb <110 g/l) taking fortified and unfortified milk between 6 and 18 months of age

	Fortified milk					*Unfortified milk*				
Age (months)	*No*	*Mean*	*SD*	*Confidence intervals (95%)*	*Hb<110 g/l*	*No*	*Mean*	*SD*	*Confidence intervals (95%)*	*Hb<110 g/l*
6	44	113·6	10·7	110·4 to 116·8	15	48	118	11·7	114·7 to 121·3	11
9	35	116·1	10·5	112·7 to 119·7	7	43	117·4	8·3	115·0 to 119·9	10
12	34	116·6	9·4	113·4 to 119·8	7	36	121·2	11	117·6 to 124·8	6
15	27	119·2	9	115·8 to 122·7	4	32	120·9	8·5	117·9 to 123·9	3
18	24	122	6·1	119·6 to 124·5	0	26	119·7	10·1	115·8 to 123·6	4

Comparison of the two treatment groups showed no significant difference in mean haemoglobin or in the proportion of infants with anaemia at any age.

foods (mg per day) at the onset of the trial (table 1), or between the graded assessment of iron in the diet between the two treatment groups at 18 months of age.

HAEMOGLOBIN

There was no difference between the mean haemoglobin concentrations of the two treatment groups throughout the period of the trial, or in the proportion of infants with anaemia (haemoglobin <110 g/l) (table 2).

SERUM FERRITIN

There was no difference in median ferritin levels between the two treatment groups throughout the period of the trial or in the proportion with low serum ferritin levels (<10 μg/l) between the two treatment groups (table 3).

Discussion

The principal finding of our study is that we could find no evidence that iron added to follow on milk had an effect on haemoglobin or serum ferritin concentrations in infants aged 6 to 18 months of age. Before these results can be accepted as reliable it is important to examine possible errors in the trial which may have led to an erroneous result.

There was bias in the selection of subjects. Infants who entered the trial were not typical of the majority of infants who fulfilled the initial entry criteria. Within the group of infants who entered the trial there was a significantly higher proportion from social class IV and V than within the group who fulfilled the initial entry criteria but did not enter the trial. The children entering the trial were from poorer socioeconomic groups and were therefore at greater risk of developing iron deficiency.[9] There was no significant difference between the groups taking the two different milks with respect to social class and other demographic criteria. Asian infants are at the greatest risk of developing iron deficiency anaemia[10] but almost all the infants in our study were Caucasian. It is possible that a different result would have been obtained if a group of infants of different ethnic origin or who were taking a different diet had been studied.

Considerable numbers of infants were withdrawn during the trial period, thereby reducing the sample size and power of the trial, but this effect was lessened by the tendency for the standard deviation and range of both haemoglobin and serum ferritin concentrations to narrow as the trial progressed. Infants who were withdrawn from the trial did not differ with respect to mean haemoglobin or median serum ferritin at entry into the trial from those who were not withdrawn from the trial. The power was therefore assessed at each examination and remained more than 80% at the 5% significant level for haemoglobin and ferritin estimations throughout the trial, assuming a minimum clinically significant difference of 7·5 g/l for haemoglobin and 20 μg/l for serum ferritin. Our study does not reliably exclude the possibility that the iron in follow on milk might produce differences in mean haemoglobin or median serum ferritin of less than 7·5 g/l and 20 μg/l respectively. If differences less than this could be shown to be clinically significant the trial would have to be repeated with a larger number of subjects.

It is possible that the trial may have altered the dietary intake of infants in ways other than just the giving of trial milk. Parents were asked not to alter the diet of their infants as a result of entry into the trial and no dietary advice was offered or given. However, fully informed consent inevitably meant that the parents were aware that the trial was designed to look into the prevention of iron deficiency anaemia and this may have altered their approach to feeding their infants. They may have become more

Table 3 Median serum ferritin levels and number of iron deficient infants (serum ferritin <10 μg/l) taking fortified and unfortified milk between 6 to 18 months of age

	Fortified milk					*Unfortified milk*				
Age (months)	*No*	*Median*	*Range*	*Confidence intervals (95%)*	*Ferritin <10 μg/l*	*No*	*Median*	*Range*	*Confidence intervals (95%)*	*Ferritin <10 μg/l*
6	44	28	5·2–176	22 to 40·4	3	40	30·5	12–128	25 to 36·7	0
9	36	18·8	5–123	13·4 to 25	5	43	21	1·9–66	16 to 25	1
12	36	23·9	4–101	16 to 32	3	37	15	7·3–66	13 to 21	5
15	24	17	4–48	10 to 23	6	33	15	6·4–61	13 to 19	3
18	25	16	5–106	14·5 to 19·9	4	27	15	4–43	10·3 to 19	7

Comparison of the two treatment groups showed no significant difference in mean serum ferritin or in the proportion of infants with iron deficiency at any age.

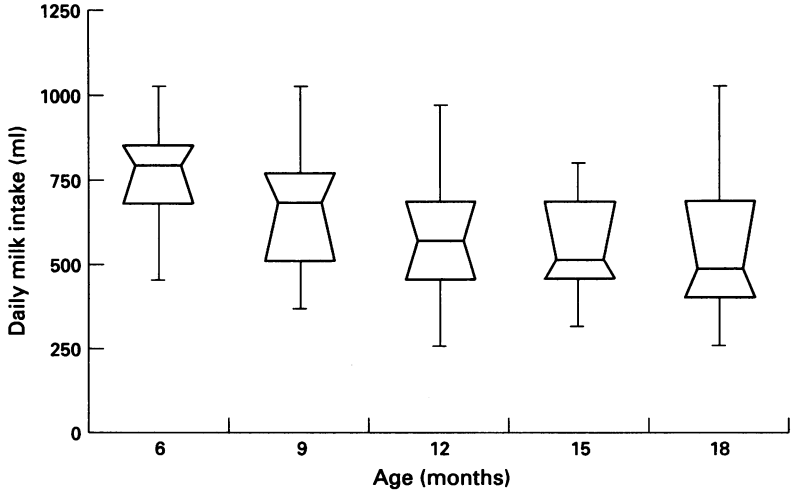

Figure 1 Volume of milk (ml/d) ingested by infants aged 6–18 months. Centre line=median; notch=25–75th centiles; whisker=5–95th centiles.

aware of the risk of iron deficiency as a result of entering the trial and thereby have taken a greater interest in their child's diet and made inquiries about ways to avoid iron deficiency. On the other hand, they may have become confused about the trial milk and shown an inappropriate reliance on the milk as a method of avoiding iron deficiency, even after the double blind nature of the study had been explained. The trial milk was supplied free of charge and delivered to the home, so it is very likely that the mothers fed their infants with more milk than if they had not entered the trial.

The double blind control method should protect the trial against unintentional observer bias. The trial was financed by a milk company but the laboratory and statistical analysis was performed independently of the milk company. The observers and hospital did not receive any expenses beyond those required to complete the trial. The hospital laboratory takes part in a national quality control audit.

There have been controlled studies[6 7] which have examined the effect of different concentrations of iron in formula milks. Bradley *et al*[7] performed a double blind controlled trial which compared a formula milk containing 0·74 mg iron per 100 ml with the same formula containing 1·27 mg/100 ml in infants up to 12 months of age. There was no significant difference in the mean haemoglobin concentrations between the two groups throughout the trial. Mean serum ferritin concentration was higher in the high iron formula group at 12 months of age in those infants who had started formula feeds at birth but not higher if the infants had been started on formula feeds between 1 week and 8 months of age. Haschke *et al*[6] found no significant difference in haemoglobin, free erythrocyte protoporphyrin, and ferritin levels at 6 and 9 months of age in infants fed with a formula with a very low iron content (0·3 mg/100 ml) compared with the same formula with an iron content of 0·6 mg/100 ml. There were no infants in either group who developed iron deficiency (serum ferritin <10 μg/l).

If follow on milk is to provide an important source of iron for the growing infant it is essential that adequate volumes of milk are ingested and that there is adequate absorption of iron. We found that the milk intake of infants diminished after 6 months of age, both as total volume per day, and, as expected and even greater, as volume per kg per day. At each age there were marked differences in the volume of milk taken by different infants in the trial but no significant difference between the two treatment groups. As there was no significant difference in the volume of milk per day ingested by infants in the two groups, the amounts per day and per kg per day ingested by all infants in the trial are shown in figs 1 and 2. Our data show that the potential importance of milk as a source of iron lessens between 6 and 18 months of age because the amount of milk ingested decreases and other forms of fluid are introduced into the diet. The wide variation in the amount of milk drunk by different infants, even when milk is supplied free of charge, further calls into question the reliability of milk as the principal source of iron in the growing infant.

Oski[4] has estimated that the daily requirements of iron from birth to 1 year of age are 0·78 mg iron/day. The reference nutritional intake (RNI) of iron[11] of 7·8 mg per day for infants aged 7–12 months and 6·9 mg per day for those aged 1–3 years is adequate if at least 10% of dietary iron is absorbed. The absorption of iron from formula milk is poor and it has been estimated that only about 4% of the iron is absorbed from formula milk containing 1·2 mg of iron per 100 ml.[4 5] The median amount of iron absorbed per day by infants in our trial, based on the volume of milk consumed and calculated on the assumption that 4% of the iron in milk is absorbed, diminished from 0·36 mg at 6 months to 0·23 mg at 18 months of age. For many infants who were taking relatively small volumes of milk the contribution of milk to the amount of iron absorbed from the diet is minimal. Vitamin C increases iron absorption and we found that 95% of infants in the fortified milk group and 93 % in the unfortified milk group were taking fruit

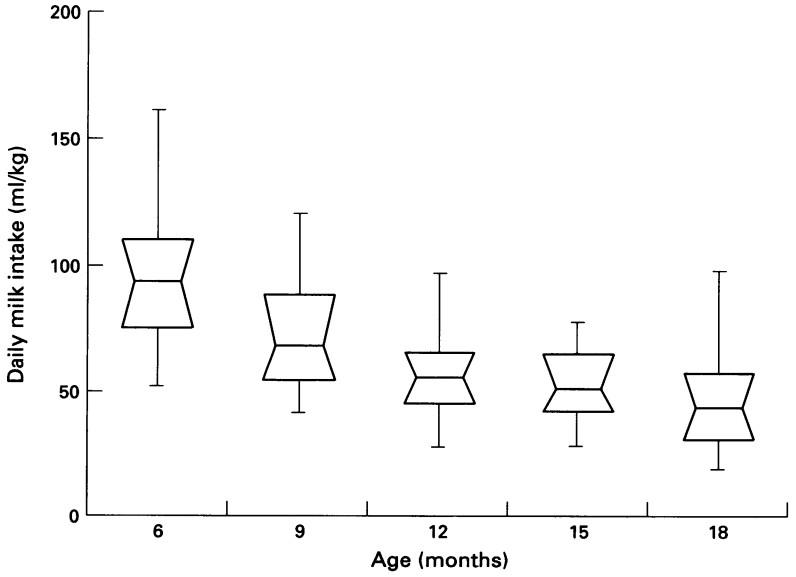

Figure 2 Volume of milk (ml/kg/d) ingested by infants aged 6–19 months. Centre line=median; notch=25–75th centiles; whisker=5–95th centiles.

drinks at 18 months of age. On the other hand 43% of the fortified group and 41% of the unfortified group were taking tea, a potent inhibitor of iron absorption, by 18 month of age.

There was a low incidence of iron deficiency anaemia (haemoglobin <110 g/l and serum ferritin <10 μg/l) in the two treatment groups but there were considerable numbers of infants in both groups who had depleted iron stores (serum ferritin <10 μg/l) without anaemia. Towards the end of the trial most infants had serum ferritin levels below 30 μg/l. It is possible that a number of these infants may have had iron deficiency anaemia because it has been shown that a serum ferritin level of 10 μg/l does not reliably exclude iron deficiency anaemia, and many infants with serum ferritin levels between 10 and 30 μg/l will show a rise of haemoglobin in response to iron therapy.[12]

There were considerable numbers of infants throughout the trial who had low haemoglobin concentrations without definite iron deficiency (serum ferritin <10 μg/l). The reason for this is not certain. Some infants may have been iron deficient with borderline serum ferritin, some may have had low normal haemoglobin values with no haematological abnormality, and some may have had temporarily reduced haemoglobin concentrations after relatively minor upper respiratory tract infections[10,13,14] or may have been iron deficient with temporarily raised serum ferritin as a result of recent mild infection.[15]

The results of this trial suggest that iron added to follow on milk was not an important source of dietary iron in the infants whom we studied. It is important to recognise that our trial does not provide any evidence to suggest that it is desirable to feed infants with unmodified cows' milk in the first 18 months of life as this question was not addressed by our study.

1 Addy DP. Happiness is: iron. [Leading article.] *BMJ* 1986; **292:** 969–70.
2 Aukett MA, Parks YA, Scott PH, Wharton BA. Treatment with iron increases weight gain and psychomotor development. *Arch Dis Child* 1986; **61:** 849–59.
3 Walter T, Dallman PR, Pizarro F, *et al.* Effectiveness of iron-fortified infant cereal in prevention of iron deficiency anaemia. *Pediatrics* 1993; **91:** 976–82.
4 Oski FA. Iron deficiency in infancy and childhood. *N Engl J Med* 1993; **329:** 190–3
5 McMillan JA, Oski FA, Lourie G, Tomarelli RM, Landaw SA. Iron absorption from human milk, simulated human milk, and proprietary formulas. *Pediatrics* 1977; **60:** 896–900.
6 Haschke F, Vanura H, Male C, *et al.* Iron nutrition and growth of breast and formula fed infants during the first 9 months of life. *J Paediatr Gastroenterol Nutr* 1993; **16:** 151–6.
7 Bradley CK, Hillman L, Sherman AR, Leedy D, Cordano A. Evaluation of two iron-fortified milk based formulas during infancy. *Pediatrics* 1993; **91:** 908–14.
8 Dawson DW, Fish DI, Shackleton P. The accuracy and clinical interpretation of serum ferritin assays. *Clin Lab Haematol* 1992; **14:** 47–52.
9 Owen GM, Lubin AH, Gary PJ. Preschool children in the United States: who has iron deficiency? *J Pediatr* 1971; **79:** 563–8.
10 Stevens D. The epidemiology of hypochromic anaemia in young children. *Arch Dis Child* 1991; **66:** 886–9.
11 Department of Health. Report on health and social subjects No 41. *Dietary reference values for food energy and nutrients for the United Kingdom.* London: HMSO, 1991.
12 Dallman PR, Reeves JD, Driggers DA, Lo EYT. Diagnosis of iron deficiency: the limitations of laboratory tests in predicting response to iron treatment in 1-year-old infants. *J Pediatr* 1981; **98:** 376–81.
13 Jansson LT, Kling S, Dallman PR. Anaemia in children with mild infections seen in a primary care pediatric outpatient department. *Pediatr Infect Dis* 1986; **5:** 424–7.
14 Dallman PR. Changing characteristics of childhood anaemia. *J Pediatr* 1989; **114:** 161–4.
15 Elin RJ, Wolff SM, Finch CA. Effects of fever on serum iron and ferritin concentrates in man. *Blood* 1977; **49:** 147–53.