

A longitudinal study of iron status in healthy Danish infants: effects of early iron status, growth velocity and dietary factors

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Michaelsen KF, Milman N, Samuelson G. A longitudinal study of iron status in healthy Danish infants: effects of early iron status, growth velocity and dietary factors. *Acta Paediatr* 1995;84:1035-44. Stockholm. ISSN 0803-5253

In a cohort of term infants ($n = 91$), followed from birth to 12 months, iron intake was examined by 24-h food records, and iron status by blood samples (haemoglobin (Hb), mean corpuscular volume (MCV), serum values for iron, ferritin and transferrin, and erythrocyte protoporphyrin) at 2, 6 and 9 months. At 9 months of age, 5% had anaemia (Hb < 105 g/l), but none had developed iron deficiency according to strict definitions used in this study (serum ferritin < 13 $\mu\text{g/l}$ and transferrin saturation < 10%). Infants with high serum ferritin, serum transferrin and erythrocyte protoporphyrin values at one blood sampling also had high values at the following sample (tracking, $r = 0.45-0.80$), suggesting that iron stores at delivery are an important determinant of iron stores during late infancy. Factors related to changes in serum ferritin were investigated by multiple linear regression. From 2 to 6 months, serum ferritin was negatively associated with knee-heel growth velocity ($p = 0.006$) and positively with intake of infant formula ($p = 0.04$). From 6 to 9 months it was negatively associated with intake of bread ($p = 0.001$), and there was a trend for a positive association with intake of meat ($p = 0.07$) and fish ($p = 0.08$) and for a negative association with intake of cow's milk ($p = 0.07$). In conclusion, those with a high growth velocity and a dietary pattern with a high intake of bread and a low intake of meat and fish had lower ferritin values and thereby an increased risk of depleting their iron stores later during infancy. □ *Erythrocyte protoporphyrin, infant nutrition, iron, iron deficiency, serum ferritin, serum transferrin*

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Iron deficiency is still common during late infancy and early childhood in industrialized countries, especially among socially disadvantaged groups and ethnic minorities (1-5). The present situation in the Scandinavian countries is, however, not well documented. It is important to prevent iron deficiency because it may be associated with impairment of mental and psychomotor development. This can be reversed by iron supplementation (6, 7), but in some cases it seems that the impairment is irreversible (7-9). Furthermore, iron deficiency has a negative influence on cell-mediated immune function (10) and may also affect appetite (11).

Iron status during infancy is determined by interaction between a number of critical factors. Iron endowment at birth is important, but it is generally agreed that the iron status of the mother has no or little influence on the iron status of the newborn infant (12, 13). However, a number of studies have shown that newborn infants of mothers with low serum ferritin values also have lower ferritin levels (14-16). In a study of healthy Danish mothers who had all been recommended an iron supplement during pregnancy, umbilical cord ferritin values

were significantly correlated with maternal values (17), and in another Danish controlled study of healthy mothers, infants of mothers receiving placebo had significantly lower umbilical cord ferritin values than infants of mothers who had received iron (18). Iron endowment at birth is needed to protect the infant from iron deficiency during the first 4-6 months of life when the iron requirement for growth is very high and iron intake from breast milk is low.

After 4-6 months of age, dietary iron intake becomes critical. Exclusive breast feeding beyond 6 months of age is associated with an increased risk of developing iron deficiency (19). Cow's milk has a negative influence on iron status (5, 20-23), because the iron content is low and poorly absorbed, and because it may provoke minor intestinal bleeding, especially during early infancy (24). Iron-fortified infant formula has therefore been recommended instead of cow's milk until 12 months of age (25, 26), but there is no consensus in the Scandinavian countries on up to which age formula should be recommended. Iron-fortified infant cereals are widely used, but the availability of the iron in

these products has been questioned (21, 27, 28), partly because of the phytate content in the cereals which reduces the availability of iron. Meat, fish and chicken are good sources of iron because they contain a high proportion of haem-iron, which is easily absorbed, as well as substances that promote the absorption of non-haem iron from other foods (29). However, advice on intake of meat, fish and chicken does not usually play a conspicuous role in recommendations for the prevention of iron deficiency during infancy. Although iron fortification is effective in the prevention of iron deficiency anaemia during infancy, caution has been advised against uncritical fortification and supplementation (30–32). Iron is a potent pro-oxidant which might cause damage if given in excess, and a high iron content in infant food can interfere with the absorption of other minerals (32–34).

The aim of the present study, which was part of the Copenhagen Cohort Study on Infant Nutrition and Growth (35, 36), was to describe the iron status and iron intake, and influence of feeding pattern and growth velocity on iron status, in Danish infants during the first year of life.

Subjects and methods

In this prospective study, a random sample of Danish infants was followed from birth to 12 months of age. This was an observational study in which the mothers were not influenced in their choice of feeding. Two hundred and fifty-one infants born at Hvidovre Hospital during 109 predetermined 24-h periods from October 1987 to February 1988 fulfilled the following inclusion criteria: parents of Danish origin, singleton birth, gestational age between 37 and 42 weeks, birth weight for gestational age between the 10th and 90th percentiles, no neonatal disease or malformation, and mother and infant admitted to maternity ward for at least 3 days. Randomization of the 251 infants allocated 139 to a study group and 112 to a control group. Ninety-one (65%) of the families in the study group agreed to participate and 84 completed the study up to 12 months of age. Fifty-nine (53%) of the families in the control group agreed to participate. The control group was included in the study in order to determine if the close observation with food registrations, test weighing, anthropometry and blood sampling had any influence on infant feeding patterns. This group was contacted and examined only at 9 months, at which time the same blood tests and food records were performed as in the examination at 9 months in the study group. There were no significant differences between duration of breast feeding in the two groups, and there were no major differences between the characteristics of the study and control groups. A more detailed description of the selection and characteristics of the groups is published elsewhere (35, 36). The study

was approved by the Ethics Committee for Copenhagen and Frederiksberg.

Food intake

Twenty-four hour food records were kept monthly by the parents from 1 to 12 months of age, using household measures. At 2, 4 and 9 months of age, intake was weighed on electronic scales for an extended period (48 h, 48 h and 5 days, respectively). Breast-feeding status was recorded monthly according to definitions published previously (35). All mothers except one started breast feeding. At 3, 6 and 9 months, 71%, 52% and 33%, respectively, were still breast feeding. A detailed report of the breast feeding pattern in this cohort has been published previously (36). Nutrient intake was calculated using the Dankost computer program (version 1.3), developed by the Danish National Food Agency.

Iron supplements and fortification

A daily iron supplementation of 15 mg is recommended in Denmark for infants between 6 and 12 months of age who are not receiving a daily minimum of 400 ml of iron-fortified formula. However, this advice is not followed by the majority of parents, including those in this study. Five study and six control infants were given an iron supplement before 9 months of age, for a median of 2 months (range 1–4 months). Infants receiving iron supplements were included in the analysis of iron status markers, but were excluded from the analysis of the influence of diet on iron status. All brands of formula given to the infants in the study were iron fortified (median value 8 mg/l formula, range 8–13 mg/l, information from manufacturer). At the time of the study most of the commercially produced infant cereals were fortified with iron (median level 16 mg/kg ready-made porridge, range 15–21 mg/kg, information from manufacturer). From 1988, iron fortification of infant cereals was abolished due to a change in legislation.

Venous blood samples

At 2, 6 and 9 months of age, 86, 79 and 125 infants, respectively, were available for blood tests. The remaining infants were absent or the parents did not agree to the blood sampling. Venepuncture was attempted only once and was successful in 83%, 73% and 77% of cases, respectively. Mean age (\pm SD) on the day of blood sampling was 61 ± 3 days, 185 ± 8 days and 276 ± 8 days, respectively. In some cases the amount of blood obtained was not sufficient for all blood tests; this is the reason why the numbers in Table 1 differ slightly. Haemoglobin (Hb) and haematocrit were measured on the day of sampling. Serum was stored at -20°C for the other analyses, which were performed within the following year. It was possible to obtain cord serum

samples from 31 infants. These samples were kept at 4°C until they were frozen at -20°C, 4 days after delivery.

Hb and mean red cell volume (MCV) were analysed on a Hemalog analyser (Technicon, Tarrytown, USA), while haematocrit was determined manually ($\text{Hb mmol/l} = \text{Hb g/l} \times 0.0621$). Serum iron was measured on a SMAC autoanalyser (Technicon, Tarrytown, USA) and serum transferrin by immunoelectrophoresis. Transferrin saturation was calculated as serum iron ($\mu\text{mol/l}$) $\times 100$ / serum transferrin ($\mu\text{mol/l}$) $\times 2$. Serum ferritin was measured with a radioimmunoassay (Ferritin RIA Amersham, Amersham, UK). Total red blood cell (RBC) protoporphyrins were quantitated in duplicate on a Hitachi F-1000 fluorescence spectrophotometer (37). Protoporphyrin concentrations were expressed as nanomoles coproporphyrin equivalents per litre red blood cells ($\text{nmol } 0.655 = \mu\text{g}$). The radioimmunoassay used for determination of serum ferritin (Ferritin RIA Amersham) is described in detail elsewhere (38). In the concentration range 13–40 $\mu\text{g/l}$, the within-assay reproducibility (coefficient of variation) is 5.6% and the between-assay variation (total assay variation) 12.0% (38). The kit has been calibrated against the international human liver ferritin standard WHO 80/602 (39). Ferritin RIA Amersham values of 13 and 16 $\mu\text{g/l}$ correspond to WHO standard ferritin values of 12 and 15 $\mu\text{g/l}$ (39). However, throughout this paper we are reporting the original uncalibrated Amersham values. There is at present no consensus as to which ferritin value should indicate absent or depleted iron stores, either in adults or children. In infants, cut-off values of 8–12 $\mu\text{g/l}$ (40, 41), 10 $\mu\text{g/l}$ (5, 19, 42) and 12 $\mu\text{g/l}$ (8, 20) have been suggested. In the present study, a critical serum ferritin value of <13 $\mu\text{g/l}$ (approximating a WHO standard value of <12 $\mu\text{g/l}$) was used. We defined iron deficiency as cases in which serum ferritin was <13 $\mu\text{g/l}$ and transferrin saturation <10%. When, in addition to this, Hb was <105 $\mu\text{g/l}$ (6.5 mmol/l), iron deficiency anaemia was considered present.

Growth

Body weight and knee–heel length were measured at 2, 6 and 9 months of age on the day of blood sampling. Knee–heel length, which allows a more accurate measurement of linear growth velocity than crown–heel length, was measured using an electronic infant knemometer (43).

Statistical methods

Paired and non-paired Student's *t*-tests (two-tailed) were used for comparison of means between groups. Pearson's correlation coefficient was employed to describe tracking of iron status indicators (i.e. the degree to which an infant with a high value at one blood sampling also had a high value at the following

sampling) and associations between different iron status indicators. All correlations were plotted and inspected for outliers. Statistical analyses of ferritin values were performed after logarithmic transformation, and geometric means were used because of the skewed distribution. The change in ferritin values over a period was calculated as the difference between the logarithmic values. This parameter was used as the dependent variable in multiple linear regression analysis of factors influencing change in iron stores. Backward elimination was used in the multiple linear regression analysis. Variables with a *p* level <0.1 were kept in the final model. This level was chosen because of the limited number of infants in the analysis. The highest possible number of infants was included in each multiple regression analysis.

Results

When the study and control groups were compared, at 9 months, iron status markers were not significantly different, and there were no differences in iron intake. However, Hb was slightly, but significantly, higher in the control group (115 g/l versus 119 g/l; *p* = 0.01). There were no significant differences between the two groups at 9 months regarding the number of infants receiving iron supplements, duration of breast feeding or the number of infants being breast fed. We have therefore added data from the control group when analysing 9-month values.

Hb and iron status markers

Values at 2, 6 and 9 months of age are shown in Table 1 and Fig. 1. Geometric mean serum ferritin in cord blood was 186 $\mu\text{g/l}$ (5th, 95th percentiles: 83 $\mu\text{g/l}$, 373 $\mu\text{g/l}$), and was significantly lower than the 2-month value (*p* < 0.001). There was a strongly significant tracking of the values for serum ferritin, serum transferrin and erythrocyte protoporphyrins, implying that infants with a high value at one blood sampling also tended to have a high value at the following blood sampling (Table 2). The associations between Hb, serum ferritin, erythrocyte protoporphyrin, serum transferrin, transferrin saturation and serum iron were examined at 2, 6 and 9 months. There was a strong negative association between serum ferritin and serum transferrin at all ages (*r* = -0.45, -0.44 and -0.40, respectively; all *p* ≤ 0.001). Hb was positively associated with serum iron (*r* = 0.43, *p* < 0.001) and transferrin saturation (*r* = 0.37, *p* < 0.01) at 2 months, but not later. Erythrocyte protoporphyrin was negatively associated with Hb values at 2 months (*r* = -0.23, borderline significant, *p* = 0.07) and 9 months (*r* = -0.24, *p* < 0.05), but not at 6 months (*r* = -0.01), when the erythrocyte protoporphyrin levels were lower than values at both 2 and 9 months. There were no consistently significant gender

Table 1. Haemoglobin and iron status indicators according to age.

	2 months			6 months			9 months		
	Mean ± SD	5-95 percentiles	n	Mean ± SD	5-95 percentiles	n	Mean ± SD	5-95 percentiles	n
Haemoglobin (g/l)	115 ± 8	101-130	68	116 ± 9	105-130	57	117 ± 7	103-129	94
(mmol/l)	7.1 ± 0.5	6.3-8.1		7.2 ± 0.6	6.5-8.1		7.3 ± 0.5	6.4-8.0	
Serum ferritin ^a (µg/l)	301	134-675	63	59	23-140	54	37	17-78	84
Erythrocyte protoporphyrin (nmol/l erythrocytes)	679 ± 185 ^b	479-1148	65	475 ± 109	304-659	48	556 ± 133 ^b	369-763	83
Serum transferrin (µmol/l)	26 ± 4 ^b	20-33	65	31 ± 4	24-38	57	35 ± 7 ^b	26-47	90
Serum iron (µmol/l)	13.1 ± 3.5 ^b	7.5-19.1	59	9.9 ± 3.1	6.3-17.3	46	8.8 ± 3.4	3.9-14.9	80
Transferrin saturation (%)	26 ± 8 ^b	15-40	59	16 ± 6	10-29	46	13 ± 6	5-25	77
Mean red cell volume (fl)	86 ± 5 ^b	78-94	64	76 ± 4	69-82	49	76 ± 6	68-85	89

^a Geometric mean values.

^b Significantly different ($p < 0.001$) from values at 6 months (Student's paired *t*-test).

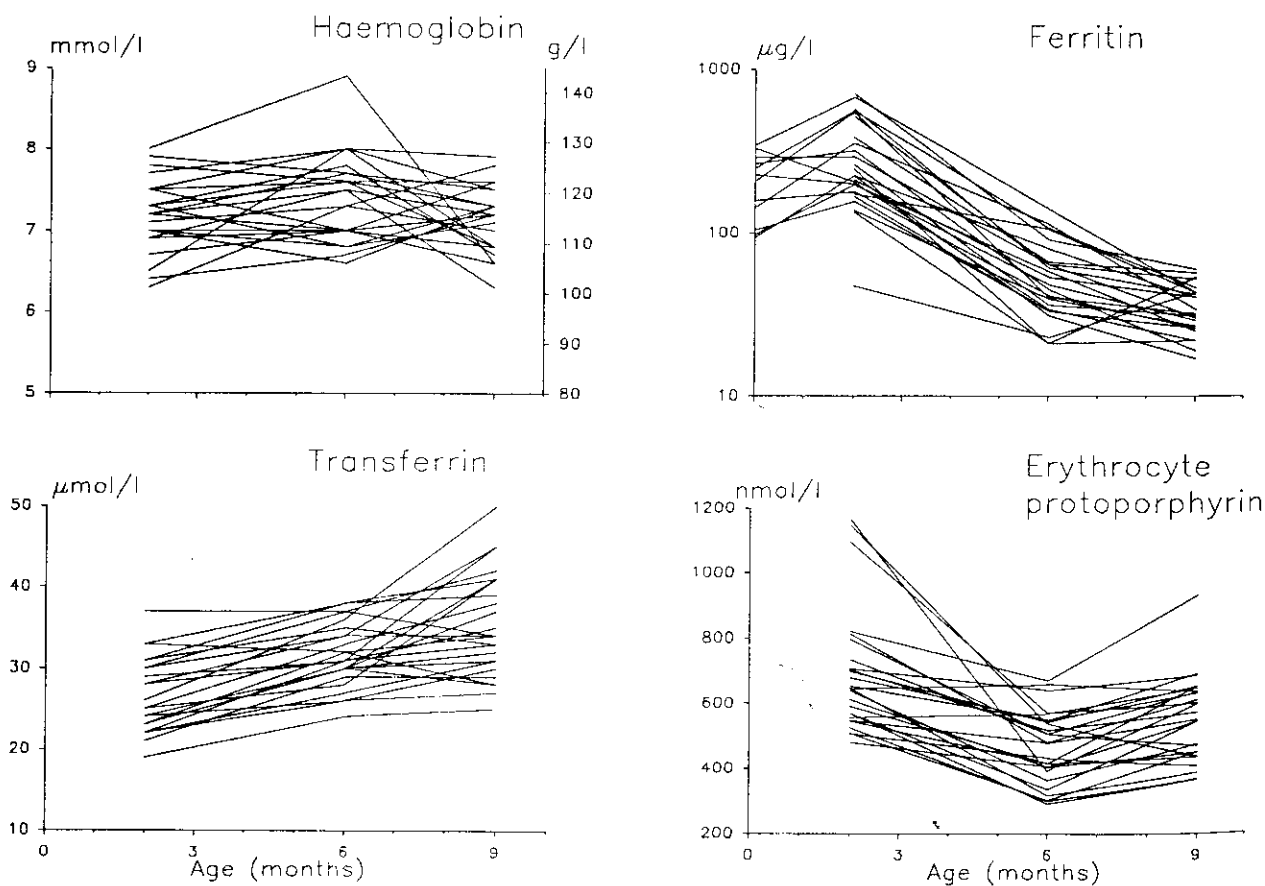


Fig. 1. Individual values for haemoglobin, serum ferritin, serum transferrin and erythrocyte protoporphyrins at 2, 6 and 9 months, and for serum ferritin in cord blood. Only infants with successful blood sampling at 2, 6 and 9 months are included.

Table 2. Tracking of haemoglobin and iron status indicators.^a

	Cord blood 2 months	2-6 months	6-9 months	2-9 months
Haemoglobin		0.50*** (48)		
Serum ferritin	0.47* (28)	0.65*** (43)	-0.02 (40)	0.08 (48)
Erythrocyte protoporphyrin		0.45** (37)	0.49** (32)	0.18 (35)
Serum transferrin		0.80*** (46)	0.77*** (31)	0.26 (42)
Serum iron		0.07 (35)	0.53*** (37)	0.34* (39)
Transferrin saturation		0.15 (35)	0.07 (29)	0.11 (33)
Mean red cell volume		0.12 (37)	-0.11 (26)	0.05 (30)
			0.22 (33)	0.24 (43)

^a Values are Pearson correlation coefficients. Number of infants in parentheses.
p* < 0.05, *p* < 0.01, ****p* < 0.001.

Table 3. Percentage of infants with anaemia.^a

	2 months (<i>n</i> = 68)	6 months (<i>n</i> = 57)	9 months (<i>n</i> = 94)
Hb < 105 g/l (≤ 6.5 mmol/l)	15% (10) ^b	5% (3) ^b	5% (5) ^b
Hb < 110 g/l (≤ 6.8 mmol/l)	29% (20)	32% (18)	20% (19)

^a Number of infants with anaemia in parentheses.
^b None of the infants had serum ferritin values < 13 μmol/l.

differences in Hb and iron status markers throughout all age groups. Hb and iron status markers were also examined according to current breast feeding status, using analysis of variance, and distinguishing between exclusive breast feeding, partial breast feeding and no breast feeding. No significant differences were found at 2, 6 or 9 months.

Iron deficiency and anaemia

The lowest serum ferritin values at 2 and 6 months were 47 and 21 μg/l, respectively. At 9 months only two

infants (2%) had values < 13 μg/l (approximating a WHO standard value of 12 μg/l), and no infant had values in the range 13-16 μg/l (approximating a WHO standard value of 15 μg/l). The percentage of infants with transferrin saturation < 10% was 2%, 4% and 30% at 2, 6 and 9 months, respectively. The number of infants with anaemia according to the haemoglobin cut-off points of 105 g/l and 110 g/l is shown in Table 3. No infant had iron deficiency or iron deficiency anaemia according to our definitions at any age.

Iron intake

Iron intake for infants not breast fed is shown in Fig. 2. The contribution of the different food groups to the total iron intake of these infants is shown in Table 4. The iron content of human milk was not analysed.

Food intake and growth velocity

The influence of growth velocity and intake of foods from different food groups on the change in serum

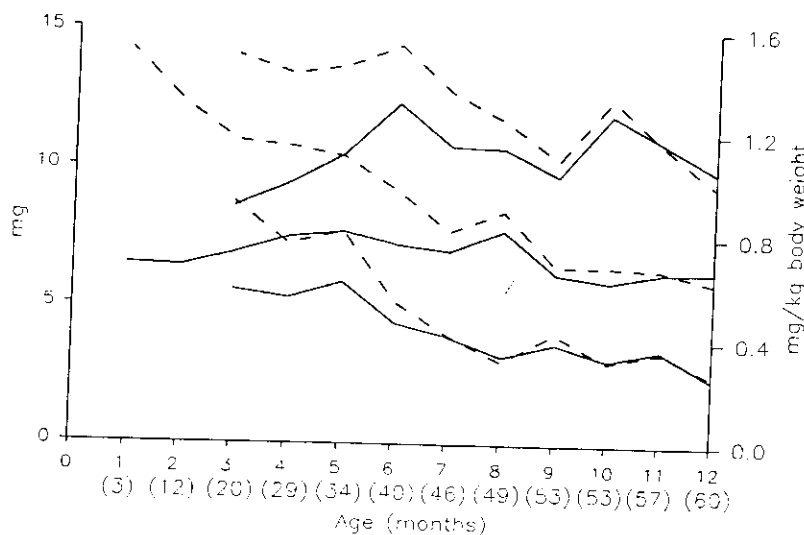


Fig. 2. Iron intake by age in infants not breast fed (10th, 50th, 90th percentiles). Solid lines show daily intake and broken lines daily intake per kg body weight. Number of infants in parentheses.

Table 4. The contribution of different food groups to iron intake in infants not breast fed.^a

	Age (months)									
	3	4	5	6	7	8	9	10	11	12
Formula follow-up	100	98	89	72	54	35	29	15	13	11
Cow's milk	---	0	0	1	2	3	4	5	4	4
Porridge, industrial	---	1	3	8	12	13	9	8	6	7
Porridge, home-made	---	0	1	5	8	10	11	11	12	11
Meat and fish	---	---	1	2	8	14	15	20	22	17
Bread	---	---	---	0	5	14	17	24	23	29
Vegetables and fruits	---	1	6	12	10	9	11	11	16	12
Other foods	---	0	0	0	1	2	4	6	4	9
Number of infants	20	22	29	33	40	44	49	53	56	57

^a Values are percentage of total iron intake. --- = no intake, 0 = intake < 0.5%.

ferritin values was examined using multiple regression analysis. The periods from 2 to 6 months and 6 to 9 months of age were analysed separately (Tables 5, 6). The influence of weight gain and knee-heel length velocity was examined in separate multivariate analyses because of the strong positive correlation between these two variables.

During the 2-6-month period there was a significant negative correlation between knee-heel length velocity and changes in serum ferritin levels. Growth velocity (both weight and knee-heel length) was positively correlated with formula intake ($p = 0.02$ and $p = 0.02$, respectively), and consequently the influence on serum ferritin of both growth and formula intake became stronger in the multivariate analysis. Knee-heel growth velocity was negatively ($p = 0.006$), and formula intake positively ($p = 0.04$), correlated with

changes in serum ferritin values in the final model. There was a highly significant tracking of serum ferritin levels from 2 to 6 months of age, as previously mentioned. When serum ferritin values at 2 months of age, knee-heel growth velocity and formula intake from 2 to 6 months were included as explanatory variables, 58% of the variation in serum ferritin levels at 6 months of age could be explained (estimates not shown).

During the 6-9-month period, weight gain, intake of bread and intake of cow's milk were all negatively associated with changes in serum ferritin in the univariate analysis, while knee-heel growth velocity was insignificant (Table 6). The influence of weight gain became insignificant in the multivariate analysis. Fish, and to a lesser degree meat, is often eaten with bread, and was therefore positively correlated with bread intake ($p = 0.003$ and $p = 0.11$, respectively). This explains

Table 5. Description of variables included in regression analysis of factors influencing change in iron stores.

	Centiles			Percentage of infants with any intake	n
	25	50	75		
Growth 2-6 months					
Weight gain (g/day)	18	21	24	---	42
Knee-heel length (mm/day)	0.19	0.22	0.24	---	41
Average intake 2-6 months ^a					
Infant formula (ml/day)	0	40	388	56	43
Growth 6-9 months					
Weight gain (g/day)	10	12	15	---	32
Knee-heel length (mm/day)	0.14	0.15	0.19	---	32
Average intake 6-9 months ^a					
Infant formula (ml/day)	0	105	426	64	28
Cow's milk (ml/day)	17	61	179	86	28
Bread (g/day)	10	16	26	93	28
Porridge, home-made (g/day)	26	54	125	93	28
Porridge, ready made (g/day)	0	20	96	64	28
Fruits and vegetables (g/day)	76	117	158	100	28
Potatoes (g/day)	22	39	60	100	28
Meat (g/day)	4	11	22	93	28
Fish (g/day)	0	3	6	71	28

^a Average of the monthly food records during the periods.

Table 6. Estimates from regression analysis of factors influencing change in serum ferritin values.

Dependent variable		Explanatory variables	Regression coefficient $\beta \pm SD$	<i>p</i>	<i>r</i> ²	<i>n</i>	
Change in serum ferritin from age 2–6 months ^a	Univariate	Weight gain ^b	-0.02 ± 0.02	0.26	0.03	42	
		Knee-heel growth velocity ^c	-4.61 ± 1.98	0.03	0.12	41	
		Infant formula ^d	0.04 ± 0.03	0.12	0.06	43	
	Multivariate	Model 1					
		Weight gain ^b	-0.03 ± 0.02	0.09	0.13	41	
		Infant formula ^d	0.06 ± 0.03	0.04	—	—	
Change in serum ferritin from age 6–9 months ^e	Univariate	Model 2					
		Knee-heel growth velocity ^c	-6.05 ± 2.08	0.006	0.21	40	
		Infant formula ^d	0.06 ± 0.03	0.04	—	—	
	Multivariate	Weight gain ^b	-0.03 ± 0.02	0.03	0.15	32	
		Knee-heel growth velocity ^c	1.02 ± 2.19	0.64	0.01	32	
		Bread ^f	-2.04 ± 0.62	0.003	0.29	28	
Cow's milk ^d		-0.13 ± 0.06	0.05	0.15	28		
Fish ^f		-1.12 ± 1.95	0.57	0.01	28		
Meat ^f		0.35 ± 1.01	0.73	0.00	28		
Bread ^f		-2.68 ± 0.71	0.001	0.48	28		
Meat ^f		1.57 ± 0.82	0.07	—	—		
Cow's milk ^d		-0.10 ± 0.05	0.07	—	—		
Fish ^f	3.37 ± 1.81	0.08	—	—			

^a The only food group examined was formula intake; ^b g/day; ^c mm/day; ^d 100 ml/day; ^e Only results from food groups with a *p* value below 0.1, either in the univariate or multivariate analysis have been shown; ^f 100 g/day.

why fish and meat became more significant in the multivariate analysis when bread was included. Since the change in serum ferritin values over a period was calculated as the difference between the logarithmic values, the effect of the explanatory variables can be estimated by taking the exponential value of the regression coefficient. For example, the univariate effect of an additional 100 ml of infant formula intake from 2 to 6 months of age changed the ferritin ratio by $\exp(0.04) = 1.04$. This means that the serum ferritin level at 6 months of age would be 4% higher than it would have been if the infant had not been given this additional 100 ml of infant formula. The average daily intake of bread was only a few grams at 6 and 7 months of age. At 8 and 9 months of age the average intakes were 22 g and 31 g, respectively. Approximately 75% of the bread intake was brown rye bread. Thirty to forty percent of the intake of meat was liver paste, the remaining being mainly minced meat. Approximately half of the intake of fish products consisted of cod roe used as sandwich spread.

Discussion

The main finding of this study was that a major part of the variation in serum ferritin values between the infants could be explained by early ferritin values, by growth velocity, which had a negative influence, and by different dietary characteristics, which had either negative or positive influences. Furthermore, up to 9 months of age, no case of iron deficiency developed, according to the strict criteria used in this study.

Serum ferritin is regarded as a reliable indicator of iron stores (30), although reservations have been expressed regarding how well it reflects iron stores during the first months of life (5, 44). However, we found that the strong tracking, together with the significant influence of growth velocity and diet on ferritin values, supported the view that serum ferritin values during early life are a useful indicator of iron stores, and thereby of the risk of developing iron deficiency later during infancy. An alternative explanation has been proposed by Morton et al., who also found significant tracking of ferritin levels during infancy (5). They suggest that ferritin levels might also indicate an individual's ability to extract iron both from the placenta and from the diet in postnatal life. Considering the day-to-day variation in serum ferritin values, which, at least in adults, is considerable (45), we were surprised that as much as 58% of the variation in serum ferritin values at 6 months of age could be explained by tracking, by differences in growth velocity, and by differences in intake of iron-fortified formula.

Serum ferritin is elevated during infectious diseases which might result in an underestimation of the true prevalence of iron deficiency. However, it is unlikely that this was a major problem in the present study, since all children were regarded as not suffering from infectious diseases at the time of blood sampling.

The negative association between growth velocity and change in serum ferritin values is in accordance with a study in which iron deficiency at 1 year of age was associated with greater weight gain (5), and can be explained by the need for iron during growth of lean tissue.

Iron stores at birth are to some extent determined by maternal iron stores (14–18), and since they consist mainly of iron from circulating haemoglobin, the amount of blood transfused from the placenta before clamping the umbilical cord is also of importance (46). We obtained no information in the present study on maternal iron status or iron supplementation, and none on the timing of umbilical cord clamping. However, it was the general recommendation that pregnant women should take iron supplements, and in uncomplicated deliveries the policy was to clamp the cord when cord pulsations stopped and to keep the infant below the level of the placenta until then.

Up to 9 months of age we did not find iron deficiency according to the definitions used in this study. Ferritin cut-off values were not critical in this study, since only two infants had values less than 16 µg/l. However, the best criterion for a diagnosis of iron is a treatment trial with iron (41). In a study of 1-year-old infants, more than 50% of the infants who responded to an iron treatment trial would have been missed if treatment had been restricted only to those with abnormal laboratory tests (47). Thus we cannot exclude the possibility of iron deficiency in our study. It is likely that iron deficiency is most prevalent from 12 to 18 months of age and it is possible that some of the infants in this study developed iron deficiency later. Blood sampling at this age would have been most interesting, but we had to limit the number of venepunctures and the main study had several other objectives requiring early blood samples.

The use of a Hb value of 105 g/l (6.5 mmol/l) as the criterion for anaemia was suggested by Siimes et al. (19) and Fuchs et al. (21). Others have suggested critical values of 107 or 110 g/l (6.8 mmol/l) (40, 42, 48), but our data indicate that a value of 110 g/l is too high, since 32% of the infants would be classified as having anaemia at 6 months of age using this value, despite none having iron deficiency according to our criteria. In a retrospective study of routine screening of 1-year-old infants in Norway, 37% had Hb values below 110 g/l (49), and in a Swedish study including healthy, breast-fed infants, the mean Hb value at 6 months was 109 g/l (32), also supporting the view that 110 g/l as a cut-off value is too high.

The data presented provide normative data for iron intake and iron status markers during infancy in healthy Danish infants. Serum ferritin values in the present study showed the same change with age and the same levels as in the classical study by Siimes et al. (50). Our 6-month values were similar to those from a recent Swedish study (32). There is no consensus on the optimal method of expressing the results of erythrocyte protoporphyrin and the preferred technique for standardization (30, 51), and there is a lack of normative data for infancy. Age-related changes, with high values during early infancy, lower values during mid-to-late infancy, and then an increase towards late infancy, which might

reflect increasing iron deficiency at this age, have also been found in other studies (3, 21, 52). Our serum transferrin values were similar to those found by Saarinen and Siimes (53), while the serum iron values, and thereby the transferrin saturation values, were lower than in other studies. Since this was also the case at 2 and 6 months, when iron deficiency is less likely, we believe that this was due to a methodological difference, rather than iron deficiency. Thus a cut off-value of 10% as a criterion for iron deficient erythropoiesis seems to be too high in our setting.

Median iron intake during the last months of infancy was ~6 mg/day or ~0.6 mg/kg/day, with 10th percentile values of ~4 mg/day and ~0.4 mg/kg/day (Fig. 2). These are somewhat lower than values from other Scandinavian countries, where the mean intake was between 8 and 11 mg/day (54–56). During these months 35–40% of iron intake comes from bread and home-made cereals, both of which have low iron availability, while ~40% comes from meat, fish and iron-fortified formula, with high availability. The recommended intake of iron from 6 to 12 months varies between 6 mg/day (57) and 10 mg/day (58). Lower limits for intake for the Nordic countries (58) and the UK (59) are 4.0 and 4.2 mg/day, respectively. Recommendations for total iron intake are, however, of limited use, since the absorption of iron varies from a few percent to 50%, depending on the composition of the meal, and iron absorption is higher in individuals with low iron stores. Stekel (13) and Fomon (42) have estimated the requirement of absorbed iron during this age at 0.9 and 0.75 mg/day, respectively. Owing to the variation in iron availability, several studies have failed to find an association between total iron intake and iron status (3, 4, 60). In a study comparing iron status in Asian and Caucasian infants in England, the Asian infants had lower serum ferritin values despite a higher iron intake (1). The main difference between the diets was the absence of meat in the Asian diet.

Instead of iron intake we examined the influence on iron status of the amount eaten of different food groups, since iron content and availability, and the content of enhancers and inhibitors of iron absorption, are usually the same within the different food groups. The results from multiple regression analysis of the association between intake from different food groups and changes in serum ferritin should be interpreted with caution. The number of infants in the analysis was limited and the amount eaten of some of the food groups was low. Furthermore, significant associations might not be causal since the intake of certain food groups might be a proxy for a dietary pattern that is affecting iron status. However, the significant associations found in the analysis (i.e. cow's milk and bread having a negative effect on iron status, and infant formula, meat, and fish having a positive effect) are in accordance with what would be expected from present knowledge. A positive effect of

infant formula on iron status from 6 to 9 months was expected because of iron fortification, but was not found.

The strong negative association between bread intake and changes in serum ferritin from 6 to 9 months suggests an inhibitory effect of bread on iron absorption. It has been shown that the inhibitory effect of bread is mainly due to the content of phytate and other inositol phosphates (61). In the present study approximately 75% of the bread intake was brown rye bread; bread made from high extraction wheat constituted a considerable part of the remaining 25%. Brown rye bread is traditionally produced with prolonged sourdough fermentation, which reduces the phytate content effectively (61), but the degree to which phytate is reduced in industrially produced rye bread is unknown, since rapid baking processes are often used. The intake of home-made porridge, which is mainly oat porridge, containing a high level of phytate, was considerably higher than bread intake. However, this was not significantly associated with changes in serum ferritin in our study. The intake of commercially produced cereals was not associated with changes in ferritin values, but the amount eaten was low: not all cereals were fortified with iron, and the number of infants in the analysis was small.

The positive effect of meat and fish intake on iron status in this study, despite the low amounts eaten, warrants further evaluation of the efficiency of meat and fish intake in the prevention of iron anaemia during the second half of infancy. Higher meat and fish consumption would also improve zinc intake. Studies suggest that zinc deficiency is not uncommon at this age (62, 63), and we found indications of mild zinc deficiency in the present cohort (64).

In conclusion, iron deficiency anaemia was not present in this cohort up to 9 months of age, but some of the infants had indications of marginal iron stores at this age. These infants might be at risk of developing iron deficiency anaemia later if their diet does not contain sufficient amounts of available iron. Those with a high growth velocity will have an increased risk of depleting their iron stores, and our data suggest that a high intake of bread and cow's milk and a low intake of meat and fish during the last half of infancy would further increase the risk. However, to conclusively suggest dietary changes in this population we need to know the prevalence of iron deficiency anaemia during the second year of life, and possible associations with dietary habit.

Acknowledgments.—Supported by a grant from Otto Monsted's Fond and from the Danish Medical Research Council (12-5957, 12-7735), the Danish Agricultural and Veterinary Research Council (13-4048), the Danish Technical Research Council (16-4338 H), the Danish Natural Research Council (11-7011), and the Research and Development Program for Food Technology (FØTEK). The authors are indebted to the families who participated in the study, to the secretaries and laboratory technicians at the Paediatric Department at Hvidovre Hospital and Research Department of Human Nutrition

who contributed considerably to the study. Furthermore, the authors thank Axel Brock for making the erythrocyte protoporphyrin analyses, and to Birthe Lykke Thomsen and Brittmarie Sandström for useful comments on the manuscript.

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Received July 25, 1994. Accepted Feb. 22, 1995